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THE STIMULATION OF HEPATIC OXIDATIVE PHOSPHORYLATION FOLLOWING DEXAMETHASONE TREATMENT OF RATS

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The effect of short-term treatment of rats with the synthetic glucocorticoid, dexamethasone, on mitochondrial oxidative phosphorylation has been examined. Treatment of rats for 3 h increased the oxidative capacity of the subsequently isolated mitochondria such that they displayed increased uncoupled and State 3 rates of respiration with NAD-linked substrates, succinate or durohydroquinone. The oxidation of ascorbate plus *N,N,N′,N′*-tetramethyl-*p*-phenylenediamine was unaffected. No change was apparent in the activity of a variety of dehydrogenase enzymes nor was there any increase in the mitochondrial content of cytochromes *a*, *b*, c_1 or *c*. The uncoupler-dependent ATPase activity of the mitochondria was slightly enhanced following hormone treatment, but not the basal or the total ATPase activity measured in the presence of Triton X-100 plus Mg^{2+}. The mitochondria prepared from dexamethasone-treated rats also displayed increased intramitochondrial concentrations of Mg^{2+}, K^+ and exchangeable adenine nucleotides but not Ca^{2+}. It is suggested that the effect of glucocorticoids on mitochondrial respiration may be both the result of a direct activation of the respiratory chain within Complex III and an elevated intramitochondrial adenine nucleotide concentration. The evidence for the de novo synthesis of mitochondrial proteins which mediate the response remains inconclusive.

Introduction

While it is clearly established that chronic administration of glucocorticoids to animals results in profound alterations in hepatic oxidative metabolism with reports of decreased rates of substrate oxidation, succinate dehydrogenase activity and ability to take up and retain calcium [1–4], there are few reports of the effects of short-term treatment. In contrast to the effect of long-term administration, the work of Haynes et al. [5,6] has demonstrated that mitochondria prepared from rats treated for 3 h with cortisol or the synthetic glucocorticoid, dexamethasone, display enhanced rates of respiration with either NAD-linked substrates or succinate, when measured under either uncoupled or State 3 conditions of respiration. A similar enhancement of oxygen uptake has been demonstrated in liver homogenates by Goetsch and co-workers [7–9] following a single injection of cortisol or prednisolone into fasted adrenalectomised rats. However, in these studies the effect of hormone treatment on mitochondrial oxygen consumption was unstable and lost upon isolation of the organelle, although the mitochondria prepared from the steroid-treated animal retained an elevated rate of ATP formation and increased P/O ratio [9]. Hughes and Barritt [10] have shown that short-term treatment with glucocorticoids is also implicated in the regulation of calcium metabolism within the mitochondria,

Abbreviations: TMPD, *N,N,N′,N′*-tetramethyl-*p*-phenylenediamine; DCIP, 2,6-dichlorophenolindophenol.

steroid administration resulting in an increased ability of the mitochondria to retain a given load of calcium. The latter effect was independent of changes in either State 3 or 4 rates of respiration, in contrast to the studies of Haynes and co-workers [5,6]. It has been proposed that the mechanism of action of the glucocorticoids at the level of the mitochondria is via a protein synthetic mechanism as the stimulation of both respiration and calcium retention was completely abolished by prior treatment of the animals with cycloheximide [6,10] and partially blocked by the co-administration of puromycin with the hormone [10].

Owing to the potential significance of alterations in oxidative metabolism in the control of anabolic pathways such as gluconeogenesis and ureogenesis [5,6,11–20], both of which are known to be stimulated by glucocorticoids [5,6,21–24], the effect of dexamethasone on mitochondrial function has been re-examined to determine if such a stimulation occurs and if so, to characterise it in greater detail.

Materials and Methods

Materials

Sodium pyruvate, malic acid, glutamic acid, sodium succinate, durohydroquinone, dexamethasone acetate, DCIP, phenazine methosulphate, sodium ascorbate, TMPD and cycloheximide were obtained from Sigma Chemical Co. (Poole, Dorset, U.K.). Glucagon was a gift from Novo Chemicals (Basingstoke, Hants, U.K.). [1-^{14}C]Pyruvate was obtained from Amersham International (Amersham, Bucks, U.K.). All other chemicals were AnalaR grade from BDH Chemicals (Poole, Dorset, U.K.).

Treatment of animals

Male Sprague-Dawley rats weighing 180–240 g were injected intraperitoneally with dexamethasone acetate (15 μmol/100 g body weight) suspended in isotonic saline or vehicle alone. The rats were maintained at a temperature of 21–24°C as described by Wakat and Haynes [6], and after 3 h the rats were killed by decapitation. The livers were rapidly removed and the mitochondria prepared as described previously [25]. Bilateral adrenalectomies were carried out under diethyl ether anaesthetic 7–14 days prior to the experiment and the animals maintained with 0.9% saline in their drinking water. Controls received a sham operation. In studies where the effects of cycloheximide were examined, the cycloheximide was injected intraperitoneally (10 mg/kg body weight) 3 h prior to killing, together with the dexamethasone or vehicle. Glucagon (0.5 mg/kg body weight) was injected intraperitoneally 20 min prior to killing.

Assays

Mitochondrial uncoupler-dependent ATPase activity and respiration were assayed as described previously [25], except that 2,4-dinitrophenol was used as the uncoupling agent at a final concentration of 0.2 mM. Succinate dehydrogenase (EC 1.3.99.1) was assayed by the reduction of DCIP after solubilising the mitochondria with 2 μg/ml of phospholipase A_2 by the method of Singer [26]. Glutamate dehydrogenase (EC 1.4.1.3) and malate dehydrogenase (EC 1.1.1.37) were measured as described in Ref. 25. The concentration of mitochondrial ions was measured in a 10% trichloroacetic acid extract using a Pye Unicam SP9 atomic absorption spectrophotometer. Protein was determined by the method of Lowry et al. [27] using bovine serum albumin (fraction V) as the standard. Adenine nucleotides were measured in protein-depleted extracts of mitochondria using bioluminescence with an ATP-monitoring reagent from LKB Wallac (Turku, Finland) following conversion of the ADP to ATP. Results were expressed as means ± S.E. with the number of different mitochondrial preparations indicated in parentheses. Statistical significance was calculated using a paired Student's *t*-test.

Results and Discussion

Effect of dexamethasone on mitochondrial respiration

Table I shows the effect of dexamethasone treatment of rats for 3 h on the rate of mitochondrial oxidative phosphorylation. The results confirm and extend the observations of Wakat and Haynes [6], but are at variance with those of Goetsch et al. [7–9] and Hughes and Barritt [10], in that dexamethasone treatment resulted in a stable general enhancement of respiration in the

TABLE I

THE EFFECT OF DEXAMETHASONE TREATMENT ON MITOCHONDRIAL RESPIRATION

Dexamethasone (15 μmol/100 g body wt) was injected intraperitoneally 3 h prior to killing. Mitochondria were prepared and assayed as described in Materials and Methods. The results shown are the means ± S.E. for six different paired groups of animals. RCR, respiratory control ratio. Results are expressed as nmol O_2/min per mg mitochondrial protein.

	Dexamethasone	State 4	State 3	Uncoupled	RCR
5 mM pyruvate +	−	10.0 ± 0.7	38.1 ± 2.2	26.2 ± 0.9	3.86
0.5 mM malate	+	11.7 ± 0.7	52.5 ± 3.8 [b]	36.4 ± 2.3 [b]	4.44
5 mM glutamate +	−	11.2 ± 1.2	55.6 ± 2.4	63.3 ± 3.5	5.04
0.5 mM malate	+	12.6 ± 0.5	78.0 ± 3.5 [c]	87.6 ± 4.6 [b]	6.21
5 mM succinate	−	18.6 ± 0.9	75.1 ± 3.5	83.8 ± 3.9	4.46
	+	22.4 ± 0.9	102.7 ± 5.3 [c]	100.7 ± 6.5 [a]	4.70
0.2 mM durohydro-	−	23.5 ± 3.7	70.0 ± 4.1	56.6 ± 2.8	3.22
quinone	+	23.3 ± 0.6	90.0 ± 2.8 [b]	74.5 ± 2.2 [a]	3.59
3 mM ascorbate	−	48.4 ± 3.0	73.1 ± 4.3	83.0 ± 4.9	1.52
+ 0.3 mM TMPD	+	52.8 ± 3.1	82.3 ± 4.2	95.9 ± 6.1	1.55

[a] $P < 0.05$.
[b] $P < 0.01$.
[c] $P < 0.001$.

isolated organelle under both State 3 and uncoupled conditions. Oxygen uptake by the mitochondria was stimulated with either NAD-linked substrates, succinate or the artificial electron and proton donor, durohydroquinone, but not with ascorbate plus TMPD. No effect was apparent with any substrate under State 4 conditions or respiration. From their observations on homogenate respiration, Bottoms and Goetsch [9] suggested that glucocorticoids enhance mitochondrial respiration at a site within Complex I of the respiratory chain, as the oxidation of succinate was unaffected by hormone treatment. However, the results presented in Table I suggest that if a direct effect of hormone treatment on the respiratory chain does occur, then it does so either at the level of Complex III or at several sites, since the oxidation of succinate and durohydroquinone, both of which feed electrons into the chain after Complex I, were enhanced.

Effect of dexamethasone treatment on mitochondrial dehydrogenase activities

The finding that glucagon pretreatment of animals resulted in a stimulation of succinate dehydrogenase activity [16,25] suggested that it might be relevant to investigate the effect of dexamethasone on the activities of a variety of mitochondrial dehydrogenase enzymes to determine whether this may in part explain the increased rates of respiration with both NAD-linked substrates and succinate. In contrast to the response to glucagon, pretreatment of the animal with glucocorticoids had no significant effect on the activities of any of the dehydrogenase enzymes measured. The respective values for the activities of succinate, malate and glutamate dehydrogenases were 0.45 ± 0.05 and 0.40 ± 0.05; 3.70 ± 0.18 and 3.82 ± 0.09; 4.22 ± 0.21 and 4.42 ± 0.24 ($n = 6$, $P > 0.05$) U/mg mitochondrial protein for control and dexamethasone-treated mitochondria, respectively. Similarly, no effect of dexamethasone treatment was apparent on either the total pyruvate dehydrogenase activity in the mitochondria or the percentage of pyruvate dehydrogenase in the active form (data not shown). If, as has been suggested, dexamethasone acts via a protein synthetic mechanism at the level of the mitochondria to stimulate respiration and calcium retention [6,10], then it is evident that the site of action is not at the level of the respective dehydrogenase enzymes, but must occur elsewhere in the respiratory chain.

The increased oxidation of durohydroquinone following hormone treatment supports the above conclusions and argues in favour of an activation of some component of the respiratory chain within the cytochrome bc_1 region, as this would not require either dehydrogenase activity, substrate

transport or the adenine nucleotide translocator when measured under uncoupled conditions. However, no effect of steroid treatment on the concentrations of either cytochromes *a*, *b*, c_1, or *c* could be detected (data not shown), indicating that if glucocorticoids influence the activity of the respiratory chain in this region then the stimulation is independent of increased synthesis of these components.

Effect of glucocorticoid treatment on the exchangeable adenine nucleotide pool size and the mitochondrial uncoupler-dependent ATPase activity

Previous studies into the mechanisms by which hormones stimulate mitochondrial oxidative phosphorylation have suggested that an enlargement of the mitochondrial adenine nucleotide pool size may contribute to the stimulation of respiration as a result of an increased rate of adenine nucleotide transport and an enhanced uncoupler-dependent ATPase activity [12,14,17,28]. Table II shows that dexamethasone treatment also resulted in a small but significant increase in the concentration of the mitochondrial exchangeable adenine nucleotides (ATP and ADP) and also the ATPase activity in the presence of the uncoupler, 2,4-dinitrophenol. No effect was observed on the activity of the ATPase measured in the absence of uncoupler, in agreement with earlier studies comparing the effects of other gluconeogenic hormones [12,17,28]. Similarly, there was no effect of steroid treatment on the total ATPase activity in the mitochondria measured in the presence of 1 mM Mg^{2+} and Triton X-100, the rates being 398.0 ± 8.4 and 393.4 ± 13.2 nmol/min per mg protein ($n = 6$).

TABLE II

THE INTRAMITOCHONDRIAL CONCENTRATIONS OF ADENINE NUCLEOTIDES, CATIONS AND ATPase ACTIVITY IN MITOCHONDRIA PREPARED FROM CONTROL AND DEXAMETHASONE-TREATED ANIMALS

ATPase activity was measured in mitochondria in the presence and absence of 0.2 mM 2,4-dinitrophenol as described in Ref. 21. The activities are expressed in nmol/min per mg mitochondrial protein. The intramitochondrial concentrations of adenine nucleotides and cations were determined in protein-depleted extracts of mitochondria as described in Materials and Methods, and are expressed in nmol/mg mitochondrial protein. Results are means ± S.E. with the number of different mitochondria preparations given in parentheses.

	Treatment	
	Control	Dexamethasone
Basal ATPase activity	21.8 ± 2.8 (12)	22.5 ± 3.0 (12)
Uncoupler-dependent ATPase activity	364.2 ± 7.2 (12)	405.2 ± 12.0 [a] (12)
Adenine nucleotides (ATP + ADP)	11.5 ± 0.5 (6)	14.5 ± 0.4 [a] (6)
Ca^{2+}	9.25 ± 0.34 (10)	9.38 ± 0.48 (10)
Mg^{2+}	33.1 ± 1.1 (10)	39.9 ± 1.0 [b] (10)
K^+	128.4 ± 3.8 (10)	144.7 ± 3.7 [b] (10)

[a] $P < 0.05$.
[b] $P < 0.01$.

While these results agree with the hypothesis that increased rates of respiration may be coupled to an enlargement of the mitochondrial adenine nucleotide pool size, the contribution of the elevated ATPase activity to the stimulation of respiration may be much less than previously postulated [17,28], The increase in respiratory rate ranged from 28 to 40% following dexamethasone treatment, while the activity of the ATPase and the size of the adenine nucleotide pool was increased by 11 and 26%, respectively. The difference in magnitude of the ATPase response between this and previous studies was the result of an increase in the activity of the control rather than a lowering of the hormone-stimulated rate. Preliminary experiments have suggested that this may reflect some degree of stress imposed upon the animal during the initial treatment as the animals were not maintained under sedation in the present studies.

In addition to contributing towards the stimulation of respiration, the elevated adenine nucleotide pool size may also explain the increased ability of the mitochondria prepared from dexamethasone-treated animals to retain calcium, as both calcium retention and uptake are known to be influenced by the mitochondrial pool size [4,7]. In light of this and the finding that chronic administration of dexamethasone to rats decreased the mitochondrial concentration of both calcium and adenine nucleotides [4], the content of calcium and also magnesium and potassium were measured in freshly isolated mitochondria. The results are shown in Table II. Despite the elevated adenine nucleotide levels, dexamethasone treatment had no effect on the total calcium content of the mitochondria, although there were small but sig-

nificant increases in the intramitochondrial concentrations of both magnesium and potassium.

Effect of cycloheximide on mitochondrial respiration stimulated by glucagon and dexamethasone

Table III shows the effect of administration of cycloheximide to rats 3 h prior to mitochondrial preparation, either alone, or in the presence of glucocorticoids or glucagon. In agreement with previous studies, both glucagon and dexamethasone treatment increased the State 3 rate of respiration by isolated mitochondria with succinate as the substrate, although neither hormone had any effect on the State 4 rate of respiration [6,12,14,20]. Treatment with cycloheximide did not alter the rate of respiration under either condition, however, it did result in a considerable elevation in the plasma glucose concentration over the 3 h period. Co-administration of cycloheximide with dexamethasone completely prevented the effect of hormone treatment on oxygen uptake in the presence of ADP, suggesting a possible involvement of de novo protein synthesis in the response, as described previously [6.10]. However, administration of cycloheximide prior to glucagon also suppressed the mitochondrial glucagon response. This is thought not to be mediated via a protein synthetic mechanism as it is apparent within 1–2 min following hormone administration and is preceded by a rise in cyclic AMP formation [17–19]. Therefore, the evidence in favour of dexamethasone acting via the de novo synthesis of a mitochondrial protein remains inconclusive as cycloheximide treatment may be producing toxic effects in addition to inhibiting protein synthesis, which prevent the action of both hormones and which may be reflected in the very high plasma glucose levels. If protein synthesis is involved in the mechanism of action of the steroids, then it is conceivable that the protein may be of extramitochondrial origin, as it is apparent that it is not at the level of dehydrogenase enzymes, the ATPase complex or the concentration of cytochromes within the mitochondria. In addition, adrenalectomising the animals for a period of 14 days prior to the experiment resulted in no significant change in either the total mitochondrial ATPase activity, the uncoupler-dependent ATPase activity, or State 3 or 4 rates of respiration (data not shown). Similarly, adrenalectomy had no significant effect on the magnitude of the dexamethasone response after this period. This would suggest that glucocorticoids, unlike the thyroid hormones [30,31], do not play a role in the normal maintenance of mitochondrial oxidative metabolism.

TABLE III

EFFECT OF CYCLOHEXIMIDE TREATMENT OF THE RAT ON MITOCHONDRIAL RESPIRATION

Cycloheximide (10 mg/kg) was injected 3 h prior to killing the rat together with dexamethasone or saline. Where appropriate glucagon (100 μg/kg) or vehicle was injected 20 min prior to death. The mitochondria were prepared and assayed as described in Materials and Methods using 5 mM succinate as the substrate. Results are expressed as means ± S.E. for six different animals in each group. Values of State 3 and 4 respiration are expressed as nmol O_2/min per mg protein.

Treatment	Plasma glucose (mM)	State 4	State 3
Control	8.4 ± 0.2	13.6 ± 1.1	56.9 ± 3.8
Glucagon	12.4 ± 0.6 [b]	14.3 ± 1.1	71.4 ± 6.8 [a]
Dexamethasone	9.7 ± 0.5	16.2 ± 1.1	75.5 ± 6.3 [a]
Cycloheximide	19.9 ± 1.7 [b]	13.4 ± 0.8	55.8 ± 4.1
Cycloheximide + glucagon	20.2 ± 2.1 [b]	13.7 ± 0.8	59.4 ± 4.2
Cycloheximide + dexamethasone	18.4 ± 1.7 [b]	15.0 ± 0.5	58.6 ± 5.6

[a] $P < 0.05$.
[b] $P < 0.001$.

In conclusion, the data presented indicate that mitochondria isolated from rats treated with-dexamethasone over the short term exhibit an enhanced oxidative capacity, as first described by Haynes and co-workers [5,6]. The mechanism of action of the steroids at the level of the mitochondria still remains unclear, but it is suggested that the effect is mediated through a stimulation of the respiratory chain in the cytochrome bc_1 region and an increased intramitochondrial concentration of adenine nucleotides. The mechanism behind the latter effect may be the result of an increased intramitochondrial pH gradient (Martin and Titheradge, unpublished observations), the more alkaline matrix encouraging accumulation [32]. Although Goetsch et al. [7–9] and Hughes and Barritt [10] were unable to demonstrate an effect of dexamethasone treatment on

respiration in the isolated organelle, their work clearly demonstrates that short-term treatment with glucocorticoids has a positive influence on mitochondrial metabolism. One possible explanation for the discrepancies apparent in this and preceding studies has come from the work of Siess et al. [33] which has suggested that some of the effects of hormones at the level of the mitochondria may be artifacts produced by the lengthy isolation procedure and the composition of the isolation medium. However, a more recent report by Jensen et al. [34] has shown that the stimulation of respiration following glucagon pretreatment of the animal also occurs in liver homogenates and that the magnitude of the response is comparable to that found in the isolated mitochondria. The effect was also independent of the medium employed to prepare the homogenates. In preliminary experiments carried out in this laboratory, we have demonstrated that the effect of dexamethasone on mitochondrial pyruvate metabolism is also apparent in homogenates and that the absolute increase in pyruvate utilisation is independent of the isolation medium (Martin and Titheradge, unpublished observations), suggesting that the effects are a true representation of hormone treatment in vivo.

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