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Ageing-Induced Alterations in Lipid/Phospholipid Profiles of Rat Brain and Liver Mitochondria: Implications for Mitochondrial Energy-Linked Functions

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Abstract Effects of ageing on the lipid/phospholipid profile of brain and liver mitochondria from rats were examined. In the brain mitochondria the contents of total phospholipid (TPL) and cholesterol (CHL) increased with simultaneous increase in the TPL/CHL (mole:mole) ratio. The proportion and contents of lysophospholipid (Lyso), sphingomyelin (SPM), phosphatidylinositol (PI), phosphatidylserine (PS) and diphosphatidylglycerol (DPG) components increased, with maximal increases seen for PS and PI; phosphatidylcholine (PC) and phosphatidylethanolamine (PE) components registered decrease. In the liver mitochondria contents of TPL and CHL increased. However, the TPL/CHL (mole:mole) ratio was not altered. Lyso, PI and PS increased. However, the magnitude of increase was competitively lower; PE and DPG decreased. SPM and PC did not change as a consequence of ageing. These changes altered the contents of individual phospholipids in the two membrane systems. Respiration with glutamate, pyruvate + malate, succinate and ascorbate + *N,N,N',N'*-tetramethyl-*p*-phenylenediamine was significantly impaired in brain mitochondria from old animals. For liver mitochondria the respiratory activity declined with glutamate and succinate. Correlation studies by regression analysis revealed that the lipid/phospholipid classes regulate respiratory function differently in the mitochondria from the two tissues. The respiration-related parameters in the brain mitochondria were dependent on multiple lipid/phospholipid components, and the process of regulation was complex compared to the liver mitochondrial functions.

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Introduction

Ageing, a unique feature of all organisms, is accompanied by impaired functional capacity of many systems. With ageing there is a gradual decline in the capacity of various cell types including neurons (Toescu, Myronova & Verkhratsky, 2000). Also, the levels of several hormones, including the thyroid hormones insulin, dehydroepiandrosterone (DHEA), dehydroepiandrosterone sulfate (DHEAS), testosterone, estradiol and leptin, as well as others, decline with ageing (Elmlinger et al., 2003; Moreira et al., 2005; Baranowska et al., 2007). It has been shown that the thyroid hormones insulin and DHEA significantly influence the lipid/phospholipid makeup of subcellular organelles including mitochondria (Pasquini et al., 1980; Ruggiero et al., 1984; Hostetler, 1991; Bangur, Howland & Katyare, 1995; Parmar et al., 1995; Dugan & Porter, 1997; Patel & Katyare, 2006a, b). It may hence be anticipated that age-related changes could occur in the lipid/phospholipid profiles of mitochondria.

It has also been reported that the oxidative energy metabolism of mitochondria from various tissues including brain and liver is significantly diminished in aged animals (Hansford, 1983; Kim et al., 1988a; Kim, Shrago & Elson, 1988b; Patel & Katyare, 2006b; Patel, Modi & Katyare, 2007). Since several components of the electron transport chain require specific lipids/phospholipids for their function (Daum, 1985), the ageing-related changes in lipid/phospholipid profiles can in turn lead to alteration(s) in the function(s) of the components of the electron transport chain.

Age-related changes in the contents of lipids in brain regions in experimental animals and in humans have been documented (Giusto, Roque & Ilincheta de Boschero, 1992; Soderberg et al., 1990; Delion et al., 1997). Changes in the enzymes involved in lipid metabolism in rat liver have also been reported (Bourre, 2004). In particular, the emphasis has been on alterations in the fatty acid composition (Ilincheta de Boschero et al., 2000; Carver et al., 2001). A few reports describe the effect of ageing on the lipid/phospholipid composition of mitochondria. However, the results are equivocal (Kim et al., 1988a, 1988b; Ruggiero et al., 1992).

Therefore, in the present study, we examined the effect of ageing on lipid/phospholipid profiles of rat brain mitochondria. Parallel studies were also carried out on liver mitochondria since liver is the major site of metabolism and lipid biosynthesis. It was felt that these studies will help to address the question as to whether the ageing-related changes are tissue-specific. We also attempted to correlate the observed changes with respiratory functions of the mitochondria. Results of our studies indicate that ageing differentially affected the mitochondrial lipid/phospholipid makeup and respiratory functions of mitochondria from brain and liver. Our studies also point out that the effects were more prominent and pronounced for brain mitochondria. Our present results also suggest that altered lipid/phospholipid profiles of brain mitochondria have a major influence on the respiratory function of the brain mitochondria.

Materials and Methods

Chemicals

ADP, rotenone, NAD^+, NADH, 4-morpholinopropanesulfonic acid (MOPS), dichlorophenolindophenol (DCIP), *N,N,N',N'*-tetramethyl-*p*-phenylenediamine (TMPD) and bovine serum albumin (BSA) fraction V were purchased from Sigma (St. Louis, MO). Sources of the substrates used for measurement of oxidative phosphorylation were as detailed previously (Patel & Katyare, 2006b; Patel et al., 2007). Silica gel G was from E. Merck (Darmstadt, Germany). All other chemicals were of analytical-reagent grade and were purchased locally.

Animals

Male young adult (8–10 weeks, body weight 220–230 g) and old (18–24 months, body weight 350–370 g) albino rats of Charles-Foster strain were used. The experimental protocol was approved by the Departmental Animal Ethics Committee.

Isolation of Mitochondria

Isolation of brain and liver mitochondria was essentially according to the procedures described previously with some modifications (Katyare, Balasubramanian & Parmar, 2003; Katewa & Katyare, 2004; Pandya, Dave & Katyare, 2004; Katyare & Rajan, 2005; Patel & Katyare, 2006a, b; Patel et al., 2007).

Lipid Analysis

The extraction of mitochondrial lipids/phospholipids with freshly prepared chloroform and methanol (2:1vol/vol) was according to the procedures described in detail previously (Folch, Lees & Sloane-Stanley, 1957; Pandya et al., 2004).

Separation of phospholipid classes by thin layer chromatography (TLC) and estimations of cholesterol (CHL) and phospholipid phosphorus were according to the procedures described earlier (Zlatkis, Zak & Boyle, 1953; Bartlett, 1954; Skipski et al., 1967; Pandya et al., 2004).

The content of individual phospholipid classes were calculated by multiplying the values of total phospholipid (TPL) with the percent composition of the individual phospholipid classes (Pandya et al., 2004).

Oxidative Phosphorylation

Measurements of oxidative phosphorylation were carried out at 25°C using a Clark-type oxygen electrode as described previously (Katewa & Katyare, 2004; Patel & Katyare, 2006b; Patel et al., 2007). Briefly, the respiration medium (total volume 1.6 ml) consisted of 225 mM sucrose, 20 mM KCl, 10 mM MOPS (pH 7.4), 5 mM potassium phosphate buffer (pH 7.4), 0.2 mM ethylenediaminetetraacetic acid (EDTA) and 160 mg BSA (i.e., 0.1 mg BSA/ml).

Determination of state 3 and state 4 respiration rates and calculation of ADP/O ratio and ADP phosphorylation rates were as described previously (Ferreira & Gil, 1984; Katyare & Satav, 1989).

Enzyme Assays

Glutamate dehydrogenase (GDH), malate dehydrogenase (MDH) and succinate DCIP reductase (SDR) activities were determined as described previously (Patel & Katyare, 2006a). The ATPase activity in the brain mitochondria was determined using the assay medium (total volume 0.1 ml) containing 350 mM sucrose, 10 mM MOPS (pH 7.4), 10 mM KCl, 0.2 mM EDTA, 2 mM $MgCl_2$ and 50 μM dinitrophenol

(DNP). For liver mitochondria the assay medium (total volume 0.1 ml) consisted of 50 mM MOPS (pH 7.4), 75 mM KCl, 0.4 mM EDTA, 6 mM $MgCl_2$ and 100 μM DNP (Katewa & Katyare, 2004; Patel & Katyare, 2006b; Patel et al., 2007). Estimation of inorganic phosphate was according to the procedure described (Katewa & Katyare, 2004). Protein estimation was by the method of Lowry et al. (1951) using BSA as the standard. Regression analysis was performed using Jandel Sigmastat Statistical Software, version 2.0 (Jandel, San Rafael, CA). Results are given as mean ± standard error of the mean (SEM). Statistical evaluation of the data was by Students' *t*-test.

Results

Data in Table 1 show that with increase in age the body and the brain weights increased, respectively, by 61% and 17%. This disproportionate increase was also reflected in the lowering of the relative brain weight (27% decrease). Compared to this, both liver weight as well as relative liver weight decreased significantly with ageing (21% and 51%, respectively).

Data in Table 2 show that with ageing the TPL and CHL content of the brain mitochondria increased by 33% and 22%, respectively. This disproportionate increase was also reflected in a small but reproducible increase in the TPL/CHL (mole:mole) ratio.

Table 1 Effect of ageing on body weight, brain weight and liver weight

Parameters	Young adult (12)	Old (10)
Body weight (g)	233.9 ± 7.43	376.5 ± 8.62*
Brain weight (g)	1.52 ± 0.03	1.78 ± 0.01*
Brain weight (% of body weight)	0.66 ± 0.02	0.48 ± 0.01*
Liver weight (g)	8.62 ± 0.30	6.79 ± 0.25*
Liver weight (% of body weight)	3.69 ± 0.06	1.80 ± 0.03*

Experimental details are given in the text. Results are mean ± SEM of the number of observations indicated in parentheses

* $P < 0.001$ compared with the corresponding young adult group

Examination of the phospholipid profile revealed that in old animals the proportion of lysophospholipid (Lyso), sphingomyelin (SPM) and diphosphatidylglycerol (DPG) components increased by 30–54%. However, the most dramatic increase was in the proportion of the acidic phospholipids (APL) phosphatidylinositol (PI) and phosphatidylserine (PS) components (2.7-fold increase in both). The phosphatidylcholine (PC) and phosphotidylethanolamine (PE) components were reduced by 19% and 27% in old animals, respectively (Table 3).

Data in Table 4 detail the content of individual phospholipids in the brain mitochondria. It is clear that in the old animals the content of Lyso and SPM increased by 99% and 74%, respectively. As is to be expected, a pronounced 3.9-fold increase in the content of PI and PS was evident; under these conditions the DPG content doubled. Interestingly, the contents of PC and PE were unchanged (Table 4).

For liver mitochondria, compared to brain mitochondria, an opposite picture was noted with respect to TPL and CHL contents. Thus, in old animals the TPL content increased by 23% and the CHL content increased by 37%. The TPL/CHL (mole:mole) ratio was somewhat lowered, but the decrease was not statistically significant (Table 2).

The phospholipid composition of liver mitochondria was also affected by age. Thus, in old animals the Lyso component increased by 82%. Of the acidic phospholipids, the increase in PI was marginal (46%), whereas PS components increased by 1.31-fold. Paradoxically, the DPG component showed a 20% decrease. SPM and PC were unchanged, while PE decreased by 9% (Table 3).

The data in Table 4 show the contents of individual phospholipids in the liver mitochondria. Thus, in old animals the contents of Lyso, SPM and PC increased, respectively, by 1.25-, 0.36- and 0.24-fold. The increase in the contents of acidic phospholipids PI and PS was 82% and 81%, respectively; the content of DPG was not changed. Also, PE content was unchanged (Table 4).

The foregoing results prompted us to evaluate if the observed changes in the lipid/phospholipid profiles correlated with and had bearing on the energy-linked functions in the mitochondria from the two tissues. With a view to

Table 2 Effect of ageing on TPL and CHL contents of rat brain and liver mitochondria

Mitochondria	Animals	TPL (μg/mg protein)	CHL (μg/mg protein)	TPL/CHL (mole:mole)
Brain	Young adult	423.4 ± 12.93	385.0 ± 6.74	0.55 ± 0.01
	Old	563.1 ± 7.76*	470.8 ± 7.49**	0.60 ± 0.02*
Liver	Young adult	180.7 ± 7.71	48.6 ± 1.63	1.86 ± 0.08
	Old	222.9 ± 6.28**	66.7 ± 3.41**	1.69 ± 0.08

Experimental details are given in the text. Results are mean ± SEM of eight independent observations in each group

* $P < 0.05$, ** $P < 0.001$ compared with the corresponding young adult group

Table 3 Effect of ageing on phospholipid composition of rat brain and liver mitochondria

Phospholipid class	Composition (% of total)			
	Brain		Liver	
	Young adult	Old	Young adult	Old
Lyso	3.59 ± 0.24	5.29 ± 0.37**	1.55 ± 0.04	2.82 ± 0.27**
SPM	6.47 ± 0.10	8.43 ± 0.57**	3.19 ± 0.09	3.50 ± 0.22
PC	41.73 ± 0.16	33.65 ±1.06**	46.21 ±1.04	46.42 ± 1.09
PI	2.41 ± 0.08	8.87 ± 0.63**	2.40 ± 0.17	3.51 ± 0.25*
PS	2.66 ± 0.08	9.77 ± 0.55**	1.83 ± 0.11	4.23 ± 0.28**
PE	39.86 ± 0.17	28.98 ± 1.60**	32.42 ± 0.59	29.62 ± 0.30**
DPG	3.27 ± 0.14	5.02 ± 0.24**	12.40 ± 0.69	9.91± 0.43*

Experimental details are given in the text. Results are mean ± SEM of eight independent observations in each group
* $P < 0.01$, ** $P < 0.001$ compared with the corresponding young adult group

Table 4 Effect of ageing on phospholipid content of rat brain and liver mitochondria

Phospholipid class	Content (μg/mg protein)			
	Brain		Liver	
	Young adult	Old	Young adult	Old
Lyso	15.06 ± 0.75	29.93 ± 2.31**	2.81 ± 0.17	6.31 ± 0.68**
SPM	27.45 ± 1.11	47.72 ± 3.76**	5.76 ± 0.32	7.83 ± 0.58*
PC	176.74 ± 5.67	189.56 ± 6.93	83.47 ± 3.85	103.47 ± 3.78*
PI	10.24 ± 0.53	49.97 ± 3.61**	4.30 ± 0.30	7.81 ± 0.58**
PS	11.25 ± 0.50	55.06 ± 3.31**	3.32 ± 0.26	9.34 ± 0.44**
PE	168.82 ± 5.39	162.73 ± 8.06	58.63 ± 2.91	6.06 ± 2.30
DPG	13.82 ± 0.67	28.17 ± 1.19**	22.38 ± 1.61	22.05 ± 1.00

Experimental details are given in the text. Results are mean ± SEM of eight independent observations in each group
* $P < 0.01$, ** $P < 0.001$ compared with the corresponding young adult group

further explore this point, we examined the effect of ageing on oxidative energy metabolism and related enzyme activities. These data are given in Tables 5–7

From the data in Table 5 it becomes evident that in the brain mitochondria state 3 respiration (in the presence of added ADP) with all the substrates tested decreased significantly (18–36%). The effect on state 4 respiration was variable; increase with pyruvate + malate (54% increase) and decrease with succinate and ascorbate + TMPD (17% and 50%, respectively). The respiratory control ratio (RCR) decreased for all the substrates, indicating that in old animals the mitochondria were less tightly coupled. Although the ADP/O ratios were unchanged, the potential for ATP synthesis, i.e., ADP phosphorylation rates, decreased significantly (24–38%). The levels of dehydrogenases also decreased significantly in old rats (21–73%), with maximal decrease seen for SDR activity. ATPase activity was somewhat low, but the change was not statistically significant (Table 7).

In liver mitochondria, state 3 respiration decreased only with glutamate and succinate (25% and 33%, respectively) and state 4 respiration decreased with glutamate by 36%. The values of RCR were unchanged. ADP phosphorylation rates with glutamate and succinate as the substrates decreased by 24% and 30%, respectively (Table 6). The trend for the dehydrogenases and ATPase activities was similar to that for the brain mitochondria. However, ATPase activity decreased drastically by 41% (Table 7).

The foregoing results thus emphasize that ageing differentially affected the oxidative energy metabolism of the mitochondria from brain and liver and that the effects were more pronounced on the cerebral mitochondria. These results are in general agreement with our previously reported observations (Patel & Katyare, 2006b; Patel et al., 2007).

We tried to correlate the changes in lipid/phospholipid profile and the respiratory functions of the mitochondria from the two tissues by regression analysis. These data are given in Tables 8 and 9. As can be noted, in brain mitochondria state 3 respiration rates in general correlated positively with PC and PE, whereas TPL, CHL, PS, PI, DPG and acidic phospholipid/basic phospholipid (APL/BPL) ratio seemed to be the negative modulators. Additionally, SPM/PC and SPM/PE ratios seemed to be negative modulators except for glutamate. State 4 respiration with glutamate was independent of lipid/phospholipid modulation, as was the case even for succinate. In the case of pyruvate + malate, TPL, CHL, PS, PI, DPG, SPM/PC and APL/BPL were positive modulators, while PE was a negative modulator. For ascorbate + TMPD system, the role was reversed and TPL, CHL, PI, PS, PC/PE, SPM/PE and APL/BPL became negative modulators; PS was a positive modulator.

Table 5 Effect of ageing on oxidative phosphorylation in rat brain mitochondria using glutamate, pyruvate+malate, succinate and acsorbate+TMPD as substrates

Substrate	Animals	ADP/O ratio	Respiration rate (nmole O_2/min/mg protein)		Respiratory control ratio	ADP phosphorylation rate (nmole/min/mg protein)
			+ ADP	-ADP		
Glutamate	Young adult (12)	3.17 ± 0.10	19.9 ± 1.0	5.2 ± 0.3	3.86 ± 0.14	125.9 ± 7.5
	Old (12)	3.07 ± 0.12	12.8 ± 0.5***	5.2 ± 0.5	2.64 ± 0.20***	77.6 ± 2.2***
Pyruvate+malate	Young adult (12)	3.17 ± 0.19	21.7 ± 1.3	5.0 ± 0.5	4.41 ± 0.54	135.8 ± 7.6
	Old (10)	2.92 ± 0.15	17.9 ± 0.8*	7.7 ± 0.7***	2.44 ± 0.17**	102.6 ± 3.2***
Succinate	Young adult (12)	2.07 ± 0.06	24.2 ± 0.8	14.0 ± 0.8	1.83 ± 0.17	99.9 ± 3.7
	Old (10)	2.07 ± 0.16	16.9 ± 0.8***	11.6 ± 1.1***	1.53 ± 0.10	69.6 ± 6.0***
Ascorbate+TMPD	Young adult (16)	0.70 ± 0.03	23.7 ± 1.7	14.3 ± 1.0	1.67 ± 0.04	32.6 ± 2.1
	Old (12)	0.75 ± 0.06	16.1 ± 0.8***	11.9 ± 0.6*	1.36 ± 0.04***	24.1 ± 2.1***

The respiration medium (total volume 1.6 ml) consisted of 225 mM sucrose, 20 mM KCl, 10 mM MOPS (pH 7.4), 5 mM potassium phosphate buffer (pH 7.4), 0.2 mM EDTA and 160 mg BSA (i.e., 0.1 mg BSA/ml). Concentration of glutamate was 10 mM. State 3 respiration rates initiated by addition of 80–200 nmoles of ADP and state 4 rates ensuing after its depletion were recorded. Other experimental details are given in the text. Results are mean ± SEM of the number of observations indicated in parentheses

* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ compared with the young adult group

Table 6 Effect of ageing on oxidative phosphorylation in rat liver mitochondria using glutamate, pyruvate+malate, succinate and ascorbate+TMPD as substrates

Substrate	Animals	ADP/O ratio	Respiration rate (nmole O_2/min/mg protein)		Respiratory control ratio	ADP phosphorylation rate (nmole/min/mg protein)
			+ ADP	-ADP		
Glutamate	Young adult (12)	3.11 ± 0.07	27.3 ± 1.1	10.2 ± 0.4	2.70 ± 0.08	168.4 ± 5.5
	Old (10)	3.11 ± 0.11	20.6 ± 0.9*	6.5 ± 0.4*	3.26 ± 0.15	128.1 ± 7.5*
Pyruvate+malate	Young adult (12)	3.25 ± 0.09	16.8 ± 0.6	7.7 ± 0.3	2.21 ± 0.09	109.1 ± 4.5
	Old (12)	3.11 ± 0.15	15.4 ± 0.7	8.1 ± 0.4	1.97 ± 0.14	97.0 ± 8.1
Succinate	Young adult (12)	2.17 ± 0.07	57.0 ± 2.6	22.1 ± 1.2	2.65 ± 0.15	246.3 ± 11.1
	Old (12)	2.30 ± 0.14	38.0 ± 2.3*	20.5 ± 1.3	1.88 ± 0.08	172.0 ± 11.9*
Ascorbate+TMPD	Young adult (14)	0.44 ± 0.03	26.7 ± 1.3	20.7 ± 1.2	1.30 ± 0.04	23.3 ± 1.5
	Old (21)	0.44 ± 0.03	27.8 ± 1.2	21.1 ± 1.1	1.33 ± 0.03	24.1 ± 1.2

The respiration medium (total volume 1.6 ml) consisted of 225 mM sucrose, 20 mM KCl, 10 mM MOPS (pH 7.4), 5 mM potassium phosphate buffer (pH 7.4), 0.2 mM EDTA and 160 mg BSA (i.e., 0.1 mg BSA/ml) Concentration of glutamate was 10 mM. State 3 respiration rates initiated by addition of 80–200 nmoles of ADP and state 4 rates ensuing after its depletion were recorded. Other experimental details are given in the text. Results are mean ± SEM of the number of observations indicated in parentheses

* $P < 0.001$ compared with the young adult group

Table 7 Effect of ageing on mitochondrial enzymes

Enzyme	Brain		Liver	
	Young adult	Old	Young adult	Old
GDH	60.2 ± 1.33	47.8 ± 2.07*	202.9 ± 5.60	145.8 ± 8.68*
MDH	4,680 ± 141	2,954 ± 146*	4,214 ± 146	2,901 ± 154*
SDR	14.6 ± 0.40	4.0 ± 0.11*	20.4 ± 0.68	5.6 ± 0.31*
ATPase	5.4 ± 0.31	4.6 ± 0.27	20.1 ± 0.86	11.8 ± 0.49*

Experimental details are given in the text. Enzyme activities are given as nanomoles of substrate transformed per minute per milligram of protein. Results are given as mean ± SEM of 12–15 observations

* $P < 0.001$ compared with the corresponding young adult group

For liver mitochondria, PE positively modulated state 3 and state 4 respiration rates with glutamate. For succinate PE and DPG were the positive modulator, whereas for ascorbate + TMPD system CHL seemed to be the positive modulator. The state 4 respiration rates with succinate correlated positively with DPG and APL/BPL ratio. On the negative modulator list of state 3 and state 4 respiration rate of glutamate were CHL, PI and APL/BPL; SPM/PE was an additional negative modulator for state 4 respiration. The state 3 and state 4 respiration rates with succinate were negatively correlated with PC, PI, PC/PE and PC. For ascorbate + TMPD system, both respiration rates correlated negatively with TPL/CHL

ratio. Interestingly, state 3 and state 4 respiration rates were independent of any lipid modulation.

Regression analysis for enzyme activities revealed that the positive and negative modulators for GDH in brain mitochondria were PE and PS. For MDH, SDR and ATPase, PC and PE were common positive modulators, whereas TPL, CHL, SPM, PS, PI, DPG, TPL/CHL, SPM/PC, SPM/PE and APL/BPL were common negative modulators. For liver mitochondria, PE emerged as the common positive modulator for MDH, SDR and ATPase. Additionally, DPG correlated positively with the latter two enzymes. SPM/PC and PI were common negative modulators for SDR and ATPase. PS and SPM/PE correlated negatively with SDR activity.

The foregoing results thus suggest that the lipid/phospholipid components of the membranes differentially modulate the respiratory function–related parameters in a tissue-specific manner. The result also points out that the regulation of brain enzymes is a more complex process than that in the liver mitochondria.

Discussion

Reports in the literature indicate that in the brain from old rats the pathways of phospholipid synthesis as well as the composition of phospholipid-specific fatty acids are altered significantly (Ilincheta de Boschero et al., 2000). The changes include decrease in docosahexaenoic acid and arachidonic acid and increase in the content of 18:1 monounsaturated fatty acid (MUFA) (Lopez et al., 1995). In the rat hippocampus, loss of docosahexaenoic acid in PE, plasmenylethanolamine (PmE) and PS with increasing age has been reported (Favreliere et al., 2003). Also, ageing significantly reduced PE levels in the frontal cortex of rat brain (Favreliere et al., 2000). The aforementioned changes can thus alter membrane fluidity. The alternative pathways for phospholipid synthesis referred to above have been suggested to represent the compensatory mechanism to provide a pool of phospholipid classes for the maintenance of cellular membrane lipid composition during ageing (Ilincheta de Boschero et al., 2000). However, no significant changes occurred in the composition or content of phospholipid classes in the whole brain (Ilincheta de Boschero et al., 2000). Interestingly, Soderberg et al. (1990) reported that in human brain the TPL content decreased marginally (5–10%) with age and the phospholipid composition changed differently in the various brain regions, whereas CHL content showed a 0–40% decrease.

The changes in liver lipid synthesis in aged rats have been suggested to relate to modifications in lipid homeostasis induced by altered hormonal balance (Ilincheta de Boschero et al., 2000; Favreliere et al., 2000; Toescu et al., 2000). Toescu et al. (2000) suggested that gradual, age-dependent impairment of mitochondrial function is an important factor in the decrease of this "homeostatic reserve." The differential changes which we observe here (Tables 2–4) may also relate to differential responses of the two tissues to hormonal changes in old rats.

The results of our present studies have shown that the TPL and CHL contents of the brain and liver mitochondria increased in old rats in a tissue-specific manner. Consequently, the TPL/CHL (mole:mole) ratio increased in the brain mitochondria but was not affected in the liver mitochondria. Our results differ from the observation of Grinna (1977a), who reported that the TPL/CHL (mole:mole) ratio decreased in liver and kidney mitochondria. The observed difference may be attributed to the differences in the strain of rats; Grinna used Sprague-Dawley rats for these studies. Strain-dependent variations in the age-related changes in lipid profiles of plasma and liver lipids of Yoshida and Wistar rats have been reported (Masella et al., 1995).

The phospholipid profiles of the mitochondria from the two tissues also changed in a tissue-specific manner (Tables 3 and 4). Although there were similarities and differences in the observed changes, it became evident that ageing influenced maximally the lipid/phospholipid profiles of the brain mitochondria (Tables 2–4). The common feature of ageing was a significant increase in the Lyso component in the mitochondria from both tissues, which may be attributed to increased phospholipase activity associated with ageing. Altered phosphatidate phosphohydrolase and phospholipase D activities in the aged brain have been reported (Pasquare, Ilincheta de Boschero & Giusto, 2001). Although SPM, PI and PS contents increased in mitochondria from both tissues, the magnitude of increase was always higher for brain mitochondria. Significantly increased levels of SPM in the whole brain as well as in specific brain regions of old rats have been reported (Delion et al., 1997; Giusto et al., 1992; Aureli et al., 2000). Also, the contents of PI and PS increased significantly in the hippocampus of old rats (Delion et al., 1997). The increased synthesis of PS in the cerebral cortex and cerebellum of aged rats has been attributed to increased serine base-exchange activity (Giusto et al., 2002). The content of DPG in brain mitochondria doubled in aged rats, whereas in liver mitochondria the proportion of DPG decreased. A similar 20% decrease in the content of DPG in liver and kidney mitochondria from old rats has been reported, although the changes were not statistically significant (Grinna, 1977a). The contents of PC and PE were unchanged in brain mitochondria, while in liver mitochondria PC content increased.

The increases in TPL and CHL contents would result in an increased ratio of lipid/protein and add to the lipid bulk in the membrane. Additionally, alterations in the

Table 8 Correlation between respiratory activity of rat brain and liver mitochondria with membrane lipid/phospholipid composition

Substrate	Respiration	Brain mitochondria		Liver mitochondria	
		Positive	Negative	Positive	Negative
Glutamate	State 3	PC (r = +0.707) PE (r = +0.696)	TPL, CHL, PS, PI, DPG, APL/BPL ($r \leq -0.650$) (range –0.797 to −0.650)	PE (r = +0.596)	CHL, PI, APL/BPL ($r \leq -0.553$) (range –0.766 to −0.553)
	State 4	–	–	PE (r = +0.580)	CHL, Lyso, PI, SPM/PE, APL/BPL ($r \leq -0.536$) (range – 0.734 to − 0.536)
Pyruvate+malate	State 3	PC (r = +0.759) PE (r = +0.696)	TPL, CHL, PS, PI, DPG, SPM/PC, APL/BPL, ($r \leq -0.621$) (range –0.688 to −0.621)	–	–
	State 4	TPL, CHL, PS, PI, DPG, SPM/PC, APL/BPL ($r \geq +0.591$) (range +0.591 to +0.701)	PE (r = −0.736)	–	–
Succinate	State 3	PC (r = +0.711) PE (r = +0.761)	TPL, CHL, PS, PI, DPG, SPM/PC, SPM/PE, APL/BPL ($r \leq -0.529$) (range –0.816 to −0.529)	PE (r = +0.596) DPG (+0.677)	PS, PI, PC/PE ($r \leq -0.598$) (range − 0.677 to −0.598)
	State 4	–	–	DPG (+0.592) APL/BPL(+0.601)	PC (− 0.614)
Ascorbate+TMPD	State 3	PC (r = +0.784) PE (r = +0.778)	TPL, CHL, SPM, PS, PI, DPG, SPM/PC, SPM/PE, APL/BPL ($r \leq -0.571$) (range –0.881 to −0.571)	CHL (r = +0.648)	TPL/CHL (r = − 0.682)
	State 4	PS (r = +0.636) DPG (+ 0.663)	TPL, CHL, PI, PE, PC/PE, SPM/PE, APL/BPL ($r \leq -0.586$) (range –0.773 to −0.586)	–	TPL/CHL (r = − 0.589)

Values in parentheses represent regression coefficient r, which is based on eight independent experiments in each group

proportion and contents of individual phospholipid classes would lead to altered charge distribution across the membrane. This in turn could affect the permeability properties of the membrane, besides affecting the catalytic activity of the specific enzymes (e.g., Table 9). Requirement of phospholipid components for the proper functioning of the component electron transport chain has been well documented (Daum, 1985). In particular, it has been shown that cytochrome oxidase and FoF_1 ATPase have a specific requirement for DPG. Similarly, succinate oxidase has requirement for bulk phospholipids for its activity (Daum, 1985). In earlier studies we reported that the cytochrome aa_3 content of brain mitochondria decreased significantly in old rats, whereas that in the liver was not affected (Patel & Katyare, 2006b; Patel et al., 2007). Similarly, with ageing the FoF_1 ATPase activity decreased significantly in liver mitochondria (Patel et al., 2007). Viewed in this context, the increased content of DPG in brain mitochondria which we observed here may represent a compensatory mechanism to aid the enzyme activity, especially cytochrome oxidase. In the liver, where only FoF_1 ATPase activity decreased, the attempt is to retain the DPG content near the young adult level.

Another interesting feature of our studies was the increased content of PS. It has been shown that in experimental animals and in human trials nutritional supplementation with PS improved memory and cognitive functions, while PC was ineffective in this respect (McDaniel, Maier & Einstein, 2003). One therefore wonders whether increased PS and PI contents represent a compensatory mechanism to improve cognitive functions. Increased content of PS, however, raises some concern since externalization of PS is a signal for apoptosis (Mourdjeva et al., 2006).

Ruggiero et al. (1992) reported that in synaptic and nonsynaptic mitochondria the cholesterol and phospholipid contents decreased by 27% and 12%, respectively. Among the phospholipids, only the cardiolipin fraction showed a

Table 9 Correlation between enzymatic parameters of brain and liver with membrane lipid/phospholipid composition

Enzymes	Brain mitochondria		Liver mitochondria	
	Positive	Negative	Positive	Negative
GDH	PE (+ 0.564)	PS (−0.565)	–	SPM/PC (−0.535)
MDH	PC (+ 0.709) PE (+ 0.851)	TPL, CHL, SPM, PS, PI, DPG, TPL/CHL, SPM/PC, SPM/PE, APL/BPL, ($r \leq -0.592$) (range –0.888 to −0.592)	PE (+ 0.585)	PI (−0.605)
SDR	PC (+ 0.809) PE (+ 0.875)	TPL, CHL, SPM, PS, PI, DPG, TPL/CHL, SPM/PC, SPM/PE, APL/BPL, ($r \leq -0.554$) (range –0.938 to −0.554)	PE (+ 0.696) DPG (+ 0.599)	TPL, CHL, PS, PI, SPM/PE ($r \leq -0.559$) (range –0.877 to −0.559)
ATPase	PC (+ 0.778) PE (+ 0.785)	TPL, CHL, SPM, PS, PI, DPG, TPL/CHL SPM/PC, SPM/PE, APL/BPL, ($r \leq -0.554$) (range –0.857 to −0.554)	PE (+ 0.524) DPG (+ 0.599)	TPL, CHL, Lyso, PI, ($r \leq -0.679$) (range –0.874 to −0.679)

Values given in parentheses indicate regression coefficient r, which is based on eight independent experiments in each group

significant decrease (26%) in the nonsynaptic mitochondria from the brains of aged rats. Our results are at variance with these observations. In this context it may be pointed out that Ruggiero et al. (1992) achieved the separation of phospholipids by high-performance liquid chromatography (HPLC), which resolves in five peaks. In our studies separation by TLC enabled us to the resolve phospholipids into seven distinct classes. It is possible that resolution of phospholipid classes by HPLC in their studies might not have given a fine resolution, probably due to intermixing of the components (Ruggiero et al., 1992).

Our present studies also emphasize that ageing caused a significant decrease in the respiratory activity of brain mitochondria with all the substrates tested, thereby significantly impairing the energy potential (Table 5). Against this, in the liver mitochondria, respiration only with glutamate and succinate decreased (Table 6). It may hence be suggested that impairment in oxidation of glutamate and succinate may be a primary event in ageing. Nevertheless, the total energy potential of the liver tissue would also decrease significantly due to drastic reduction in the tissue weight. It may hence be suggested that hormonal imbalance in ageing may affect the energy metabolism of tissues in a differential manner (Tables 1, 5, 6).

The interesting observation that emerged from our attempts to correlate the changes in lipid/phospholipid profiles with respiratory functions was that the regulation of these functions is also tissue-specific. It also became apparent that, compared to liver mitochondria, the regulation of respiratory functions in brain mitochondria is a complex process involving several lipid/phospholipid components and molar ratios (Tables 8 and 9). As referred to above, requirement of specific lipid/phospholipid classes for function of several mitochondrial enzymes has been reported (Daum, 1985). These studies were carried out mainly with liver or heart mitochondria as the model system (Daum, 1985). Our present studies, however, suggest that, indeed, no common rule applies and the requirements seem to be tissue-specific. For example, while PE was important for SDR activity in mitochondria from both tissues, brain mitochondria had an additional requirement for PC. By contrast, for liver mitochondria, DPG was an additional requirement (Table 9). Also, the negative modulators for these two enzyme systems for mitochondria from the two tissues varied widely. A similar picture of involvement of different phospholipid classes for GDH and MDH also became evident (Table 9). The results thus suggest that these requirements, more likely than not, are tissue-specific and there may not be a general rule of thumb.

Morphological and biochemical alterations are associated with a progressive age-related cognitive deficit (Pasquare et al., 2001). Also, the turnover rates of the individual phospholipids are affected differentially with ageing in the synaptic membrane and liver mitochondria (Grinna, 1977b; Ando et al., 2002). When such changes, including the compositional changes, occur in the brain, the susceptibility to neurodegenerative diseases amplifies considerably. Although some of the neurodegenerative diseases are also associated with some degree of mitochondrial dysfunction, it is not yet clear if these changes are due to the underlying process of normal physiological ageing or to the specific pathophysiological agents responsible for the neurodegenerative processes. Furthermore, it was proposed that important differences exist between normal ageing and neurodegeneration (Toescu

et al., 2000). Abnormalities in lipid/phospholipid profiles and activation of phospholipase A_2 are known to affect cerebral and mitochondrial functions in pathological conditions such as Parkinson disease, neural trauma, neurodegenerative disease and Alzheimer disease (Farooqui, Ong & Horrocks, 2004). Decrease in the levels of PI 18% and a 22% increase in SPM have been reported in Down syndrome patients (Murphy et al., 2000). It has been suggested that free radical also contribute to brain ageing by promoting lipid peroxidation, thus introducing modifications in membrane fluidity (Viani et al., 1991; Poon et al., 2004).

In conclusion, our present results show that as a consequence of ageing very major changes occur in the lipid/phospholipid components and respiratory functions of brain mitochondria. Compared to these changes the alterations in liver mitochondrial membranes are of lesser magnitude. The results thus suggest that brain mitochondria may be the primary target in the normal ageing process.

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