Chapter 7

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

Introduction

Results from the previous chapter indicated that DHEA treatment had a grater stimulatory effect on the respiratory functions in brain mitochondria from old rats than in the young animals. In the old rats DHEA treatment was able to restore respiratory parameters close to those seen in untreated young adults or beyond this level.

The foregoing study is to examine 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.Therefore the effects of DHEA treatment on energy metabolism of liver mitochondria from the old rats in comparison with the young animals were examined.

Materials and methods

Chemicals

Chemicals used were same as described in chapter 2.

Animals and treatment with DHEA

All treatments were described in chapter 6.

Other methods are described in chapter 2.

All results are given as mean ± SEM.

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

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). In 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).

Oxidative phosphorylation

General

The results on effect of DHEA treatment on oxidative energy metabolism are summarized in Tables 2-5. 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 2-5) which is consistent with the earlier reports by other researchers (Marcus, Ibrahim and Freedman, 1982; Sastre et al., 1996; Nakahara et al., 1998; Navarro and Boveris , 2004).

Effects of DHEA treatment

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 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 increase 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 aa_3, b and $c+c_1$ 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 cytochromes and the increase 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, Mg^{2+}-stimulated, DNP-stimulated and Mg^{2+} + 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 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 activities of the dehydrogenases 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 1.0 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).

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

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

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 (nmole O_2/ min/mg protein)		Respiratory Control Ratio	ADP phosphorylation rate (nmole /min/ mg protein)
			+ ADP	-ADP		
Young Adult	Untreated (12)	3.24 ± 0.07	27.88 ± 0.95	10.80 ± 0.34	2.72 ± 0.07	180.8 ± 7.82
	0.2 mg DHEA (12)	3.06 ± 0.11	38.34 ± 1.53[b]	14.49 ± 0.68[b]	2.67 ± 0.05	231.8 ± 9.07[b]
	1.0 mg DHEA (12)	3.10 ± 0.09	32.90 ± 0.73[b]	18.38 ± 0.87[b]	1.86 ± 0.09	204.0 ± 6.92[b]
Old	Untreated (19)	3.23 ± 0.19	23.27 ± 1.03**	7.78 ± 0.46**	3.07 ± 0.11	149.4 ± 10.08*
	0.2 mg DHEA (15)	3.11 ± 0.13	25.73 ± 1.46	11.59 ± 0.46[b]	2.23 ± 0.11	161.3 ± 8.51
	1.0 mg DHEA (9)	3.15 ± 0.12	30.71 ± 2.17[a]	12.09 ± 0.82[b]	2.59 ± 0.21	193.1 ± 11.72[a]

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.
* $p<0.01$ and ** $p<0.001$ compared with the untreated young adult 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 (nmole O_2/ min/mg protein)		Respiratory Control Ratio	ADP phosphorylation rate (nmole /min/ mg protein)
			+ ADP	-ADP		
Young Adult	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 ± 0.32a	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. 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.

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 (nmole O_2/ min/mg protein)		Respiratory Control Ratio	ADP phosphorylation rate (nmole /min/ mg protein)
			+ ADP	-ADP		
Young Adult	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. 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 (nmole O_2/ min/mg protein)		Respiratory Control Ratio	ADP phosphorylation rate (nmole /min/ mg protein)
			+ ADP	-ADP		
Young Adult	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. 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.

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

Age group	Treatment	Cytochrome content (pmol/mg protein)		
		aa_3	b	$c+c_1$
Young Adult	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 (µmol Pi liberated/h/mg protein)			
		Basal	$+Mg^{2+}$	+DNP	$+Mg^{2+}$+DNP
Young Adult	Untreated (12)	2.01 ± 0.13	6.33 ± 0.14	19.11 ± 0.76	22.08 ± 1.03
	0.2 mg DHEA (12)	5.11 ± 0.10[d]	8.80 ± 0.46[d]	32.51 ± 1.09	33.24 ± 1.19[d]
	1.0 mg DHEA (12)	4.96 ± 0.07[d]	8.12 ± 0.63[a]	25.36 ± 0.98	22.98 ± 0.86
Old	Untreated (12)	1.49 ± 0.76*	2.16 ± 0.18**	11.37 ± 0.83**	12.09 ± 0.46**
	0.2 mg DHEA (12)	2.20 ± 0.18[c]	2.29 ± 0.12	16.76 ± 0.84[d]	15.89 ± 0.95[c]
	1.0 mg DHEA (12)	2.21 ± 0.16[d]	3.26 ± 0.19[d]	14.94 ± 0.62[b]	17.34 ± 1.06[d]

Experimental details are as given in the text. Results are given as mean ± SEM of the number of observations indicated in the parentheses.
a $p<0.02$; 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

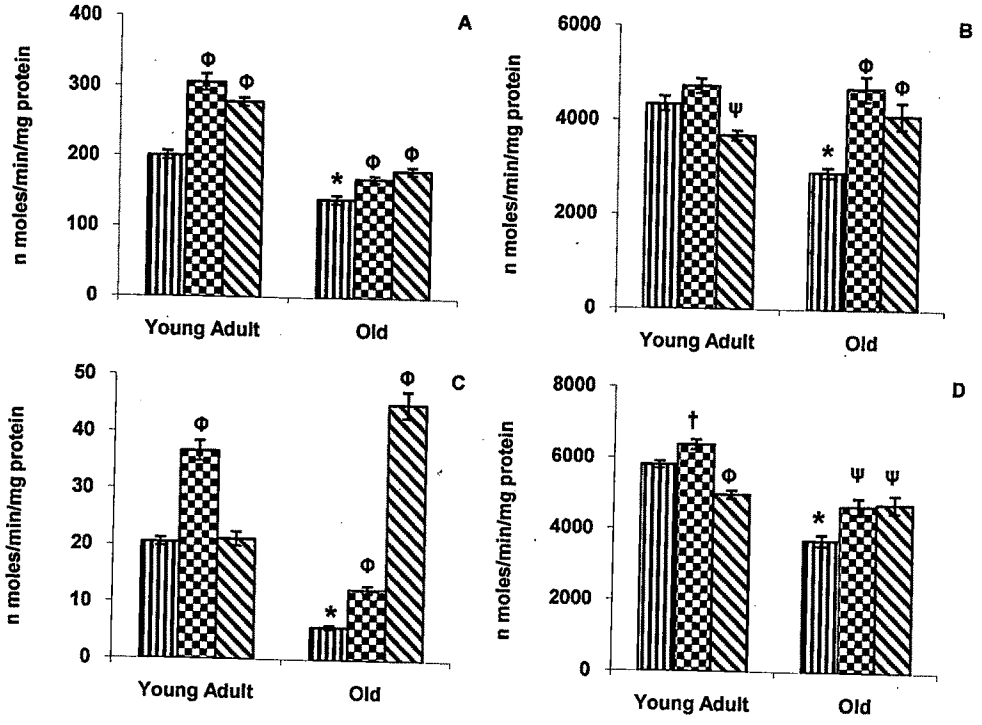

Fig. 1. Effect of DHEA treatment on mitochondrial and cytosolic dehydrogenases activities in rat liver. The results are given as mean ± SEM of 12 independent observations. (A) Glutamate dehydrogenase; (B) Malate dehydrogenase (Mitochondrial); (C) Succinate DCIP reductase and (D) Malate dehydrogenase (cytosolic); ▥ Untreated; ▩ 0.2 mg DHEA and ▨ 1.0 mg DHEA †, $p<0.01$; ψ, $p<0.002$ and Φ, $p<0.001$ compared with the corresponding untreated group. *, $p<0.001$ compared with the untreated young adult group.

Discussion

The present study was undertaken to examine if exogenous supplementation with DHEA has beneficial effect on oxidative energy metabolism of liver mitochondria in old rats. Studies were carried out with normal animals to study normal aging process rather than using a rat model of aging such as Fischer 344. Complications and limitations associated with Fischer 344 strain have been documented (Shimokawa et al., 1993). As is evident, the data of present study on the various respiratory parameters in the young animals (Tables 2-7, Fig. 1) are consistent with previous observations (chapter 2 and 5). The results of present study 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, activities of the dehydrogenases and the ATPase activities were stimulated in the liver mitochondria of 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 2-7). 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 ATPase activity.

The differential increase in the contents of cytochrome aa_3, b and $c+c_1$ and ATPase and dehydrogenases activities in old versus young animals is of interest. It is well recognized that while the dehydrogenases and cytochrome $c + c_1$ are coded by the nuclear genes, crucial polypeptides of cytochrome aa_3, 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 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 study 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 2-5) 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 total content cytochromes and dehydrogenases activities. Thus the results of the present study indicate that DHEA treatment specifically stimulates the proliferative potential of the liver cells in the old rats.

As cited above, in humans the plasma levels of DHEA reach a peak in young adults and decline substantially in the older population (Hinson and Raven, 1999; Parker, 1999). Viewed in this context, data of present study 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 (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 2-7). 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 (chapter 2). 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; whereas in rats it is 60 days (Alberts et al., 1994).

The steroids DHEA and DHEA-S are synthesized in the highest concentrations by the adrenals. Additionally, these steroids are also synthesized in the brain (Racchi, Balduzzi and Corsini, 2003). However, there are no known receptors for either of the steroids (Natawa et al., 2002). DHEA-S 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 hydroxyl DHEA is considered as active metabolite (Steckelbroeck et al., 2002; Weill-Engerer et al., 2003). Thus based on these tudies it may also be suggested that the metabolism of DHEA-S may be differentially affected in aging.

In conclusion, the overall results of present study 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 greater 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.

So far we have seen that DHEA treatment positively influenced mitochondrial respiration rates, cytochrome content and activity of ATPaes, GDH, SDR, and MDH from developing and old rats (chapter 4-7). The effects were age-dependent, dose-dependent and tissue-specific. We have also seen that lipid/phospholipid profile was altered in young adult rats after DHEA treatment (chapter 3). So it further raised the question on whether DHEA treatment also alters lipid/phospholipid profile in developing and old rats similarly. This was studied in next chapter.

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