CHAPTER - VI

EFFECTS OF CORTICOSTERONE TREATMENT ON LIPID/PHOSPHOLIPID COMPOSITION/CONTENT AND ^{3}H ACETATE INCORPORATION INTO LIVER MITOCHONDRIAL PHOSPHOLIPIDS AND MEMBRANE FLUIDITY DURING DEVELOPMENT.

Besides affecting the mitochondrial function (Chapter V), glucocorticoids also have numerous effects on lipid metabolism in liver. Glucocorticoid effects on membrane composition include changes in cholesterol, phospholipids, polysaccharides and proteins (1).

The effects of glucocorticoids on cholesterol synthesis have been investigated both in vivo and in vitro by many researchers. However, the results are often contradictory. Glucocoticoids have been shown to cause marked decrease in cholesterol synthesis (2); this action of glucocorticoids has been shown to be linked to an inhibition of synthesis of 3 hydroxy 3 methylglutaryl coenzyme A (HMG-CoA) synthase (1). In contrast, Lin and Snodgrass (3) have reported that dexamethasone increases the HMG-CoA reductase activity by 2 to 4 fold in cultured liver cells. Cholesterol synthesis, measured by ^{14}C acetate or $^{3}H_2O$ incorporation, increased by 3 fold after treatment with 1 μM dexamethasone.

Glenny and Brindly (4) have reported effects of cortisol treatment for 5 days on rates of glycerolipid synthesis in rat liver. Cortisol treatment increased the rate of triglyceride synthesis from ^{3}H glycerol in phosphatidate and increased the flux of ^{14}C palmitate and ^{3}H glycerol from phosphatidate to diacylglycerol. The activity of phosphatidate phosphohydrolase was increased by about 2.5 fold in liver. Cortisol treatment decreased significantly the proportion of ^{14}C palmitate

incorporation in phosphatidylcholine and phosphatidyl-ethanolamine and incorporation of ^{3}H glycerol in phosphatidylethanolamine.

In vivo cortisol treatment caused significant reduction in phosphatidylcholine, phosphatidylethanolamine and sphingomyelin in rat liver microsomes. The levels of palmitic, stearic, oleic, linoleic and arachidonic acids in phosphatidylcholine and of palmitic acid in phosphatidylserine and phosphatidylinositol were also affected (2). Glucocorticoids by affecting lipid composition of membranes, regulate/modulate the activity of the lipid requiring enzymes (2,5,6).

Kaur et al. (7) have reported effects of dexamethasone treatment for 8 days on lipid metabolism in several rat organs including liver. Dexamethasone treatment significantly increased the turnover of phosphatidylcholine and phosphatidylethanolamine in liver tissue. In hepatic mitochondrial membranes, the contents of phospholipids and cholesterol/ g tissue had decreased in dexamethasone treatment group (8).

Thus by affecting the mitochondrial lipid composition glucocorticoids could alter the activity of mitochondrial membrane - bound enzymes, which have lipid requirement (8). As mentioned earlier (Chapter V), glucocorticoid effects on the

mitochondrial functions in liver are well documented. Since there were no systematic studies available on glucocorticoid effects on the composition/contents and synthesis of lipids/ phospholipids, the studies from our laboratory (9) were extended to have better understanding about corticosterone effects on membrane lipids and lipid - protein interactions in liver mitochondria. The objective of the present study was to examine the effects of <u>in vivo</u> acute and chronic corticosterone treatments on the following parameters of liver mitochondria from rats of different age groups: 1) Mitochondrial content of total phospholipids (TPL), cholesterol (CHL) and their molar ratios (TPL / CHL), 2) Mitochondrial composition and content of individual phospholipid classes, 3) ^{3}H acetate incorporation into different phospholipid compoments of mitochondria and 4) Fluidity of mitochondrial membranes.

MATERIALS AND METHODS

The sources of the chemicals and the methods used were the same as described in earlier Chapters II, IV and V.

RESULTS

The results of corticosterone effects on lipids and membrane fluidity of liver mitochondria from rats of different

age groups are summarized in this Chapter.

The data on effects of corticosterone treatments on total phospholipid (TPL) and cholesterol (CHL) contents and TPL/CHL molar ratios are given in Table 1. In 14-day group, both the corticosterone treatments had no significant effect either on TPL or CHL contents or their molar ratios. In the 21-day-old rats, acute treatment caused more than 2 fold increase in TPL content, while chronic treatment caused about 45% decrease in CHL content consequently both the treatments significantly increased TPL/CHL molar ratios, the extent of increase being higher upon acute treatment (170% increase) compared to the chronic treatment (106% increase). Acute treatment to 35-day-old rats increased the TPL content by about 30% but the CHL content decreased by 33%. Chronic treatment had no effect on TPL content but CHL content was decreased by 45%. Hence both the treatments caused about 2 fold increase in TPL/CHL molar ratio. In adult animals, only chronic treatment led to 40% increase in TPL. Both the corticosterone treatments had no effect on CHL content but caused a small (20-30%) increase in TPL/CHL molar ratios.

Results on corticosterone effects on phospholipid composition of liver mitochondria from animals of different age groups are shown in Tables 2 to 5. Corticosterone treatments produced maximum changes in 35-day-old animals while the adults were least affected.

Table 1

Effect of corticosterone treatment on total phospholipid (TPL) and cholesterol (CHL) content and TPL/CHL molar ratios of rat liver mitochondria during development.

Age	Treatment	Total phospholipid content (µg/mg protein)	Cholesterol content (µg/mg protein)	Total phospholipid: cholesterol (mol:mol)
	Control	231.5 ± 19.9(8)	22.4 ± 2.06(8)	5.3 ± 0.31 (8)
14 Day	Acute	258.8 ± 26.5(11)	24.2 ± 1.84(8)	5.4 ± 0.29 (8)
	Chronic	257.6 ± 15.5(11)	22.7 ± 2.21(8)	5.6 ± 0.26 (8)
	Control	250.1 ± 18.2(10)	34.5 ± 3.45(8)	3.6 ± 0.21 (8)
21 Day	Acute	531.4 ± 18.3^{c}(6)	27.3 ± 3.20(8)	9.7 ± 0.23^{c}(6)
	Chronic	281.8 ± 6.5(11)	19.4 ± 2.62^{a}(8)	7.4 ± 0.34^{c}(8)
	Control	218.4 ± 4.6(16)	42.4 ± 3.47(12)	2.6 ± 0.15(12)
35 Day	Acute	285.5 ± 11.4^{c}(12)	28.4 ± 1.60^{b}(12)	5.1 ± 0.32^{c}(12)
	Chronic	250.2 ± 21.5(12)	23.5 ± 1.18^{c}(8)	5.2 ± 0.34^{c}(8)
	Control	215.8 ± 15.1(10)	19.8 ± 1.78(8)	5.4 ± 0.26(8)
Adults	Acute	201.1 ± 12.6(10)	14.7 ± 2.19(8)	7.2 ± 0.86^{b}(8)
	Chronic	309.4 ± 21.3^{b}(12)	24.1 ± 2.51(8)	6.5 ± 0.25^{a}(8)

Results are given as mean ± SEM of number of independent observations for each group as indicated in parentheses.

$^{a}P < 0.01$; $^{b}P < 0.002$ and $^{c}P < 0.001$ compared to the corresponding controls.

Table 2

Effect of corticosterone treatment on phospholipid composition of liver mitochondria from 14-day-old rats.

Phospholipid class	Phospholipid composition (% of total)		
	Control(12)	Acute(12)	Chronic(6)
LYSO	1.25 ± 0.37	1.00 ± 0.37	1.44 ± 0.55
SPM	2.95 ± 0.44	3.85 ± 0.25	3.43 ± 0.73
PC	43.33 ± 1.14	45.37 ± 0.62	45.56 ± 0.54
PI	2.89 ± 0.58	3.25 ± 0.32	1.18 ± 0.30[a]
PS	2.12 ± 0.30	6.08 ± 1.19[b]	0.42 ± 0.21[c]
PE	37.90 ± 1.30	33.74 ± 0.71[a]	33.99 ± 1.57
DPG	9.60 ± 0.84	6.70 ± 0.51[b]	14.03 ± 0.41[c]

Results are given as mean ± SEM of number of independent observations for each group as indicated in parentheses.

[a] $P < 0.02$; [b] $P < 0.01$ and [c] $P < 0.001$ compared to control.

Table 3

Effect of corticosterone treatment on phospholipid composition of liver mitochondria from 21-day-old rats.

Phospholipid class	Phospholipid composition (% of total)		
	Control(10)	Acute(10)	Chronic(5)
LYSO	3.18 ± 0.74	2.34 ± 0.85	0.15 ± 0.05[c]
SPM	3.40 ± 0.81	5.59 ± 1.34	8.85 ± 0.52[d]
PC	47.55 ± 1.63	46.17 ± 1.41	40.14 ± 4.59
PI	3.49 ± 0.60	3.92 ± 0.32	5.31 ± 1.96
PS	0.63 ± 0.25	2.39 ± 0.42[b]	3.05 ± 1.29
PE	33.08 ± 1.56	29.67 ± 1.68	29.59 ± 2.97
DPG	8.66 ± 0.93	9.92 ± 0.61	12.87 ± 1.21[a]

Results are given as mean ± SEM of number of independent observations for each group as indicated in parentheses.

[a]$P < 0.02$; [b]$P < 0.01$; [c]$P < 0.002$ and [d]$P < 0.001$ compared to control

Table 4

Effect of corticosterone treatment on phospholipid composition of liver mitochondria from 35-day-old rats.

Phospholipid class	Phospholipid composition (% of total)		
	Control(8)	Acute(8)	Chronic(6)
LYSO	10.99 ± 0.48	2.61 ± 1.07[b]	0.00 ± 0.00[b]
SPM	8.09 ± 0.46	3.26 ± 0.66[b]	1.74 ± 0.42[b]
PC	38.08 ± 0.55	55.03 ± 1.67[b]	46.00 ± 0.58[b]
PI	8.82 ± 0.49	4.62 ± 0.42[b]	5.17 ± 0.81[a]
PS	7.74 ± 0.59	1.28 ± 0.23[b]	4.21 ± 0.30[b]
PE	20.84 ± 1.05	25.87 ± 0.95[a]	31.30 ± 0.79[b]
DPG	7.01 ± 0.33	7.34 ± 0.49	11.63 ± 0.99[b]

Results are given as mean ± SEM of number of independent observations for each group as indicated in the parentheses.
[a]$P < 0.01$ and [b]$P < 0.001$ compared to control.

Table 5

Effect of corticosterone treatment on phospholipid composition of liver mitochondria from adult rats.

phospholipid class	Phospholipid composition (% of total)		
	Control(10)	Acute(8)	Chronic(8)
LYSO	2.22 ± 0.67	1.51 ± 0.46	1.40 ± 0.38
SPM	5.80 ± 0.75	6.16 ± 0.43	5.48 ± 0.79
PC	40.31 ± 1.77	43.28 ± 1.79	48.62 ± 0.94
PI	4.85 ± 0.69	4.14 ± 0.41	3.84 ± 0.52
PS	4.04 ± 0.94	2.84 ± 0.29	2.57 ± 0.60
PE	32.20 ± 0.98	29.87 ± 1.60	28.35 ± 1.05[a]
DPG	10.57 ± 0.35	9.99 ± 0.87	8.93 ± 0.98

Results are given as mean ± SEM of number of independent observations for each group as indicated in parentheses.

[a] $P < 0.02$; and [b] $P < 0.001$ compared to control.

Thus, in case of 14-day-old rats (Table 2) acute treatment caused about 3 fold increase in PS while PE and DPG had decreased by 11 and 30% respectively. Upon chronic treatment, the PS showed about 5 fold decrease and DPG had increased by 46%; PI was about 40% of the control.

In the 21-day-old animals (Table 3) PS increased by about 4 fold upon acute treatment; this increase is similar to that noted for the 14-day group mentioned above. Upon chronic treatment a significant reduction (95% decrease) in lysophospholipid can be noted, whereas the SPM and DPG had increased by 160% and 50% respectively.

In 35-day-old rats (Table 4), acute treatment caused drastic reduction in LYSO, SPM, PI and PS components. Maximum (4 to 6 fold) decrease was observed in LYSO and PS ; SPM and PI also showed 2 to 2.5 fold decrease. In acute treatment group the PC and PE components showed significant (25 to 45%) increase. Chronic treatment also caused drastic reduction in LYSO , SPM , PI and PS. As it was observed in acute treatment group, the SPM had decreased by 4.5 fold and the lysophspholipid component disappeared totally. PC, PE and DPG showed 20 to 65% increase upon chronic treatment.

Acute corticosterone treatment to adults (Table 5) had no effect on the phospholipid composition of liver mitochondria (Table 5). Chronic treatment resulted in

increased PC content by 20% whereas the PE had decreased by 12%.

Actual contents of different phospholipids of liver mitochondria from animals of different age groups, as affected by the corticosterone treatments are given in Tables 6 to 9. Thus in 14-day-old animals (Table 6), acute treatment caused 45% and 200% increase in SPM and PS respectively. The contents of other phospholipids remained unaltered. Chronic treatment led to significant (15 to 60%) increase in PC and DPG contents. On the other hand, the contents of PI and PS had decreased by 55 and 78% respectively (Table 6).

In 21 - day - old animals, acute treatment caused 2 to 8 fold increase in content of the various phospholipid components except the lysopholipid. The maximum (8 fold) increase was in the PS content. Chronic treatment increased the contents of SPM, PS and PDG by 3, 5.5 and 1.7 folds respectively; the contents of LYSO (-95%) and PC (-14%) has decreased significantly (Table 7).

In 35 - day group, acute treatment led to significant (37 to 89%) increase in contents of PC, PE and DPG ; the maximum (89%) increase was observed in PC content. The contents of LYSO, SPM, PI and PS decreased significantly in acute treatment group, the extent of decrease in PS content was maximum (-78%) compared to LYSO (- 69%), SPM (- 47%) and

Table 6

Effect of corticosterone treatment on phospholipid content of liver mitochondria from 14-day-old rats.

Phospholipid class	Phospholipid content (µg/mg protein)		
	Control(12)	Acute(12)	Chronic(6)
LYSO	2.89 ± 0.55	2.56 ± 0.59	3.69 ± 0.82
SPM	6.84 ± 0.81	9.97 ± 0.83[b]	8.78 ± 1.20
PC	100.30 ± 5.63	116.65 ± 6.77	117.49 ± 4.23[a]
PI	6.70 ± 0.95	8.39 ± 0.84	3.04 ± 0.48[c]
PS	4.90 ± 0.56	14.98 ± 2.23[d]	1.08 ± 0.30[d]
PE	86.83 ± 4.7	87.27 +_ 5.39	87.59 ± 4.66
DPG	22.21 ± 1.92	17.31 ± 1.55	36.12 ± 1.61[d]

Results are given as mean ± SEM of number of independent observations for each group as indicated in parentheses.

[a]$P < 0.05$; [b]$P < 0.02$; [c]$P < 0.01$ and [d]$P < 0.001$ compared to control.

Table 7

Effect of corticosterone treatment on phospholipid content of liver mitochondria from 21-day-old rats.

Phospholipid class	Phospholipid content (µg/mg protein)		
	Control(10)	Acute(10)	Chronic(5)
LYSO	7.96 ± 1.22	12.44 ± 2.47	0.42 ± 0.21[b]
SPM	8.51 ± 1.31	29.76 ± 4.07[b]	24.94 ± 1.02[b]
PC	117.96 ± 6.32	245.20 ± 7.91[b]	101.10 ± 4.33[a]
PI	8.68 ± 1.05	20.81 ± 1.20[b]	14.68 ± 2.88
PS	1.57 ± 0.37	12.65 ± 1.33[b]	8.67 ± 1.94[b]
PE	82.74 ± 4.97	157.64 ± 7.14[b]	83.40 ± 5.15
DPG	21.73 ± 1.96	51.68 ± 2.47[b]	36.27 ± 2.12[b]

Results are given as mean ± SEM of number of independent observations for each group as indicated in parentheses.

[a]$P < 0.05$ and [b]$P < 0.001$ compared to control.

Table 8

Effect of corticosterone treatment on phospholipid content of liver mitochondria from 35-day-old rats

Phospholipid class	Phospholipid content (µg/mg protein)		
	Control(8)	Acute(8)	Chronic(8)
LYSO	24.02 ± 0.78	7.42 ± 1.67[a]	0.00 ± 0.00[a]
SPM	17.65 ± 0.69	9.33 ± 1.13[a]	4.34 ± 0.72[a]
PC	83.21 ± 1.48	157.07 ± 5.52[a]	115.06 ± 5.67[a]
PI	19.25 ± 0.74	13.17 ± 0.86[a]	12.87 ± 1.56[a]
PS	16.74 ± 0.82	3.65 ± 0.40[a]	10.51 ± 0.83[a]
PE	48.11 ± 2.17	73.83 ± 2.82[a]	78.40 ± 4.36[a]
DPG	15.29 ± 0.52	20.94 ± 1.11[a]	29.17 ± 2.50[a]

Results are given as mean ± SEM of number of independent observations for each group as indicated in parentheses.

[a] $P < 0.001$ compared to control.

Table 9

Effect of corticosterone treatment on phospholipid content of liver mitochondria from adult rats.

Phospholipid class	Phospholipid content (µg/mg protein)		
	Control(10)	Acute(8)	Chronic(8)
LYSO	4.80 ± 0.89	3.03 ± 0.55	4.32 ± 0.73
SPM	12.50 ± 1.24	12.35 ± 0.81	17.00 ± 1.80
PC	87.00 ± 4.95	87.02 ± 4.52	150.40 ± 6.60[b]
PI	10.47 ± 1.11	8.33 ± 0.68	11.90 ± 1.21
PS	8.72 ± 1.31	5.70 ± 0.46[a]	7.96 ± 1.20
PE	69.49 ± 3.50	60.07 ± 3.48	87.61 ± 4.62[b]
DPG	22.80 ± 1.18	20.09 ± 1.50	27.52 ± 2.45

Results are given as mean ± SEM of number of independent observations for each group as indicated in parentheses.

[a]$P < 0.05$ and [b]$P < 0.001$ compared to control.

PI (-32%). Chronic treatment also caused significant increase in contents of PC, PE and DPG, the increase in DPG was maximum (2 fold increase). The contents of LYSO, SPM, PI and PS decreased significantly ; SPM decreased by 75% and LYSO had disappeared completely upon chronic treatment (Table 8).

In adult animals both the corticosterone treatments did not have much effect on the individual phospholipid content of liver mitochondria. Acute treatment caused 35% decrease only in PS. On the other hand, chronic treatment caused increase in contents of PC and PE by 73 and 26% respectively (Table 9).

Data on age-dependent effects of the corticosterone treatments on ^{3}H acetate incorporation into phospholipid subclasses of liver mitochondria are given in Table 10. Both the corticosterone treatments caused reduction in label incorporation into different phospholipids. The extent of decrease for each phospholipid being variable depending on age of the animal and treatment employed.

Acute treatment to 35-day-old rats completely abolished the counts in lysophospholipids. Upon chronic treatment no detectable counts were obtained in lysophospholipids in liver mitochondria from animals of all the age groups.

Table 10

Effect of corticosterone treatments on ^{3}H acetate incorporation into mitochondrial phospholipids of rat liver during development.

Age/ Treatment	Label incorporatiom (DPM/μmol) in different phospholipids						
	LYSO	SPM	PC	PI	PS	PE	DPG
14 Days							
Control	450	511	121	228	411	48	2478
Acute	413	306	95	40	---	51	1341
Chronic	---	---	40	---	---	28	382
21 Days							
Control	69	384	380	1014	257	313	8985
Acute	78	17	190	849	---	118	5759
Chronic	---	---	150	---	---	60	608
35 Days							
Control	103	213	198	57	204	112	5286
Acute	---	154	231	39	120	132	957
Chronic	---	241	139	65	209	76	818
Adult							
Control	859	326	192	709	1175	192	2664
Acute	693	176	90	350	605	168	2208
Chronic	---	124	166	542	784	104	1617

Results for each data point are average of two independent observations in duplicate.

Incorporation of ^{3}H acetate into SPM was decreased by about 20 to 95% in acutely treated animals of all the age groups. Upon chronic treatment no label incorporation was detected in SPM in animals of all the age groups except adults.

PC showed about 20 to 50% decrease in label incorporation in acute treatment groups. Chronic treatment also had more or less similar effect on PC (30 to 65% decrease).

In case of PI, acute treatment caused 15 to 80% decrease in label incorporation into this phospholipid; the maximum (80%) decrease was observed in 14-day age group. In liver mitochondria from 14- and 21-day-old rats, the incorporation of label into PI was totally abolished.

Both the corticosterone treatments completely inhibited the incorporation of ^{3}H acetate into PS in liver mitochondria from 14- and 21-day-old rats. In case of 35-day-old and adult animals, both the treatments decreased label incorporation into PS by 30 to 50%.

Acute treatment did not have much effect on label incorporation into PE except in 21-day group where it was decreased by 60%. Chronic treatment caused about 30 to 80% decrease; maximum decrease in label incorporation was found in 21-day-old rats.

Table 11

Effect of corticosterone treatment on membrane fluidity of liver mitochondria from 14-day-old rats.

Parameter	Treatment		
	Control	Acute	Chronic
Fluorescence Polarization (P)	0.130 ± 0.003	0.142 ± 0.002[a]	0.149 ± 0.004[b]
Fluorescence anisotropy (r)	0.091 ± 0.002	0.100 ± 0.002[a]	0.104 ± 0.003[a]
Limited hindered anisotropy (r∞)	0.021 ± 0.003	0.033 ± 0.002[a]	0.039 ± 0.004[a]
Order parameter (S)	0.465 ± 0.029	0.572 ± 0.013[a]	0.604 ± 0.025[a]

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

[a] $P < 0.01$ and [b] $P < 0.002$ compared to control.

Table 12

Effect of corticosterone treatment on membrane fluidity of liver mitochondria from 21-day-old rats.

Parameter	Treatment		
	Control	Acute	Chronic
Fluorescence Polarization (P)	0.159 ± 0.002	0.156 ± 0.005	0.142 ± 0.006[a]
Flurescence anisotropy (r)	0.112 ± 0.001	0.110 ± 0.004	0.100 ± 0.004[a]
Limited hindered anistropy (reζ)	0.049 ± 0.002	0.046 ± 0.005	0.033 ± 0.005[a]
Order parameter (S)	0.660 ± 0.009	0.641 ± 0.023	0.589 ± 0.053

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

[a] $P < 0.02$ compared to control.

Table 13

Effect of corticosterone treatment on membrane fluidity of liver mitochondria from 35-day-old rats.

Parameter	Treatment		
	Control	Acute	Chronic
Fluorescence Polarization (P)	0.161 ± 0.003	0.154 ± 0.003	0.126 ± 0.004[a]
Fluorescence anisotropy (r)	0.113 ± 0.003	0.108 ± 0.002	0.088 ± 0.003[a]
Limited hindered anisotropy (r_∞)	0.051 ± 0.004	0.044 ± 0.003	0.018 ± 0.004[a]
Order parameter (S)	0.668 ± 0.015	0.636 ± 0.014	0.415 ± 0.052[a]

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

[a] $P < 0.001$ compared to control.

Table 14

Effect of corticosterone treatment on membrane fluidity of liver mitochondria from adult rats.

Parameter	Treatment		
	Control	Acute	Chronic
Fluorescence Polarization (P)	0.140 ± 0.004	0.130 ± 0.005	0.147 ± 0.004
Fluorescence anisotropy (r)	0.098 ± 0.003	0.091 ± 0.004	0.103 ± 0.003
Limited hindered anisotropy (r_∞)	0.030 ± 0.004	0.021 ± 0.005	0.037 ± 0.004
Order parameter (S)	0.548 ± 0.028	0.419 ± 0.062	0.590 ± 0.028

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

Label incorporation into DPG decreased by 20 to 80% in the acute treatament groups; maximum decrease was observed in 35-day group. The extent of decrease in ^{3}H acetate incorporation in to DPG was much more upon chronic treatment (85 to 95% decrease) in animals of all the age groups except adults. Adults showed about 40% decrease.

Data on effects of corticosterone treatments on membrane fluidity of liver mitochondria (Tables 11 to 14) suggested that except for 14-day-old animals, acute treatment had no significant effects on fluidity. Chronic treatment significantly altered the fluidity in all the age groups except adults. Thus in the adult animals both the treatments did not cause any change in membrane fluidity.

In liver mitochondria from 14-day-old rats, both the treatments caused significant decrease in fluidity. But in case of 21- and 35-day-old rats, chronic treatment led to significant increase in membrane fluidity. These results suggested that corticosterone effects on membrane fluidity of liver mitochondria are age-dependent and treatment specific.

DISCUSSION

In an age-dependent and treatment specific manner, corticosterone treatments to rats caused significant increase in TPL contents of liver mitochondria, whereas the CHL content

had decreased significantly. As a result, the TPL/CHL molar ratios showed greater increase in both the treatment groups. The relative proportion of phospholipids and cholesterol is one of the important factors governing the membrane fluidity (1), and could also affect the catalytic properties of the membrane-bound enzymes (10). The observed alterations in Arrhenius parameters (Chapter V) could be attributed to this factor. Increase in cholesterol content of the membrane can abolish the transition temperature (10). In 14- and 35-day-old controls no break could be detected in Arrhenius plots of ATPase activity (Chapter V) suggesting an increase in cholesterol. In fact in case of 35-day-old control rats cholesterol content of mitochondria was much higher compared to other age groups (Table 1).

The reported glucocorticoid mediated decrease in HMG-CoA synthase and HMG-CoA reductase activities and cholesterol synthesis (1,3) support our findings showing decreased cholesterol content of liver mitochondria from corticosterone treated animals. Additionaly, Kaur et al. (7) have also shown that dexamethasone treatment to rats for 8 days caused significant reduction in cholesterol content/g tissue in hepatic mitochondria. Melby et al. (2) also reported similar findings for liver microsomes. Adrenalectomy increased whereas cortisol treatment decreased the cholesterol content of hepatic microsomes.

Corticosterone significantly altered the composition and contents of individual phospholipids of liver mitochondria in age-dependent manner. 35-day-old rats were most affected by the two corticosterone treatments. Kaur et al. (7) have reported that chronic dexamethasone treatment to adult rats did not alter phospholipid composition of liver mitochondria. In contrast, the present studies have clearly shown that in adult animals, chronic corticosterone treatment caused significant changes in composition/ content of PC and PE in liver mitochondria.

Decreased lysophospholipids in the liver mitochondria from corticosterone treated animals indicates a decreased phospholipid breakdown. This could be one of the reasons for significant increase in TPL content; increased synthesis does not seem to be the case, as the incorporation studies revealed that corticosterone treatments decreased the rates of phospholipid synthesis. Increased transport of phospholipids from microsomes is an interesting possibility.

Phospholipid polar head groups in the vicinity of ATPase may have specific electrostatic interactions with this enzyme. Net ionic charge on phospholipid molecules is directly responsible for activation of mitochondrial ATPase in the reconstituted liposomes. When fatty acyl chain composition is constant, increase in ATPase activation is directly

proportional to the net negative charge of phospholipid polar head groups (11). Thus, distribution of phospholipid classes within the membrane plays an important role in modulating catalytic activity of mitochondrial ATPase by dictating ionic interactions between protein and lipid matrix. The observed decrease in activity and altered kinetic properties of ATPase in liver mitochondria from corticosterone treated animals (Chapter V) could be attributed to the altered phospholipid content and composition reported here and/or the possible alterations in fatty acid composition of mitochondrial phospholipids. Mitochondrial ATPase has negatively charged phospholipid requirement for its function (11). The data presented in this Chapter clearly show the decreased synthesis of negatively charged phospholipids upon corticosterone treatments and the composition/content of these phospholipids affect the overall negative charge in the vicinity of ATPase and hence result in decreased activity observed here (Chapter V).

^{3}H acetate incorporation studies have revealed that rate of label incorporation into individual phospholipids of liver mitochondria from control animals follows a specific developmental pattern. This is totally different from that of the brain mitochondria mentioned earlier in Chapter IV. Corticosterone treatments in general significantly decreased ^{3}H acetate incorporation into all the phospholipid classes of

liver mitochondria in age dependent manner. Kaur et al. (7) were unable to show any significant effects of in vivo chronic dexamethasone treatment on ^{14}C acetate incorporation into phospholipids in liver tissue of adult rats.

Although corticosterone treatment: especially chronic- significantly increased the composition/content of DPG, both the corticosterone treatments caused several fold reduction in label incorporation into DPG in liver mitochondria. The observed increase in DPG composition/content could be due to decreased breakdown of DPG. This was evident from the fact that the composition /content of lysophospholipid decreased significantly upon corticosterone treatment. Secondly, the incorporation studies revealed that upon chronic corticosterone treatment no label was detected inlysophospholipids in liver mitochondria from animals of all the age groups.

It was interesting to note here that chronic corticosterone treatment completely inhibited the counts not only in the lysophospholipids but also completely blocked the label incorporation into SPM, PI and PS in 14- and 21-day groups. Both the treatments decreased label incorporation into PC and PE. Similarly Glenny and Brindley (4) reported that in vivo cortisol treatment to rats for five days caused significant decrease in the incorporation of ^{14}C palmitate in PC and PE and ^{3}H glycerol into PE in the liver tissue.

As a result of host of changes in hepatic mitochondrial lipids, the corticosterone treatments also altered significantly the membrane fluidity. Except for 14-day group, acute treatment had no effect on membrane fluidity. Chronic treatment caused significant alterations in membrane fluidity of liver mitochondria in age-dependent manner.

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SUMMARY

1. Corticosterone treatments caused significant increase in TPL content of rat livear mitochondria while the CHL content had decreased significantly. As a result the TPL/CHL molar ratios showed increase in both the corticosterone treatment groups.

2. Corticosterone treatments caused significant alterations in composition and content of individual phospholipids of liver mitochondria in age-dependent manner. Corticosterone treatment in general significantly decreased ^{3}H acetate incorporation into different phospholipids classes in age-dependent manner. Upon chronic treatment no label was found in LYSO component in animals of all the age groups. In case of 14-day and 21-day groups, after chronic treatment label incorporation into SPM, PI and PS was also completely inhibited. Chronic corticosterone treatment significantly increased composition/content of DPG. However, both the corticosterone treatments caused several fold reduction in label incorporation in DPG.

3. Effects of corticosterone treatment on membrane fluidity were age-dependent and treatment specific. Acute treatment had no effect on mitochondrial membrane fluidity except in 14-day group. Chronic treatment caused significant alterations in membrane fluidity.