Effects of corticosterone on muscle mitochondria identifying different sensitivity to glucocorticoids in Lewis and Fischer rats

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Effects of corticosterone on muscle mitochondria identifying different sensitivity to glucocorticoids in Lewis and Fischer rats. *Am J Physiol Endocrinol Metab* 286: E159–E167, 2004. First published September 9, 2003; 10.1152/ajpendo.00281.2003.—Previous studies in rat have demonstrated decreased number of mitochondria and uncoupling of oxidative phosphorylation after administration of glucocorticoids but at supraphysiological doses and using synthetic glucocorticoids. To analyze the relationships between corticosterone levels (the natural glucocorticoid in rat) and muscle mitochondrial metabolism, Lewis and Fischer 344 rats were bilaterally adrenalectomized and implanted with different corticosterone pellets (0, 12, 50, 100, and 200 mg of corticosterone). Rats bearing a corticosterone pellet delivering corticosterone at concentrations in the range of chronic stress-induced levels presented a lower amount of functional muscle mitochondria with a decrease in cytochrome c oxidase and citrate synthase activities and a depletion of mitochondrial DNA. Moreover, a strain difference in tissue sensitivity to corticosterone was depicted both in end-organ sensitive to glucocorticoids (body, thymus, and adrenal weights) and in muscle mitochondrial metabolism (Lewis > Fischer). Interestingly, this strain difference was also observed in the absence of corticosterone, with a deleterious effect on muscle mitochondrial metabolism in Fischer rats, whereas no effects were observed in Lewis rats. We therefore postulate that corticosterone is necessary for muscle mitochondrial metabolism exerting its effects in Fischer rats with an inverted U curve, whereby too little (only Fischer) or too much (Fischer and Lewis) corticosterone is deleterious to muscle mitochondrial metabolism. In conclusion, we propose a general model of coordinate regulation of mitochondrial energetic metabolism by glucocorticoids.

skeletal muscle; cytochrome c oxidase; tissue sensitivity to glucocorticoids

GLUCOCORTICOIDS (GC), hormones released by the adrenal cortex, have numerous homeostatic and stress-response functions. A delicate balance exists between the protective effects of adrenal steroids secreted in response to stressful experiences and the negative consequences that GC may have for many processes (20). For example, in skeletal muscles, GC insufficiency leads to debilitating fatigue, myalgia, and general muscle weakness (27), whereas excess GC causes muscular atrophy and a decreased rate of muscle protein synthesis (2). Among several mechanisms leading to muscle wasting in GC-induced myopathy, defects in mitochondrial function appear to be involved (21, 22, 35). With the administration of supraphysiological doses of GC in rat, electron microscopic and biochemical studies have demonstrated enlarged mitochondrial volume, decreased number of mitochondria, decreased substrate utilization, and uncoupling of oxidative phosphorylation (21). Studies of the effects of GC on mitochondrial oxidative phosphorylation (ATP production) have been relatively numerous, consisting of reports describing the in vitro direct effects of GC on mitochondrial energy metabolism (22, 33). However, high concentrations of synthetic GC (dexamethasone, triamcinolone, methylprednisolone, etc.) have been used in these studies (21, 22, 35). To determine the effects of endogenous increases in plasma corticosterone, the natural GC in rat, on muscle mitochondrial metabolism, in a previous study (9) we submitted rats to 8 wk of daily stress (novel environment or forced exercise), and we demonstrated that plasma corticosterone concentrations were negatively correlated with mitochondrial metabolism. Although such correlations do not necessarily indicate a direct effect of the change in corticosterone concentration on mitochondrial function, this does suggest that plasma corticosterone and functional muscle mitochondrial amount are related. The present experiment was designed to demonstrate that corticosterone at physiological concentrations (from 25 to 210 ng/ml) is involved in the regulation of muscle mitochondrial metabolism in the plantaris muscle. Rats were adrenalectomized and implanted with subcutaneous pellets with various amounts of corticosterone to maintain constant corticosterone concentrations (24). Moreover, because in the previous study we reported a strain effect in the response of muscle mitochondrial amount to variations in plasma corticosterone levels, Lewis and Fischer strains were selected because they differ considerably in the response of their hypothalamo-pituitary-adrenal (HPA) axis to acute or chronic stress. Fischer rats are more reactive than standard rat lines to a variety of stressful stimuli, whereas the Lewis HPA axis is hypersensitive to a wide range of acute or chronic stress compared with Sprague-Dawley rats (7). The Fischer and Lewis strains could be thought of as representing two ends of a spectrum of HPA axis responsiveness to stress. For these reasons, we were interested in determining whether similar plasma corticosterone concentrations in Lewis and Fischer strains could be differentially translated at the level of muscle

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mitochondrial metabolism. Two other markers were used in support of the different sensitivity to corticosterone of Lewis and Fischer strains of rat: body weight changes and thymus weight. Indeed, thymus is a corticosteroid target tissue, and thymus weight is sensitive to integrated corticosterone secretion of the previous days (1) and a good index of corticosterone levels (3).

In this study, we confirm our previous results (9) demonstrating a dose effect of corticosterone from low levels to those reached after stressful stimuli on functional muscle mitochondrial amount and a strain effect on tissue sensitivity to glucocorticoids.

MATERIALS AND METHODS

Animals

Experiments were conducted on 37 male Lewis and 36 male Fischer rats (IFFA CREDO, Les Oncins, France). The animals were 4 wk old upon arrival at our laboratory and were housed one per cage in an animal quarter at constant temperature (23–25°C) with a 12:12-h light-dark cycle (lights on at 0700). Food and water were provided ad libitum. All rats were allowed to adapt to the animal room for 2 wk before the beginning of the experiments. This study was conducted in conformance with the French recommendations on animal experimentation.

Experimental Design

Lewis and Fischer rats were randomly assigned to experimental groups and either bilaterally adrenalectomized (ADX) or sham-adrenalectomized under intraperitoneal pentobarbital sodium anesthesia (day 0). In ADX rats, subcutaneous pellets of 100 mg of wax or a fused mixture of corticosterone (12, 50, or 100 mg) and cholesterol were inserted at the time of surgery ~2 cm rostral to the skin incision in the nape of the neck. Pellets were made using an established technique (1). Briefly, corticosterone (Sigma-Aldrich, St Quentin Fallavier, France) was liquefied by heating and pipetted into a mold designed specifically for manufacturing the pellets. Therefore, to obtain a final weight of 100 mg, the 12-, 50-, and 100-mg corticosterone pellets consisted of 12, 50, and 100 mg of corticosterone fused with 88, 50, and 0 mg of cholesterol (Sigma-Aldrich), respectively. Consequently, there was in each strain six experimental groups: sham operated (wax pellet), ADX with wax pellet, or ADX plus 12, 50, 100, 88, 50, and 0 mg of cholesterol (Sigma-Aldrich), respectively.

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Body weight and food intake were measured daily in all rats (initial weight: Fischer, 111 ± 10 g; Lewis, 142 ± 16 g). Twelve days after adrenal surgery and pellet insertion, the animals were killed by decapitation in an adjacent room (between 0900 and 1200) within 30 s of removal from their home cage. Immediately after decapitation, trunk blood (4 ml) was collected in 10-ml polyethylene tubes containing 0.1 ml of 10% EDTA and stored in ice until centrifugation. Plasma samples were stored at −80°C until analysis. Thymus was collected and weighed. The superficial portion of left and right plantaris muscles was removed, and muscle samples were immediately frozen at −80°C. The superficial portion of the plantaris, a fast-twitch muscle, was chosen because this region contains predominantly type II (fast-twitch) fibers, whereas the region close to the bone (deep region) contains a mixture of fiber types (10). Because fast-twitch muscles are highly susceptible to the atrophying actions of GC whereas little or no effects are observed in slow-twitch muscles such as the soleus, which are more resistant to GC treatment (5), we hypothesized that the effects of corticosterone on mitochondrial metabolism would be more marked in fast-twitch fibers.

Plasma Corticosterone

Plasma corticosterone was measured after alcohol extraction by a competitive protein-binding assay (4) using rhesus monkey serum as the source of transcortin, [3H]corticosterone as the tracer, and dextran-coated charcoal to absorb the unbound fraction (sensitivity 4 ng/ml, specificity >95%, interassay coefficient of variation 8.0%). Samples were analyzed in duplicate.

Mitochondrial Enzymatic Activities

Analysis of mitochondrial metabolism. To understand the variations of mitochondrial activity and amount of functional mitochondria, we monitored two specific activities: 1) cytochrome c oxidase (COX) activity, which represents one of the activities of oxidative phosphorylation and can be used to evaluate the mitochondrial ability to produce ATP; and 2) succinate dehydrogenase (SDH) activity, which is specific for mitochondrial metabolism (TCA cycle) but independent of oxidative phosphorylation. We could therefore use these activities to evaluate the amount of functional mitochondria in cells (16, 17). The normalization of COX activity by SDH allowed us to distinguish two types of adaptation mechanisms: 1) a variation in the amount of functional mitochondria (in this case, COX/SDH ratio remains constant) and 2) a direct effect on the COX kinetic parameters (modification of the COX/SDH ratio while SDH remains constant).

Tissue preparation. Left muscles were cut in thin pieces and homogenized at 4°C in 10 volumes of a buffer (in mM: 225 mannitol, 75 sucrose, 10 Tris HCl, and 0.1 EDTA, pH 7.2). Homogenates were then centrifuged for 20 min at 2,500 g (4°C). Supernatants were frozen at −80°C. The enzymatic activities of COX and SDH were measured on the supernatant by observing the transfer of electrons from a natural or artificial substrate to a natural or artificial respiratory chain acceptor (16, 17). The rate of appearance of the reduced acceptor was measured spectrophotometrically (Kontron 922). All of the enzymatic activities were measured at 37°C in a final volume of 1 ml and expressed in nanomoles of product formed per minute per milligram of protein. Protein concentrations were determined spectrophotometrically by the procedure of Lowry (18).

COX activity (complex IV). Complex IV activity was determined by monitoring the cytochrome c oxidation, measured spectrophotometrically by the decrease of absorbency at 550 nm, for 1 min (38). The kinetics of cytochrome c oxidation were linear for 1 min. Activity was calculated using ε (cytochrome c) = 18,500 M−1 cm−1. Cytochrome c from horse heart (Sigma C-7752) at 1% (0.8 mM) was diluted 1:20 in phosphate buffer (25 mM KH2PO4, pH 7.4). One milliliter of this solution was reduced by a light excess of sodium dithionite, and 1 ml was completely oxidized by an excess of potassium ferricyanide to serve as a reference. The value of the optical density ratio (reduced cytochrome c) at 550 nm was ~0.7. Then, small quantities of the completely reduced solutions were added to the 8 ml of the initial 50 μM oxidized solution until 80–90% reduction was reached (checked spectrophotometrically). This ensured that there was no excess dithionite in the solution that could interfere with the reaction itself.

SDH activity. SDH activity was measured by the following method. In phosphate buffer (60 mM KH2PO4, pH 7.4) with succinate (100 mM) as the substrate, and phenazine methosulfate (PMS, Sigma P-9625, 130 mM) and 2,6-dichloroindophenol (DCIP, Sigma D-1878, 7.2 mM) as electron “acceptors,” enzymatic activity was determined by monitoring the absorbency increase at 600 nm for 2 min at 37°C, which corresponds to the DCIP reduction. For the calculation of the activity, ε (DCIP) = 21,000 M−1 cm−1.

Southern Blot Analysis

Total genomic DNA was isolated from homogenates of plantaris muscle according to standard procedures. Approximately 5 μg of DNA were digested with XhoI, a restriction enzyme that linearizes the
covalently closed circular mitochondrial DNA molecule by cutting at a unique site. Samples were resolved on 0.7% agarose gels by electrophoresis, transferred to nylon membranes, and hybridized simultaneously with 32P-labeled probes for mitochondrial DNA (mtDNA) and the nuclear 18S rRNA gene to correct for uneven loading of the samples (25). Signals were quantified with a PhosphorImager using ImageQuant software (Molecular Dynamics). To correct for quantitative variations among the samples, mtDNA signals were normalized relative to nuclear DNA signals. The results of mtDNA were expressed as means ± SE or as a percentage of ADX + corticosterone rats compared with sham-operated rats.

Statistics

The final number of rats analyzed was 67 instead of 73 rats due to the exclusion of six rats: two Lewis and four Fischer (see Table 1; two rats died after ADX, loss of its 100-mg pellet in one rat, and bad corticosterone diffusion from three 200-mg pellets). Data are presented as means ± SE. Body weight and thymus weight were analyzed by means of a two-way ANOVA with strain (2 levels) and experimental group (6 levels) as between-subject factors. For the comparisons of plasma corticosterone values and muscle mitochondrial metabolism between experimental groups, sham-operated rats were not used for calculation because the plasma corticosterone levels measured in these rats do not correspond to their 24-h mean levels but instead only to their nadir (morning) levels. Therefore, for the above-mentioned parameters, between-subject factors included 5 levels (experimental groups). When significant, ANOVA was followed by post hoc individual comparisons (Newman-Keuls test). Relationships between corticosterone levels and enzymatic activity or thymus weight were examined by computing logarithmic nonlinear regression and the Pearson product-moment correlation coefficient (r), respectively. Statistical significance was accepted at P < 0.05.

RESULTS

Effectiveness of Corticosterone Pellets

Plasma corticosterone. Plasma corticosterone levels in samples collected from the six experimental groups of Lewis and Fischer rats at 1500 h by decapitation on day 12 are shown in Table 1. In ADX rats, ANOVA revealed an experimental group effect (F4,45 = 146.19, P < 10−6) and a strain by experimental group effect (F4,45 = 4.04, P < 0.005). Plasma corticosterone levels were significantly higher in Fischer ADX + 12 mg and ADX + 100 mg subgroups compared with their Lewis counterparts (P = 0.03 and 0.04, respectively), whereas ADX + 200 mg yielded lower plasma corticosterone levels in Fischer than in Lewis rats (P = 0.001). Moreover, in both strains, serum corticosterone concentrations were roughly proportional to pellet composition for 50, 100, and 200 mg of corticosterone (present study and Refs. 1, 3, 24). However, the serum level produced by the 12-mg corticosterone pellet was only 50% lower than that produced by a 50-mg corticosterone pellet. This may be due to differences in diffusion and/or peripheral metabolism of corticosterone between the 12- and 50-mg pellets. Such differences have also been reported by Akana et al. (1). As expected, after implantation of the 12-mg corticosterone pellet, serum corticosterone levels were within the range found in sham-operated rats in the morning in both Lewis and Fischer rats (11, 30), whereas the 50-mg corticosterone pellet led to corticosterone levels similar to those reached during the first hours of lights off in Lewis rats (62.3 vs. 80 ng/ml, Lewis ADX + 50 mg vs. Ref. 11, respectively) but not in Fischer rats (72.9 vs. 130 ng/ml, Fischer ADX + 50 mg vs. Ref. 30). Consequently, in Lewis rats, taking into account the entire 24-h period, plasma corticosterone levels obtained after implantation of the 12-mg pellets were lower than the mean 24-h range of corticosterone found in sham-operated rats, as they are equivalent to the nadir (lights on) levels of corticosterone of rats with normal circadian variation of plasma corticosterone (sham-operated rats). On the other hand, the 50-mg pellets produced plasma corticosterone levels that were higher than the 24-h mean corticosterone levels produced during the normal circadian rhythm as equivalent to the peak (dark) levels of corticosterone found in sham-operated rats. This was also true

Table 1. Plasma corticosterone, COX, and SDH activities and COX/SDH ratio in sham, ADX + 0 mg, ADX + 12 mg, ADX + 50 mg ADX + 100 mg, and ADX + 200 mg Lewis and Fischer rats

<table>
<thead>
<tr>
<th></th>
<th>Plasma Corticosterone, ng/ml</th>
<th>COX Activity, nmol-min−1·mg protein−1</th>
<th>SDH Activity, nmol-min−1·mg protein−1</th>
<th>COX/SDH Ratio</th>
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<tr>
<td>Sham</td>
<td></td>
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<tr>
<td>Lewis (n = 6)</td>
<td>19.29 ± 3.67</td>
<td>481.65 ± 38.02</td>
<td>83.65 ± 4.75</td>
<td>5.75 ± 0.29</td>
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<td>Fischer (n = 6)</td>
<td>17.11 ± 2.97</td>
<td>322.83 ± 25.68</td>
<td>59.32 ± 2.97f</td>
<td>5.44 ± 0.30</td>
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<td>ADX + 0 mg</td>
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<tr>
<td>Lewis (n = 5)</td>
<td>ND</td>
<td>388.82 ± 20.07</td>
<td>57.30 ± 4.61</td>
<td>6.94 ± 0.64e</td>
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<td>Fischer (n = 5)</td>
<td>ND</td>
<td>164.76 ± 5.66c,d,e</td>
<td>25.90 ± 2.75c,d,e</td>
<td>6.60 ± 0.59e</td>
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<td>ADX + 12 mg</td>
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<td>Lewis (n = 7)</td>
<td>25.47 ± 2.65c,d,e</td>
<td>326.33 ± 35.59</td>
<td>69.70 ± 6.33e</td>
<td>4.69 ± 0.32a</td>
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<td>Fischer (n = 6)</td>
<td>41.70 ± 6.49c,d,e</td>
<td>260.68 ± 18.30c,e</td>
<td>46.65 ± 2.44d,e,d,f</td>
<td>5.60 ± 0.23</td>
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<td>ADX + 50 mg</td>
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<td>Lewis (n = 6)</td>
<td>62.29 ± 3.03b,d,e</td>
<td>383.53 ± 33.12e</td>
<td>69.33 ± 5.15e</td>
<td>5.60 ± 0.45</td>
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<td>Fischer (n = 6)</td>
<td>72.87 ± 5.87b,d,e</td>
<td>273.10 ± 34.42e</td>
<td>47.07 ± 3.72d,e,d,f</td>
<td>5.76 ± 0.51</td>
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<td>ADX + 100 mg</td>
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<td>Lewis (n = 6)</td>
<td>101.57 ± 9.38b,c,d,e</td>
<td>351.92 ± 45.58</td>
<td>63.43 ± 4.20</td>
<td>5.51 ± 0.54</td>
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<tr>
<td>Fischer (n = 5)</td>
<td>129.49 ± 6.27b,c,d,e</td>
<td>306.19 ± 26.99c,e</td>
<td>60.52 ± 4.38b,c,e</td>
<td>5.08 ± 0.34</td>
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<td>ADX + 200 mg</td>
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<tr>
<td>Lewis (n = 5)</td>
<td>215.74 ± 22.76c,d,e</td>
<td>261.79 ± 53.54b,c,d</td>
<td>47.05 ± 6.83b,c,d</td>
<td>5.88 ± 0.78a</td>
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<td>Fischer (n = 4)</td>
<td>176.77 ± 8.33b,c,d,e</td>
<td>118.34 ± 27.61b,c,d</td>
<td>31.94 ± 3.36b,c,d</td>
<td>4.08 ± 0.46a</td>
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Values are means ± SE; n = 6. COX, cytochrome c oxidase; SDH, succinate dehydrogenase; sham, sham operated; ADX + 0 mg, ADX + 12 mg, ADX + 50 mg, ADX + 100 mg, and ADX + 200 mg, adrenalectomized with indicated amounts of corticosterone; ND, not detectable. *P < 0.05 with ADX + 0 mg rats of the same strain; **P < 0.05 with ADX + 12 mg rats of the same strain; ***P < 0.05 with ADX + 50 mg rats of the same strain; ****P < 0.05 with ADX + 100 mg rats of the same strain; *****P < 0.05 with ADX + 200 mg rats of the same strain; ******P < 0.05 with Lewis rats (same group).
for Fischer rats, albeit less marked as concerns the 50-mg corticosterone pellet.

**Body weight variation.** The time course of body weight variation is shown in Fig. 1A for sham-operated and ADX rats implanted with different corticosterone pellets. Before surgery (day −1 and day 0) both strains displayed the same body weight gain independently of the experimental group. After surgery, ANOVA revealed a day effect (day effect: $F_{1,5825} = 827.29, P < 0.0001$), a strain effect ($F_{1,55} = 17.58, P < 0.0001$), an experimental group effect ($F_{5,55} = 21.59, P < 0.0001$), and a day × strain × group interaction ($F_{75,825} = 5.86, P < 0.0001$). In Lewis rats, from day 3 to the end of the experiment, body weight increase in sham-operated rats was significantly higher than in all the other groups ($P < 0.01$).

Moreover, ADX + 0 mg, ADX + 100 mg, and ADX + 200 mg corticosterone groups showed significantly less body weight increase than their counterparts with 12- and 50-mg corticosterone pellets after day 2 ($P < 0.01$). In Fischer rats, no significant intergroup difference could be observed up to the fourth postsurgical day. Compared with their sham-operated counterparts, the ADX + 100 mg and ADX + 200 mg corticosterone groups showed less body weight increase after day 4 and day 6, respectively ($P < 0.05$). Finally, the ADX + 50 mg corticosterone group displayed less body weight gain than their sham-operated counterparts only on days 11 and 12.

To determine the effects of variations of corticosterone concentration on body weight gain and taking into account the differences obtained in plasma corticosterone levels between strains within the same corticosterone pellet, body weight variations were also calculated comparatively with the group with no corticosterone (ADX + 0 mg) and are represented with reference to the plasma corticosterone levels actually reached (Fig. 1B). For plasma corticosterone values $>100$ ng/ml, weight loss induced by increased plasma corticosterone levels was lower in Fischer rats compared with their Lewis counterparts.

**Thymus weight.** Strain ($F_{1,55} = 16.19, P < 0.01$) and experimental group ($F_{5,55} = 90.06, P < 0.01$) had significant effects on relative thymus weight (Fig. 2A). No interstrain thymus weight difference was observed in sham-operated rats, but post hoc comparisons revealed, compared with sham-operated rats, a significant increase in thymus weight of ADX + 0 mg and ADX + 12 mg groups in Lewis rats only ($P < 0.01$). In both strains, 50-, 100-, and 200-mg corticosterone pellet insertion resulted in a significant decrease in thymus weight. Moreover, a significant negative correlation was measured between plasma corticosterone and thymus weight in Lewis rats ($r = -0.41, P < 0.01$) and in Fischer rats ($r = -0.61, P < 0.01$).

When the variation of thymus weight was calculated with regard to the thymus weight obtained in the condition with no corticosterone (ADX + 0 mg), thymolysis induced by increased plasma corticosterone levels was lower in Fischer rats compared with their Lewis counterparts (Fig. 2B).

It is clear from the data presented in Figs. 1 and 2 that, in the Lewis strain, 50-, 100-, and 200-mg corticosterone pellet insertion resulted in significant decreases in body weight gain and in thymus weight, suggesting that the 50-, 100-, and 200-mg pellets provide larger than physiological amounts of corticosterone. This is also true for Fischer rats, albeit less marked as concerns the 50-mg pellet. Finally, as previously reported (1), the absence of corticosterone replacement (ADX + 0 mg) or insufficient corticosterone replacement (ADX + 12 mg) resulted in a decreased rate of body weight gain and a marked increase in thymus weight in Lewis only.

**Muscle Mitochondrial Metabolism**

**COX activity.** As Table 1 shows, ANOVA revealed a strain effect ($F_{4,45} = 29.61, P < 10^{-5}$), with higher COX activities in Lewis rats than in Fischer rats and a group effect ($F_{4,45} = 4.94, P < 0.005$) on COX activity.

**SDH activity.** A strain effect ($F_{4,45} = 42.09, P < 10^{-6}$) with higher SDH activities in Lewis rats than in Fischer rats, a group effect ($F_{4,45} = 9.58, P < 10^{-4}$), and a tendency toward a
strain × group interaction ($F_{4.45} = 2.29, P = 0.07$) were noted on SDH activity.

**COX/SDH activity.** A group effect ($F_{4.45} = 3.83, P < 0.01$) was noted on COX/SDH activity. Corticosterone replacement of 200 mg induced a significant decrease in the COX/SDH ratio of ADX rats ($P < 0.05$).

**Relationships Between Plasma Corticosterone and Muscle Mitochondrial Metabolism**

When the Fischer rats are considered as individuals instead of different experimental groups, plasma corticosterone is correlated with COX activity ($P = 0.002$; Fig. 3A) and SDH activity ($P = 0.0008$; Fig. 4A). In Lewis rats, no significant correlation was found between plasma corticosterone and COX activity (Fig. 3B) or SDH activity (Fig. 4B). In contrast, when only supraphysiological values of corticosterone are taken into account, plasma corticosterone was negatively correlated with COX activity ($r = -0.44, P < 0.001$) and SDH activity ($r = -0.54, P < 0.001$) in Lewis rats (pooled data from ADX + 50-, 100-, and 200-mg groups). In Fischer rats of >100 ng/ml plasma corticosterone concentrations (ADX + 100 mg and ADX + 200 mg groups), a strong negative correlation was also evidenced (COX: $r = -0.91, P = 0.0005$; SDH: $r = -0.85, P = 0.003$).

In the absence of or in low plasma corticosterone levels (ADX + 0 and ADX + 12 mg), COX and SDH activities were decreased in Fischer (Table 1 and Figs. 3A and 4A) but not in Lewis rats (Table 1 and Figs. 3B and 4B). Finally, no correlation was found between COX/SDH ratio and plasma corticosterone in either strain (Fischer: $r = -0.40, P = 0.07$; Lewis: $r = 0.022, P = 0.92$). These results suggest that, in Fischer and in Lewis rats, plasma corticosterone at physiological ranges (in low to high concentrations) are correlated with significant changes in the amount of functional mitochondria in plantaris muscle.
Because the biochemical data showed a partial deficiency of enzyme complexes containing subunits encoded by mtDNA, we examined mtDNA for rearrangements and determined mtDNA levels by Southern blot hybridization only for the subgroups with high corticosterone plasma concentrations (ADX/H11001 50, 100, and 200 mg; Fig. 5). The rat muscle contained mtDNA of the same size as sham-operated but the mtDNA signal relative to the nuclear DNA signal was, in the cases of Lewis ADX/H11001 50, ADX/H11001 100, and ADX/H11001 200 mg rats, respectively, 89, 74, and 42% of the mean sham-operated values (Fig. 5). For Fischer rats, the relative mtDNA signals were 89, 94, and 33% (for ADX/H11001 50, ADX/H11001 100, and ADX/H11001 200 mg rats, respectively) compared with sham-operated rats (Fig. 5).

**DISCUSSION**

**Methodological Considerations**

In the present experiments, the rats were adrenalectomized and implanted with subcutaneous pellets of various concentrations of corticosterone, the rat natural GC. The advantage of the pellet method includes the establishment of constant corticosterone concentrations with no evidence of circadian variation (24). Therefore, in our experimental conditions, the only difference between experimental groups was the plasma corticosterone concentrations. One criticism of this model may be that it induces a constant 24-h plasma corticosterone concentration, which does not mimic the nycthemeral rhythm of plasma corticosterone concentration. On the other hand, this absence of nycthemeral rhythm is similar to pathological conditions such as Cushing’s syndrome or, more interestingly, exogenous corticosteroid treatment.

Data of plasma corticosterone concentrations, body weight gain, and thymus weight obtained in the different experimental groups show that, in Lewis rats, the levels of corticosterone achieved by the 12-mg corticosterone pellets were in the low physiological range, whereas the corticosterone levels obtained after the implantation of the 50-, 100-, and 200-mg pellets were in the high physiological range. This is also true for Fischer rats, albeit less marked for the 50-mg pellet. It is important to note that the plasma corticosterone values of the ADX + 200-mg pellets remained in the range of plasma corticosterone observed in chronically stressed rats (9), suggesting that the range of corticosterone concentrations achieved in the present experiment represents reference values ranging from low HPA activity to high (stress-induced) HPA axis activity.

A strain difference between plasma corticosterone concentrations obtained with the same corticosterone pellets was also depicted (Table 1). To our knowledge, this is the first description of such a difference between Lewis and Fischer rats. This suggests a strain difference in absorption and/or metabolism of corticosterone.

**Tissue Sensitivity to Corticosterone**

**Mitochondrial metabolism.** We evaluated the amount of functional mitochondria in cells (16, 17) through the normalization of COX activity by SDH. This allowed us to distinguish two types of adaptation mechanisms: 1) a variation in the amount of functional mitochondria (in this case, the COX/SDH ratio remains constant) and 2) a direct effect on the COX kinetic parameters (modification of the COX/SDH ratio while SDH remains constant) (16, 17).

The present results provide evidence for the first time that corticosterone at physiological concentrations (25–210 ng/ml) is involved in the regulation of muscle mitochondrial function.
Compared with ADX + 12 mg and ADX + 50 mg rats, corticosterone concentrations in the range of chronic stress-induced levels (ADX + 200 mg corticosterone) are associated with a lower amount of functional muscle mitochondria in both Lewis and Fischer rats. In addition, we also report deleterious effects of the absence of GC on muscle mitochondrial metabolism (ADX + 0 mg group). In Fischer rats, ADX induces a huge decrease in COX and SDH activities, indicating a strong dependence of mitochondrial function on corticosterone levels, the lack of corticosterone having the same effect as high corticosterone levels on SDH levels (Fig. 4A). In the Lewis rat, ADX induces a weak decrease in SDH activity with no variation in COX activity. Therefore, we postulate that corticosterone is necessary for muscle mitochondrial metabolism exerting its effects with an inverted U curve, whereby too little (Fischer only) or too much corticosterone (Fischer and Lewis) is deleterious to muscle mitochondria metabolism. These dual actions of corticosterone have also been reported for body weight gain and feeding efficiency (6) as well as for cognitive process (19).

In a previous study (9), we demonstrated that chronic stress-induced (novel environment or forced exercise for 8 wk) increases in endogenous corticosterone concentrations were negatively correlated with functional muscle mitochondrial amount. These previous results are confirmed by the present study, wherein the only manipulated variable was the concentration of corticosterone delivered by the pellet borne by rats of the different experimental groups. Compared with sham-operated rats, corticosterone concentrations in the range of chronic stress-induced levels (ADX + 200 mg corticosterone) are associated with lower amounts of functional muscle mitochondria in both Lewis and Fischer rats. Moreover, in Lewis rats, we report a linear regression curve between high plasma corticosterone concentrations (≥50 ng/ml) and functional muscle mitochondria amount; the higher the corticosterone levels the lower the muscle functional mitochondria amount (Figs. 3 and 4). Although such correlations do not necessarily indicate a direct effect of the change in corticosterone concentration on mitochondrial function, they do suggest that plasma corticosterone and functional muscle mitochondrial amount are related. These results were confirmed by the direct measure of the mitochondrial DNA by Southern Blot analysis (Fig. 5).

These results are in agreement with earlier reports on the in vitro effects of high synthetic GC (dexamethasone, triamcinolone, methylprednisolone, etc.) concentrations on mitochondrial oxidative phosphorylation (ATP production) (21, 22, 35). In vivo, defects in mitochondria function have been involved in GC-induced myopathy (21, 22, 35). With the administration of high doses of GC in rat, electron microscopic and biochemical studies have demonstrated enlarged mitochondrial volume, decreased numbers of mitochondria, decreased substrate utilization, and uncoupling of oxidative phosphorylation (21).

Our findings are in disagreement with those of Weber et al. (36), who investigated the in vivo and in vitro effects of dexamethasone on mitochondrial biogenesis in rats over a period of 3 days. They showed that high levels of GC stimulate mitochondrial biogenesis in skeletal muscle. Nevertheless, their results are difficult to compare with ours because of differences in the GC used [dexamethasone vs. the rat’s natural GC, corticosterone (36), vs. present study], the amounts of GC [higher dexamethasone doses than those used in GC therapy (15) vs. plasma corticosterone levels of chronically stressed rats (1)], and the duration of GC treatment (3 vs. 12 days).

One mechanism for the relationship reported in the present experiment between corticosterone and muscle mitochondrial biogenesis could be the presence of GC receptor (GR) acting via hormone response elements (HRE) in muscle mitochondria. In liver mitochondria (34) and in several cell types (31), the GC receptor has been detected, and its interactions with putative mitochondrial HRE have been demonstrated (34). However, the action of GR on mitochondrial gene expression remains to be determined.

The present study also demonstrates that the absence of corticosterone (ADX + 0 mg group) also induces a deleterious effect on mitochondrial function in Fischer rats. GC have been reported to be essential for the postnatal rise in mitochondrial enzymes in the developing rat kidney (8). However, to our knowledge, there is no report on the effects of the absence of corticosterone on muscle-functional mitochondria in adult rats. In Fischer rats, ADX induces a large decrease in COX and SDH activities with a further decrease in COX activity in ADX + 200 mg group. In Lewis rats, ADX induced no variation in COX or SDH activities (ADX + 0 mg group), whereas in the ADX + 200 mg group the decrease in muscle mitochondrial content was associated with both decreased COX and decreased SDH activities. This suggests that mitochondrial function is impaired probably by two different mechanisms: a direct action on enzymatic activity and a decreased number of functional mitochondria. A possible answer may lie in corticosterone’s dual receptor population; the two glucocorticoid receptors (GR and MR) may instigate differential mitochondrial actions, with MR-mediated effects on low GC concentrations and GR-mediated effects on high GC concentrations (6, 29).

Interstrain Differences

The second purpose of this study was to examine the effects of similar concentrations of corticosterone on target tissues in Lewis and Fischer rats. We found that tissue sensitivity to GC was higher in Lewis than in Fischer rats. Indeed, identical corticosterone concentrations induce a more pronounced thymus decrease in Lewis than in Fischer rats (Fig. 2B). This is the first report of such a strain difference in tissue sensitivity to corticosterone. This point is also exemplified by the data on decrement of body weight, offered in support of the greater sensitivity of Lewis rats to corticosterone. We also report a higher muscle mitochondrial sensitivity to high GC levels between Fischer and Lewis rats (Figs. 3 and 4). In sham-operated rats, SDH and COX activities were significantly higher in Lewis compared with Fischer rats (similar effects were obtained with citrate synthase activity; data not shown). In contrast, their COX/SDH ratios were similar. This suggests that the mitochondria are similar between these two strains of rats and that Lewis rats have more mitochondria in their plantaris muscle than Fischer rats. These results are in agreement with the interstrain relationship between plasma corticosterone concentration and muscle mitochondria functional amount, as in Fischer rats the range in plasma corticosterone levels that occurs during a 24-h period is significantly higher than in the Lewis rat (12).
In contrast, Karalis et al. (14) have not reported differences in tissue sensitivity to GC between Fischer and Lewis rats. Possible explanations might be differences in the age of the rats (6 wk vs. 6 mo, present study vs. Ref. 14), the type of GC used (corticosterone vs. dexamethasone) and its mode of administration (pellet vs. intraperitoneal injection, the latter being responsible for a stress effect), and the duration of the treatment (7 vs. 12 days). Another potential consideration concerns the role of corticosterone-binding globulin (CBG). In plasma, >90% of corticosterone is bound to CBG, and only the free fraction of the steroid represents the biologically active form of corticosterone (37). In contrast, dexamethasone is almost exclusively bound to albumin in plasma (28). It has been shown that Fischer rats displayed higher CBG levels in plasma, thymus, and spleen, resulting in less free and bioactive corticosterone (7). This could explain on the one hand the lesser effectiveness of similar corticosterone levels in Fischer compared with Lewis rats in the present study and, moreover, the similar sensitivity to dexamethasone in the study of Karalis et al. (14). Whether such a strain difference is directly linked to similar sensitivity to dexamethasone in the study of Karalis et al. (14) has not been reported differences in tissue sensitivity to GC between Fischer and Lewis rats. Moreover, we report that mitochondria are another corticosteroid-sensitive physiological target, as physiological corticosterone concentrations from low to high levels influence muscle functional mitochondria amount. This new track has to be considered when studying the pathologies induced by GC and, notably, the molecular mechanisms linking corticosterone and the muscle functional mitochondrial amount.

REFERENCES


