Stimulation of oxidative energy metabolism in liver mitochondria from old and young rats by treatment with dehydroepiandrosterone (DHEA). A comparative study

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Abstract Effects of treatment with DHEA (0.2 or 1.0 mg/kg body weight for 7 days) on oxidative energy metabolism of rat liver mitochondria from old (18–24 month old) and young (8–10 weeks old) male albino rats belonging to Charles-Foster strain were examined. Treatment with 1.0 mg DHEA resulted in increased body weights of the young rats without change in the liver weight. In the old animals the liver weight increased progressively with increasing dose of DHEA without affecting body weight. The state 3 respiration rates in liver mitochondria from old animals were, in general, lower than those in the young rats. The state 3 and state 4 respiration rates increased following DHEA treatment in dose-dependent manner bringing them close to values for young animals or beyond that with the effect being more pronounced at 1.0 mg dose. Treatment with DHEA also stimulated state 3 and state 4 respiration rates in young rats in dose-dependent manner. Contents of cytochrome aa₃, b and c+c₁ increased significantly in old animals in dose-dependent manner. In the young rats the lower dose (0.2 mg) of DHEA was more effective in bringing about a maximum increase in the contents of the cytochromes; the effect declined at the higher dose (1.0 mg). DHEA treatment also stimulated the mito-

chondrial ATPase activity in the old as well as in the young rats. The dehydrogenases activities were considerably low in the old rats compared to the values for the young animals. Treatment with DHEA stimulated dehydrogenases activities in old rats in dose-dependent manner bringing them close to values for the young animals or beyond. Treatment with lower dose (0.2 mg) of DHEA maximally stimulated dehydrogenases activities in young animals.

Key words ATPase activity · cytochromes · dehydroepiandrosterone (DHEA) · dehydrogenases · liver mitochondria · oxidative energy metabolism

Introduction

Dehydroepiandrosterone (DHEA) and its sulfated ester DHEA-S show an age related pattern of synthesis and secretion in humans. The levels are low at young age, peak at adulthood and decline thereafter (Hinson and Raven 1999; Parker 1999). Based on this characteristic pattern DHEA is considered to be a youth hormone (Hinson and Raven 1999; Celec and Starka 2003). It is well recognized that the respiratory activity of mitochondria from different tissues declines with aging (Marcus et al. 1982; Sastre et al. 1996; Nakahara et al. 1998; Navarro and Boveris 2004). Earlier studies from our laboratory have shown that DHEA treatment stimulated respiratory functions in liver and brain mitochondria from young rats (Patel and Katyare 2007).

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Additionally we also found that DHEA treatment had a grater stimulatory effect on the respiratory functions in brain mitochondria from old rats than in the young animals (Patel and Katyare 2006a). The foregoing studies prompted us to examine as to whether treatment with DHEA would also influence the energy metabolism of liver mitochondria in old rats in a similar manner. These studies assume importance in view of the fact that the levels of DHEA decrease significantly in the older population and beneficial effects of exogenous supplementation with DHEA in elderly population have been claimed (Hinson and Raven 1999; Milgrom 1990; Buvat 2003). It is possible that observed beneficial effects may result due to enhancement in the energy potential of the liver which is the major site of metabolism. Thus, if DHEA is indeed a youth hormone (Hinson and Raven 1999; Celec and Starka 2003) it may be anticipated that the effects should also be manifested on the energy metabolism of liver mitochondria from the old rats.

We therefore examined the effects of DHEA treatment on energy metabolism of liver mitochondria from the old rats in comparison with the young animals. The results are summarized in the present communication.

Materials and methods

Chemical

Dehydroepiandrosterone (DHEA), (3 β -Hydroxy-5-androsten-17-one (+)-dehydroisoandrosterone) was obtained from Sigma-Aldrich Corporation, St. Louis, MO, USA. Sodium salts of succinic acid, pyruvic acid,

L-malic acid and ADP, rotenone, bovine serum albumin fraction V (BSA), 4-morpholinopropanesulfonic acid (MOPS), dichlorophenolindophenol (DCIP), NAD⁺, NADH, and oxaloacetic acid were purchased from Sigma Chemical Co. St. Louis, MO, USA. Sodium salt of L-glutamic acid was purchased from E Merck, Darmstadt, Germany. N,N,N',N'-Tetramethyl-*p*-phenylenediamine (TMPD) was obtained from British Drug Houses, Dorset, Poole, England. All other chemicals were of analytical-reagent grade and were purchased locally.

Animals and treatment with DHEA

Male young (8–10 week old) and old (18–24 month old) albino rats of Charles-Foster strain were used. At the start of the experiments the body weights of young rats was in the range of 220–230 g while that of the old rats was in the range of 350–370 g (Table 1). The animals received 0.2 or 1.0 mg DHEA/Kg body weight subcutaneously (s.c.) for 7 consecutive days. This dose regimen was decided on the basis of our earlier observation (Patel and Katyare 2007). Suspension of DHEA was prepared fresh in saline prior to injection. The controls received equivalent volume of saline vehicle. The animals were killed on the 8th day of treatment. Daily record of body weight was maintained. The experimental protocol was approved by the Departmental Animal Ethics Committee.

Isolation of mitochondria

Isolation of liver mitochondria was essentially according to the procedures described previously with some

Table 1 Effect of DHEA treatment on body weight and liver weight

Age group	Treatment	Body weight, g	Liver weight	
			g	% of body wt.
Young	Untreated (12)	243.1 $\pm$ 6.33	8.66 $\pm$ 0.56	3.50 $\pm$ 0.08
	0.2 mg DHEA (12)	251.6 $\pm$ 7.01	8.70 $\pm$ 0.31	3.51 $\pm$ 0.04
	1.0 mg DHEA (12)	268.3 $\pm$ 5.98 ^a	9.01 $\pm$ 0.63	3.62 $\pm$ 0.06
Old	Untreated (12)	378.2 $\pm$ 7.26	6.73 $\pm$ 0.21	1.72 $\pm$ 0.03
	0.2 mg DHEA (15)	372.1 $\pm$ 6.95	9.92 $\pm$ 0.41 ^b	2.66 $\pm$ 0.09 ^b
	1.0 mg DHEA (15)	381.7 $\pm$ 10.0	12.35 $\pm$ 0.59 ^b	3.24 $\pm$ 0.13 ^b

Experimental details are as given in the text. Results are given as mean $\pm$ SEM of the number of observations indicated in the parentheses.

^a $p < 0.01$ and ^b $p < 0.001$ compared with the corresponding control.

modifications (Katewa and Katyare 2004; Patel and Katyare 2007). The mitochondria were sedimented by centrifugation of the nuclei-free supernatant at $7000\times g$ for 10 min and were washed once by suspending in the isolation medium (250 mM sucrose containing 5 mM MOPS and 1 mM EDTA all at pH 7.4 and 0.25 mg BSA/ml of isolation medium) and resedimenting. Finally the pellet was suspended in the isolation medium to give a protein concentration in the range of 25–30 mg/ml. Isolation of cytosolic fraction was as described previously (Kaushal et al. 1999).

Oxidative phosphorylation

Measurements of oxidative phosphorylation were carried out at 25°C using a Clark-type oxygen electrode as described previously (Katewa and Katyare 2004; Patel and Katyare 2007). State 3 respiration rates initiated by the addition of 80–200 n moles of ADP and state 4 rates ensuing after its depletion were recorded. Calculations of ADP/O ratio and ADP phosphorylation rates were as described previously (Ferreira and Gil 1984; Katyare and Satav 1989).

Cytochrome content

The contents of cytochromes were quantified from the difference spectra of dithionite reduced versus ferri-

cyanide oxidized samples using the wavelength pairs and millimolar extinction coefficients as detailed previously. (Katewa and Katyare 2004; Patel and Katyare 2007; Subramaniam and Katyare 1990).

Assay of dehydrogenases

Glutamate dehydrogenase (GDH), malate dehydrogenase (MDH) and succinate DCIP reductase (SDR) activities were determined by the procedures described earlier (Katewa and Katyare 2004; Patel and Katyare 2007).

Assay of ATPase

The ATPase activity was determined using the assay medium (total volume 0.1 ml) consisting of 50 mM MOPS pH 7.4, 75 mM KCl and 0.4 mM EDTA. Activity determinations were carried out in the absence and presence of $MgCl_2$ (6 mM) and/or 100 μM DNP as detailed earlier (Katewa and Katyare 2004; Patel and Katyare 2007). Estimation of inorganic phosphate was according to the procedure described by Katewa and Katyare (2003).

Protein estimation was by the method of Lowry et al. (1951) using bovine serum albumin as the standard.

Results are given as mean $\pm$ SEM.

Statistical evaluation of the data was by Students' t-test.

Table 2 Effect of DHEA treatment on oxidative phosphorylation in rat liver mitochondria using glutamate as the substrate

Age group	Treatment	ADP/O ratio	Respiration rate (n mole O_2 /min/mg protein)		Respiratory control ratio	ADP phosphorylation rate (n mole/min/mg protein)
			+ADP	–ADP		
Young	Untreated (12)	3.24 $\pm$ 0.07	27.88 $\pm$ 0.95	10.80 $\pm$ 0.34	2.72 $\pm$ 0.07	180.8 $\pm$ 7.82
	0.2 mg DHEA (12)	3.06 $\pm$ 0.11	38.34 $\pm$ 1.53 ^b	14.49 $\pm$ 0.68 ^b	2.67 $\pm$ 0.05	231.8 $\pm$ 9.07 ^b
	1.0 mg DHEA (12)	3.10 $\pm$ 0.09	32.90 $\pm$ 0.73 ^b	18.38 $\pm$ 0.87 ^b	1.86 $\pm$ 0.09	204.0 $\pm$ 6.92 ^b
Old	Untreated (19)	3.23 $\pm$ 0.19	23.27 $\pm$ 1.03**	7.78 $\pm$ 0.46**	3.07 $\pm$ 0.11	149.4 $\pm$ 10.08*
	0.2 mg DHEA (15)	3.11 $\pm$ 0.13	25.73 $\pm$ 1.46	11.59 $\pm$ 0.46 ^b	2.23 $\pm$ 0.11	161.3 $\pm$ 8.51
	1.0 mg DHEA (9)	3.15 $\pm$ 0.12	30.71 $\pm$ 2.17 ^a	12.09 $\pm$ 0.82 ^b	2.59 $\pm$ 0.21	193.1 $\pm$ 11.72 ^a

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 of BSA (i.e. 0.1 mg BSA/ml). Concentration of glutamate was 10 mM. State 3 respiration rates initiated by the addition of 80–200 n moles of ADP and state 4 rates ensuing after its depletion were recorded. Other experimental details are as given in the text. Results are given as mean $\pm$ SEM of the number of observations indicated in the parentheses.

^a $p < 0.01$ and ^b $p < 0.001$ compared with the corresponding untreated group.

* $p < 0.01$ and ** $p < 0.001$ compared with the untreated young group

Table 3 Effect of DHEA treatment on oxidative phosphorylation in rat liver mitochondria using pyruvate + malate as the substrate

Age group	Treatment	ADP/O ratio	Respiration rate (n mole O ₂ /min/mg protein)		Respiratory control ratio	ADP phosphorylation rate (n mole/min/mg protein)
			+ADP	−ADP		
Young	Untreated (12)	3.19±0.07	16.49±0.80	7.59±0.26	2.18±0.10	105.2±5.89
	0.2 mg DHEA (12)	3.00±0.08	17.36±0.82	9.43±0.32 ^a	1.85±0.04	104.1±6.09
	1.0 mg DHEA (12)	3.09±0.07	33.95±2.18 ^a	17.33±1.53 ^a	2.04±0.13	211.0±15.00 ^a
Old	Untreated (17)	3.14±0.15	15.25±0.97	7.76±0.45	2.03±0.12	94.2±5.69
	0.2 mg DHEA (12)	3.24±0.21	14.61±0.82	8.33±0.58	1.78±0.08	95.1±7.10
	1.0 mg DHEA (10)	3.23±0.25	22.81±1.47 ^a	13.75±0.57 ^a	1.66±0.08	147.1±9.31 ^a

Experimental details are as given in the text and in Table 2. Concentrations of pyruvate and malate were 10 and 1 mM respectively. Results are given as mean ± SEM of the number of observations indicated in the parentheses.

^a $p < 0.001$ compared with the corresponding untreated group.

Results

The data in Table 1 show that in the young rats treatment only with higher dose (1.0 mg) of DHEA resulted in 18% increase in the body weight without any change in the liver weight (Table 1). By contrast, in the old rats treatment with DHEA had no effect on the body weights but the liver weight increased progressively with increasing dose of DHEA (47% and 84% increase respectively by the two dose regimens).

The results on effect of DHEA treatment on oxidative energy metabolism are summarized in Tables 2, 3, 4 and 5. As can be noted, the state 3 respiration rates with glutamate, pyruvate+malate, succinate and ascorbate+TMPD were generally low (8–32% lower) in the old rats (Tables 2, 3, 4 and 5) which is consistent

with the earlier reports by other researchers (Marcus et al. 1982; Sastre et al. 1996; Nakahara et al. 1998; Navarro and Boveris 2004). Treatment with 0.2 mg DHEA stimulated state 3 respiration rates in mitochondria from young rats with glutamate by 38%. However, the effect declined at higher dose (Table 2). In the old animals maximum stimulatory effect (32% increase) was obtained with 1.0 mg dose and the value became comparable to untreated young rats (Table 2). When pyruvate+malate was used as the substrate pair, in young rats state 3 and state 4 respiration rates almost doubled following treatment with 1.0 mg DHEA. Even in the old animals treatment with 1.0 mg DHEA resulted in 50% and 77% increase in state 3 and state 4 respiration rates (Table 3). With succinate as the substrate, treatment with 0.2 mg DHEA was able to

Table 4 Effect of DHEA treatment on oxidative phosphorylation in rat liver mitochondria using succinate as the substrate

Age group	Treatment	ADP/O ratio	Respiration rate (n mole O ₂ /min/mg protein)		Respiratory control ratio	ADP phosphorylation rate (n mole/min/mg protein)
			+ADP	−ADP		
Young	Untreated (12)	2.34±0.10	56.88±2.92	22.55±1.29	2.56±0.09	264.1±15.34
	0.2 mg DHEA (12)	2.55±0.09	69.81±2.82 ^b	33.72±2.72 ^c	2.22±0.19	358.3±21.74 ^c
	1.0 mg DHEA (12)	2.33±0.12	53.91±2.96	33.55±2.62 ^c	1.67±0.10	252.7±21.35
Old	Untreated (18)	2.24±0.13	38.82±3.28*	23.04±0.68	1.69±0.09	174.2±11.39*
	0.2 mg DHEA (12)	2.17±0.12	45.37±3.21	24.67±1.85	1.87±0.06	189.5±12.25
	1.0 mg DHEA (10)	2.27±0.11	49.95±2.15 ^b	26.15±1.04 ^a	1.92±0.07	226.6±15.01 ^a

Experimental details are as given in the text and in Table 2. Concentration of succinate was 10 mM. Rotenone (1 μM) was included in the respiration medium. Results are given as mean ± SEM of the number of observations indicated in the parentheses.

^a $p < 0.02$; ^b $p < 0.01$ and ^c $p < 0.002$ compared with the corresponding untreated group.

* $p < 0.001$ compared with the untreated young group.

Table 5 Effect of DHEA treatment on oxidative phosphorylation in rat liver mitochondria using ascorbate + TMPD as the substrate

Age group	Treatment	ADP/O ratio	Respiration rate (n mole O ₂ /min/mg protein)		Respiratory control ratio	ADP phosphorylation rate (n mole/min/mg protein)
			+ADP	−ADP		
Young	Untreated (12)	0.44±0.03	28.12±1.90	21.46±1.65	1.33±0.03	24.52±1.57
	0.2 mg DHEA (12)	0.41±0.03	36.02±1.84 ^b	26.36±2.19	1.43±0.10	29.99±1.89 ^a
	1.0 mg DHEA (12)	0.42±0.02	45.24±2.08 ^d	31.76±1.52 ^d	1.44±0.07	38.61±2.06 ^d
Old	Untreated (21)	0.40±0.02	24.71±1.12	19.27±0.80	1.28±0.02	19.92±1.18 [*]
	0.2 mg DHEA (15)	0.42±0.03	27.92±0.86 ^a	21.67±0.72 ^a	1.30±0.05	23.64±1.59
	1.0 mg DHEA (12)	0.43±0.02	32.01±1.38 ^d	24.40±1.18 ^c	1.32±0.02	26.50±1.63 ^b

Experimental details are as given in the text and in Table 2. Concentrations of ascorbate and TMPD were 10 and 0.1 mM respectively. Rotenone (1 μM) was included in the respiration medium. Results are given as mean ± SEM of the number of observations indicated in the parentheses.

^a $p < 0.05$; ^b $p < 0.01$; ^c $p < 0.002$ and ^d $p < 0.001$ compared with the corresponding untreated group.

^{*} $p < 0.05$ compared with the untreated young group.

bring about 23% and 50% increase respectively in state 3 and state 4 respiration rates in the young rats; effect on state 4 respiration rate persisted at the higher dose (1.0 mg) of DHEA. Under these conditions, in the old animals there was a dose-dependent 17% and 29% increase in state 3 respiration rate. Corresponding increases in the state 4 respiration rates, respectively, were 7% and 14% (Table 4). With ascorbate+TMPD used as electron donor system, treatment with increasing doses of DHEA brought about progressive increase in the state 3 and state 4 respiration rates (23 to 61% increase) in the young animals. In the old rats also a similar trend with 13–30% increase was evident (Table 5).

The contents of cytochrome aa₃, b and c+c₁ were comparable for the young and the old rats. Treatment

with 0.2 mg DHEA resulted in 15–31% increase in the contents of the three cytochrome classes in young rats; the effect declined at higher dose (1.0 mg) of DHEA. On the other hand, in the old rats treatment with 1.0 mg DHEA brought about significant increase in the contents of all the cytochromes which ranged from 26–47%. The most important point was that at the highest dose employed (1.0 mg) the observed increase in the contents of the cytochromes in the old rats was of greater magnitude than that seen in the young rats (Table 6). The basal, Mg²⁺-stimulated, DNP-stimulated and Mg²⁺ + DNP-stimulated, ATPase activities were significantly low in the old rats. DHEA treatment was able to stimulate the ATPase activities by 6–51%. As against this, in the young animals treatment with 0.2 mg DHEA resulted in substantial

Table 6 Effect of DHEA treatment on the cytochrome content of rat liver mitochondria

Age group	Treatment	Cytochrome content (pmoles/mg protein)		
		aa ₃	b	c+c ₁
Young	Untreated (6)	135.1±3.11	277.8±10.93	326.2±15.94
	0.2 mg DHEA (6)	167.7±3.97 ^b	364.8±12.90 ^b	375.3±7.10 ^b
	1.0 mg DHEA (6)	151.7±7.44	310.5±21.00	313.0±13.10
Old	Untreated (17)	136.6±6.09	285.9±9.81	331.4±12.71
	0.2 mg DHEA (19)	137.5±10.37	309.3±9.93	364.5±15.90
	1.0 mg DHEA (12)	169.3±9.57 ^a	421.1±18.89 ^b	436.4±19.90 ^b

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

^a $p < 0.01$; and ^b $p < 0.001$ compared with the corresponding untreated group.

Table 7 Effect of DHEA treatment on ATPase activity in rat liver mitochondria

Age group	Treatment	Activity (μ mole Pi liberated/hr/mg protein)			
		Basal	+Mg ²⁺	+DNP	Mg ²⁺ + DNP
Young	Untreated (12)	2.01 $\pm$ 0.13	6.33 $\pm$ 0.14	19.11 $\pm$ 0.76	22.08 $\pm$ 1.03
	0.2 mg DHEA (12)	5.11 $\pm$ 0.10 ^d	8.80 $\pm$ 0.46 ^d	32.51 $\pm$ 1.09	33.24 $\pm$ 1.19 ^d
	1.0 mg DHEA (12)	4.96 $\pm$ 0.07 ^d	8.12 $\pm$ 0.63 ^a	25.36 $\pm$ 0.98	22.98 $\pm$ 0.86
Old	Untreated (12)	1.49 $\pm$ 0.76*	2.16 $\pm$ 0.18**	11.37 $\pm$ 0.83**	12.09 $\pm$ 0.46**
	0.2 mg DHEA (12)	2.20 $\pm$ 0.18 ^c	2.29 $\pm$ 0.12	16.76 $\pm$ 0.84 ^d	15.89 $\pm$ 0.95 ^c
	1.0 mg DHEA (12)	2.21 $\pm$ 0.16 ^d	3.26 $\pm$ 0.19 ^d	14.94 $\pm$ 0.62 ^b	17.34 $\pm$ 1.06 ^d

Experimental details are as given in the text. Results are given as mean $\pm$ SEM of the number of observations indicated in the parentheses.

^a $p < 0.02$; ^b $p < 0.01$; ^c $p < 0.002$ and ^d $p < 0.001$ compared with the corresponding untreated group.

* $p < 0.01$ and ** $p < 0.001$ compared with the untreated young group

39% to 2.5 fold increases in the ATPase activities. However, the effect declined at the higher dose of 1.0 mg (Table 7). The dehydrogenases activities were generally low in the old rats and DHEA treatments were able to restore the GDH and mitochondrial MDH activities near the level of untreated young animals. While the SDR activity was stimulated by 78% in young animals, interestingly, in the old rats treatment with 1 mg DHEA brought about a substantial 7.8 fold increase in the SDR activity. The cytosolic MDH activity increased marginally in young rats after treatment with 0.2 mg DHEA; higher dose of 1.0 mg had an adverse effect. By contrast, both the doses of DHEA resulted in 26–28% increase in cytosolic MDH activity in old rats (Fig. 1).

Discussion

The present studies were undertaken to examine if exogenous supplementation with DHEA has beneficial effect on oxidative energy metabolism of liver mitochondria from old rats. Studies were carried out with normal animals rather than using a rat model of aging such as Fischer 344 since we were interested in normal aging process. Complications and limitations associated with Fischer 344 strain have been documented Shimokawa et al. (1993). As is evident, the data of our present studies on the various respiratory parameters in the young animals (Tables 2, 3, 4, 5, 6 and 7, Fig. 1) are consistent with our previously published observations (Patel and Katyare 2006b, 2007). The results of our present studies also show that the respiratory functions of liver mitochondria, in general, declined in the

old rats, although the respiration rates with pyruvate+malate and ascorbate+TMPD were not affected. Variable and equivocal effects on respiratory activity of mitochondria, depending on the strain of the animals have been documented (Hansford 1983).

From the data presented it is clear that the respiratory activities, contents of cytochromes, dehydrogenases activities and the ATPase activities were stimulated in the liver mitochondria from both young as well as old rats in a dose-dependent manner after treatment with DHEA. In general, the higher dose of DHEA seemed to have a greater stimulatory effect in the old rats (Tables 2, 3, 4, 5, 6 and 7). This may perhaps relate to the declining levels of DHEA in the old animals (Kazihnitkova et al. 2004; Ren and Hou 2005; Vallee et al. 2000; Weill-Engerer et al. 2003). From the data presented one may also tend to think that with respect to respiratory activities the effect was more pronounced in the young animals than in the old animals. A similar conclusion may be drawn even for the ATPase activity.

The differential increase in the contents of cytochrome aa₃, b and c+c₁ and ATPase and dehydrogenases activities in old versus young animals is of interest and deserves some comment. It is well recognized that while the dehydrogenases and cytochrome c+c₁ are coded by the nuclear genes, crucial polypeptides of cytochrome aa₃, cytochrome b and mitochondrial ATPase are mitochondrial gene products (Poyton and Mc Ewen 1996). It may hence be suggested that DHEA may have differential effects on activation of the nuclear and mitochondrial genes in the young and old rats. It has been reported that in the old animals initially there is up-regulation of the genes encoding

Fig. 1 Effect of DHEA treatment on mitochondrial and cytosolic dehydrogenases activities in rat liver. The results are given as mean $\pm$ SEM of 12 independent observations. **a** Glutamate dehydrogenase; **b** Malate dehydrogenase (Mitochondrial); **c** Succinate DCIP

reductase and **d** Malate dehydrogenase (cytosolic); $\square$, Untreated; $\square$, 0.2 mg DHEA and $\square$, 1.0 mg DHEA $\dagger$, $p < 0.01$; ψ , $p < 0.002$ and Φ , $p < 0.001$ compared with the corresponding untreated group. *, $p < 0.001$ compared with the untreated young group

peptides in complex I, III, IV and V of the respiratory chain which is followed by down-regulation at later stage (Manczak et al. 2005).

The most significant observation of the present studies is the progressive increase in the liver weight in the old rats following treatment with DHEA (Table 1). Because of the significant increase in the liver weight the total potential of the tissue for respiratory activity and rates of ATP synthesis i.e. ADP-phosphorylation rates (Tables 2, 3, 4 and 5) would be significantly high in the old animals. Although these data are not given, an approximate estimation of respiratory potential and potential for ATP synthesis can be calculated by multiplying corresponding values with the respective liver weights. A similar picture would emerge even for the cytochromes contents and dehydrogenases activities. Thus the results of the present studies indicate that DHEA treatment specifically stimulates the proliferative potential of the liver cells in the old rats.

As cited above, in the humans the plasma levels of DHEA reach a peak in young adults and decline sub-

stantially in the older population (Hinson and Raven 1999; Parker 1999). Viewed in this context, data of our present studies would suggest that the plasma level of DHEA in the young rats receiving 0.2 mg dose of DHEA may represent the safe highest threshold value beyond which at higher dose of 1.0 mg the adverse effects become evident (e.g. see Tables 2 and 4). By contrast, in the old rats the maximum stimulatory effect was seen at the higher (1.0 mg) dose of DHEA (Tables 2, 3, 4, 5, 6 and 7). This is consistent with the reported low levels of DHEA (10% of adult value) in the old population (Hinson and Raven 1999; Parker 1999) and in rats (Kazihnitkova et al. 2004; Ren and Hou 2005; Vallee et al. 2000; Weill-Engerer et al. 2003). Of interest to note in this context is our earlier observation that high dose of 2.0 mg had adverse affects on respiratory activities of the liver as well as the brain mitochondria (Patel and Katyare 2007). This may relate to toxicity of DHEA given in higher doses. The age-dependent changes in the plasma and tissue levels of DHEA in the humans are well documented

(Hinson and Raven 1999; Parker 1999). However, no such data are available for plasma levels in the rats. What has been reported is that the plasma and tissue levels of DHEA in rats are comparatively very low and that the levels of DHEA in the brains of old rats decrease significantly (Kazihnitkova et al. 2004; Ren and Hou 2005; Vallee et al. 2000; Weill-Engerer et al. 2003). The low DHEA levels in the rat may possibly relate to high metabolic rate which could result in rapid turnover of the steroid. For example, it is well recognized that the life-span of erythrocytes in humans is 120 days; in rats the life-span is 60 days (Alberts et al. 1994).

The steroids DHEA and DHEAS are synthesized in the highest concentrations by the adrenals. Additionally, these steroids are also synthesized in the brain (Racchi et al. 2003). However, there are no known receptors for either of the steroids (Natawa et al. 2002). DHEAS is metabolized to 7α hydroxy DHEA and δ 5 androstene 3 β , 17 β diol (Steckelbroeck et al. 2002; Weill-Engerer et al. 2003) and 7α hydroxy DHEA is considered as active metabolite (Steckelbroeck et al. 2002; Weill-Engerer et al. 2003). Thus based on our studies it may also be suggested that the metabolism of DHEAS may be differentially affected in aging.

In conclusion, the overall results of our present studies point out that DHEA treatment significantly stimulated the respiratory activity in the liver mitochondria from young rats as well as old rats. Although the effects were of lesser magnitude in old rats, the total potential of oxidative energy metabolism increased to a grater extent because of significant dose-dependent increase in the liver weight. The results thus suggest that treatment with DHEA can have beneficial effect on energy transduction potential in liver mitochondria even in the old rats.

References

- Alberts B, Bray D, Lewis J, Raff M, Roberts K, Watson JD (1994) Molecular biology of the cell, 3rd edn. Garland, New York
- Buvat J (2003) Androgen therapy with dehydroepiandrosterone. *World J Urol* 21:346–355
- Celec P, Starka L (2003) Dehydroepiandrosterone - Is the fountain of youth drying out? *Physiol Res* 52:397–407
- Ferreira J, Gil L (1984) Nutritional effects on mitochondrial bioenergetics: alterations in oxidative phosphorylation by rat liver mitochondria. *Biochem J* 218:61–67
- Hansford RG (1983) Bioenergetics in ageing. *Biochim Biophys Acta* 726:41–80
- Hinson JP, Raven PW (1999) DHEA deficiency syndrome: a new term for old age? *J Endocrinol* 163:1–5
- Katewa SD, Katyare SS (2003) A simplified method for inorganic phosphate determination and its application for phosphate analysis in enzyme assays. *Anal Biochem* 323: 180–187
- Katewa SD, Katyare SS (2004) Treatment with antimalarials adversely affects the oxidative energy metabolism in rat liver mitochondria. *Drug Chem Toxicol* 27:41–53
- Katyare SS, Satav JG (1989) Impaired mitochondrial energy metabolism following paracetamol-induced hepatotoxicity in the rat. *Br J Pharmacol* 96:51–58
- Kaushal R, Dave KR, Katyare SS (1999) Paracetamol hepatotoxicity and microsomal function. *Environ Toxicol Pharmacol* 7:67–74
- Kazihnitkova H, Tejkalova H, Benesova O et al (2004) Simultaneous determination of dehydroepiandrosterone, its 7-hydroxylated metabolites, and their sulfates in rat brain tissues. *Steroids* 69:667–674
- Lowry OH, Rosebrough NJ, Farr AL et al (1951) Protein measurement with Folin-phenol reagent. *J Biol Chem* 193:265–272
- Manczak M, Jung Y, Park BS et al (2005) Time-course of mitochondrial gene expressions in mice brains: implications for mitochondrial dysfunction, oxidative damage, and cytochrome c in aging. *J Neurochem* 92:494–504
- Marcus DL, Ibrahim NG, Freedman ML (1982) Age-related decline in the biosynthesis of mitochondrial inner membrane proteins. *Exp Gerontol* 17:333–341
- Milgrom E (1990) Steroid hormones. In: Baulieu EE, Kelly PA (eds) *Hormones. From molecules to disease*. Hermann and Hall, New York, pp 387–438
- Nakahara H, Kanno T, Inai Y et al (1998) Mitochondrial dysfunction in the senescence accelerated mouse (SAM). *Free Radic Biol Med* 24:85–92
- Natawa H, Toshihiko Y, Kiminobu G, Taijiro OKA (2002) Mechanism of action of anti-aging DHEA-S and the replacement of DHEA-S. *Mech Ageing Dev* 123:1101–1106
- Navarro A, Boveris A (2004) Rat brain and liver mitochondria develop oxidative stress and lose enzymatic activities on aging. *Am J Physiol Regul Integr Comp Physiol* 287: R1244–R1249
- Parker CR Jr (1999) Dehydroepiandrosterone and dehydroepiandrosterone sulfate production in the human adrenal during development and aging. *Steroids* 64:640–647
- Patel MA, Katyare SS (2006a) Treatment with Dehydroepiandrosterone (DHEA) stimulates oxidative energy metabolism in the cerebral mitochondria. A comparative study of effects in old and young adult rats. *Neurosci Lett* 402:131–136
- Patel MA, Katyare SS (2006b) Treatment with Dehydroepiandrosterone (DHEA) stimulates oxidative energy metabolism in the liver mitochondria from developing rats. *Mol Cell Biochem* 293:193–201
- Patel MA, Katyare SS (2007) Effect of Dehydroepiandrosterone (DHEA) treatment on oxidative energy metabolism in rat liver and brain mitochondria. A dose-response study. *Clin Biochem* 40:57–65
- Poyton RO, Mc Ewen JE (1996) Crosstalk between nuclear and mitochondrial genomes. *Ann Rev Biochem* 65:563–607

- Racchi M, Balduzzi C, Corsini E (2003) Dehydroepiandrosterone (DHEA) and the aging brain: flipping a coin in the “fountain of youth”. *CNS Drug Rev* 9:21–40
- Ren JM, Hou YN (2005) Determination of unconjugated neurosteroids in rat brain regions by liquid chromatography-negative atmospheric pressure ionization mass spectroscopy. *Yao Xue Xue Bao* 40:262–266 (Taken from Abstract, Article in Chinese)
- Sastre J, Pallardo FV, Pla R et al (1996) Aging of the liver: age-associated mitochondrial damage in intact hepatocytes. *Hepatology* 24:1199–1205
- Shimokawa I, Higami Y, Hubbard GB et al (1993) Diet and the suitability of the male Fischer 344 rat as a model for aging research. *J Gerontol* 48:B27–B32
- Steckelbroeck S, Watzka M, Lutjohann D et al (2002) Characterization of the Dehydroepiandrosterone (DHEA) metabolism via oxysterol 7 α -hydroxylase and 17-ketosteroid reductase activity in the human brain. *J Neurochem* 83:713–726
- Subramaniam M, Katyare SS (1990) Oxidative phosphorylation in mouse liver mitochondria during weaning. *Mech Ageing Dev* 54:121–129
- Vallee M, Rivera JD, Koob GF et al (2000) Quantification of neurosteroids in rat plasma and brain following swim stress and allopregnanolone administration using negative chemical ionization gas chromatography/mass spectrometry. *Anal Biochem* 287:153–166
- Weill-Engerer S, David JP, Szadovitch V et al (2003) In vitro metabolism of Dehydroepiandrosterone (DHEA) to 7 α -hydroxy-DHEA and Delta 5 androstene 3 β , 17 β -diol in specific regions of the aging brain from Alzheimer’s and non-demented patients. *Brain Res* 969:117–125