Chapter 5

Treatment with dehydroepiandrosterone (DHEA) stimulates oxidative energy metabolism in the liver mitochondria from developing rats

Introduction

Results of the previous chapter suggested positive correlation of DHEA treatment on cerebral mitochondrial development and maturation. However efffects of exogenous DHEA on several energy-linked functions in mitochondria have been reported (Miller et al., 1988; Cleary, 1990; McIntosh, Pan and Berdanier, 1993). So it is good to know whether DHEA treatment also affects liver mitochondria from developing rats.

There are some studies which give information about different effects of DHEA treatment on liver. In case of male F-344 rats pharmacological administration of dehydroepiandrostenone for 2 weeks significantly increased the activity of hepatic peroxisomal beta-oxidation of fatty acids. It accelerated lipid catabolism by direct regulation of hepatic lipid metabolism and by induction of relevant gene expression. DHEA decreased the expression of hepatic lipogenic genes and suppressed triglycerols transport, by which the deposition of fat in adipose tissue in broiler chickens during embryonic development and hatching (Khan, 2008; Tang et al., 2007; Zhao, 2007). DHEA in pharmacological doses produced a significant increase in protein kinase C (PKC)-δ and -Є mRNA levels in the human hepatoma HepG2 cell line (Rypka et al., 2005). It suppresses cell growth by altering mitochondrial gene expression, morphology and functions of hepatoma cells (Ho et al., 2008). Mitochondrial palmitic acid oxidation in liver homogenates and isolated hepatocytes was increased in DHEA-treated rats (Imai et al., 2003). It has been shown that when the experimental animals are fed diets supplemented with DHEA, this results in proliferation of mitochondria in the liver and stimulation of mitochondrial functional parameters (Min Kyung et al., 1989; Bellei et al., 1992). However, since there are no known receptors for DHEA or DHEA-S the mechanism of action remains unclear (Natawa et al., 2002).

The results of previous chapters prompted us to examine the possibility as to whether DHEA levels relate to the development of mitochondrial function in liver. To achieve this aim we treated the rats belonging to different age group viz. 2 week, 4 week and

young adults with DHEA for one week and evaluated the effects on energy-linked functions.

Materials and methods

Chemicals

All detail is given in chapter 2.

Animals and treatment with DHEA

It is according to chapter 4.

Other methods are described in chapter 2.

All results are given as mean ± SEM.

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

Results

Data in Table 1 show that in the 3 week group at the end of the treatment period the final body weight of the DHEA treated animals was comparable to untreated controls. However, the liver weight showed a dose-dependent 8% and 19% increase. In the 5 week animals also the body weight was unchanged by DHEA treatment but the liver weight showed a dose-dependent 8 and 22% increase. In the young adult animals treatment with 1.0 mg DHEA resulted in about 19% increase in body weight. However, DHEA treatment had practically no effect on the liver weight. These observations are consistent with our earlier reported observations (chapter 2).

Effects on oxidative phosphorylation

General

In the untreated rats the state 3 respiration rates with glutamate increased progressively with age. Thus compared to the 3 week old animals in the young adults the state 3 respiration rates had almost doubled. A similar trend was seen for state 4 respiration rates.

The state 3 respiration rate with pyruvate + malate was relatively very low in 3 week old pups, increased 3.6 fold by the 5^{th} week of life, and in the young adults the increase amounted to 4.4 folds. Generally a similar trend was seen for state 4 respiration rates.

With succinate as the substrate, compared to the three week group, the increase in state 3 respiration rates was about 1.63 and 2.1 fold respectively in the 5 week old and the young adult animals. State 4 respiration rates had already reached young adult value by 5^{th} week.

For ascorbate + TMPD as the electron donor system the state 3 respiration rates increased (19%) marginally by the 5^{th} week; in the young adults the rate had tripled. A similar trend was seen for state 4 respiration rate (Tables 2-5). We have noted similar trend earlier for rat and mouse liver and rat brain mitochondria (Pandya, Agarwal and Katyare, 2004; Subramaniam and Katyare, 1990; Jani, Telang and Katyare, 1991; Katyare, Balasubramanian and Parmar, 2003; Rajan and Katyare, 1991).

The contents of cytochrome aa_3, b and $c+c_1$ increased progressively up to the young adult stage (Table 6).

A similar trend was seen even for the ATPase activities (Table 7).

The GDH activity also increased progressively with age whereas mitochondrial MDH activity increased only by 59% in the young adults. As against this the cytosolic MDH

activity increased progressively with age. Increase in SDR activity amounted to 1.75 and 2.36 folds under these conditions (Fig 1).

Effects of DHEA treatment

Treatment with DHEA resulted in progressive increase in state 3 respiration rates with glutamate in a dose-dependent manner. Thus in 3 week pups after treatment with DHEA state 3 respiration rate became comparable to that in the 5 week old untreated animals. Likewise when the 5 week old animals were treated with DHEA, the state 3 respiration rates was stimulated by 20% and 42% respectively by the two dose regimens employed. Interestingly, at higher dose (1.0 mg) of DHEA the value was very close to that in the untreated young adults. A parallel trend was seen even for state 4 respiration rates. In the young adult rats, 0.2 mg dose proved to be more efficacious (Table 2) which is consistent with our earlier observation (chapter 2).

DHEA treatment was less effective in improving state 3 or state 4 respiration rates with pyruvate + malate in 3 week old pups. Although the respiratory activity increased significantly, the values were substantially low compared to those in 5 week old untreated controls. By contrast, in the 5 week old group treatment with both the doses of DHEA increased the state 3 as well as state 4 respiration rates near or above the values for untreated young adults. In the case of the young adults the state 3 as well as state 4 respiration rates almost doubled only by 1.0 mg dose of DHEA (Table 3).

For succinate, only 1.0 mg DHEA treatment was effective in stimulating the state 3 and state 4 respiration rates. Especially, in the 5 week old animals the values became comparable to untreated control. In the young adults, 0.2 mg DHEA treatment only had stimulatory effect on state 3 respiration rate (Table 4) which is consistent with our earlier observations (chapter 2).

With ascorbate + TMPD system, treatment with DHEA caused a progressive dose-dependent stimulation of respiratory activities.

Interestingly, the magnitude of increase was always higher in developing animals. Thus in 3 week group the state 3 respiration rates increased by 1.71 and 2.75 fold while state 4 respiration rates increased by 1.89 and 2.84 fold. In 5 week group the increase amounted to 2.73 and 3.5 fold and 2.59 and 3.10 fold respectively for the state 3 and state 4 respiration rates. Under these experimental conditions the increase in state 3 and state 4 respiration rates in young adult group ranged from 24-58% (Table 5). DHEA treatment also resulted in significant increase in the contents of all the cytochrome. The lower dose of DHEA (0.2 mg) was more effective in 3 week group. In the 5 week animals both the doses were more or less equally effective. In the young adults only 0.2 mg dose had a greater stimulatory effect (Table 6).

DHEA treatment in general resulted in increase in the basal and Mg^{2+}-dependent ATPase activities in the developing animals. Under these conditions the DNP- and Mg^{2+}+DNP-dependent ATPase activities were also stimulated maximally with the effect being more pronounced in 3 week old group. In the young adults also all the ATPase activities increased significantly (Table 7).

DHEA treatment brought the GDH values in developing animals close to untreated young adults, whereas the mitochondrial MDH activity increased by 2.6 and 2.9 fold only in the 3 week animals. In the 3 week old pups DHEA treatment caused 3.6 fold increases in SDR activity. A similar 3.73 and 3.83 fold increase was seen even in the 5 week group. In the young adults only 0.2 mg DHEA treatment caused about 2.4 fold increase in SDR activity. The DHEA treatment also significantly stimulated cytosolic MDH activity in 3 week group; only 1.0 mg dose was effective in increasing the cytosolic MDH activity in 5 week animals. By contrast, this dose had a negative effect in the young adults where only a marginal increase of 11% was seen with 0.2 mg dose (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
3 week	Untreated (24)	39.00 ± 0.87	1.33 ± 0.03	3.42 ± 0.04
	0.2 mg DHEA (24)	39.83 ± 0.60	1.44 ± 0.03[a]	3.64 ± 0.08[a]
	1.0 mg DHEA (24)	38.81 ± 0.71	1.58 ± 0.05[b]	4.08 ± 0.11[b]
5 week	Untreated (18)	79.59 ± 2.24	3.54 ± 0.10	4.47 ± 0.10
	0.2 mg DHEA (18)	79.57 ± 3.76	3.83 ± 0.12	4.92 ± 0.24
	1.0 mg DHEA (18)	79.96 ± 4.08	4.30 ± 0.15[b]	5.38 ± 0.16[b]
Young Adult	Untreated (12)	254.6 ± 5.28	8.90 ± 0.46	3.45 ± 0.07
	0.2 mg DHEA (12)	263.6 ± 8.03	9.10 ± 0.51	3.45 ± 0.06
	1.0 mg DHEA (12)	281.9 ± 7.93[a]	9.60 ± 0.56	3.41 ± 0.06

Experimental details are as given in the text. Results are given as mean ± SEM of the number of observations indicated in the parentheses. a, $p < 0.02$; and b, $p < 0.001$ compared with the corresponding untreated group.

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		
3 week	Untreated (6)	3.12 ± 0.16	13.46 ± 0.77	5.68 ± 0.37	2.41 ± 0.21	84.07 ± 6.72
	0.2 mg DHEA (9)	3.11 ± 0.09	16.18 ± 0.89[a]	7.60 ± 0.49[b]	2.20 ± 0.18	101.17 ± 7.00
	1.0 mg DHEA (8)	3.06 ± 0.18	18.23 ± 0.35[d]	9.16 ± 0.56[d]	2.00 ± 0.11	111.90 ± 8.32[a]
5 week	Untreated (9)	3.11 ± 0.16	17.63 ± 0.94	6.87 ± 0.34	2.59 ± 0.13	110.80 ± 9.23
	0.2 mg DHEA (14)	3.09 ± 0.13	21.09 ± 1.38	8.74 ± 0.74[a]	2.52 ± 0.14	128.30 ± 7.40
	1.0 mg DHEA (9)	3.22 ± 0.11	24.99 ± 1.40[d]	9.24 ± 0.57[b]	2.77 ± 0.19	160.90 ± 10.30[b]
Young Adult	Untreated (12)	3.11 ± 0.10	28.87 ± 1.24	10.77 ± 0.43	2.70 ± 0.10	179.70 ± 9.39
	0.2 mg DHEA (12)	3.22 ± 0.14	37.00 ± 1.84[c]	13.60 ± 0.73[b]	2.73 ± 0.05	236.10 ± 11.13[b]
	1.0 mg DHEA (12)	3.18 ± 0.11	33.10 ± 0.81[b]	18.08 ± 1.13[d]	1.91 ± 0.12[d]	210.20 ± 7.78

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.

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		
3 week	Untreated (7)	3.01 ± 0.14	3.56 ± 0.21	2.67 ± 0.19	1.32 ± 0.06	21.29 ± 1.94
	0.2 mg DHEA (8)	3.02 ± 0.11	4.80 ± 0.26[c]	3.07 ± 0.26	1.63 ± 0.09[b]	28.47 ± 1.40[b]
	1.0 mg DHEA (9)	3.25 ± 0.17	5.98 ± 0.34[e]	3.52 ± 0.26[b]	1.76 ± 0.14[b]	39.09 ± 2.45[e]
5 week	Untreated (9)	3.22 ± 0.13	12.69 ± 0.41	6.59 ± 0.58	2.05 ± 0.11	81.43 ± 3.72
	0.2 mg DHEA (7)	3.05 ± 0.10	14.47 ± 0.95	7.52 ± 0.43	1.95 ± 0.09	87.82 ± 5.87
	1.0 mg DHEA (9)	3.07 ± 0.15	16.97 ± 1.09[d]	10.29 ± 0.96[c]	1.75 ± 0.08[a]	102.90 ± 6.29[c]
Young Adult	Untreated (12)	3.19 ± 0.07	15.82 ± 0.59	7.33 ± 0.21	2.17 ± 0.07	101.00 ± 4.36
	0.2 mg DHEA (12)	3.05 ± 0.08	17.74 ± 0.60[a]	9.51 ± 0.32[e]	1.87 ± 0.03[e]	107.90 ± 4.39
	1.0 mg DHEA (12)	3.16 ± 0.06	30.97 ± 1.93[d]	16.37 ± 1.11[e]	1.94 ± 0.09	195.40 ± 12.19[e]

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, $p < 0.02$; c, $p < 0.01$, d, $p < 0.002$ and e, $P < 0.001$ 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		
3 week	Untreated (8)	2.26 ± 0.12	27.09 ± 1.20	13.98 ± 1.06	1.96 ± 0.12	120.2 ± 6.64
	0.2 mg DHEA (9)	2.24 ± 0.13	30.00 ± 1.06	16.36 ± 0.62	1.85 ± 0.08	133.5 ± 6.54
	1.0 mg DHEA (6)	2.19 ± 0.19	32.95 ± 2.33[a]	17.71 ± 0.88[b]	1.87 ± 0.13	143.8 ± 5.01[b]
5 week	Untreated (9)	2.21 ± 0.11	44.18 ± 2.01	21.59 ± 1.57	2.13 ± 1.43	183.3 ± 7.90
	0.2 mg DHEA (8)	2.14 ± 0.17	44.22 ± 1.87	23.36 ± 1.62	1.96 ± 0.12	187.5 ± 10.90
	1.0 mg DHEA (9)	2.23 ± 0.16	52.07 ± 3.05[a]	29.09 ± 1.29[d]	1.84 ± 0.09	230.2 ± 12.02[c]
Young Adult	Untreated (12)	2.38 ± 0.08	56.43 ± 2.00	21.64 ± 1.05	2.67 ± 0.11	267.1 ± 11.71
	0.2 mg DHEA (12)	2.39 ± 0.09	71.31 ± 2.15[e]	36.99 ± 2.51[e]	2.08 ± 0.15[c]	340.7 ± 16.77[d]
	1.0 mg DHEA (12)	2.35 ± 0.09	55.23 ± 2.34	33.79 ± 1.91[e]	1.69 ± 0.08[e]	259.7 ± 15.01

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, $p < 0.02$; c, $p < 0.01$, d, $p < 0.002$ and e, $P < 0.001$ compared with the corresponding untreated 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		
3 week	Untreated (11)	0.42 ± 0.02	9.52 ± 0.31	6.05 ± 0.43	1.55 ± 0.03	8.10 ± 0.62
	0.2 mg DHEA (12)	0.41 ± 0.03	16.26 ± 1.19[d]	11.43 ± 0.75[d]	1.42 ± 0.05[a]	13.23 ± 1.15[d]
	1.0 mg DHEA (12)	0.41 ± 0.02	26.15 ± 0.99[d]	17.18 ± 0.91[d]	1.81 ± 0.06[a]	21.14 ± 1.46[d]
5 week	Untreated (11)	0.42 ± 0.03	11.33 ± 0.81	8.20 ± 0.75	1.42 ± 0.04	9.12 ± 0.45
	0.2 mg DHEA (11)	0.41 ± 0.02	30.80 ± 1.93[d]	21.24 ± 1.54[d]	1.47 ± 0.05	24.34 ± 1.33[d]
	1.0 mg DHEA (11)	0.43 ± 0.03	39.65 ± 2.96[d]	25.37 ± 1.06[d]	1.61 ± 0.11	31.05 ± 1.52[d]
Young Adult	Untreated (12)	0.43 ± 0.03	28.34 ± 1.38	22.04 ± 1.16	1.30 ± 0.03	23.72 ± 1.41
	0.2 mg DHEA (12)	0.41 ± 0.02	36.51 ± 1.15[d]	27.34 ± 1.38[c]	1.39 ± 0.06	29.74 ± 1.58[c]
	1.0 mg DHEA (12)	0.43 ± 0.02	44.88 ± 1.44[d]	30.50 ± 1.33[d]	1.52 ± 0.08[b]	38.94 ± 2.40[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, $p < 0.05$; b, $p < 0.02$; c, $p < 0.01$ and d, $p < 0.001$ compared with the corresponding untreated 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	$c+c_1$
3 week	Untreated (12)	102.7 ± 3.02	154.2 ± 4.51	202.9 ± 5.91
	0.2 mg DHEA (12)	153.1 ± 5.36[c]	176.6 ± 7.55[a]	362.2 ± 9.07[c]
	1.0 mg DHEA (12)	138.8 ± 6.19[c]	169.4 ± 3.26[a]	283.6 ± 3.99[c]
5 week	Untreated (18)	138.8 ± 6.19	199.8 ± 8.60	281.7 ± 11.70
	0.2 mg DHEA (18)	162.6 ± 11.21	303.9 ± 14.35[c]	442.2 ± 21.87[c]
	1.0 mg DHEA (15)	177.1 ± 9.89[b]	291.1 ± 15.74[c]	397.4 ± 18.28[c]
Young Adult	Untreated (10)	136.0 ± 2.46	278.7 ± 7.77	320.0 ± 14.21
	0.2 mg DHEA (10)	166.3 ± 3.88[c]	372.7 ± 11.83[c]	394.0 ± 16.73[b]
	1.0 mg DHEA (10)	153.4 ± 5.83[a]	339.9 ± 20.70[c]	327.4 ± 16.00

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, $p < 0.001$ 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
3 week	Untreated (12)	0.96 ± 0.24	2.16 ± 0.18	7.42 ± 0.22	7.78 ± 0.19
	0.2 mg DHEA (12)	2.41 ± 0.20[d]	2.96 ± 0.11[c]	13.37 ± 0.74[d]	12.21 ± 0.50[d]
	1.0 mg DHEA (12)	3.83 ± 0.26[d]	3.44 ± 0.21[d]	15.18 ± 0.66[d]	14.01 ± 0.72[d]
5 week	Untreated (12)	1.56 ± 0.09	2.67 ± 0.11	9.31 ± 0.74	9.44 ± 0.36
	0.2 mg DHEA (12)	3.80 ± 0.34[d]	3.09 ± 0.16[a]	10.27 ± 0.52	9.59 ± 0.58
	1.0 mg DHEA (12)	2.21 ± 0.12[d]	3.26 ± 0.19[b]	14.94 ± 0.62[d]	11.56 ± 0.71[b]
Young Adult	Untreated (12)	1.94 ± 0.08	5.43 ± 0.26	17.89 ± 0.87	20.08 ± 0.71
	0.2 mg DHEA (12)	4.63 ± 0.04[d]	8.92 ± 0.65[d]	31.32 ± 1.18[d]	32.48 ± 1.09[d]
	1.0 mg DHEA (12)	4.15 ± 0.22[d]	8.54 ± 0.66[d]	24.93 ± 0.58[d]	21.83 ± 0.94

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, $p < 0.02$; c, $p < 0.01$ and d, $p < 0.001$ compared with the corresponding untreated group.

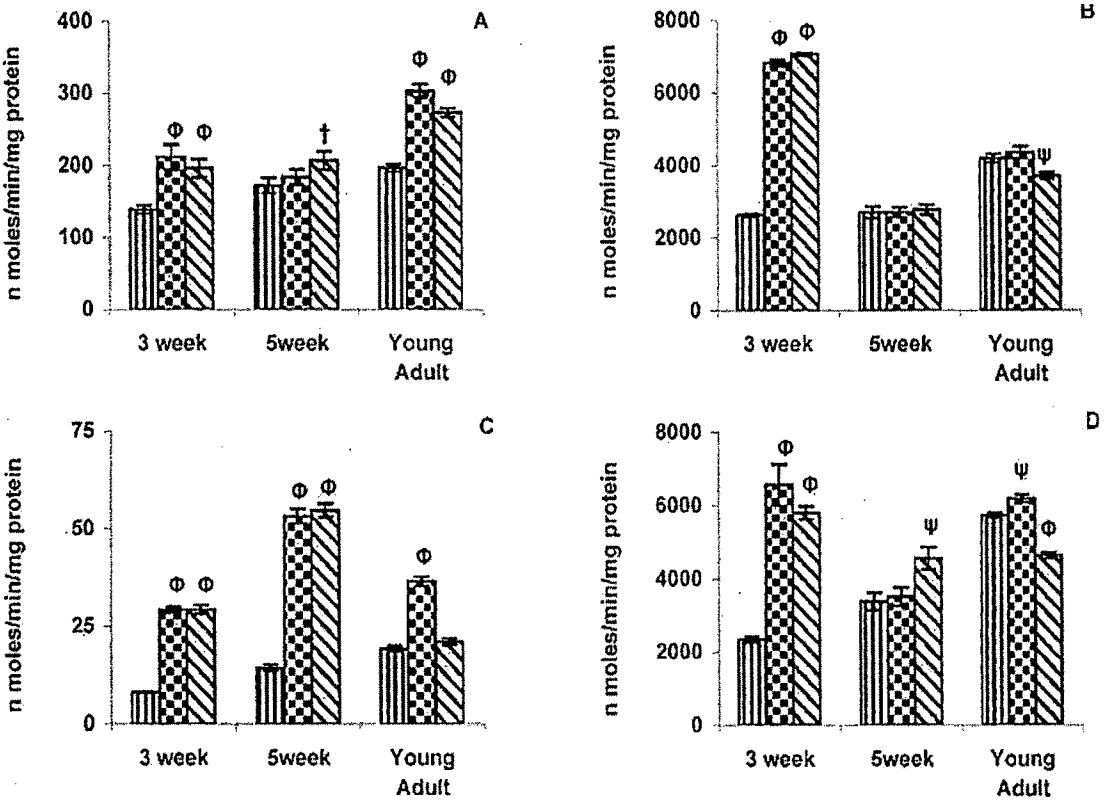


Fig. 1. Effect of DHEA treatment on mitochondrial and cytosolic dehydrogenases activities in rat liver. The results are given as mean ± SEM of 12 independent observations. A, Glutamate dehydrogenase; B, Malate dehydrogenase (Mitochondrial); C, Succinate DCIP reductase and D, Malate dehydrogenase (cytosolic); Untreated; 0.2 mg DHEA and 1.0 mg DHEA; †, $p < 0.05$; ψ, $p < 0.01$ and **Φ**, $p < 0.001$ compared with the corresponding untreated group.

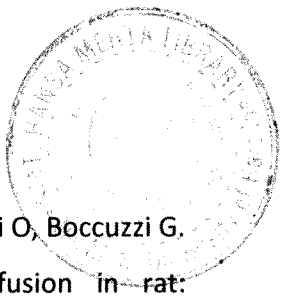
Discussion

The present study was initiated to find out if treatment with exogenous DHEA can accelerate the process of mitochondrial maturation and development in the developing animals. From the data presented this seems to be indeed the case. Thus treatment with DHEA regimen was able to stimulate the respiration rates with all the substrates employed bringing them closer to or beyond the next higher age group. Under these conditions the ADP/O ratios were not altered (Tables 2-5) which is consistent with our earlier observations (chapter 2). In this connection it is interesting to note that earlier in our lab we have observed that treatment with exogenous corticosterone or synthetic glucocorticoid, dexamethasone resulted in significant uncoupling of oxidative phosphorylation in rat liver and brain mitochondria (Pandya, Agarwal and Katyare, 2004; Jani, Telang and Katyare, 1991; Katyare, Balasubramanian and Parmar, 2003). Therefore the action of DHEA should be considered as unique because it stimulated respiration rates without affecting the ADP/O ratios thereby increasing the bioenergetics potential of mitochondria. This is very well reflected in the increase in the ADP phosphorylation rates (Tables 2-5). As is well recognized, all the developmental processes are energy dependent (Golovachev and Nadal'yak, 1975; Okada, 1994; Rust, 1994). Viewed in this context, the effects of DHEA treatment seem to be beneficial and conducive to development and maturation of mitochondrial functions. The enhancement of respiratory functions seems to be aided by increase in the contents of cytochromes. It may be pointed out here that DHEA treatments were more effective in increasing the contents of cytochromes aa_3 and $c + c_1$ in the 3 week animals. In the 5 week old animals the effect was more pronounced on the increases in the content of cytochromes b and $c + c_1$. The most important aspect was that the levels of cytochromes became comparable to untreated young adults or increased beyond these values. In the young adults, although contents of cytochromes increased, the increase was of lesser magnitude (Table 6). The observed enhancement in respiration rates was also accompanied by significant increases in the dehydrogenase activities (Fig. 1). Interestingly, DHEA treatments also stimulated the ATPase activity in the developing animals which became somewhat comparable to the untreated young adults values (Table 7). However, most substantial stimulatory effect

of DHEA treatment was evident only in the young adults. It is well recognized that the mitochondrial dehydrogenases and cytochrome c + c_1 are coded by the nuclear genes whereas crucial peptides of cytochromes aa_3 and b, as well as ATPase are coded by mitochondrial DNA (Poyton and Mc Ewen, 1996). The differential stimulatory effects of DHEA treatment on rates of substrate oxidation and on increase in the contents of cytochromes which we observe here seem to be consistent with age-related profile of DHEA synthesis and plasma levels of DHEA (Hinson and Raven, 1999; Parker, 1999). In other words the results of our present study suggest that DHEA may play crucial role in activating specific mitochondrial and nuclear genes during mitochondrial development and maturation. The mechanism of action of DHEA still remains unclear since there are no known receptors of DHEA (Natawa et al., 2002). However, recently it has been shown that DHEA is metabolized to 7α hydroxyl DHEA and δ 5 androstene 3 β, 17β diol, the former is considered to be active metabolite (Steckelbroeck et al., 2002; Weill-Engerer et al., 2003). Thus based on our studies it may be suggested that DHEA and or its active metabolite may influence the process of development and/or aging by activation or silencing specific nuclear and/or mitochondrial genes in an age-dependent manner. It has been shown that in aging mice in the initial stages there is up regulation of genes encoding mitochondria complex I, III, IV and V which is followed by down-regulation at the later stage (Mamczka et al., 2005). It may hence be suggested that DHEA may play an age-dependent role in gene regulation in development and aging. However, further experiments are needed to verify and substantiate this possibility. It has been shown that DHEA increases synthesis of nitric oxide (NO) by stimulating inducible NO synthase (*i*NOS) especially in the endothelial cells (Formoso et al., 2006) although inhibitory effects in BV-2 microglia have also been reported (Wang et al., 2001). It is unlikely that the enhanced respiration rates together with increased contents of cytochromes and increased levels of dehydrogenases as well as ATPase activities which we observe here could have been mediated via NO. Especially of interest to note here is the recent finding that NO binds at the active site of cytochrome oxidase and modulates its activity (Kadenbach, 2003; Brunori et al., 2005). DHEA has been shown to afford protection from oxidative stress (Aragno et al., 2003). However, implication of the same in the process of maturation of mitochondrial function during development is unclear at this stage.

In conclusion, our results showed that treatment with DHEA stimulated the respiratory activity and/or accelerated the developmental process of maturation of mitochondrial function in liver from developing rats.

Data from chapter 4 and 5 have shown that DHEA treatment positively affects brain and liver mitochondria from developing rats. We have also seen in introduction chapter 1 that DHEA showed characteristic age-related pattern and so is considered to be the youth hormone. All this information leads to the idea of studying a role of exogenous DHEA in aging in next chapter.



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