CHAPTER - V

EFFECTS OF CORTICOSTERONE TREATMENT ON ACTIVITIES OF MITOCHONDRIAL ATPase AND PRIMARY DEHYDROGENASES AND KINETIC PROPERTIES OF ATPase IN LIVER DURING DEVELOPMENT.

Glucocorticoids under both *in vivo* and *in vitro* conditions are knwon to have effect on mitochondrial respiration and oxidative phosphorylation in tissues such as liver, heart and muscles (1-24). Glucocorticoid effects on brain mitochondria are described and discussed in chapter II. Most of the researchers have used liver mitochondria as a model system to study glucocorticoid effects on energy - linked funtions (10-23).

Glucocorticoid hormones are known to increase the volume of mitochondria from liver and muscles (1-3). cortisone treatment to adult rats for 6 - days resulted in four fold increased in the average mitochondrial volume with a commensurate decrease in the number of mitochondria per cell in the liver. The changes were observed throughout the hepatic lobule, but were most marked in midzonal cells. The total mitochondrial volume per cell and the percent of the total cytoplasmic volume occupied by mitochondria and total cristae surface per cell remained relatively unaltered (1). Study by Kimberg and Loeb (2) has shown that cortisone - induced increase in mitochondrial size and approximately reciprocal decrease in mitochondrial number in liver was due to mitochonrial fusion rather than loss of pre-existing mitochondria. Bullock *et al*. (3) have reported that triamcinolone acetonide treatment to adult rats results in enlargement of muscles mitochondria.

Treatment of rats with glucocorticoids has been observed to diminish both the substrate and ATP- dependent calcium accumulation by isolated liver mitochondria (4,5). Similarly administration of dexamethasone to rats markedly diminished the initial rate and maximum extent of substrate dependent calcium uptake in subsequently isolated liver mitochondria and enhanced the release of calcium. The liver mitochondria from dexamethasone treatment group showed significantly low cytochrome c oxidase activity, and translocated ATP from inside to outside faster than those from controls. The decrease in calcium transport and retension of calcium in liver mitochondria from dexamethasone treated rats was found to be regulated by intramitochondrial ATP content and not due to alterations in calcium carrier system (6). Hughes and Barritt (7) have shown that single injection of dexamethasone to intact rats causes 6 fold increase in the time to accumulate calcium by isolated liver mitochondria. These authors have also shown interactions between glucocorticoids and glucogon in hormonal regulation of calucium retension by liver mitochondria.

Hydrocortisone selectively inhibited oxidative metabolism of liver mitochondria from control rats under <u>in vitro</u> conditions. This glucocorticoid at concentration above 0.1 mM inhibited the oxidation of citrate, L-malate, L-glutamate and octanoate but had no effect on succinoxidase system. The

inhibition of oxidative metabolism with pyridine nucleotide requiring dehydrogenases by hydrocortisone was due to destruction of selective permeability of mitochondria, resulting in loss of pyridine nucleotides (8).

In vitro studies by Jenson and Neuhard (9) have shown that corticosterone inhibits oxidation of NADH by heart sarcosome fragments. Liljeroot and Hall (10) found that in vitro addition of cortisone increased P : O ratios in liver mitochondria. In contrast, results of some other in vitro studies have shown that incubation of isolated liver mitochondria with high concentrations of glucocorticoid caused uncoupling and decreased state 3 repiration rates (8,11-13).

In intact rat skeletal muscle, liver and heart mitochondria, in vitro incubation with methyl prednisolone caused inhibition of state 3 respiration rates with succinate and NAD^+ - linked substrates. Inhibition of oxidation of NAD^+- linked substrates was suggested to be at the level of respiratory chain between the NADH dehydrogenase flavoprotein and coenzyme Q (14). In vitro studies by McIntosh et al. (5) have also shown that glucocorticoids caused decrease in state 3 mitochondrial respiration, respiratory control ratio (RCR) and oxidative phosphorylation capacity (ADP/O) ratio) in a dose - dependent manner with pyruvate + malate as substrate.

Deoxycorticosterone when added in vitro, increased ATPase activity of lymphosarcoma and liver mitochondria in a dose - dependent manner. This glucocorticoid did not cause leakage of proteins from mitochondria and caused expansion of mitochondrial volume (15).

As far as in vitro studies are concerned a question arises about the physiological significance of these direct actions of the glucocorticoids at the membrane level, given the fact that most of the direct actions of the steroids on membranes are usually demonstrated at high concentrations, which are several orders of magnitude higher than the normal circulating concentrations. It is generally accepted that these effects represent non-specific pharmacological actions (17). Additional factors which are not taken into consideration in in vitro studies are penetration of added hormone into cells and subcellular organelles, rate of possible degradation and inactivation of the hormone. Above all the major limitations of in vitro studies would be that they will give idea only about the non-genomic actions of glucocorticoids and the classical receptor mediated genomic actions would not be observed under in vitro conditions. Although in vivo studies may have some limitations, these could be physiologically more relevant.

Studies using liver homogenates have shown that single injections of prednisolone or hydrocortisone increased oxygen uptake, whereas chronic injections decreased the oxygen uptake in homogenenates (18,19) Clark and Pesch (11) found that chronic administration of cortisone lowered oxygen uptake but had no effect on oxidative phosphorylation in rat liver mitochondria. In contrast, Kerpolla (12) reported tht chronic injections of cortisone inhibited oxidative phosphorylation and caused uncoupling of the rat liver mitochondria. Strickland (20) reported no alteration in P:O ratios of liver mitochondria or homogenates following adrenalectomy but Liljeroot and Hall (10) found that adrenalectomy decreases P:O ratio in rat liver mitochondria. Bottoms and Goestch (21) have reported that single injection of corticosterone to rats enhanced oxidative metabolism in liver homogenates and increased P:O ratios in liver mitochondria using malate as the substrate. Treatment of rats with dexamethasone for 3 hours increaed state 3 respiration rates with NAD^{+}-linked substrates and succinate in liver mitochondria. The activities of various dehydrogenases and cytochrome contents remained unaffected (22). By contract Kimberg *et al*. (1) have reported that chronic cortisone treatment to rats results into 14 to 40% decrease in hepatic mitochondrial oxygen consumption with various substrates. In addition to this the oxidative phosphorylation was uncoupled with lowering of P:O ratios.

From the foregoing it is clear that glucocorticoid effects on hepatic mitochondrial energy metabolism are variable depending on the type of glucocorticoid used, dose and duration of the treatment. This makes it difficult to arrive at a generalized conclusion regarding glucocorticoid effects on hepatic mitochondrial function.

With a view to understanding the in vivo glucocorticoid effect on liver mitochondrial energy metabolism, Jani et al. (23) studied effects of corticosterone (a principal glucocorticoid in rat) treatment -acute and chronic- on repiration and oxidative phophorylation in liver mitochondria from 20-, 35- and 60-day old rats. Acute treatment caused generalized decrease in mitochondrial state 3 respiration rates without having much effect on ADP/O ratios with glutamate, succinate and ascorbate + TMPD as substrates. Chronic treatment resulted in uncoupling of oxidative phosphorylation without having significant effects on respiration rates. Both the corticosterone treatments significantly lowered the rates of ATP synthesis in hepatic mitochondria. The effects of corticosterone treatments were also age-dependent; young animals were more susceptible whereas older animals showed increased resistance to deleterious effects of corticostrone

The substrates gain entry into the mitochondrial ETC via specific primary dehydragenases. The activity of

dehydrogenases is a rate limiting step for mitochondrial state 3 respiration (24). The reported decerase in mitochondrial state 3 respiration rates in liver from corticosterone treated animals (25) could be due to the effect of this glucocorticoid on dehydrogenase activities. Glucocorticoids under in vitro conditions are known to have stimulatory effects on the mitochondrial ATPase (16), an enzyme which in situ functions as ATP synthase and couples the transport of protons across the membrane to the synthesis of ATP inside the mitochondrial matrix. However, no information is available regarding in vivo glucocorticoid effects on activities and kinetic properties of this enzyme in liver mitochondria.

In a systematic studies the effect of in vivo acute and chronic corticosterone treatments on the following parameters in developing rats were examined using liver mitochondria as a model system: 1) Specific activities of primary dehydrogenases i.e. glutamate dehydrogenase (GDH), malate dehydrogenase (MDH) and succinate - DCIP reductase (SDR), 2) Mitochondrial ATPase activities under basal and Mg^{2+} and/or DNP - stimulated conditions and 3) Temperature kinetic studies (Arrhenius kinetics of ATPase to find out energies of activation and phase transition temperature.

MATERIALS AND METHODS

Chemicals

The sources of chemicals were the same as mentioned in Chapter II and III.

Isolation of liver mitochondria

Isolation of rat liver mitochondria was carried out according to the procedure described by Katyare and Rajan (25) with some modifications. The animals were killed by decapitation and the livers were quickly removed, blotted on filter paper, weighed and transferred into the beakers containing chilled (0-4°C) isolation medium (0.25 M sucrose, 10 mM tris.HCl, 1 mM EDTA all at pH 7.4 and 250 µg of BSA /ml). Livers were washed several times to get rid of adhering blood. Ten percent (w/v) homogenates of liver were prepared in isolation medium using a Potter-Elvehjem type homogenizer with a tight fitting teflon pestle (wall clearance: 0.18 mm). The homogenates were subjected to centrifugation in a Sorvall RC 5 refrigerated centrifuge (0 - 4°C) at 650 g for 10 minutes to sediment nuclei and cell debries. The post-nuclear supernatant was centrifuged at 6500 g for 10 minutes to sediment mitochondria. The mitochondrial pellets thus obtained were washed once by resuspending in the isolation medium and resedimenting at 6500 g for 10 minutes. Finally the washed

mitochondrial pellets were suspended in isolation medium to give about 10 to 15 mg mitochondrial proteins/ml. All the operations were carried out at 0-4°C.

For studies on mitochondrial lipids, the mitochondria were washed thrice by resuspending and resedimenting as described above, before making the final mitochondrial suspension. This procedure gives mitochondria practically free from microsomal contamination. As assessed in terms of glucose-6-phosphatase activity (a microsomal marker enzyme) in mitochondrial suspension comes to be less than 5% (26).

Assay of liver mitochondrial ATPase

ATPase activities were measured in freshly prepared intact mitochondria. The assay medium (final volume 0.5 ml) consisted of 50 mM tris.HCl, pH 7.4, 75 mM KCl and 0.4 mM EDTA; 6.0 mM MgCl and/or 0.1 mM 2,4-dinitrophenol (DNP) were included wherever indicated. After pre-incubating 200 to 250 µg of mitochondrial proteins (as a source of enzyme) in a reaction mixture for 2 minutes at 37°C in a water bath, the reaction was started by adding 6.0 mM ATP and carried out for 10 minutes. At the end of the incubation period, the reaction was terminated by adding 0.1 ml of 5% (w/v) sodium dodecyl-sulfate (SDS) (27). The liberated inorganic phosphorous was estimated according to the method of Fiske and Subba Row (28).

All other methods/procedures were essentially similar to those described for brain mitochondria in Chapter II and III.

RESULTS

Results of effects of corticosterone treatments on liver mitochondrial enzymes are summarized in this chapter. Table 1 shows the effects of corticosterone treatments on glutamate dehydrogenase (GDH) activity during postnatal development. Thus in 14-day-old animals, the GDH activity was found to be low but increased by 4 fold in the adults. Acute treatment With corticosterone increased the GDH activity from 40 to 110% in all the age groups except adults which showed 25% decrease. The maximum increase was observed in 21-days group. Chronic treatment caused 75% decrease in GDH activity in 14-day-group but in 21-day-old animals the activity had increased by about 2 fold and in other two age groups chornic treatment had no significant effect (Table 1).

Both the corticosterone treatments in general caused significant decrease in MDH activity in all the age groups (Table 2). The extent of decrease was higher in chornic treatment than in acute treatment groups. Acute treatment had maximum effect (45% decrease) in mitochondria from adults, whereas chronic treatment had maximum effect (50% decrease) in 21-day group.

Table 1

Effect of corticosterone treatment of glutamate dehydrogenase activity in rat liver mitochondria during development.

Age	GDH activity (n moles/ min/mg protein)		
	Control	Acute	Chronic
14 Days	21.36 ± 1.41	29.59 ± 1.18[b]	5.25 ± 0.26[b]
21 Days	48.67 ± 1.99	102.88 ± 9.52[b]	107.16 ± 2.28[b]
35 Days	33.45 ± 4.95	49.03 ± 3.87[a]	43.73 ± 1.65
Adult	85.63 ± 4.50	62.59 ± 1.94[b]	92.71 ± 3.30

Results are given as mean ± SEM of 8 independent observations in each group.

[a]$P < 0.05$ and [b]$P < 0.001$ compared to the corresponding controls.

Table 2

Effect of corticosterone treatment of malate dehydrogenase activity in rat liver mitochondria during development.

	MDH activity (n moles/ min/mg protein)		
Age	Control	Acute	Chronic
14 Days	7198 ± 172	4693 ± 230[b]	4209 ± 193[b]
21 Days	9672 ± 219	7391 ± 303[b]	4656 ± 272[b]
35 Days	14352 ± 655	13999 ± 724	9214 ± 208[b]
Adult	7466 ± 537	4038 ± 339[b]	5056 ± 268[a]

Results are given as mean ± SEM of 8 independent observations in each group.

[a]P < 0.002 and [b]P < 0.001 compared to the corresponding controls.

Table 3

Effect of corticosterone treatment on succinate-DCIP reductase activity in rat liver mitochondria during development.

	SDR activity (n moles/ min/mg protein)		
Age	Control	Acute	Chronic
14 Days	9.77 ± 0.42	10.84 ± 0.44	7.45 ± 0.48[c]
21 Days	23.16 ± 1.33	30.15 ± 2.03[b]	32.03 ± 0.96[d]
35 Days	31.54 ± 2.64	35.52 ± 2.74	32.65 ± 0.89
Adult	60.70 ± 4.36	53.29 ± 2.71	50.84 ± 1.78[a]

Results are given as mean ± SEM of 8 independent observations in each group.

[a]P < 0.05 and [b]P < 0.02 ; [c]P < 0.01 and [d]P < 0.001 compared to the corresponding controls.

Succinate - DCIP reductase (SDR) activity in liver mitochondria from control animals also displayed more or less similar developmental pattern as noted previously for GDH. The SDR activity was low in 14-day-old animals and increased gradually with advancement of age and by adult stage the increase was about 6 fold (Table 3). Acute corticosterone treatment had no much effect except for 30% increase in the 21-day-old animals. On the other hand, chronic treatment increased the SDR activity in 21-day group by about 20% but in the case of 14-day-old and adult rats the activity had decreased significantly (15 to 20% decrease) upon chronic treatment (Table 3).

Data on mitochondrial ATPase activities in control animals revealed that in young pups the activity was low and gradually increased with age to reach the adult value. Compared to 14-day-old rats, the adults showed about 3 to 10 fold increase in mitochondrial ATPase activities under basal and Mg^{2+} and/or DNP stimulatory conditions (Table 4). The effects of corticosterone treatments on mitochondrial ATPase activities were age-dependent and treatment specific. The maximum effects were observed in 21-day-old animals, where both the treatments caused 20 to 50% decrease in basal as well as Mg^{2+} and/or DNP - stimulated ATPase activities. Similarly adults also shwoed generalized decrease in ATPase activities. Acute treatment caused significant reduction in basal and Mg^{2+}

Table 4

Effect of corticosterone treatment on liver mitochondrial ATPase activities during development

Treatment	ATPase activity (μmoles/hr/mg protein)			
	Basal	Mg^{2+}	DNP	DNP + Mg^{2+}
		14 Days		
Control	4.33 ± 0.30	5.31 ± 0.33	6.16 ± 0.55	6.43 ± 0.45
Acute	4.00 ± 0.11	5.77 ± 0.35	5.98 ± 0.24	6.54 ± 0.30
chronic	5.96 ± 0.20[e]	4.43 ± 0.30	6.49 ± 0.25	5.54 ± 0.20
		21 Days		
Control	9.96 ± 1.14	22.92 ± 1.73	30.69 ± 1.90	39.15 ± 2.85
Acute	6.63 ± 0.48[a]	12.89 ± 1.42[e]	16.85 ± 0.47[e]	24.17 ± 3.33[c]
chronic	7.73 ± 0.68	12.68 ± 1.64[d]	20.72 ± 1.39[d]	20.67 ± 1.39[e]
		35 Days		
Control	5.99 ± 0.68	14.73 ± 1.77	31.87 ± 2.97	24.05 ± 2.05
Acute	8.01 ± 0.84	12.57 ± 0.77	24.07 ± 1.96	28.60 ± 1.58
chronic	9.07 ± 0.40[c]	18.28 ± 0.74	24.44 ± 2.27	30.81 ± 1.41[a]
		Adult		
Control	12.12 ± 0.60	25.94 ± 2.19	56.74 ± 5.23	63.34 ± 5.92
Acute	9.95 ± 0.53[b]	20.81 ± 0.61[a]	48.96 ± 0.72	53.75 ± 1.73
chronic	7.43 ± 0.39[e]	21.70 ± 0.50	35.32 ± 1.50[c]	41.98 ± 2.81[c]

Results are given as mean ± SEM of 8 independent observations in each group.

[a]$P < 0.05$; [b]$P < 0.02$; [c]$P < 0.01$; [d]$P < 0.002$ and [e]$P < 0.001$ compared to the corresponding controls.

stimulated activity whereas chronic treatment reduced significantly the basal, and DNP and DNP + Mg^{2+} stimulated ATPase activities.

In 14- and 35-day-old rats, acute corticosterone treatment did not affect the ATPase activity. Chronic treatment increased significantly only the basal ATPase activity in 14-day-group, but in case of 35-day-old animals chronic treatment caused significant increase in basal as well as DNP + Mg^{2+} - stimulated ATPase activities.

Temperature kinetics studies of ATPase from liver SMP from control and corticosteone treated animals of different age groups were carried out and the typical Arrhenius plots are shown in Figure 1. The values of phase transition temperature (Tt) and energies of activation (E_1 and E_2) for ATPase from each group were derived from respective Arrhenius plots and data on these kinetic parameters are given in Table 5.

In control animals belonging to 14-and 35-day age groups, the phase transition temperature (Tt) is abolished and upon corticosterone treatments again the Tt was observed in rats of age groups. In 21-day-old animals both the treatment -acute and chronic decreased the Tt by 3.8°C and 4.4°C respectively. In case of adult animals, no significant change in Tt was observed after both the corticosteone treatments.

Figure 1

Arrhenius plots of liver SMP ATPase from control and corticosterone-treated animals of different age groups.

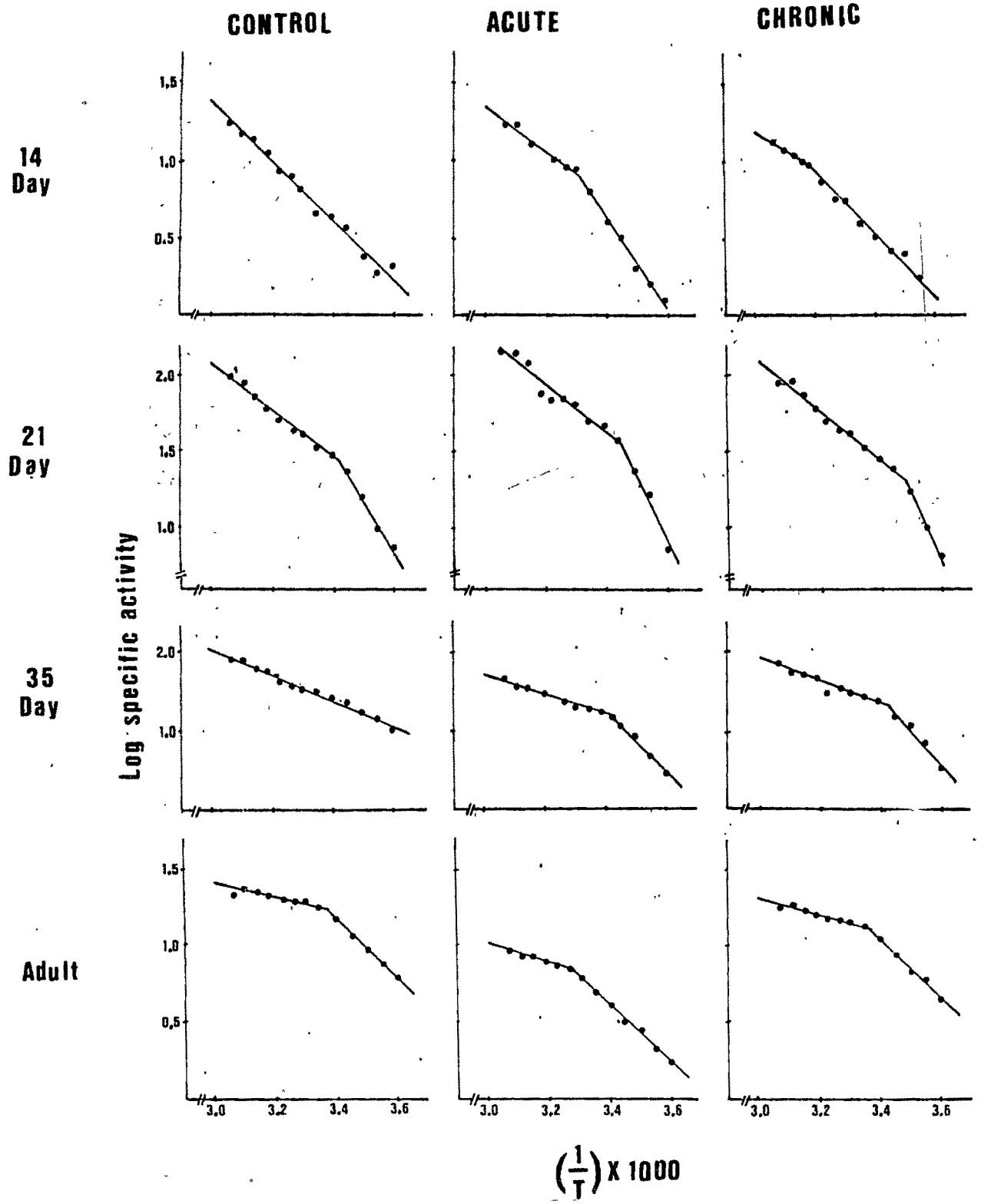

Table 5

Effect of corticosterone treatment on Arrhenius kinetics parameters of liver SMP ATPase during development.

Treatment	Phase transition temperature (Tt), °C	Energy of activation (KJ/mol)	
		E_1	E_2
		14 Days	
Control	-----	-----	37.9 ± 3.16
Acute	31.0 ± 1.94[b]	17.9 ± 2.34[b]	50.1 ± 2.29[a]
chronic	35.1 ± 4.05[d]	22.8 ± 1.76[d]	44.1 ± 2.08
		21 Days	
Control	19.2 ± 0.47	30.5 ± 0.85	65.6 ± 1.38
Acute	16.4 ± 0.27[b]	32.2 ± 0.51	79.0 ± 1.60[c]
chronic	14.8 ± 0.27[c]	33.0 ± 0.83	86.9 ± 0.88[d]
		35 Days	
Control	-----	29.6 ± 1.85	------
Acute	18.6 ± 0.03[d]	24.2 ± 1.08	70.0 ± 2.36[d]
chronic	18.9 ± 1.62[d]	26.2 ± 0.77	82.4 ± 1.18[d]
		Adult	
Control	23.7 ± 3.57	18.0 ± 3.35	33.7 ± 5.86
Acute	29.5 ± 2.60	8.8 ± 0.69[c]	32.1 ± 1.18
chronic	22.2 ± 1.66	14.7 ± 2.32	31.5 ± 2.95

Result are given as mean ± SEM of 4 independent obsevations in each group.

[a]$P < 0.1$; [b]$P < 0.02$; [c]$P < 0.01$ and [d]$P < 0.001$ compared to the corresponding controls.

The activation energy E_1 remained unaltered by both the corticosterone treatments in 21-and 35-day-old rats. In adults, acute treatment led to significant (50%) decrease in E1, whereas chronic treatment had no effect. Acute treatment to 14-day-old animals resulted in 32% increase in activation energy E_2 for ATPase from liver SMP, chronic treatment had no effect on E_2 in this age group. In 21-day group, both the treatments; acute and chronic caused increase in E_2 by 20% and 33% respectively. Acute as well as chronic corticosterone treatment to adult rats caused no significant change in E_2.

DISCUSSION

The previous studies have shown that in vivo corticosterone treatments to rats adversely affected the oxidative energy metabolism in liver mitochondria. This involved significant reduction in state 3 respiration rates, ADP/O ratios and ADP phosphorylation rates in subsequently isolated mitochondria from livers of corticosterone treated animals. The effects of the two corticosterone treatments (acute and chronic) were quite different and also age-dependent (23). The present studies were undertaken to critically evaluate the effects of in vivo corticosterone treatments on various enzymes involved in mitochondrial oxidative energy metabolism.

From the data presented it is clear that specific activity of GDH in liver mitochondria increased gradually with advancement of age. However, it is knwon that the mitochondrial state 3 respiration rate with glutamate as the substrate does not change in liver during the development from weaning age to adult stage (23). The increase in GDH activity need not cause increase in mitochondrial state 3 respiration rates as it would also depend on other components of ETC.

Acute treatment significantly increased GDH acvity in animals of all the age groups except adults. It has been reported that acute corticosterone treatment caused significant reduction in state 3 respiration rates in rat liver mitochondria with glutamate as the substrate (23). These observations suggest that GDH activity is not a factor responsible for reported decrease in state 3 respiration rates with glutamate as the substrate. Chronic treatment also caused significant alterations in GDH activity in young animals, but chronic treatment has been reported not to alter the state 3 respiration rates in liver mitochondria from 20-day-old and adult rats (23).

Both the corticosterone treatments (acute and chronic) significantly reduced MDH activity in subsequently isolated liver mitochondria from animals of all the age groups studied. Glucocorticoids are known to alter the permeability of mitochondrial membrane to cause selective loss of pyridine

nucleotides from mitochondria (8). Since MDH is a pyridine nucleotide requiring enzyme of mitochondrial matrix, the observed decrease in MDH activity upon corticosterone treatments could be attributed to this factor.

As GDH is also a pyridine nucleotide dependent enzyme, it's activity should have decreased in the hepatic mitochondria from corticosterone treated animals. Suprisingly, the GDH activity increased in the acute treatment group. The reason for this apparent discrepancy is not clear and further investigations on these line are necessary.

The age-dependent increase in specific activity of SDR in liver mitochondria agrees well with the reported developmental increase in state 3 respiration rates with succinate as the substrate. Both the corticosterone treatments significantly altered the SDR activity in animals of all the age groups except 35-day-old rats. Acute corticosterone treatment to rats is known to cause significant reduction in state 3 respiration rates with succinate as the substrate in liver mitochondria from 20-, 35- and 60-day-old rats (23).

The basal as well as Mg^{2+} and/or DNP-stimulated ATPase activity of liver mitochondria increased with the age. In general both corticosterone treatments significantly lowered the basal and Mg^{2+} and/or DNP-stimulated ATPase activities, maximum effects were observed in 21-day-old animals. These

observations are in general agreement with the findings reported from our laboratory that ADP phopshorylation rates of liver mitochondria using different substrates, increased with advancement of age and both the corticosterone treatments significantly lowered the rates of ATP synthesis. Young animals were more susceptible to deleterious effects of corticosterone overload compared to the adults (23). In contrast, Allan et al (22) have reported that adrenalectomy had no effect either on basal or on DNP - stimulated ATPase activity in rat liver mitochondria. In vivo dexamethasone treatment (single injection) to these animals had no effect on basal ATPase activity but DNP - stimulated ATPase activity was increased in liver mitochondria.

In vitro studies by Blecher and White (16) have shown that glucocorticoids and variety of other steroids stimulated the mitochondrial ATPase activity. The physiologic relevance of these observations is questionable and these effects could be termed as non-specific membrane effects of steroids; these studies were performed in vitro and additionally the concentration of the steroids used was very high (0.6 mM). The results of in vivo studies with the major glucocorticoid of rat (corticosterone) reported here would seem to be physiologically more relevent.

Data on Arrhenius kinetics parameters of ATPase from liver SMP of control animals revealed that in 14-and 35-day-

old animals the phase transition temperature was abolished. This could be attributed to changes in composition and content of different lipids. Thus in liver mitochondria from 35-day-old rats the cholesterol content was highest among all the age groups (chapter VI) and increase in cholesterol in the membrane is known to abolish the phase transition temperature (29). Besides causing significant reduction in mitochondrial ATPase activities, both the corticosterone treatments caused significant alterations in phase transition temperature and energies of activiation E_1 and E_2 of liver SMP ATPase. The effect of corticosterone treatments on the kinetic properties of ATPase in liver were age-dependent and treatment specific. These changes could possibly be due to the alterations in the mitochondrial lipid/phospholipid composition/content (Chapter VI). Phospholipids are known to be important for the catalytic function of mitochondrial ATPase (30).

REFERENCES

1. Kimberg,D.V., Loud,A.V. and Wiener,J.(1968) cortisone-induced alterations in mitochondrial function and structure. J.Cell Biol. 37,63-79.

2. Kimberg,D.V. and Loeb,J.N. (1972) Effects of cortisone administration on rat liver mitochondria : Support for the concept of mitochondrial fusion. J. Cell Biol. 55, 635-643.

3. Bullock,G.R., Christian,R.A., Peters,R.F. and White,A.M. (1971) Rapid mitchondrial enlargement in muscle as a response to triamcinolone acetonide and its relationship to the ribosomal defect. Biochem.Pharmacol. 20, 943-953.

4. Kimberg,D.V. and Goldstein,S.A. (1966) Binding of calcium by liver mitochondria of rats treated with steriod hormones. J.Biol.Chem. 241, 95 - 103.

5. Kimberg,D.V. and Goldstein,S.A. (1967) Binding of calcium by liver mitochondria : an effect if steroid hormones in vitamin D - depleted and parathyroidectomized rats. Endocrinology 80, 89 - 97.

6. Kimura,S. and Rasmussen,H. (1977) Adrenal glucocorticoids, adenine nucleotide translocation and mitochondrial calcium accumulation. J.Biol.Chem. 252, 1217 - 1225.

7. Hughes,B.P. and Barritt,G.J. (1979) Interaction between glucocorticoids and glucagon in hormonal modification of calcium retention by isolated rat liver mitochondria. Biochem.J. 180, 291-295.

8. Gallagher,C.H. (1960) The mechanism of action of hydrocortisone on mitochondrial metabolism. Biochem.J. 74, 38 - 43.

9. Jenson,P.K. and Neuhard,J. (1961) Steroid inhibition of reduced diphosphopyridine nucleotide oxidase activity in electron-transport particles. Biochim.Biophys.Acta 52, 97-105.

10. Liljeroot,B.S. and Hall,J.C. (1965) Oxidative phosphorylation in liver mitochondria from adrenalectomized rats and the response to hormones added in vitro. J.Biol.Chem. 240, 1446 - 1452.

11. Clark,J.H. and Pesch,D. (1956) Effects of cortisone upon liver enzymes and protein synthesis. J.Pharmacol.Exptl. Therap. 117, 202 - 207.

12. Kerpolla,W. (1960) Uncoupling of oxidative phosphorylation with cortisone in liver mitochondria. Endocrinology. 67, 252 - 263.

13. Gomez - Puyou,A., Pena - Diaz,A., Guzman - Gracia,J. and Laguma,J. (1963) Biochem. Pharmacol. 12, 331 - 340. (cited from ref. No, 14).

14. Martens,M.E., Peterson,P.L. and Lee,C.P. (1991) In vitro effects of glucocorticoid on mitochondrial energy metabolism. Biochim.Biophys.Acta 1058, 152 - 160.

15. McIntosh,M.K., Pan,J.S. and Berdanier,C.D.(1993) In vitro studies on the effects of dehydroepiandrosterone and corticosterone on hepatic steroid receptor binding and mitochondrial respiration. Comp.Biochem.Physiol. 104A, 147-153.

16. Blecher,M. and White,A. (1960) Alterations produced by steroids in adenosine triphosphatase activity and volume of lymphosarcoma and liver mitochondria. J.Biol.Chem. 235, 3404 - 3412.

17. Duval,D., Durant,S. and Homo-Delarche,F. (1983) Non - genomic effects of steroids : Interactions of steroid molecules with membrane structures and functions. Biochim.Biophys. Acta 737, 409 - 442.

18. Goetsch,D.D. and McDonald,L.E.(1962) Glucocorticoid effects in respiration and metabolism by rat liver homogenates. Am.J.Physiol. 202, 343 - 346.

19. Goetsch,D.D. (1963) Glucocorticoid effects on respiration and metabolism of a goat liver homogenates. Am.J.Vet.Res. 24, 867 - 870.

20. Strickland, H. (1962) Respiration and phosphorylation in liver mitochondria and homogenates from adrenalectomized rats. Arch.Biochem.Biopnys. 100, 110 - 118.

21. Bottoms,G. and Goetsch,D.D. (1968) Effects of corticosterone on oxidative metabolism in different tissues of the rat. General and Comparative Endocrinology 10, 310- 314.

22. Allan,E.H., Chisholm,A.B. and Titheradage,M.A. (1983) The stimulation of hepatic oxidative phosphorylation following dexamethasone treatment of rats. Biochim.Biophys.Acta 725, 71- 76.

23. Jani,M.S., Telang,S.D. and Katyare,S.S.(1991) Effect of corticosterone treatment on energy metabolism in rat liver mitochondria. J.Steroid Biochem. Molec. Biol. 38, 587- 591.

24. Roosevelt,T.S., Ruhmann-Wennhold,A. and Nelson,D.H.(1973) Adrenal corticosteroid effects upon rat brain mitochondrial metabolism. Endocrinology 53, 619- 625.

25. Katyare,S.S. and Rajan,R.R. (1988) Enhanced oxidative phospharylation in rat liver mitochondria following in vivo treatment with imipramine. Br.J.Pharmacol. 95, 914- 922.

26. Satav,J.G., Rajwade,M.S., Katyare,S.S., Netrawali,M.S., Fatterpaker,P. and Sreenivasan,A. (1973) The significance of promitochondrial structures in rat liver for mitochondrial biogenesis. Biochem.J. 134, 687- 695.

27. Katyare,S.S. and Satav,J.G. (1989) Impaired mitochondrial oxidative energy metabolism following paracetamol - induced hepatotoxicity in the rat. Br.J. Pharmacol. 96, 51- 58.

28. Fiske,C.H. and Subba Row,Y. (1925) The colorimetric determination of phosphorous. J.Biol.Chem. 66,375-400.

29. Demel,R.A. and Kruyff,B.D.(1976) The funtion of sterols in membranes. Biochim.Biophys Acta. 437, 109- 132.

30. Brown,R.E. and Cunningham,C.C. (1982) Negatively charged phospholipid requirement of the oligomycin - sensitive mitochondrial ATPase. Biochim.Biophys.Acta 684, 141-145.

SUMMARY

1. In control animals, GDH activity of liver mitochondria from 14-day-group was low but increased gradually with advancement of age. The corticosterone treatments caused generalized increase in GDH activity. MDH activity had decreased significantly in liver mitochondria from acute as well as chronic corticosterone treatment groups. SDR activity in liver mitochondria showed developmental increase as observed for GDH in control animals. Except the 35-day group, both the corticosterone treatments caused significant alterations in SDR activity.

2. Basal as well as Mg^{2+} and/or DNP - stimulated ATPase activities in liver mitochondria showed age-dependent increase. In general both the corticosterone treatments significantly lowered basal as well as Mg^{2+} and/or DNP-stimulated ATPase activities. Maximum effects of corticosterone treatments were observed in 21-day-old rats.

3. Arrhenius kinetics studies have shown that ATPase from liver SMP of control animals of 14- and 35-day group, did not show any phase transition temperature. This could be attributed to increased cholesterol content in mitochondria from animals of these age groups. Both the corticosterone treatments caused significant

alterations in transition temperature and activation energies E_1 and E_2 in an age-dependent and treatment specific manner.