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EFFECT OF CORTICOSTERONE TREATMENT ON ENERGY METABOLISM IN RAT LIVER MITOCHONDRIA

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Summary—1. Effect of *in vivo* treatment (40 mg/kg body wt) with corticosterone on energy metabolism in rat liver mitochondria was examined under acute and chronic conditions in 20-, 35- and 60-day-old rats.

2. Acute treatment did not affect body or liver weight. However, chronic treatment caused increased liver weight in the former two age groups; in the 60-day-old animals the liver weight decreased.

3. Acute treatment resulted in a generalized decrease in state 3 respiration rates and state 4 respiration rates without having any significant effect on ADP/O ratios with glutamate, succinate and ascorbate + TMPD as substrates. However, rates of ATP synthesis decreased significantly. The effect was age-dependent, older animals showed increased resistance.

4. Chronic treatment resulted in uncoupling of oxidative phosphorylation without having significant effects on respiration rates. Once again, the effects were age-dependent. Consequently, the ATP synthesis rates were significantly lowered. However, it was apparent that the underlying mechanisms were entirely different.

5. With succinate as the substrate the state 3 respiration rates increased with age to reach adult values by day 60. The coupling efficiency was also exhibited via maturational changes.

INTRODUCTION

The steroid hormones, glucocorticoids and mineralocorticoids, elicit a wide range of biological effects which include anti-inflammatory and immuno-supressive action, stimulation of erythropoiesis and regulation of cardiac output [1]. The glucocorticoids affect the metabolism of carbohydrate, lipids, proteins and nucleic acids [2–5]. The effect of glucocorticoids on mitochondrial functions has been a subject of interest for many years. Thus, a survey of the literature indicates that various research workers have reported diverse findings with respect to the effect of steroid hormones on mitochondrial function. These include either stimulation or inhibition of respiration in mitochondria [6] and increased ATPase activity associated with the uncoupling of oxidative phosphorylation [7], the effects were observed when different steroids were incubated with mitochondria at high (1×10^{-5}–1×10^{-4} M) concentrations under *in vitro* conditions. In addition, the effects were of a variable nature since different researchers used different steroids in their studies [8, 9]. Kimberg *et al.* [10] studied the effects of cortisone treatment *in vivo* on rat liver mitochondria and reported an interesting finding that within 2 h of hormone administration there was increased mitochondrial volume with a decrease in its number [11], which was interpreted as resulting from mitochondrial fusion [12]. This was accompanied by uncoupling of oxidative phosphorylation [13].

In the rat, the principal glucocorticoid is corticosterone [14]. The levels of corticosterone in serum follow a circadian rhythm with the lowest and highest concentrations being seen between 6.00–9.00 a.m. and 7.00 p.m.–midnight, respectively; there are about 9 episodic bursts [15]. The serum levels of the hormone also show age-dependent changes. Thus, in the 15-day fetus the hormone levels are known and increase to a maximum by the 22nd day of gestation. The values decrease to a minimum by the 3rd day postnatally and continue to rise from 15 to 35 days to reach adult levels [16].

Since corticosterone is the major hormone of the rat and since it shows a circadian rhythm and age-dependent changes, this offers an opportunity to examine the effects of corticosterone overload on mitochondrial functions by injecting the hormone at a time when its levels are lowest and at different age-groups. Such

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studies using the major hormone of the rat can only give meaningful results rather than the earlier studies referred to above where a wide variety of steroids, both synthetic and natural, were employed, mostly for *in vitro* studies [6–13].

MATERIALS AND METHODS

Animals

Male albino rats of Charles foster strain of different age groups, viz. 20, 35 and 60 days, were used. The animals received either acute or chronic corticosterone treatment at a dose of 40 mg/kg body wt [17]; all the injections were given subcutaneously (s.c.) in the neckfold between 6.00–7.00 a.m. Corticosterone suspensions were made in 0.3% (w/v) carboxymethyl cellulose (CMC) by vigorous homogenization in a Potter–Elvehjem type glass–Teflon homogenizer.

The acute treatment consisted of a single injection and the animals were killed 2 h later. The chronic treatment consisted of injecting the animals for 3 consecutive days and the animals were killed on the 4th day which correspond to their exact age group, i.e. the chronic treatment of the animals in the 20-day age group started on day 17 and was continued up to the 19th day. The animals were then killed on the 20th day. The controls received only the vehicle

Isolation of mitochondria and measurement of oxidative phosphorylation

The animals were killed by decapitation and their livers were quickly removed and placed in beakers containing chilled (0–4°C) isolation medium: 250 mM sucrose, for isolation of mitochondria by the procedure described previously [18]. All operations were carried out at 0–4°C. Records of body and tissue weights at the time of death were made.

Measurement of oxidative phosphorylation was carried out at 30°C using a Clark-type oxygen electrode in a total volume of 1.6 ml, as described earlier [19]. Protein estimations were carried out according to the procedure described by Lowry *et al.* [20], with bovine serum albumin (BSA) as the standard.

Chemicals

All fine chemicals and substrates were purchased from Sigma Chemical Co. (St Louis, Mo., U.S.A.). Other chemicals were of the highest purity grade available commercially.

RESULTS

Table 1 summarizes data on the effect of corticosterone treatment on body and liver weights. It can be noted that acute treatment with the hormone did not affect the body or tissue weights. However, when the animals were given chronic treatment, the liver weights increased in the 20- and 35-day-old animals (20 and 28% increase, respectively) with no change in the body weight. By contrast, in the 60-day-old animals the liver weight decreased significantly (26% decrease), the body weight was not affected. The results thus show that the effects of corticosterone overload are treatment- and age-dependent.

The results on oxidative phosphorylation in rat liver mitochondria with glutamate as the substrate as affected by corticosterone treatment are summarized in Table 2. It can be noted that in the control group the state 3 respiration rate had reached a more-or-less steady-state level by day 20, which was also true for the state 4 respiration rate. When the animals were given acute treatment, this resulted in 75, 68 and 56% decreases in the state 3 respiration rate. A similar trend was seen even for state 4 respir-

Table 1. Effects of corticosterone treatment on body and liver weight

Age (days)	Parameter	Treatment		
		Control (10)	*Acute (8)*	*Chronic (12)*
20	Body wt (g)	35.0 0.89	32.1 ± 1.01 NS	33.6 ± 0.81 NS
	Liver wt (g)	1.20 ± 0.08	1.21 ± 0.07 NS	1.35 ± 0.03 NS
	Liver as % body wt	3.39 ± 0.15	3.74 ± 0.16 NS	3.99 ± 0.09***
		Control (8)	*Acute (8)*	*Chronic (10)*
35	Body wt (g)	85.9 ± 3.01	87.3 ± 4.20 NS	79.3 ± 2.91 NS
	Liver wt (g)	3.20 ± 0.13	3.44 ± 0.25 NS	3.84 ± 0.20**
	Liver as % body wt	3.74 ± 0.09	3.87 ± 0.11 NS	4.82 ± 0.15****
		Control (4)	*Acute (4)*	*Chronic (4)*
60	Body wt (g)	175.0 ± 19.9	165.3 ± 10.7 NS	180.8 ± 2.4 NS
	Liver wt (g)	5.75 ± 0.53	5.84 ± 0.35 NS	4.25 ± 0.21*
	Liver as % body wt	3.29 ± 0.08	3.55 ± 0.21 NS	2.39 ± 0.22***

Results are given as mean ± SEM of the number of observations indicated in parentheses.
*$P < 0.05$, **$P < 0.02$, ***$P < 0.01$, ****$P < 0.001$, NS = not significant.

Table 2. Effect of corticosterone treatment on oxidative phosphorylation with glutamate as the substrate

Age (days)	Parameter[a]	Treatment		
		Control (6)	*Acute (8)*	*Chronic (8)*
20	ADP/O ratio	2.10 ± 0.33	2.25 ± 0.18 NS	1.14 ± 0.17*
	State 3 respiration rate	13.0 ± 3.18	3.30 ± 0.74**	8.0 ± 1.72 NS
	State 4 respiration rate	6.3 ± 1.40	2.0 ± 0.31*	4.5 ± 1.20 NS
	ADP-phosphorylation rate	48.5 ± 9.0	16.1 ± 4.40***	15.3 ± 1.83***
		Control (11)	*Acute (8)*	*Chronic (8)*
35	ADP/O ratio	2.38 ± 0.20	2.06 ± 0.08 NS	1.55 ± 0.13***
	State 3 respiration rate	12.2 ± 1.76	3.90 ± 0.45****	7.5 ± 1.04*
	State 4 respiration rate	4.2 ± 0.75	1.4 ± 0.25***	5.4 ± 0.80 NS
	ADP-phosphorylation rate	59.3 ± 10.3	15.5 ± 1.4****	22.0 ± 1.9***
		Control (5)	*Acute (8)*	*Chronic (10)*
60	ADP/O ratio	2.27 ± 0.21	1.83 ± 0.19 NS	1.82 ± 0.17 NS
	State 3 respiration rate	14.7 ± 1.55	6.4 ± 0.75***	12.7 ± 1.47 NS
	State 4 respiration rate	7.8 ± 1.36	4.2 ± 0.10*	7.8 ± 1.13 NS
	ADP-phosphorylation rate	59.2 ± 7.96	21.8 ± 1.79***	45.0 ± 6.04 NS

Results are given as mean ± SEM of the number of observations indicated in parentheses.
[a]Respiration rates and ADP-phosphorylation rate are expressed as nmol O_2 consumed and nmol ADP-phosphorylated (respectively)/min/mg protein.
*$P < 0.05$, **$P < 0.02$, ***$P < 0.01$, ****$P < 0.001$, NS = not significant.

ation. ADP/O ratios were not altered significantly, however, the ADP-phosphorylation rate decreased from 66 to 73%. In the chronically treated animals, state 3 respiration was inhibited by 38% in 20- and 35-day-old animals with no effect being observed in the 60-day-old rats. The state 4 respiration rate, in general, was not affected. However, ADP/O ratios decreased by 55 and 35% in two early age groups; this effect was not observed in the 60-day-old animals. Consequently, the ADP-phosphorylation rate decreased by 63 and 68% in the former two groups.

When succinate was used as the substrate (Table 3), acute treatment significantly inhibited state 3 respiration from 46 to 62%, the maximum effects being seen in the younger animals as in the case of glutamate. A similar trend was seen even for state 4 respiration rates. ADP/O ratios were not affected but ADP-phosphorylation rates showed a proportionate decrease. In the chronically treated animals, the state 3 respiration rates were comparable with the control in the 20- and 60-day-old animals and actually increased by 50% in the 35-day-old animals. This may perhaps represent a compensatory mechanism since it was exactly at this point that state 3 respiration rates were significantly inhibited as described above with glutamate (Table 2). However, the ADP/O ratios decreased by 40% in the latter two age groups. In the 20-day-old animals the ADP/O ratio in the control itself was lower than the expected theoretical value, which may reflect the developmental pattern of the maturation of the second coupling site. Also, neither acute nor chronic treatment significantly affected the ADP/O ratio in this age group.

The results in Table 4 summarize the effects of acute and chronic treatment on oxidative

Table 3. Effect of corticosterone treatment on oxidative phosphorylation with succinate as the substrate

Age (days)	Parameter[a]	Treatment		
		Control (4)	*Acute (8)*	*Chronic (8)*
20	ADP/O ratio	0.76 ± 0.09	0.75 ± 0.04 NS	0.61 ± 0.05 NS
	State 3 respiration rate	20.3 ± 1.83	7.6 ± 0.41****	25.0 ± 2.59 NS
	State 4 respiration rate	13.7 ± 2.36	5.7 ± 52*	19.9 ± 2.26 NS
	ADP-phosphorylation rate	30.9 ± 4.63	11.4 ± 0.74**	2.98 ± 2.90 NS
		Control (4)	*Acute (9)*	*Chronic (8)*
35	ADP/O ratio	1.02 ± 0.16	1.16 ± 0.09 NS	0.61 ± 0.06*
	State 3 respiration rate	30.9 ± 3.60	16.3 ± 1.95***	46.2 ± 5.3*
	State 4 respiration rate	20.4 ± 1.79	10.0 ± 1.47****	32.3 ± 5.13 NS
	ADP-phosphorylation rate	61.0 ± 8.34	36.2 ± 4.29*	54.5 ± 5.41 NS
		Control (10)	*Acute (6)*	*Chronic (6)*
60	ADP/O ratio	1.42 ± 0.14	1.21 ± 0.05 NS	0.84 ± 0.08*
	State 3 respiration rate	45.1 ± 5.90	24.1 ± 3.31*	36.5 ± 4.96 NS
	State 4 respiration rate	31.9 ± 4.50	17.1 ± 3.2**	21.9 ± 2.45 NS
	ADP-phosphorylation rate	120.9 ± 14.91	58.6 ± 8.77**	59.1 ± 1.69***

Results are given as mean ± SEM of the number of observations indicated in parentheses.
[a]Respiration rates and ADP-phosphorylation rate are expressed in nmol O_2 consumed and nmol ADP-phosphorylated (respectively)/min/mg protein.
*$P < 0.05$, **$P < 0.02$, ***$P < 0.01$, ****$P < 0.001$, NS = not significant.

Table 4. Effect of corticosterone treatment on oxidative phosphorylation with ascorbate + TMPD as substrate

Age (days)	Parameter[a]	Treatment		
		Control (8)	*Acute (9)*	*Chronic (8)*
20	ADP/O ratio	0.31 ± 0.05	0.21 ± 0.01 NS	0.22 ± 0.01 NS
	State 3 respiration rate	38.8 ± 3.42	20.1 ± 1.60****	41.2 ± 3.36 NS
	State 4 respiration rate	22.0 ± 2.33	10.1 ± 0.65****	28.9 ± 1.78*
	ADP-phosphorylation rate	25.2 ± 4.99	8.2 ± 0.54***	17.9 ± 1.31 NS
		Control (8)	*Acute (8)*	*Chronic (8)*
35	ADP/O ratio	0.43 ± 0.02	0.40 ± 0.01 NS	0.19 ± 0.01****
	State 3 respiration rate	42.3 ± 3.70	30.1 ± 1.80***	52.8 ± 5.62 NS
	State 4 respiration rate	19.5 ± 2.41	14.0 ± 0.68*	25.0 ± 3.95 NS
	ADP-phosphorylation rate	36.9 ± 4.20	23.8 ± 1.69**	20.6 ± 2.73***
		Control (8)	*Acute (8)*	*Chronic (6)*
60	ADP/O ratio	0.45 ± 0.03	0.49 ± 0.02 NS	0.21 ± 0.01****
	State 3 respiration rate	43.8 ± 2.96	41.4 ± 4.69 NS	50.5 ± 7.78 NS
	State 4 respiration rate	28.9 ± 2.43	23.6 ± 2.21 NS	34.0 ± 4.79 NS
	ADP-phosphorylation rate	40.3 ± 4.68	39.3 ± 3.54 NS	21.7 ± 3.65***

Results are given as mean ± SEM of the number of observations indicated in parentheses.
[a]Respiration rates and ADP-phosphorylation rates are given as nmol O_2 consumed and nmol ADP-phosphorylated (respectively)/min/mg protein.
*$P < 0.05$, **$P < 0.02$, ***$P < 0.01$, ****$P < 0.001$, NS = not significant.

phosphorylation with ascorbate + TMPD as the substrate. For the acute treatment group the pattern for inhibition of state 3 and state 4 respiration was similar to the other two substrates tested (Tables 2 and 3), except for the fact that the effects were seen only in 20- and 35-day-old animals and the extent of inhibition was much lower (28–54%). The ADP-phosphorylation rates decreased by 35 and 67%. Chronic treatment did not affect the respiration at all, but the ADP/O ratios decreased by 53 and 55% in the latter two age groups. As a result, the ADP-phosphorylation rates decreased from 30 to 46%. The ADP/O ratio was not affected in the 20-day-old animals which may be related with the maturational changes noted earlier (Table 3) for succinate.

DISCUSSION

The present studies were undertaken to critically evaluate the effects of corticosterone overload under *in vivo* conditions on energy metabolism in mitochondria. These studies assume importance because the hormone used is the major circulating hormone in the rat. In the other studies reported in the literature, several other steroid hormones have been used to examine their effects on mitochondrial energy metabolism [6, 7]. However, as far as we are aware there are no reported studies where corticosterone treatment was employed. Ours is, therefore, the first report where the major circulating hormone of the rat is employed for the *in vivo* studies.

The present studies have shown that the effects of acute and chronic treatment with corticosterone were different and substrate specific. Thus, acute treatment, in general, resulted in inhibition of respiration rates without affecting the ADP/O ratios. The net result was impairment of the ATP synthesis rate. In the chronically treated rats, on the other hand, major lesions occurred at the phosphorylation sites. Thus, the ADP/O ratios decreased considerably, the extent of the decrease depending on the substrate employed and the age of the animal. Once again, the net effect was impairment of the ATP synthesis rate, although the underlying mechanism was quite different from that in the acute treatment group. Looking at the overall picture of the chronic treatment (Tables 2–4), it would seem that the first site is more susceptible to corticosterone action.

Earlier workers have shown that incubation of mitochondria under *in vitro* conditions results either in stimulation or inhibition of respiration [6], stimulation of ATPase activity and uncoupling [7], as mentioned above in the Introduction section. There are, however, no reports on *in vitro* effects of corticosterone on mitochrondrial energy metabolism. It is possible that the short-term effects that we observed under the acute treatment experiment may reflect interaction of corticosterone *per se* with the respiratory chain components. This, however, needs to be verified by either doing *in vitro* incubation experiments or by studying the reversal of inhibition at different time intervals, postcorticosterone treatment.

In the case of the chronically treated groups the animals received corticosterone treatment for 3 consecutive days but were killed 24 h after the last injection. It is possible that some of the

corticosterone effects might have been reversed in these animals. Nevertheless, the treatment of the animals was for a sufficiently long period of 3 days or so, which can bring about genomic changes in the mitochondrial function. In this connection, it is interesting to note that most of the mitochondrial proteins are coded for by the nuclear genome, while about 10% of the most crucial peptides are mitochondrial gene products [21]. Interestingly, it has been reported that the half-life of the mitochondrial proteins in the liver is about 3.5–4.0 days [22]. Viewed in the context of this information, the chronic treatment that we have employed seems to be sufficiently long to bring about genomic changes which can lead to altered composition of the respiratory chain. This, however, needs to be verified experimentally.

Nevertheless, the results of our present study have shown for the first time that both acute as well as chronic treatment *in vivo* with corticosterone can lead to impaired energy metabolism in mitochondria; albeit the underlying mechanisms are entirely different.

REFERENCES

1. Exton J. H., Friedmann N., Wong E. H. A., Brineaux J. P., Corbin J. D. and Park C. R.: Interaction of glucocorticoids with glucagon and ephinephrine in the control of gluconeogenesis and glycogenolysis in liver and lipolysis in adipose tissue. *J. Biol. Chem.* **247** (1972) 3579–3588.
2. Kreutner W. and Goldberg N. D.: Dependence on insulin of the apparent hydrocortisone activation of hepatic glycogen synthetase. *Proc. Natn. Acad. Sci. U.S.A.* **58** (1967) 1515–1519.
3. Kenny F. T.: Hormonal regulation of synthesis of liver enzymes. In *Mammalian Protein Metabolism* (Edited by H. N. Munro). Academic Press, New York, Vol. IV (1970) pp. 137–177.
4. Dellorco R. T. and Melnylcovych G.: Effect of prednisolone on phospholipid metabolism in tissue culture. *Expl Cell Res.* **60** (1970) 257–261.
5. Dausse J. P., Duval D., Meyer P., Gaignault J. C., Marchandeall C. and Raynaud J. P.: Cited from: Duval D., Durant S. and Homo-Delarche F.: Non-genomic effects of steroids. Interactions of steroid molecules with membrane structures and functions. *Biochim. Biophys. Acta* **737** (1983) 409–442.
6. Tomkins G. and Maxwell E. S.: Some aspects of steroid hormone action. *A. Rev. Biochem.* **12** (1963) 677–708.
7. Gomez-Puyou A., Tuena M., Guzman-Garcia J. and Pena A.: *Biochem. Pharmac* **12** (1963) 669–677. Cited from: Duval D., Durant S. and Homo-Delarche F.: Non-genomic effects of steroids. Interactions of steroid molecules with membrane structures and functions. *Biochim. Biophys. Acta* **737** (1983) 409–442.
8. Yielding K. L. and Tomkins G. M.: Inhibition of the enzymatic oxidation of DPNH by steroid hormones. *Proc. Natn. Acad. Sci. U.S.A.* **45** (1959) 1731–1739.
9. Yielding K. L., Tomkins G. M., Hunday J. S. and Kowley I. J.: The effect of steroids on electron transport. *J. Biol. Chem.* **235** (1960) 3413–3416.
10. Kimberg D. V., Loud A. V. and Wiener J.: Cortisone-induced alterations in mitochondrial function and structure. *J. Cell Biol.* **37** (1968) 63–79.
11. Bullock G. R., Christian R. A., Petters R. T. and White A. M.: Rapid mitochondrial enlargement in muscle as a response to triamcinolone acetonide and its relation to the ribosomal defect. *Biochem. Pharmac.* **20** (1971) 943–953.
12. Kimberg D. V. and Loeb J. N.: Effect of cortisone administration on rat liver mitochondria. *J. Cell Biol.* **55** (1972) 635–643.
13. Karpolla W.: Uncoupling of the oxidative phosphorylation with cortisone in liver mitochondria. *Endocrinology* **67** (1960) 252–263.
14. Carpenter W. T. and Bunney W. R. Jr: Adrenal cortical activity in depressive illness. *Am. J. Psychiat.* **128** (1971) 31–40.
15. Forsham P. H.: The adrenals. In *Textbook of Endocrinology* (Edited by R. H. Williams). Saunders, Philadelphia, Pa, 3rd edn (1962).
16. Sapolsky R. M. and Meaney M. J.: Maturation of the adrenocortical stress response: neuroendocrine control mechanisms and the stress hyporesponsive period. *Brain Res. Rev.* **11** (1968) 65–76.
17. Patel A. J., Weir M. D. and Anthony C. S. M.: Regulation of *in vivo* glutamate synthetase activity by glucocorticoids in the developing rat brain. *Dev. Brain Res.* **10** (1983) 83–91.
18. Katyare S. S. and Rajan R. R.: Enhanced oxidative phosphorylation in rat liver mitochondria following prolonged *in vivo* treatment with imipramine. *Br. J. Pharmac.* **95** (1988) 914–922.
19. Katyare S. S. and Satav J. G.: Impaired mitochondrial oxidative energy metabolism following paracetamol-induced hepatotoxicity in the rat. *Br. J. Pharmac.* **96** (1989) 51–58.
20. Lowry O. H., Rosebrough N. J., Farr A. L. and Randall R. J.: Protein measurement with Folin phenol reagent. *J. Biol. Chem.* **193** (1951) 265–275.
21. Schatz G. and Mason T. L.: The biosynthesis of mitochondrial proteins. *A. Rev. Biochem.* **43** (1974) 51–87.
22. Katyare S. S. and Shallom J. M.: Altered cerebral protein turnover in rats following prolonged *in vivo* treatment with nicotine. *J. Neurochem.* **50** (1988) 1356–1363.