Chapter 4

Dehydroepiandrosterone (DHEA) treatment stimulates oxidative energy metabolism in the cerebral mitochondria from developing rats

Introduction

In chapter 2 and 3 we found that treatment with DHEA influenced respiratory rates and lipid/phospholipid profile in tissue-specific and dose-dependent manner in young adult rats. So, the next question addressed was how exogenous DHEA would affect the developmental process as it is known that development is an energy requiring process.

The respiratory activity of cerebral mitochondria has been shown to have a characteristic pattern of maturation in rat. Thus, the respiratory activity is low in the early neonatal life but increases significantly in the postnatal period up to the age of 5 weeks where the values reach those of young adults (Milstein, White and Swaiman, 1968; Holtzman and Moore, 1975; Rajan and Katyare, 1991; Katyare, Balasubramanian and Parmar, 2003). This offers an opportunity to evaluate the role of DHEA in regulating postnatal development and maturation of the cerebral mitochondria.

Several studies have shown that DHEA is present in significant quantities in the human as well as the rat brain although the levels are lower for the rat (Corpechot et al., 1981; Vallee et al., 2000; Steckelbroeck et al., 2002; Racchi, Balduzzi and Corsini, 2003; Ren and Hou, 2005). Also, age-dependent decline in the content of DHEA in the brain has been reported (Weill-Engerer et al., 2002; Kazihnitkova et al., 2004). While the brain derives its DHEA partly from the adrenals, the brain itself is capable of synthesizing DHEA and DHEA-S as well as pregnenolone (Racchi, Balduzzi and Corsini, 2003). Hence, DHEA, DHEA-S and pregnenolone are considered to be neurosteroids (Racchi, Balduzzi and Corsini, 2003).

So we carried out experiments to examine if exogenous supplementation with DHEA in neonatal rat can accelerate the process of maturation of cerebral mitochondria. To achieve this objective we treated developing rats with DHEA and examined the effects on the respiratory parameters, in comparison with young adult rats treated in a similar manner.

From the dose dependent study (chapter 2) we found that treatment with DHEA stimulated mitochondrial respiratory rates, increased cytochrome content, activity of ATPase and activity of GDH, SDR and MDH. These changes were dose dependent and tissue specific. It was seen that at lower dose effects were marginal while higher dose of DHEA showed adverse effects. Maximum effects were seen with 0.2 mg and 1.0 mg dose of DHEA. Changes in lipid/phospholipid profile (chapter 3) support these results. So, further studies were carried out with these two doses of DHEA.

Materials and methods

Chemicals

Chemicals used were same as described in chapter 2.

Animals and treatment with DHEA

Male albino rats of Charles-Foster strain, 2 and 4 weeks and young adults (8-10 weeks old) were used. At the start of the experiments the initial body weight of the animals in the individual age group were matched (e.g. see Table 1). The animals were injected subcutaneously (s.c.) with 0.2 or 1.0 mg DHEA/kg body weight for 7 consecutive days. Suspensions of DHEA were prepared fresh in saline prior to use. The controls received equivalent volume of saline vehicle. The animals were sacrificed on the 8^{th} day for isolation of mitochondria. Thus, at the time of presentation of the results the age of the developing animals was 3 and 5 weeks, respectively. In a given batch, depending on the age group 12-24 animals were used.

Isolation of mitochondria

Isolation of brain mitochondria was essentially according to the procedure described previously in chapter 2.

Oxidative phosphorylation

Measurements of oxidative phosphorylation were carried out at 25°C using a Clark-type oxygen electrode. Calculations of ADP/O ratio and ADP phosphorylation rates were as described previously in chapter 2.

Cytochrome content

The content of cytochromes was quantified from the difference in spectra of sodium dithionite reduced versus potassium ferricyanide oxidized samples as described previously. The difference spectra were recorded in a JASCO UV/VIS spectrophotometer model V-530. The contents of cytochromes aa_3, b and $c+c_1$ were calculated using the wavelength pairs 604-624, 559-580 and 535-552 nm and millimolar extinction coefficients 24, 23.4 and 18.7, respectively (Katewa and Katyare, 2004).

Assay of dehydrogenases

Glutamate dehydrogenase (GDH), malate dehydrogenase (MDH) and succinate DCIP reductase (SDR) activities were determined according to the procedures described (Katewa and Katyare, 2004).

Assay of ATPase

It is according to procedures described in chapter 2.

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

All results are given as mean ± SEM.

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

Results

Treatment with DHEA had no effect on the body weight of animals in the 3 and 5 week groups. However, in the 5 week group, treatment with 1.0 mg DHEA brought about a small but significant increase (11% on body weight basis) in brain weight. In the young adult animals treatment with 1.0 mg DHEA resulted in 14% increase in the body weight (Table 1).

Effects on oxidative phosphorylation

General

In the untreated animals the state 3 respiration rates increased progressively up to the adult stage. The contents of the cytochromes also increased progressively up to the adult stage. Also, the ATPase activity and the levels of dehydrogenases showed increase with development. The observed pattern (Tables 2-7 and Fig. 1) is consistent with earlier observations reported by other researchers (Milstein, White and Swaiman, 1968; Holtzman and Moore, 1975; Rajan and Katyare, 1991; Katyare, Balasubramanian and Parmar, 2003).

Effects of DHEA treatment

Treatment with the two doses (0.2 and 1.0 mg) of DHEA resulted in 2.7 and 3.1-fold increase in state 3 respiration rate with glutamate in the 3 week old group thus making these values comparable to untreated young adults. In the 5 week old group the increase amounted to 2.6-2.8-fold, respectively, and the values became comparable to those of young adult animals receiving DHEA treatment. In the young adult animals the increase was of smaller magnitude ranging from 53 to 69%. Under these conditions, in the developing animals the effect on state 4 respiration rate was

generally of lesser magnitude and the mitochondria were tightly coupled as is evident from the respiratory control ratios. By contrast, in the young adult rats receiving 1.0 mg DHEA an opposite effect was seen where increase in state 4 respiration rate was significantly high which resulted in lowering of respiratory control ratio. ADP/O ratios were unchanged following DHEA treatments (Table 2).

Similar trend was seen in the 3 and 5 week old group of animals with pyruvate + malate as the respiratory substrates whereas in the case of the young adult rats, treatment with DHEA had only marginal effect on state 3 respiration rate (Table 3).

With succinate as the substrate, treatment of developing rats treated with 0.2 mg DHEA resulted in bringing the state 3 respiration rates to the level noted for untreated young adults. Higher dose, i.e. 1.0 mg had no further beneficial effect. Thus, the increase in the state 3 respiration rates ranged from 2.47-2.90-fold. In the young adult rats treatment with 0.2 mg DHEA caused 35% increase in the state 3 respiration rates; higher dose of 1.0 mg had adverse effect in that the increase amounted to only about 15%. The pattern for effects on state 4 respiration rate was similar to that noted for state 3 respiration rates. As a consequence, the respiratory control ratios were generally unchanged (Table 4).

When ascorbate + TMPD was used as a electron donor system, in the 3 week old group state 3 and state 4 respiration rates increased by 2.2-2.4-fold. In the 5 week old group the increase ranged from 45 to 122%. In the young adults, once again the corresponding increase ranged only from 16 to 37%. The trend for state 4 respiration rates was comparable to that noted above for state 3 respiration rates. Consequently, the respiratory control ratios were generally unchanged (Table 5).

The contents of cytochrome aa_3 and b increased after DHEA treatment in a dose-dependent manner in the animals from the 3 week old group while in the 5 week group 0.2 mg DHEA seemed to elicit maximum response. In the young adults also there was a dose-dependent increase in the content of both the cytochrome classes. Content of cytochrome $c+c_1$ seemed to be marginally influenced by treatment with 0.2

or 1.0 mg dose of DHEA and the observed changes were not statistically significant (Table 6).

DHEA treatments significantly increased the ATPase activity in developing animals bringing it close to untreated young adult values. Increments in the ATPase activity in the young adult group were comparatively of lower magnitude, except for the basal ATPase activity (Table 7).

DHEA treatment increased the levels of GDH in all the groups which ranged from 19 to 90% with maximum effect being seen in the young adult animals. By contrast, the mitochondrial MDH activity increased by 3.6-3.9-fold only in the 3 week group; in the young adults 1.0 mg DHEA had adverse effect. DHEA treatment caused substantial 3.3-6.8- fold increase in the SDR activity with the effect being more pronounced in the 5 week old group. In the young adult rats, treatment with 1.0 mg DHEA had adverse effect. The effects on cytosolic MDH were marginal and 26 and 38% increase could be noted in the developing animals (Fig. 1).

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

Age group	Treatment	Body weight (g)		Brain weight	
		Initial	Final	g	% of body weight
3 week	Untreated (24)	21.82 ± 0.77	37.35 ± 0.97	1.24 ± 0.02	3.33 ± 0.07
	0.2 mg DHEA (24)	22.79 ± 0.52	38.84 ± 0.59	1.26 ± 0.01	3.25 ± 0.04
	1.0 mg DHEA (24)	24.19 ± 0.50	37.75 ± 0.82	1.28 ± 0.02	3.41 ± 0.07
5 week	Untreated (18)	51.77 ± 2.26	80.50 ± 2.54	1.34 ± 0.01	1.69 ± 0.05
	0.2 mg DHEA (18)	49.17 ± 1.95	79.44 ± 2.39	1.38 ± 0.02	1.78 ± 0.07
	1.0 mg DHEA (18)	53.31 ± 2.61	80.79 ± 4.84	1.45 ± 0.01[c]	1.89 ± 0.03[b]
Young adult	Untreated (12)	256.06 ± 6.72	261.10 ± 9.13	1.71 ± 0.09	0.65 ± 0.04
	0.2 mg DHEA (12)	255.06 ± 5.79	273.10 ± 10.4	1.71 ± 0.06	0.63 ± 0.02
	1.0 mg DHEA (12)	266.78 ± 6.81	296.30 ± 9.03[a]	1.80 ± 0.08	0.61 ± 0.02

Experimental details are as given in the text. Results are given as mean ± S.E.M. of the number of observations indicated in the parentheses.
a, $p < 0.02$; b, $p < 0.002$; c, $p < 0.001$ compared with the corresponding untreated group.

Table 2: Effect of DHEA treatment on oxidative phosphorylation in rat brain 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 (9)	3.00 ± 0.23	8.15 ± 0.67	4.89 ± 0.41	1.69 ± 0.09	47.94 ± 4.49
	0.2 mg DHEA (9)	3.05 ± 0.13	22.29 ± 1.18[a]	8.06 ± 0.44[a]	2.78 ± 0.11	134.87 ± 7.57[a]
	1.0 mg DHEA (9)	3.06 ± 0.17	25.06 ± 1.80[a]	9.48 ± 0.55[a]	2.63 ± 0.07	150.33 ± 9.13[a]
5 week	Untreated (9)	3.01 ± 0.17	11.09 ± 0.93	5.51 ± 0.26	1.99 ± 0.07	64.57 ± 3.44
	0.2 mg DHEA (9)	3.14 ± 0.21	28.97 ± 1.24[a]	8.38 ± 0.20[a]	3.62 ± 0.19	172.50 ± 9.98[a]
	1.0 mg DHEA (6)	3.09 ± 0.18	30.79 ± 2.17[a]	8.08 ± 0.15[a]	4.07 ± 0.27	186.00 ± 9.01[a]
Young adult	Untreated (12)	3.09 ± 0.08	19.15 ± 0.85	4.08 ± 0.22	3.92 ± 0.13	117.60 ± 5.53
	0.2 mg DHEA (12)	3.10 ± 0.08	29.08 ± 1.60[a]	7.53 ± 0.31[a]	3.85 ± 0.11	180.80 ± 10.89[a]
	1.0 mg DHEA (12)	3.08 ± 0.09	32.28 ± 0.60[a]	18.12 ± 0.57[a]	1.81 ± 0.06	198.50 ± 5.69[a]

Experimental details are as given in the text. Results are given as mean ± S.E.M. of the number of observations indicated in the parentheses.
a, $p < 0.001$ compared with the corresponding untreated group.

Table 3: Effect of DHEA treatment on oxidative phosphorylation in rat brain 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)	3.07 ± 0.23	7.10 ± 0.60	3.03 ± 0.15	2.14 ± 0.12	42.41 ± 2.65
	0.2 mg DHEA (9)	3.08 ± 0.21	19.03 ± 1.07[a]	9.68 ± 0.41[a]	1.96 ± 0.05	114.60 ± 5.70[a]
	1.0 mg DHEA (8)	3.06 ± 0.23	21.56 ± 1.51[a]	9.10 ± 0.66[a]	2.38 ± 0.06	128.10 ± 7.28[a]
5 week	Untreated (9)	3.08 ± 0.20	8.45 ± 0.52	3.36 ± 0.27	2.44 ± 0.15	51.29 ± 3.36
	0.2 mg DHEA (7)	2.96 ± 0.24	22.83 ± 2.04[a]	8.19 ± 0.52[a]	3.00 ± 0.24	87.82 ± 5.87[a]
	1.0 mg DHEA (6)	3.01 ± 0.29	27.36 ± 2.20[a]	7.51 ± 0.44[a]	3.98 ± 0.20	159.10 ± 7.88[a]
Young adult	Untreated (12)	3.08 ± 0.08	21.36 ± 1.09	4.83 ± 0.21	4.47 ± 0.19	129.90 ± 5.71
	0.2 mg DHEA (12)	3.09 ± 0.06	22.64 ± 0.69	7.76 ± 0.29[a]	2.98 ± 0.14	139.80 ± 4.57
	1.0 mg DHEA (12)	3.07 ± 0.08	26.48 ± 0.81[a]	7.78 ± 0.18[a]	3.53 ± 0.21	162.80 ± 7.34[a]

Experimental details are as given in the text. Results are given as mean ± S.E.M. 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 brain 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 (9)	2.07 ± 0.11	7.98 ± 0.52	5.76 ± 0.41	1.40 ± 0.06	31.13 ± 2.75
	0.2 mg DHEA (9)	2.09 ± 0.15	22.73 ± 0.54[c]	17.05 ± 0.49[c]	1.34 ± 0.03	93.91 ± 5.33[c]
	1.0 mg DHEA (9)	2.06 ± 0.14	23.18 ± 1.19[c]	16.35 ± 0.91[c]	1.43 ± 0.06	93.82 ± 4.93[c]
5 week	Untreated (9)	2.08 ± 0.14	8.86 ± 0.50	6.00 ± 0.32	1.52 ± 0.06	34.87 ± 1.66
	0.2 mg DHEA (9)	2.03 ± 0.08	22.80 ± 1.88[c]	14.14 ± 0.99[c]	1.60 ± 0.04	91.50 ± 6.39[c]
	1.0 mg DHEA (9)	2.03 ± 0.14	21.91 ± 1.16[c]	14.09 ± 0.95[c]	1.58 ± 0.06	87.57 ± 4.95[c]
Young adult	Untreated (12)	2.07 ± 0.06	24.86 ± 1.01	13.52 ± 0.85	1.97 ± 0.15	102.60 ± 4.73
	0.2 mg DHEA (12)	2.09 ± 0.08	33.64 ± 0.77[c]	18.12 ± 0.69[c]	1.89 ± 0.07	140.20 ± 5.77[c]
	1.0 mg DHEA (12)	2.06 ± 0.07	28.58 ± 0.85[b]	17.25 ± 0.67[b]	1.68 ± 0.05	116.70 ± 3.04[a]

Experimental details are as given in the text. Results are given as mean ± S.E.M. of the number of observations indicated in the parentheses. a, $p < 0.02$; b, $p < 0.01$; c, $p < 0.001$ compared with the corresponding untreated group.

Table 5: Effect of DHEA treatment on oxidative phosphorylation in rat brain 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 (10)	0.69 ± 0.04	7.25 ± 0.61	5.18 ± 0.62	1.48 ± 0.09	9.69 ± 0.59
	0.2 mg DHEA (12)	0.71 ± 0.03	15.74 ± 0.87[d]	11.91 ± 0.90[d]	1.38 ± 0.13	22.43 ± 1.58[d]
	1.0 mg DHEA (12)	0.71 ± 0.06	17.30 ± 1.01[d]	12.55 ± 0.76[d]	1.38 ± 0.03	23.39 ± 1.27[d]
5 week	Untreated (11)	0.72 ± 0.03	14.70 ± 1.14	8.72 ± 0.64	1.68 ± 0.06	20.96 ± 1.79
	0.2 mg DHEA (12)	0.73 ± 0.05	21.24 ± 1.93[d]	13.27 ± 1.04[d]	1.60 ± 0.04	29.19 ± 1.80[b]
	1.0 mg DHEA (12)	0.74 ± 0.05	29.45 ± 1.78[d]	19.38 ± 1.21[d]	1.55 ± 0.08	43.00 ± 2.09[d]
Young adult	Untreated (12)	0.75 ± 0.03	23.80 ± 1.43	14.38 ± 0.91	1.67 ± 0.03	34.62 ± 1.87
	0.2 mg DHEA (12)	0.74 ± 0.02	27.71 ± 1.21[a]	19.71 ± 0.89[d]	1.42 ± 0.04	41.14 ± 2.14[a]
	1.0 mg DHEA (12)	0.73 ± 0.03	29.81 ± 0.74[c]	19.67 ± 0.66[d]	1.53 ± 0.04	43.83 ± 1.80[c]

Experimental details are as given in the text. Results are given as mean ± S.E.M. of the number of observations indicated in the parentheses. a, $p < 0.05$; b, $p < 0.01$; c, $p < 0.002$; d, $p < 0.001$ compared with the corresponding untreated group.

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

Age group	Treatment	Cytochrome content (pmol/mg protein)		
		aa_3	b	$c+c_1$
3 week	Untreated (12)	126.0 ± 6.35	152.2 ± 6.65	192.2 ± 9.18
	0.2 mg DHEA (11)	165.6 ± 7.41[b]	176.4 ± 6.67[a]	191.4 ± 11.06
	1.0 mg DHEA (12)	213.2 ± 7.71[b]	222.0 ± 9.74[b]	169.5 ± 12.42
5 week	Untreated (18)	143.8 ± 4.04	168.7 ± 5.94	231.5 ± 9.23
	0.2 mg DHEA (18)	209.1 ± 11.00[b]	259.8 ± 11.90[b]	264.8 ± 14.21
	1.0 mg DHEA (12)	187.1 ± 4.83[b]	222.6 ± 8.09[b]	238.0 ± 8.20
Young adult	Untreated (12)	156.1 ± 5.16	175.8 ± 6.37	221.3 ± 8.21
	0.2 mg DHEA (12)	180.4 ± 3.80[b]	196.9 ± 5.25[a]	200.3 ± 6.60
	1.0 mg DHEA (12)	269.1 ± 5.07[b]	354.4 ± 6.57[b]	196.4 ± 9.04

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

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

Age group	Treatment	Activity (µmol Pi liberated/h/mg protein)			
		Basal	$+Mg^{2+}$	+DNP	$+Mg^{2+}$+DNP
3 week	Untreated (12)	0.18 ± 0.01	1.79 ± 0.07	0.19 ± 0.01	1.80 ± 0.08
	0.2 mg DHEA (12)	0.32 ± 0.03[e]	3.92 ± 0.24[e]	0.39 ± 0.02[e]	4.07 ± 0.16[e]
	1.0 mg DHEA (12)	0.35 ± 0.02[e]	5.59 ± 0.22[e]	0.38 ± 0.02[e]	5.68 ± 0.18[e]
5 week	Untreated (12)	0.23 ± 0.02	2.25 ± 0.16	0.35 ± 0.02	2.65 ± 0.16
	0.2 mg DHEA (12)	0.35 ± 0.03[a]	5.07 ± 0.29[e]	0.46 ± 0.03[c]	5.25 ± 0.35[e]
	1.0 mg DHEA (12)	0.44 ± 0.02[e]	4.76 ± 0.20[e]	0.47 ± 0.02[e]	5.54 ± 0.24[e]
Young adult	Untreated (12)	0.31 ± 0.04	5.64 ± 0.16	0.43 ± 0.02	6.49 ± 0.21
	0.2 mg DHEA (12)	0.74 ± 0.06[e]	7.58 ± 0.18[e]	0.78 ± 0.04[e]	7.48 ± 0.16[d]
	1.0 mg DHEA (12)	0.67 ± 0.03[e]	6.26 ± 0.16[b]	0.60 ± 0.04[c]	8.79 ± 0.17[e]

Experimental details are as given in the text. Results are given as mean ± S.E.M. of the number of observations indicated in the parentheses. a, $p < 0.05$; b, $p < 0.02$; c, $p < 0.01$; d, $p < 0.002$; e, $p < 0.001$ compared with the corresponding untreated group.

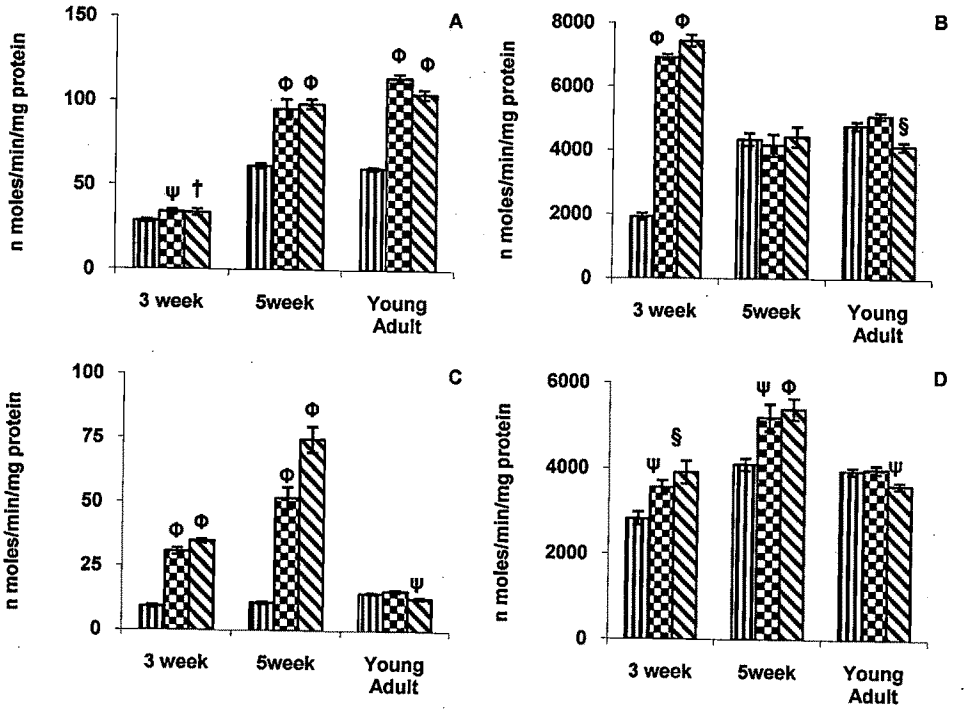

Fig. 1. Effect of DHEA treatment on mitochondrial and cytosolic dehydrogenases activities in rat brain. The results are given as mean ± S.E.M. of 15 independent observations. (A) Glutamate dehydrogenase, (B) malate dehydrogenase (mitochondrial), (C) succinate DCIP reductase and (D) malate dehydrogenase (cytosolic) ▥,untreated; ▩, 0.2 mg DHEA; ▨, 1.0 mg DHEA. †, $p < 0.02$; ψ, $p < 0.01$; §, $p < 0.002$ and Φ, $p < 0.001$ compared with the corresponding untreated group.

Discussion

Present investigations were undertaken to find out if treatment with exogenous DHEA would accelerate the process of maturation and development of cerebral mitochondria in developing rats. This assumes importance in view of the fact that brain contains significant amounts of DHEA and it also synthesizes DHEA (Corpechot et al., 1981; Vallee et al., 2000; Steckelbroeck et al., 2002; Racchi Balduzzi and Corsini, 2003; Ren and Hou, 2005). Besides, it has been shown that the concentration of DHEA in the brain declines with aging (Weill-Engerer et al., 2002; Kazihnitkova et al., 2004). This parallels the known decline in the respiratory activity of mitochondria from different tissues including the brain (Deshmukh and Patel, 1980; Ferrandiz et al., 1994; Cocco et al., 2005; Navarro et al., 2005).

The data of present study indeed suggests that DHEA may play a significant role in regulating the respiratory activity in the brain mitochondria especially during developmental stages. This assumption is substantiated by the fact that treatment with DHEA significantly stimulated the state 3 respiration rates in the mitochondria from the developing animals with glutamate, pyruvate + malate and ascorbate + TMPD while the effect on succinate-linked respiratory activity was of lesser magnitude. The ADP/O ratios were not affected by treatment with DHEA and the respiratory control ratios improved indicating that the mitochondria were structurally intact. The possibility that DHEA by itself improved the respiratory activity and/or stability of mitochondria seems unlikely. It has been shown that incubation in vitro with DHEA resulted in deterioration of all respiratory parameters in the brain mitochondria (Morin et al., 2002). In the present study, the animals received DHEA treatment for 7 consecutive days. The route of administration of DHEA was s.c. which ensures slow and sustained release of the steroids (Jani, Telang and Katyare, 1991; Katyare, Balasubramanian and Parmar, 2003; Pandya, Agarwal and Katyare, 2004). Therefore, it is likely that the observed effects could be attributed to activation of specific nuclear and mitochondrial genes following chronic exposure to DHEA. This indeed seems to be the case when one considers the significant increases in the contents of cytochromes aa_3 and b, ATPase and dehydrogenases activities (Tables 6

and 7 and Fig. 1). However, DHEA treatment had practically no effect on the content of cytochrome $c+c_1$ (Table 6). It is now well recognized that the crucial peptides of cytochromes aa_3 and b and of ATPase are mitochondrial gene products whereas cytochrome $c+c_1$ and the dehydrogenases are coded by the nuclear genes (Poyton and Mc Ewen, 1996). In view of this, it may be suggested that DHEA action may be mediated by activating specific mitochondrial and nuclear genes. It may also be suggested that DHEA may not have a role in regulating expression of cytochrome $c+c_1$ components. The increase in the contents of cytochromes aa_3 and b and of the dehydrogenases agrees well with observed increase in the respiratory activities in the cerebral mitochondria isolated from the developing rats. The net consequence was increase in the ADP phosphorylation rates which is an index of energy potential of the mitochondria (Katewa and Katyare, 2004). It may be pointed out here that the level of dehydrogenases and cytochrome aa_3 were lower in the developing animals. Lower levels of dehydrogenases and cytochrome aa_3 could be the rate limiting step in electron transport and oxidative capacity in the developing animals thereby restricting the energy potential, i.e. ADP phosphorylation rates (Tables 2-5). This indeed seems to be the case when one considers the substantial increase in state 3 respiration rates and ADP phosphorylation rates as pointed out above.

It may also be pointed out that the effects of DHEA treatments on the mitochondria from young adult animals were either of the lower magnitude or seemed to have adverse effects. Thus, treatment with 0.2mg DHEA had marginal stimulatory effects whereas higher dose, i.e. 1.0 mg had occasionally adverse effects. It has been demonstrated that DHEA is rapidly converted to 7α hydroxy DHEA and δ5-androstene-3β, 17β-diol (Steckelbroeck et al., 2002; Weill-Engerer et al., 2003). The former is considered to be the active metabolite which mediates the effects of DHEA (Steckelbroeck et al., 2002; Weill-Engrer et al., 2003). Presence of 7α hydroxylase in the brain tissue has been demonstrated (Akwa et al., 1992) The enzyme activity is low in newborn rats, reaches a value of about 1.5 pmol/min/ mg microsomal protein in the weaning rats (i.e. 3 weeks old), increases about two-fold by the 5^{th} week and reaches optimum level thereafter (Akwa et al., 1992). These authors also reported that the enzyme has Km of 13.8 mM and Vmax of 332 pmol/min/ mg microsomal protein

(Akwa et al., 1992). However, the activity measurements were carried out using suboptimal concentrations of the substrate DHEA. It may hence be suggested that when DHEA is supplied in excess exogenously, it may be able to saturate the enzyme and thereby there may be increased conversion of DHEA to 7α hydroxy DHEA in the developing rat brain. This could then be responsible for observed stimulatory effect. By contrast, in the young adult animals, the enzyme may be generating sufficient quantites of 7α hydroxy DHEA to maintain homeostasis and exogenously supplied DHEA may not have any additional beneficial effect. However, the possibility that the effects are indeed mediated by increased amount of 7α hydroxy DHEA need to be verified by more direct experiment using 7α hydroxy DHEA. Our results also show that in the young adult animals the higher dose (1.0 mg) of DHEA had at times adverse effect. This is not really surprising since catabolic effects of higher doses of thyroid hormones in intact euthyroid and hypothyroid rats have been demonstrated (Satav and Katyare, 1982; Katyare and Rajan, 2005).

As is well recognized, development is an energy-dependent process (Golovachev and Nadal'yak, 1975; Okada, 1994; Rust, 1994). As is evident from the data presented, oxidative energy potential, i.e. ADP phosphorylation rates increased substantially in the developing animals following DHEA treatment. The results thus clearly demonstrate a positive correlation and role of DHEA in the process of development and maturation of cerebral mitochondria. This data further suggests that there could be a role of DHEA supplementation on development of liver mitochondria. This will be looked at in chapter 5.

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