Chapter 2

Effect of dehydroepiandrosterone (DHEA) treatment on oxidative energy metabolism in rat liver and brain mitochondria- A dose-response study

Introduction

Dehydroepiandrosterone (DHEA) and its sulfated conjugate DHEA-S are the two steroids synthesized in highest concentrations (DHEA, 6-8 mg/day; DHEA-S, 15-18 mg/day) by the adrenal cortex in the human adults (Milgrom, 1990; Buvat, 2003). The pattern of secretion is characteristically age related. Thus, the concentration of DHEA in the serum is low in early age, starts increasing two years before the onset of puberty, peaks at adult stage and declines after the age of 35-40 years (Hinson and Raven, 1999; Parker, 1999). Based on this characteristic age-related pattern DHEA is considered to be the youth hormone (Hinson and Raven, 1999; Celec and Starka, 2003). In the past few years a number of studies have been carried out to find usefulness of exogenous DHEA supplementation in health, disease, human well being and improvement of cognitive functions in the elderly population (Celec and Starka, 2003). Although DHEA is freely available in the U.S. without prescription (Hinson and Raven, 1999), claims for its beneficial effects in health, disease and improvement of memory and behaviour in elderly persons have not been very clearly established and also seem to be unequivocal (Hinson and Raven, 1999; Oliver and Clemens, 1999).

It has been reported that treatment with DHEA resulted in increased concentration of NAD+ and NADP+ in liver (Swiercynski et al., 2001) and elevated the rate of malic enzyme gene transcription (Min Kyung et al., 1989). Hypertrophy of hepatocytes following treatment with DHEA has been attributed to proliferation of peroxisomes and mitochondria (Bellei et al., 1992). Antioxidant effects of DHEA have also been demonstrated (Abdulkadir, 2003).

DHEA and DHEA-S are present in the brains of adult rats and DHEA-S has been shown to play a major role in brain development and aging by influencing the migration of neurons, arborization of dendrites and formation of new synapses (Hoffman et al., 2003). In neural precursor culture nanomolar concentration of DHEA decreased apoptosis and activated serine-threonine protein kinase (Akt) (Lei et al., 2002). DHEA treatment also stimulated the process of neurogenesis in a fraction of older rats (Karishma and Herbert, 2002).

The foregoing reports thus indicate that treatment with DHEA significantly influences metabolic activities and energy-dependent functions in the liver and the brain. However, in the studies cited above, various researchers have used different dose regimens and the routes of administration also differed (Swiercynski et al., 2001; Bellei et al., 1992; Abdulkadir et al., 2003; Karishma and Herbert, 2002; Su and Lardy, 1991). There are no reports on the direct effect of DHEA on mitochondrial respiration.

Therefore, it is important to carry out systematic studies to evaluate simultaneously the possible effects of DHEA treatment on oxidative energy metabolism potential of the liver and the brain mitochondria. Outcome of such studies can have implications for therapeutic application of exogenous DHEA supplementation since the metabolic activities, in general, are energy dependent (Celec and Starka, 2003; Katyare Balasubramanian and Parmar, 2003; Pandya, Agarwal and Katyare, 2004).

In this chapter investigation on the effects of treatment with various doses of DHEA on oxidative energy metabolism in liver and brain mitochondria from adult male rats were summarized. This aspect assumes importance since most of the cellular processes are energy-dependent and also because effects of DHEA on mitochondrial metabolism and proliferation have already been demonstrated (Min Kyung et al., 1989; Bellei et al., 1992; Su and Lardy, 1991).

Materials and methods

Chemicals

3β-Hydroxy-5-androsten-17-one (+)-dehydroisoandrosterone (DHEA) was purchased from Sigma-Aldrich, USA. Sodium salt of L-glutamic acid was obtained from E Merck, Germany. 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, oxaloacetic acid and disodium salt of ethylenediaminetetraacetic acid (EDTA) were purchased from Sigma Chemical Co. USA. N, N, N′, N′-Tetra methyl-p-phenylenediamine (TMPD) was purchased from

British Drug Houses, Dorset, Poole, England. Ascorbic acid was from Sarabhai Chemicals, Vadodara. All other chemicals were of analytical-reagent grade and were purchased locally.

Animals and treatment with DHEA

Adult male albino rats (8-10 weeks old) of Charles-Foster strain weighing between 200 and 250 g were used. The animals received daily injections of 0.1, 0.2, 1.0 or 2.0 mg DHEA/kg body weight subcutaneously (s.c.) for 7 consecutive days. Daily records of body weight were maintained. Suspension of DHEA was prepared fresh in saline prior to injection. The controls received equivalent volume of saline. The animals were killed on the 8^{th} day. The experimental protocol was approved by the Departmental Animal Ethics Committee.

Isolation of mitochondria

Isolation of liver and brain mitochondria was done according to the procedures described previously with some modifications (Katewa and Katyare, 2004). At the end of the experimental period, the animals were killed by decapitation and the liver and brain were quickly removed and placed in beakers containing chilled (0-4°C) isolation medium which consisted of 250mM sucrose containing 5mM MOPS and 1mM EDTA all at pH 7.4; 0.25 mg BSA/mL of isolation medium was included. Liver tissue was minced with a pair of scissors. The tissues were washed repeatedly with the isolation medium to remove adhering blood. The tissues were then homogenized using a Potter-Elvehjem-type glass-Teflon homogenizer to obtain 10% and 20% (W/v) homogenates, respectively, for liver and brain. After removal of nuclei and cell debris at 650×g for 10 min, the mitochondria were sedimented by centrifugation at 7500×g for 10 min, washed once by gently suspending in the isolation medium and resedimenting and were finally suspended in the isolation medium to give a protein concentration in the range of 25-30 mg/mL or 10-15 mg/mL, respectively, for liver and brain mitochondria. All the steps in the isolation procedure were carried out at 0-4°C in a Sorvall RC 5B plus

centrifuge. Cytosolic fraction was isolated from post-mitochondrial supernatant as described previously (Bangur, Howland and Katyare, 1995). We have previously demonstrated that mitochondria isolated by these procedures are practically free from microsomal and/or cytosolic contaminations (Bangur, Howland and Katyare, 1995; Satav et al., 1976).

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). Briefly, the respiration medium (total volume 1.6mL) consisted of 225mM sucrose, 20mM KCl, 10mM MOPS pH 7.4, 5mM potassium phosphate buffer pH 7.4, 0.2mM EDTA and 160µg of BSA (i.e., 0.1 mg BSA/mL). After introducing mitochondria (4-8 mg protein depending on the substrate used) in the electrode chamber, respiration was induced by addition of substrates. Final concentrations of the substrates used were glutamate (10mM), pyruvate + malate (10mM+1mM), succinate (10mM) and ascorbate + TMPD (10mM+0.1mM). Measurements with the latter two substrates were performed in the presence of 1µM rotenone. State 3 respiration rates initiated by the addition of 80-200 nmol of ADP and state 4 respiration rates ensuing after the depletion of added ADP 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 calculated from the difference spectra as described previously (Pandya, Agarwal and, Katyare, 2004; Katewa and Katyare, 2004). Briefly, 6-8 mg mitochondrial protein was taken up in potassium phosphate-buffered sucrose and solubilized by adding 0.25mL of 10% Triton X-100. The total volume was made up to 2.5mL. The sample was then transferred to two 1mL cuvettes. The sample in the reference cuvette was oxidized by adding small amount of potassium ferricyanide and

the sample in the experimental cuvette was reduced by adding a few mg of sodium dithionite. The different spectra of reduced versus oxiõdized samples were recorded in a JASCO UV/VIS spectrophotometer model V-530. The contents of aa_3, b and $c+c_1$ cytochromes 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 (Subramaniam and Katyare, 1990).

Assay of dehydrogenases

Glutamate dehydrogenase (GDH), malate dehydrogenase (MDH) and succinate DCIP reductase (SDR) activities were determined as described previously (Pandya, Agarwal and, Katyare, 2004; Katewa and Katyare, 2004).

Assay of ATPase

The ATPase activity in the liver mitochondria was measured using the following assay medium (total volume 0.1mL) containing 50mM MOPS pH 7.4, 75mM KCl and 0.4mM EDTA. The assays were performed in the absence and presence of $MgCl_2$ (6mM) and DNP 100µM, or a combination of both. After preincubating the mitochondrial protein (40-60 µg) in the assay medium at 37°C for 1 min, the reaction was initiated by the addition of ATP at the final concentration of 5mM. The reaction was carried out for 15 min and then terminated by the addition of 0.1mL of 5% (W/v) SDS (Pandya, Agarwal and, Katyare, 2004; Katewa and Katyare, 2004).

The assay mixture for ATPase activity in brain mitochondria (total volume 0.1mL) contained 350mM sucrose, 10mM MOPS pH 7.4, 10mM KCl and 0.2mM EDTA. The assays were performed in the absence and presence of $MgCl_2$ (2mM) and DNP 50µM, or a combination of both. After pre-incubating the mitochondrial protein (40-60 µg) in the assay medium at 37°C for 1 min, the reaction was initiated by the addition of ATP at the final concentration of 2mM. The reaction was carried out for 15 min and then terminated by the addition of 1.1mL of 5% W/v TCA (Swegert, Dave and Katyare,

1999). The tubes were allowed to stand on ice and then centrifuged at 3000 rpm for 10 min and 0.8mL aliquots of supernatant fluid were taken up for the estimation of liberated inorganic phosphate. Estimation of inorganic phosphate was according to the procedure described by Katewa and Katyare (2003).

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 carried out by Tukey test using Sigmastat version 2.0.

Results

Treatment with increasing doses of DHEA resulted in progressive increase in the body weight compared to the untreated controls, with maximum increase (23%) being noted for 1 mg dose. At higher dose (2.0 mg) of DHEA the increase in the body weight declined. Under these conditions, the liver and brain weights did not change (data not given).

Effect on oxidative phosphorylation

Liver mitochondria

Effects of DHEA treatment with different doses on oxidative phosphorylation in liver mitochondria are summarized in Tables 1-4. Thus, treatment only with 0.2 mg DHEA resulted in 26% increase in state 3 respiration rate with glutamate as the substrate. However, the increase was not statistically significant. By contrast, the state 4 respiration rate was stimulated by 73% at 1.0 mg dose. The ADP/O ratios were unchanged following DHEA treatment. The ADP phosphorylation rate increased by

25% in animals treated with 0.2 mg DHEA, which agrees well with the similar increase in state 3 respiration rate (Table 1).

When pyruvate + malate were used as the substrate couple, maximum and greater stimulation of state 3 respiration rate (59% increase) was obtained in animals treated with 1.0 mg dose. Under these conditions the state 4 respiration rate was stimulated by 69%. The ADP/O ratios were not affected. An increase in ADP phosphorylation rate corresponding to a similar increase in state 3 respiration rates in animals treated with 1.0 mg dose was also noted (Table 2).

When succinate was used as the respiratory substrate the maximum stimulation of state 3 respiration rate (21% increase) was seen in animals treated with 0.2 mg DHEA. The state 4 respiration rate was also maximally stimulated at this dose (54%); the stimulatory effect declined at higher doses. ADP/O ratios were unaffected by DHEA treatment. ADP phosphorylation rate increased by 21% in animals treated with 0.2 mg DHEA although the increase was no statistically significant (Table 3).

With ascorbate + TMPD as the electron donor system, the stimulation of state 3 respiration rate (53% increase) was noted at a higher dose of DHEA, i.e. 1 mg. A similar trend was also seen for state 4 respiration rate (40% increase). The ADP/O ratios were unchanged and ADP phosphorylation rates increased by 39% in animals treated with 1 mg DHEA although the increase was no statistically significant (Table 4).

Treatment with 0.2 and 1.0 mg doses of DHEA resulted in significant increase in the content of cytochrome aa_3 (35-44% increase). Similarly, following the treatment with 0.2-2.0 mg doses of DHEA the content of cytochrome b increased from 15% to 23%. The content of cytochrome $c+c_1$ increased (11% increase) only in animals treated with 0.2 mg doses of DHEA (Table 5).

Treatment with DHEA, in general, resulted in stimulation of basal ATPase activity while the Mg^{2+}-stimulated ATPase activity increased (37%) only in the animals treated with 0.1 mg dose. Maximum stimulation (58%) of the DNP-stimulated ATPase activity was

observed in the animal treated with 0.2 mg DHEA. Also, the DNP+ Mg^{2+}-stimulated ATPase activity increased by 31% in 0.2 mg DHEA treated animals (Table 6).

Treatment with 0.1 mg and 0.2 mg DHEA stimulated GDH activity by 36% and 50%. The SDR activity was stimulated by 80% following treatment with 0.2 mg DHEA. The MDH activities both mitochondrial as well as cytosolic were generally unaffected (Fig. 1).

Brain mitochondria

Effects of treatment with DHEA on oxidative energy metabolism in brain mitochondria are summarized in Tables 1-4. Thus, when glutamate was used as the respiratory substrate treatment with DHEA (0.1 mg to 1.0 mg) brought about significant stimulation (36-44% increase) in state 3 respiration rate. Under these conditions, the state 4 respiration rate was stimulated by 3.2-fold in animals treated with 1.0 mg DHEA. The ADP/O ratios were not affected by DHEA treatment. Changes in state 3 respiration rates were reflected in corresponding increase in the ADP phosphorylation rates (35-52% increase) (Table 1).

As opposed to the above, when pyruvate + malate was used as substrate couple the changes in state 3 respiration rates were marginal; only in animal treated with 1.0 mg DHEA the rate increased by 17%. However, the change was not statistically significant. Higher doses of DHEA (1.0 mg and 2.0 mg) stimulated state 4 respiration rates by 40% and 27%, respectively; the latter change was not statistically significant. The ADP phosphorylation rates did not change significantly by DHEA treatment (Table 2).

When succinate was used as substrate the state 3 and state 4 respiration rates were generally unchanged by treatment with DHEA. Similarly, the ADP phosphorylation rates were also generally unaltered (Table 3).

With ascorbate + TMPD as the electron donor system only the state 4 respiration rate increased (22-28% increase) in the animals treated with 0.1 mg to 1.0 mg DHEA. The ADP phosphorylation rates were somewhat high but the changes were not statistically significant (Table 4).

DHEA treatment (0.2-2.0 mg) resulted in increase in the content of cytochrome aa_3 and b with the optimum increase (44% and 80% increase, respectively) seen at 1 mg dose. The effect declined at higher dose (2.0 mg) of DHEA. The content of cytochrome $c+c_1$ increased by 15% only in the animals treated with 0.1 mg DHEA. However, the change was not statistically significant. At the highest dose employed (2.0 mg), the content actually decreased by 24% (Table 5).

DHEA treatment had marginal effect on basal and Mg^{2+}- stimulated ATPase activities but significantly enhanced DNP stimulated and DNP+ Mg^{2+}-stimulated ATPase activities. This effect was noted up to the dose of 1.0 mg DHEA (Table 6).

The GDH activity increased by 89% and 114%, respectively, following treatment with 0.1 and 0.2 mg dose of DHEA with the effect declining at higher doses. The SDR activity increased progressively with maximum effect (1.74-fold increase) being seen at the highest dose (2.0 mg) of DHEA. The mitochondrial MDH activity increased (28% increase) in the animals treated with 1.0 mg DHEA whereas the cytosolic MDH activity was unaltered (Fig. 1).

Table 1: Effect of DHEA treatment on oxidative phosphorylation in rat liver and brain mitochondria using glutamate as the substrate

Tissue	Treatment	ADP/O ratio	Respiration rate (nmole O_2/ min/mg protein)		Respiratory Control Ratio	ADP phosphorylation rate (nmole /min/ mg protein)
			+ ADP	-ADP		
Liver	Untreated (12)	3.13 ± 0.15	28.45 ± 1.35	10.30 ± 0.77	2.90 ± 0.17	178.1 ± 7.74
	0.1 mg DHEA (10)	3.14 ± 0.29	27.81 ± 2.47	12.77 ± 1.85	2.24 ± 0.18	174.6 ± 9.50
	0.2 mg DHEA (10)	3.11 ± 0.16	35.88 ± 1.89	12.81 ± 1.59	2.88 ± 0.17	223.2 ± 12.74[a]
	1.0 mg DHEA (12)	2.97 ± 0.27	31.10 ± 2.70	17.77 ± 2.08[a]	1.75 ± 0.17[a,b]	184.7 ± 11.69
	2.0 mg DHEA (10)	3.18 ± 0.25	28.01 ± 1.85	13.26 ± 1.49	2.19 ± 0.17[a]	179.0 ± 18.04
***P* value between groups**		–	–	**0.022**	**<0.001**	–
Brain	Untreated (14)	3.21 ± 0.22	20.12 ± 1.02	5.40 ± 0.79	4.92 ± 0.62	127.1 ± 8.90
	0.1 mg DHEA (8)	3.26 ± 0.24	28.46 ± 2.28[a]	5.78 ± 0.76	5.36 ± 0.60	184.4 ± 15.98[a]
	0.2 mg DHEA (12)	3.16 ± 0.19	27.32 ± 1.11[a]	5.74 ± 1.01	4.61 ± 0.80	171.4 ± 10.16[a]
	1.0 mg DHEA (12)	3.26 ± 0.23	28.98 ± 1.88[a]	17.01 ± 1.74[a,b,c]	1.85 ± 0.11[a,b,c]	192.6 ± 12.50[a]
	2.0 mg DHEA (10)	3.16 ± 0.16	21.52 ± 1.02[b,d]	6.02 ± 0.62	3.85 ± 0.29	133.4 ± 4.75[b,d]
***P* value between groups**		**<0.001**	**<0.001**	**<0.001**	**<0.001**	–

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

a $p<0.05$ compared with control.

b $p<0.05$ compared with 0.1 mg DHEA.

c $p<0.05$ compared with 0.2 mg DHEA.

d $p<0.05$ compared with 1 mg DHEA groups.

Table 2: Effect of DHEA treatment on oxidative phosphorylation in rat liver and brain mitochondria using pyruvate + malate as the substrate

Tissue	Treatment	ADP/O ratio	Respiration rate (nmole O_2/ min/mg protein)		Respiratory Control Ratio	ADP phosphorylation rate (nmole /min/ mg protein)
			+ ADP	-ADP		
Liver	Untreated (12)	3.11 ± 0.27	17.72 ± 1.08	8.58 ± 0.77	2.16 ± 0.12	110.0 ± 11.68
	0.1 mg DHEA (10)	3.02 ± 0.10	16.85 ± 0.64	7.95 ± 0.45	2.18 ± 0.14	102.3 ± 6.58
	0.2 mg DHEA (10)	3.14 ± 0.24	16.42 ± 0.58	8.93 ± 0.23	1.85 ± 0.09	103.6 ± 9.98
	1.0 mg DHEA (12)	3.14 ± 0.18	28.16 ± 2.25[a,b,c]	14.50 ± 1.22[a,b,c]	1.97 ± 0.16	177.3 ± 18.43[a,b,c]
	2.0 mg DHEA (10)	3.18 ± 0.24	18.53 ± 0.89[d]	11.06 ± 0.32[d]	1.69 ± 0.10	119.1 ± 13.78[d]
***p* value between groups**		–	**<0.001**	**<0.001**	–	**<0.001**
Brain	Untreated (12)	3.13 ± 0.15	22.27 ± 1.24	5.67 ± 0.49	3.93 ± 0.25	138.9 ± 9.19
	0.1 mg DHEA (12)	3.23 ± 0.16	24.52 ± 1.33	6.21 ± 0.42	3.95 ± 0.24	157.8 ± 10.11
	0.2 mg DHEA (12)	3.19 ± 0.17	22.19 ± 1.21	6.14 ± 0.58	3.92 ± 0.28	141.5 ± 6.80
	1.0 mg DHEA (12)	3.17 ± 0.24	26.00 ± 1.31	7.93 ± 0.40[a]	3.80 ± 0.22	164.8 ± 7.69
	2.0 mg DHEA (10)	3.17 ± 0.23	21.45 ± 0.81	7.20 ± 0.35	3.59 ± 0.20	136.0 ± 8.62
***p* value between groups**		–	–	**0.007**	–	–

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

a $p<0.05$ compared with control.

b $p<0.05$ compared with 0.1 mg DHEA.

c $p<0.05$ compared with 0.2 mg DHEA.

d $p<0.05$ compared with 1 mg DHEA groups.

Table 3: Effect of DHEA treatment on oxidative phosphorylation in rat liver and brain mitochondria using succinate as the substrate

Tissue	Treatment	ADP/O ratio	Respiration rate (nmole O_2/ min/mg protein)		Respiratory Control Ratio	ADP phosphorylation rate (nmole /min/ mg protein)
			+ ADP	-ADP		
Liver	Untreated (12)	2.15 ± 0.11	59.52 ± 2.83	24.62 ± 1.45	2.47 ± 0.10	256.2 ± 9.90
	0.1 mg DHEA (9)	2.26 ± 0.19	61.79 ± 2.25	26.18 ± 1.73	2.45 ± 0.18	279.6 ± 25.18
	0.2 mg DHEA (11)	2.15 ± 0.18	71.83 ± 4.92	37.97 ± 3.04[a,b]	1.98 ± 0.12	309.3 ± 17.20
	1.0 mg DHEA (9)	2.21 ± 0.17	59.75 ± 4.08	31.37 ± 2.49	1.94 ± 0.12[a]	264.0 ± 17.50
	2.0 mg DHEA (11)	2.25 ± 0.11	56.56 ± 5.00	29.90 ± 1.34	1.99 ± 0.13	251.2 ± 22.66
***p* value between groups**		–	–	**<0.001**	**0.004**	–
Brain	Untreated (13)	2.15 ± 0.18	26.70 ± 1.18	15.07 ± 1.27	1.91 ± 0.16	114.8 ± 6.90
	0.1 mg DHEA (12)	2.02 ± 0.18	28.24 ± 1.42	17.39 ± 0.89	1.63 ± 0.05	114.1 ± 8.19
	0.2 mg DHEA (10)	2.15 ± 0.17	32.86 ± 2.38	19.16 ± 1.31	1.83 ± 0.13	141.3 ± 6.03
	1.0 mg DHEA (12)	2.11 ± 0.15	29.22 ± 1.88	18.32 ± 1.67	1.67 ± 0.08	125.5 ± 10.8
	2.0 mg DHEA (9)	1.88 ± 0.11	26.12 ± 2.19	17.24 ± 1.09	1.58 ± 0.09	98.1 ± 9.71[c]
p value between groups		–	–	–	–	**0.022**

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

a $p<0.05$ compared with control.

b $p<0.05$ compared with 0.1 mg DHEA.

c $p<0.05$ compared with 0.2 mg DHEA groups.

Table 4: Effect of DHEA treatment on oxidative phosphorylation in rat liver and brain mitochondria using ascorbate + TMPD as the substrate

Tissue	Treatment	ADP/O ratio	Respiration rate (nmole O_2/ min/mg protein)		Respiratory Control Ratio	ADP phosphorylation rate (nmole /min/ mg protein)
			+ ADP	-ADP		
Liver	Untreated (12)	0.47 ± 0.03	29.11 ± 1.84	23.15 ± 1.61	1.27 ± 0.03	26.5 ± 1.58
	0.1 mg DHEA (13)	0.44 ± 0.07	30.10 ± 1.28	22.10 ± 1.24	1.38 ± 0.03	26.0 ± 3.58
	0.2 mg DHEA (14)	0.45 ± 0.04	34.26 ± 2.47	24.95 ± 1.50	1.37 ± 0.04	29.9 ± 2.81
	1.0 mg DHEA (12)	0.42 ± 0.02	44.42 ± 2.63[a,b,c]	32.49 ± 1.82[a,b,c]	1.37 ± 0.04	36.6 ± 2.69
	2.0 mg DHEA (12)	0.41 ± 0.05	36.05 ± 3.04	26.15 ± 1.91	1.38 ± 0.03	28.6 ± 4.06
***p* value between groups**		–	**<0.001**	**<0.001**	–	–
Brain	Untreated (12)	0.77 ± 0.07	25.34 ± 1.34	16.30 ± 0.94	1.59 ± 0.06	38.9 ± 3.09
	0.1 mg DHEA (13)	0.78 ± 0.05	29.77 ± 1.18	19.81 ± 1.27	1.49 ± 0.03	45.9 ± 2.20
	0.2 mg DHEA (12)	0.76 ± 0.06	29.27 ± 1.43	20.56 ± 0.91[a]	1.42 ± 0.04	44.3 ± 3.25
	1.0 mg DHEA (12)	0.75 ± 0.04	28.94 ± 1.30	20.82 ± 0.76[a]	1.39 ± 0.03[a]	44.1 ± 2.23
	2.0 mg DHEA (13)	0.76 ± 0.04	25.62 ± 1.40	17.71 ± 0.90	1.46 ± 0.06	37.5 ± 2.11
***p* value between groups**		–	**0.047**	**0.007**	**0.041**	–

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

a $p<0.05$ compared with control.

b $p<0.05$ compared with 0.1 mg DHEA.

c $p<0.05$ compared with 0.2 mg DHEA groups.

Table 5: Effect of DHEA treatment on the cytochrome content of rat liver and brain mitochondria

Tissue	Treatment	Cytochrome content (pmol/mg protein)		
		aa_3	b	$c+c_1$
Liver	Untreated (8)	117.6 ± 8.17	257.5 ± 8.58	327.0 ± 4.07
	0.1 mg DHEA (6)	119.4 ± 4.56	255.2 ± 8.63	338.1 ± 8.52
	0.2 mg DHEA (8)	169.7 ± 6.58[a]	317.1 ± 12.54[a,b]	366.0 ± 10.24[a]
	1.0 mg DHEA (10)	158.8 ± 15.99[a,b]	298.1 ± 7.19[a,b]	344.4 ± 5.16
	2.0 mg DHEA (8)	177.8 ± 13.02	295.9 ± 5.86[a,b]	352.4 ± 6.71
***p* value between groups**		**0.002**	**<0.001**	**0.007**
Brain	Untreated (6)	146.5 ± 4.41	162.1 ± 5.41	218.8 ± 8.00
	0.1 mg DHEA (5)	177.3 ± 4.38[a]	194.2 ± 8.85[a]	251.8 ± 11.33
	0.2 mg DHEA (8)	172.4 ± 5.49[a,b]	215.9 ± 10.45[a,b]	205.7 ± 17.55
	1.0 mg DHEA (9)	211.6 ± 3.45[a,b,c]	291.3 ± 8.13[a,b,c]	203.8 ± 15.19
	2.0 mg DHEA (5)	204.6 ± 4.18[a,b,c,d]	215.0 ± 10.89[a,b,c,d]	166.2 ± 7.96[b]
***p* value between groups**		**<0.001**	**<0.001**	**0.022**

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

a $p<0.05$ compared with control.

b $p<0.05$ compared with 0.1 mg DHEA.

c $p<0.05$ compared with 0.2 mg DHEA.

d $p<0.05$ compared with 1 mg DHEA groups.

Table 6: Effect of DHEA treatment on ATPase activity in rat liver and brain mitochondria

Tissue	Treatment	Activity (µmol Pi liberated/h/mg protein)			
		Basal	$+Mg^{2+}$	+DNP	$+Mg^{2+}$+DNP
Liver	Untreated (12)	1.67 ± 0.13	5.14 ± 0.20	18.98 ± 1.75	21.42 ± 1.46
	0.1 mg DHEA (8)	3.74 ± 0.30[a]	7.03 ± 0.61[a]	23.28 ± 1.38	20.89 ± 0.69
	0.2 mg DHEA (12)	4.43 ± 0.40[a]	7.39 ± 0.68	30.00 ± 2.64[a]	28.07 ± 2.01[a,b]
	1.0 mg DHEA (14)	4.38 ± 0.29[a]	6.29 ± 0.45	22.58 ± 1.18[b]	22.45 ± 0.93
	2.0 mg DHEA (8)	4.03 ± 0.37[a]	6.12 ± 0.56	17.81 ± 1.68[b]	21.60 ± 2.44
***p* value between groups**		**<0.001**	**0.027**	**<0.001**	**0.012**
Brain	Untreated (12)	0.47 ± 0.02	6.06 ± 0.40	0.39 ± 0.02	5.76 ± 0.49
	0.1 mg DHEA (8)	0.59 ± 0.04	7.53 ± 0.24	0.62 ± 0.04[a]	7.75 ± 0.46[a]
	0.2 mg DHEA (12)	0.88 ± 0.05[a,c]	6.70 ± 0.17	0.94 ± 0.05[a,c]	7.32 ± 0.29
	1.0 mg DHEA (14)	0.53 ± 0.03[b]	5.93 ± 0.49	0.82 ± 0.04[a,c]	7.59 ± 0.46[a]
	2.0 mg DHEA (8)	0.54 ± 0.02[b]	7.15 ± 0.57	0.35 ± 0.02[a,c,d]	6.35 ± 0.33
***p* value between groups**		**<0.001**	**0.048**	**<0.001**	**0.007**

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

a $p<0.05$ compared with control.

b $p<0.05$ compared with 0.2 mg DHEA.

c $p<0.05$ compared with 0.1 mg DHEA.

d $p<0.05$ compared with 1 mg DHEA groups.

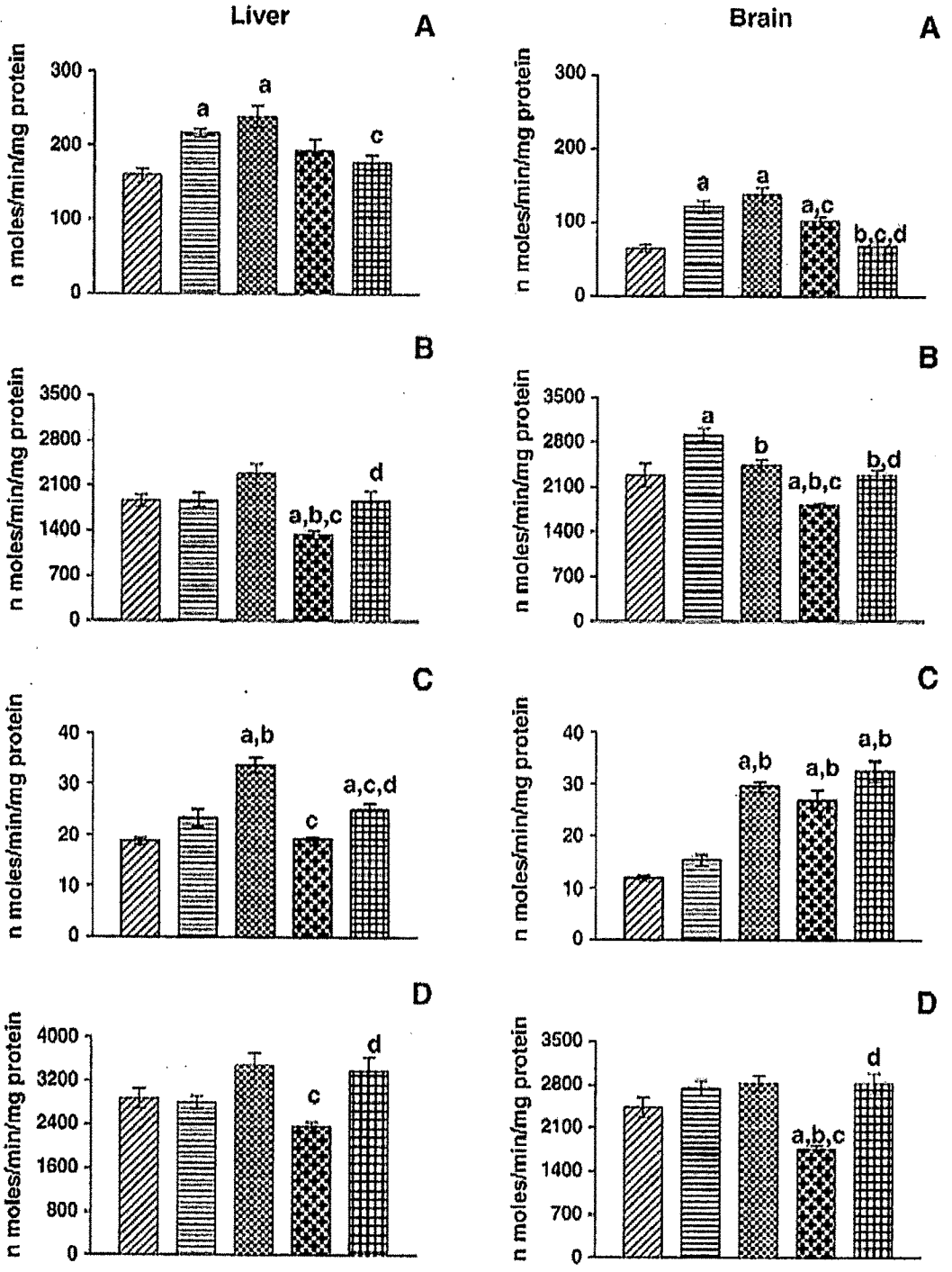

Fig. 1. Effect of DHEA treatment on mitochondrial and cytosolic dehydrogenases activities in rat liver and brain. The results are given as mean±SEM of 10-12 independent observations. (A) Glutamate dehydrogenase; (B) malate dehydrogenase (mitochondrial); (C) succinate DCIP reductase; and (D) malate dehydrogenase (cytosolic).

 Untreated,  0.1 mg DHEA,  0.2 mg DHEA,  1.0 mg DHEA and  2.0 mg DHEA. **a**, p <0.05 compared with control; **b**, p<0.05 compared with 0.1 mg DHEA; **c**, p<0.05 compared with 0.2 mg DHEA and **d**, p<0.05 compared with 1 mg DHEA groups. The p values between the groups in liver as well as brain were: GDH, p=0.001; MDH (Mitochondrial), p<0.001; SDR, p<0.001 and MDH (cytosolic), p<0.001.

Discussion

The present study was undertaken to evaluate the possible effects of treatment with DHEA on oxidative energy metabolism in rat liver and brain mitochondria. The dose regimen was essentially based on the report of Su and Lardy (Su and Lardy, 1991). These authors reported that when the rats were fed a diet containing 0.01-0.2% DHEA for seven days the hepatic mitochondrial glycerophosphate dehydrogenase activity increased 3-5 times (Su and Lardy, 1991). We decided to use s.c. route for administration of DHEA based on our earlier experiments with corticosterone and dexamethasone, where the steroids were injected s.c. to ensure a slow release in circulation (Katyare, Balasubramanian and Parmar, 2003; Pandya, Agarwal and Katyare, 2004). This aspect is important especially in view of the short half-life of DHEA (Belisle, Schiff and Tulchinsky, 1980; Tagawa et al., 2000). The additional advantage is that this eliminates the problem of variations in the absorption of DHEA ingested by oral route.

From the data presented it is clear that the treatment regimen that we followed significantly influenced the energy metabolism parameters in mitochondria from the liver as well as the brain. The effects were tissue specific and dose dependent. Broadly speaking, treatment with 0.1-2.0 mg DHEA affected respiration rates, cytochrome contents, ATPase activities and dehydrogenases levels in mitochondria from the two tissues in different manner. Most importantly with highest concentration of DHEA (2.0 mg), the effects, in general, declined.

Thus, in the liver mitochondria state 3 respiration with glutamate was maximally stimulated following treatment with 0.2 mg dose; in the brain mitochondria doses from 0.1 to 1.0 mg stimulated state 3 respiration rates with the extent of stimulation being greater than that observed for liver mitochondria. State 3 respiration rates with pyruvate + malate were stimulated maximally in liver mitochondria after treatment with 1 mg DHEA whereas only marginal changes were noted for the brain mitochondria which were not statistically significant. In the liver as well as brain mitochondria with succinate as the substrate, the state 3 respiration rates increased

by about 20% with 0.2 mg dose of DHEA although the increase was not statistically significant State 3 respiration rate with ascorbate + TMPD was only marginally affected in the brain mitochondria whereas in the liver mitochondria about 50% stimulation was obtained with 1 mg dose. DHEA treatment had no uncoupling effect and ADP/O ratios were unchanged. Observed increases in state 3 respiration rate were also reflected in corresponding increases in ADP phosphorylation rates, which is indicative of increased energy potential (Tables 1-4).

DHEA treatment also resulted in significant increase in the contents of cytochrome aa_3 and b in mitochondria from both the tissues. The increase in the content of $c+c_1$ cytochromes was marginal. Once again the effects were tissue specific. In the case of liver mitochondria 0.2 mg dose was more effective whereas for brain mitochondria the effects were evident with the lowest dose (0.1 mg) employed. Also DHEA treatment was more effective in increasing the contents of cytochrome aa_3 in liver mitochondria, by contrast in the brain mitochondria more pronounced effect was seen on the content of cytochrome b (Table 5).

Likewise the DHEA treatment differentially influenced GDH and SDR activities in the liver and brain mitochondria. Paradoxically, however, a negative effect on mitochondrial and cytosolic MDH was seen after treatment with 1.0 mg DHEA (Fig. 1). Stimulation of malic enzyme following treatment with DHEA has been reported by other workers (Min Kyung et al., 1989; Su and Lardy, 1991). However, in our studies we obtained an opposite effect. The reason for this discrepancy remains unclear at this stage. It is possible that this may be related to the dose, route of administration as well as duration of treatment (Min Kyung et al., 1989; Su and Lardy, 1991). DHEA treatment also differentially stimulated the ATPase activities in liver and brain mitochondria (Table 6). The increased respiratory activities in mitochondria correlate well with observed increases in the contents of the aa_3 and b cytochromes and the dehydrogenases activities.

Although DHEA and DHEA-S are known to be synthesized in the highest quantity by adrenal gland, there are no known receptors demonstrated for these two steroids

(Natawa et al., 2002). The mechanism for action of DHEA therefore remains unclear. Based on its stimulatory effect on malic enzyme synthesis, it has been suggested that DHEA action is mediated via thyroid hormones, which are known to stimulate MDH synthesis (Min Kyung et al., 1989; Su and Lardy, 1991). Other suggested mechanism is the direct interaction of DHEA with macromolecules (Natawa et al., 2002). However, inhibition of state 3 respiration rates and FoF1 ATPase activity in mitochondria by addition of DHEA and DHEA-S in vitro (McIntosh, Pan and Berdanier, 1993; Chance and McIntosh, 1995; Zheng and Ramirez, 1999) would argue against this possibility. Alternative mechanism that has been proposed is that secondary metabolites, steroid in nature, may mediate DHEA action (Lardy, Marwah and Marwah, 2002). More recently, it has been demonstrated that DHEA is metabolized to 7α-hydroxy-DHEA and δ5-androstene-3β,17β-diol. The former is considered to be the active metabolite (Steckelbroeck et al., 2002; Weill-Engerer et al., 2003).

In the present study, we observed that the exposure to DHEA resulted in increase in the contents of cytochrome aa_3, b and ATPase activity but not of content of cytochrome $c+c_1$. These observations deserve some comments. It is well established that crucial polypeptide of cytochrome oxidase, cytochrome b and FoF1 ATPase are coded by mitochondrial DNA (Poyton and Mc Ewen, 1996). By contrast, cytochrome $c+c_1$ is a nuclear gene product (Poyton and Mc Ewen, 1996). It may hence be suggested that DHEA action may be mediated by activating specific mitochondrial genes coding for polypeptide subunits of cytochrome aa_3, b and FoF1 ATPase. In this connection, it is interesting to note thatpresence of dexamethasone binding site in COX II region of mitochondrial genome has been demonstrated (Demonacos et al., 1995; Tsiriyotis, Sandidos and Sekeris, 1997; Simon et al., 1998). It would be interesting to know if similar DHEA binding site(s) exists on mitochondrial genome. Likewise, increase in the GDH and SDR activities suggests that DHEA action may also be specific for activating these nuclear genes.

It is possible that DHEA treatment-induced changes in the respiratory activities in mitochondria from the two tissues could influence the cellular reactive oxygen species

(ROS) parameters. This interesting possibility needs to be examined by separate experiments.

In conclusion, our present study has shown that treatment with DHEA resulted in stimulation of respiratory activity and increase in the energy potential of the liver and the brain mitochondria. However, the effects were dose dependent and tissue specific for a given parameter. Also, higher dose (2.0 mg) of DHEA had adverse effects. Thus, our results point out that although exogenous DHEA can stimulate the energy related functions in the tissues such as liver and brain which can have beneficial effects for the elderly population (Celec and Starka, 2003), one has to exercise caution in therapeutic application of DHEA. Especially one has to take into consideration the fact that an overdoseof DHEA can have adverse effects.

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