Effects of Perinatal High Dose Dexamethasone on Skeletal Muscle Development in Rats

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ABSTRACT

Five litters of suckling rats were given either dexamethasone (DEX), 1 mg/kg, subcutaneously, three times daily (n=4/litter) or vehicle control (n=4/litter) from day 3 through day 7 after birth. Rats were weighed weekly and were weaned on day 30. On day 60, rats were killed and the soleus (SOL) and extensor digitorum longus (EDL) were removed for the following analyses: 1) wet weight, 2) light microscopic examination of hematoxylin and eosin stained transverse sections, 3) quantitative morphometric analysis of myosin ATPase stained transverse sections (fiber numbers, fiber type percentages and mean fiber diameters), and 4) DNA (total and mg/g wet weight). The following parameters were significantly reduced in treated rats: 1) body weight, 2) wet weight of SOL and EDL, and 3) mean diameter of SOL type I fibers. There was a trend for total DNA of SOL and EDL to be decreased in treated rats but this was not statistically significant.

In a second experiment, pregnant rats (n=4) were given DEX, 1 mg/kg, subcutaneously, twice daily, on days 17 and 18 of gestation. Two rats served as vehicle controls. The prenatally DEX-exposed rats weighed significantly less on weeks 3, 4, 6, 7 and 8. There were significant reductions in the following parameters for treated rats: 1) SOL wet weight, and 2) total number of SOL type I fibers. There was a trend for SOL DNA to be reduced but this was not statistically significant.

RÉSUMÉ

Cette expérience impliquait cinq portées de ratons nouveau-nés qui en comptaient chacune huit. Du troisième au septième jour après leur naissance, quatre sujets de chacune des portées reçurent, trois fois par jour, une injection sous-cutanée de dexaméthasone, à raison de 1 mg/kg, alors que les quatre autres ne reçurent, de la même façon, que l'excipient. On pesa hebdomadairement les ratons et on les sevra à l'âge de 30 jours. On les sacrifia, 30 jours plus tard, et on en disséqua les muscles *soleus* et *extensor digitorum longus*, pour les soumettre ensuite aux analyses suivantes: 1-leur poids à l'état frais; 2-l'examen de coupes transversalles, colorées à l'hématoxyline et à l'éosine, au microscope photonique; 3-l'analyse morphométrique quantitative de sections transverses, colorées par l'adénosine-triphosphatase; cette analyse incluait le nombre de fibres, le pourcentage des types de fibres et leur diamètre; 4-la détermination de l'acide désoxyribonucléique, total et en mg/g de tissu musculaire frais. Les paramètres suivants affichèrent une réduction significative, chez les ratons qui avaient reçu de la dexaméthasone: 1-le poids corporel; 2-le poids du *soleus* et de *l'extensor digitorum longus*; 3-le diamètre moyen des fibres du type I du *soleus*. L'acide désoxyribonucléique total des muscles précités afficha une tendance à une diminution non significative, du point de vue statistique, chez les ratons qui avaient reçu de la dexaméthasone.

Dans une autre expérience, quatre rates reçurent, deux fois par jour, aux 17e et 18e jours de leur gestation, une injection sous-cutanée de dexaméthasone, à raison de 1 mg/kg, tandis que deux autres rates ne reçurent, de la même façon, que l'excipient. Les ratons ainsi exposés à la dexaméthasone, avant leur naissance, affichèrent un poids significativement moins élevé, à l'âge de trois, quatre, six, sept et huit semaines. Ils présentèrent aussi une réduction significative, relativement aux paramètres suivants: 1-le poids du *soleus*, à l'état frais; 2-le nombre total de fibres du type I de ce muscle. L'acide désoxyribonucléique total de ce muscle afficha aussi une tendance vers une baisse non significative, du point du vue statistique.

INTRODUCTION

High concentrations of dexamethasone (DEX) alter skeletal muscle growth *in vitro*. Myoblast proliferation, myotube formation and beta-adrenergic responsiveness are inhibited (1,2). A deleterious effect of DEX on skeletal muscle regeneration has also been suggested (3). From these reports, one might speculate that high concentrations of DEX could have a negative effect on myogenesis. Indeed, Jirmanova (4) found acute muscle necrosis after corticosteroid administration to newborn rabbits. These findings are pertinent to situations in which neonatal animals or humans are treated with corticosteroids. There are potential implications

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TABLE I. Results from a Fast-twitch Muscle (Extensor Digitorum Longus) and Slow-twitch Muscle (Soleus) of 60 Day Old Rats Given Dexamethasone, 1 mg/kg, Three Times Daily, from Days 3 to 7 After Parturition

	Extensor Digitorum Longus		Percentage	Soleus		Percentage
	Control	Treated	Difference	Control	Treated	Difference
Wet weight (mg)	178 ± 26[a]	154 ± 12	-13[b]	132 ± 27	104 ± 14	-21[b]
$\frac{\text{Wet weight (g)}}{\text{Body weight (g)}} \times 100$	0.051 ± 0.004	0.054 ± 0.004	+6[c]	0.038 ± 0.004	0.036 ± 0.004	-5
DNA (mg/g wet wt)	0.65 ± 0.14	0.61 ± 0.10	-6	0.79 ± 0.14	0.79 ± 0.18	0
DNA, total (μg)	114.46 ± 31.48	91.79 ± 18.27	-20	103.47 ± 32.05	83.14 ± 27.42	-20

[a]Mean ± SD
[b]$p < 0.001$
[c]$p < 0.05$
n = 10-15/group

for babies who have been exposed to DEX in the course of treatment of respiratory distress syndrome (5). The purpose of the present study was to search for longer-term (60 day) effects of pharmacological doses of DEX on fast-twitch muscle (extensor digitorum longus, EDL) and slow-twitch muscle (soleus, SOL). Rats received DEX parenterally during the first week after birth (experiment 1) or prenatally, by administration to pregnant females during late gestation (experiment 2).

MATERIALS AND METHODS

EXPERIMENT 1 (POSTNATAL DEX ADMINISTRATION)

Five timed pregnant Sprague-Dawley rats (VAF+, specific pathogen free) were obtained from Charles Rivers Laboratories (Raleigh, North Carolina). Rats were housed individually in shoe box cages and were fed water and pellets (Lab Chow, Purina Mills Inc., St. Louis, Missouri) *ad libitum*. Two days after parturition, litters were culled to eight males per litter, unless females were needed to maintain a litter size of eight. Data are reported for males only. At that time, all rats were randomly marked by amputating the fifth digit of either the left (four controls/litter) or right (four treated rats/litter) hind foot with sterile scissors. Treatment with DEX (Azium, Schering Corp., Bloomfield, New Jersey) diluted to 0.2 mg/kg with vehicle started on day 3, at 1 mg/kg, subcutaneously, three times daily for five days. Controls were injected with equal volumes of vehicle solution.

On the day following the last injection, one control and one treated rat were randomly chosen from each litter, and were killed with intraperitoneal (IP) T61 (Hoechst Corp., Somerville, New Jersey) for general necropsy. Necropsy included gross and histological examination of the lungs, liver, spleen, adrenal glands, trachea, kidneys, intestines, heart, thymus, thyroids, reproductive tract, urinary bladder, stifle joint and bone marrow.

Starting on day 7, the remaining rats were weighed weekly. Rats were weaned at 30 days of age and then housed singly in suspended wire cages. They were killed at 60 days of age, with T61, IP. Immediately afterward, the EDL and SOL muscles were excised from the left side, weighed, placed in gauze moistened in physiological saline, held at 4°C for 2 h, frozen in isopentane cooled in liquid nitrogen and stored at -80°C for histochemistry (3). From the right side, the EDL and SOL were removed, weighed, frozen in liquid nitrogen and stored at -80°C for DNA assay. Whole brains also were removed and weighed.

Transverse serial sections (8 μm) were cut at the mid-point of the muscles with a cryostat microtome at -20°C and stained with hematoxylin and eosin (H & E) and myosin ATPase, pH 4.3 (6,7). The H & E slides were examined for evidence of pathological changes. For quantitative analysis, ATPase slides were projected onto a monitor using a computerized video-display image analysis system (Optomax semi-automatic image analysis system, Optomax Inc., Hollis, New Hampshire). The mean lesser fiber diameters of types I and II fibers were calculated at a magnification of 700x. Where more than 200 fibers of a given type were available, fibers were randomly selected from across the section. To determine fiber type percentages, all type I and II fibers were counted. Their sum represents the total fiber count.

Deoxyribonucleic acid determination was performed according to the method described by Martin *et al* (8). Absorbance was read at 595 nm.

EXPERIMENT 2 (PRENATAL DEX ADMINISTRATION)

Six timed pregnant rats were given either DEX, 1 mg/kg, subcutaneously, twice daily (n=4), or vehicle control (n=2) for two days. Injections started 17 days postbreeding. Females were kept to maintain a litter size of eight, but results were calculated for males only. Other procedures were the same as in experiment 1.

STATISTICAL ANALYSIS

Differences between the mean values for body weight, and the various muscle parameters were tested for significance at the 0.05 level using the general linear model of SAS (GLM) for analysis of variance (ANOVA) and Scheffe's method of multiple comparisons (9). Student's *t*-test was used to evaluate differences between treatment and control groups at each week.

RESULTS

EXPERIMENT 1

Treated rats were visibly smaller than controls by the third day of DEX injections. Treated rats maintained significantly decreased body weights ($p < 0.05$) throughout the experiment (Fig. 1). However, the mean body

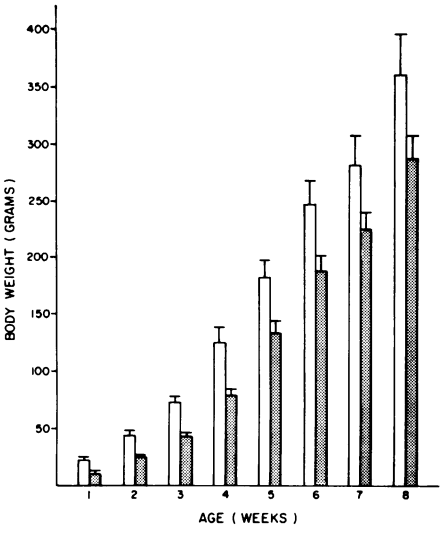

Fig. 1. Body weights (mean ± SD) for control (open bars) and treated (dark bars) rats given dexamethasone, 1 mg/kg, three times daily, from days 3 to 7 after parturition. Treated rats weighed significantly less on all weeks ($p < 0.05$).

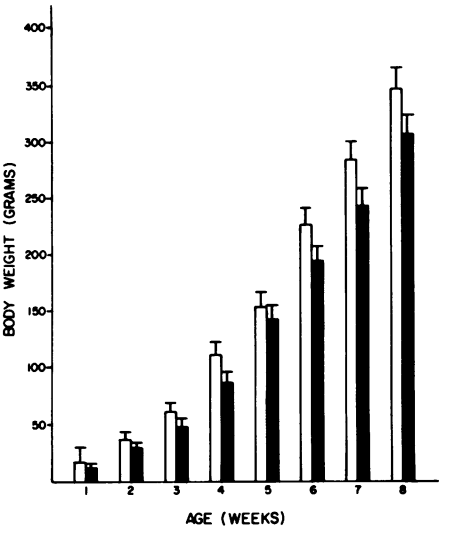

Fig. 2. Body weights (mean ± SD) for control (open bars) and treated (dark bars) rats exposed to dexamethasone during late gestation, by administration to dams at 1 mg/kg, twice daily, for two days, starting at day 17 postbreeding. Treated rats weighed significantly less on weeks 3, 4, 6, 7 and 8 ($p < 0.05$).

weight of treated rats compared to controls rose from 48% (week 1) to 87% (week 8).

The control and treated rats which were necropsied on the day after the last DEX injection had no histopathological evidence of infectious disease.

Mean wet weights of the SOL and EDL were decreased ($p < 0.001$) in treated rats at day 60 (Table I). Since there was no significant difference between the mean wet weights of the muscles from the left and right legs ($p > 0.05$, paired *t*-test), values of both sides were pooled. The mean value for the ratio of the muscle wet weight:body weight was significantly higher in treated rats for the EDL ($p < 0.05$) but not the SOL (Table I). Light microscopic examination of these muscles revealed no substantial pathological changes. The mean diameter of SOL type I fibers was diminished in treated rats ($p < 0.05$). There were no other significant differences in the morphometric analysis (Table II). There was a trend for total DNA in SOL and EDL to be decreased in treated rats (80% of control, for both muscles), but this was not statistically significant.

Brain weights (mean ± SD) were 2.027 ± 0.051 g in controls and 1.737 ± 0.086 g in treated rats; values for the treated group were significantly less ($p < 0.0001$). However, the ratio of brain weight:body weight was similar for controls (0.606 ± 0.115, mean ± SD) and treated rats (0.604 ± 0.035) ($p > 0.05$).

EXPERIMENT 2

Control litters were born on days 20 and 21 postbreeding; treated litters were born on days 23 and 24. Some rats in the DEX-treated litters were stillborn, while others died within 24 h after parturition. Survival rates for the four treated litters were 8/12, 0/6, 10/11 and 8/9. Preliminary studies indicated that DEX injections for more than two days, or more frequently than twice daily were associated with high mortality in the litters. The mean body weight of treated rats rose from 71% of control values at week 1 to 88% of controls at week 8 (Fig. 2). The mean body weight was significantly less in the treated rats on weeks 3, 4, 6, 7 and 8 ($p < 0.05$).

At day 60, the mean wet weight of the SOL but not EDL was decreased ($p < 0.005$) in treated rats compared to controls (Table III). Values for left and right sides were pooled since they were not significantly different ($p > 0.05$, paired *t*-test). The mean value for the ratio of the muscle wet weight:body weight was significantly higher in treated rats for the EDL ($p < 0.0001$) but not SOL. Light microscopic examination indicated no significant muscle pathology in any rats. The mean of the total number of type I fibers of SOL muscles was decreased in DEX-treated rats ($p < 0.05$). No other significant differences were found in the morphometric analyses (Table IV). Deoxyribonucleic acid (mg/g wet weight) and total

TABLE II. Morphometric Analysis of a Fast-twitch (Extensor Digitorum Longus) and Slow-twitch Muscle (Soleus) of Rats Given Dexamethasone, 1 mg/kg, Three Times Daily, from Days 3 to 7 After Parturition

	Fiber Number			Percentage		Fiber Diameter		
	Control	Treated	Percentage Difference	Control	Treated	Control	Treated	Percentage Difference
Extensor digitorum longus								
Fiber type I	122 ± 36[a]	64 ± 22	-48	3.8	2.6	26.2 ± 2.7	26.0 ± 3.0	-1
II A and B	3079 ± 366	2358 ± 365	-23	95.5	96.2	37.1 ± 3.3	35.8 ± 4.0	-4
II C	23 ± 9	30 ± 25	+30	0.7	1.2	27.2 ± 3.1	26.9 ± 3.5	-1
Soleus								
I	2089 ± 456	2468 ± 316	+18	96.2	97.1	45.1 ± 4.1	38.4 ± 2.5	-15[b]
II A	73 ± 93	45 ± 52	-38	3.3	2.0	46.4 ± 4.6	40.2 ± 4.8	-13
II C	11 ± 11	23 ± 19	+109	0.5	0.9	39.9 ± 6.0	36.9 ± 6.6	-8

[a] Mean ± SD
[b] $p < 0.05$

TABLE III. Results in 60 Day Old Rats Exposed to Dexamethasone During Late Gestation

	Extensor Digitorum Longus			Soleus		
	Control	Treated	Percentage Difference	Control	Treated	Percentage Difference
Wet weight (mg)	180 ± 16[a]	185 ± 14	+3	141 ± 15	128 ± 14	-9[b]
$\frac{\text{Wet weight (g)}}{\text{Body weight (g)}} \times 100$	0.052 ± 0.005	0.061 ± 0.004	+17[c]	0.041 ± 0.004	0.042 ± 0.004	+2
DNA (mg/g wet wt)	0.43 ± 0.10	0.43 ± 0.06	0	0.83 ± 0.10	0.71 ± 0.09	-14
DNA, total (μg)	78.88 ± 25.38	81.11 ± 5.90	+3	111.95 ± 10.34	90.41 ± 27.82	-19

[a]Mean ± SD
[b]$p < 0.005$
[c]$p < 0.0001$
n = 5-10/group

DNA were decreased in treated SOL muscle (86% and 81% of control, respectively), but this decrease was not statistically significant.

DISCUSSION

The consequences of undernutrition in rats during gestation and early life can be compared to the results of the present study, especially since undernutrition is associated with increased glucocorticoid levels (10). Perinatal undernutrition has been reported to cause persistent decreases in: 1) EDL and SOL wet weight and DNA content (11), 2) areas of all EDL fiber types plus a decreased percentage of type I fibers (12) and 3) EDL and SOL total fiber numbers, fiber areas (except EDL red fibers), body and muscle weights (13). Analysis of the ratio of muscle wet weight to body weight has suggested that muscle growth is compromised less by undernutrition than some other tissues (14). However, different susceptibilities may exist for fast and slow muscles. In one study, the EDL:body weight ratio remained permanently low whereas the SOL:body weight ratio was not different from controls (13). In this study, the SOL:body weight ratio in treated rats was not different from controls, whereas the EDL:body weight ratio was significantly higher. This suggests that the growth of other organ systems may be more affected by DEX than muscle, and that the growth of EDL is less susceptible to DEX than the growth of SOL.

Directly comparing the results of studies on dietary restriction to studies on glucocorticoid administration is difficult because of differences in timing, species, muscles examined and analytic methods (13). Nevertheless, the general picture that emerges from studies on early undernutrition, and which appears to be applicable to the effects of early DEX exposure also, is that skeletal muscle has a period in its development when it is especially vulnerable. In the adult, undernutrition and corticosteroids primarily affect muscle by decreasing fiber size but this is reversible, unlike the effects of early exposure.

Postnatal DNA accumulation, which is due to satellite cell proliferation, is a factor that controls subsequent muscle growth (15). Muscle fiber diameter in growing animals is directly related to the number of muscle fiber nuclei (15). Satellite cells have been shown to degenerate in response to DEX given to rabbits during the first week postpartum (4). Since SOL contains more satellite cells on a per milligram basis than does EDL (16), one would expect the growth of SOL to be more susceptible than EDL to DEX. The results of the present study are consistent with this hypothesis. Similarly, undernutrition in rats has been shown to cause a decrease in muscle DNA accretion which ultimately limits muscle growth (14,15).

The effects of corticosteroids are much better known for mature than growing animals. Steroid myopathies in adults are well documented. One experiment in adult rats indicated that DEX caused atrophy of type II fibers in SOL and EDL plus necrosis of SOL type I fibers (17). Most reports support the concept that type I fibers are resistant to corticosteroid-induced atrophy (4). For example, administration of cortisone for five days did not

TABLE IV. Morphometric Analysis in 60 Day Old Rats Exposed to Dexamethasone During Late Gestation

	Fiber Number			Percentage		Fiber Diameter		
	Control	Treated	Percentage Difference	Control	Treated	Control	Treated	Percentage Difference
Extensor digitorum longus								
Fiber type I	107 ± 37[a]	165 ± 57	+54	3.6	6.6	26.4 ± 1.0	26.4 ± 1.4	0
II A and B	2823 ± 645	2342 ± 267	-17	96.2	93.2	36.8 ± 3.4	38.4 ± 1.4	+4
II C	6 ± 4	5 ± 5	-17	0.2	0.2	26.2 ± 1.7	26.6 ± 1.6	+2
Soleus								
I	2468 ± 393	1751 ± 449	-29[b]	91.6	85.1	42.5 ± 1.5	44.8 ± 2.8	+5
II A	180 ± 94	258 ± 148	+43	6.7	12.5	43.1 ± 3.2	42.8 ± 2.3	-1
II C	47 ± 30	50 ± 60	+ 6	1.7	2.4	37.7 ± 2.0	38.2 ± 6.4	+1

[a]Mean ± SD
[b]$p < 0.05$

affect SOL weights in one study (18), and DEX caused atrophy of the EDL but not SOL in another study (19). In growing rats, DEX caused atrophy of fast-twitch muscle and decreased protein accumulation in slow-twitch muscle (20).

Few quantitative histological studies have been done on the musculature of animals given DEX or other corticosteroids in early life. In one published report on the effects of corticosteroids on muscle growth *in vivo*, in rabbits injected from days 1 to 9 after birth, the *biceps femoris* muscle had decreased fiber diameters, fiber degeneration and satellite cell degeneration (4). However, fiber types were not distinguished, and the long-term effects of corticosteroid administration were not studied.

In this study, SOL type I fibers showed significant growth inhibition. Following postnatal DEX administration, mean SOL type I fiber diameters were smaller; after prenatal DEX, the total number of SOL type I fibers was decreased. These findings from studies using pharmacological doses of corticosteroids can be compared to *in vitro* effects of relatively high concentrations of DEX: inhibition of myoblast fusion and beta-adrenergic responsiveness, and inhibition of myoblast differentiation and suppression of cell proliferation (8,22). It should be remembered, however, that the pre- and postnatal DEX exposures were not the same with respect to daily dosage and number of days administered, so that comparisons between prenatal and postnatal exposure need to be made with caution.

As well as muscle weights, body and brain weights are decreased in DEX-treated animals (21). Dexamethasone given on day 7 after birth, at 1 mg/kg, produced a transient decrease in body weight, which returned to normal within two to three months (21,22). In this study, the administration of DEX on either schedule was associated with persistent decreases in mean body weight and mean EDL and SOL wet weights. Early undernutrition has also been associated with irreversible decreases in body weight (11,13). Mice exposed to DEX for several days during the first week after birth had poor cortical dendritic development (23). The development and differentiation of muscle fiber types depends on the nervous system, which is affected by early administration of DEX. Whether muscle is only directly affected by DEX or whether indirect effects including hormonal factors are also important, remains to be determined.

The fiber type proportions found in this study are similar to those reported for the rat by Bedi *et al* (13) of 98.5% fast-twitch in EDL and 93% type I and 7% type IIA in SOL, which does not contain IIB fibers. Estimates of muscle fiber composition can vary depending on the laboratory and staining techniques (24). With myosin ATPase pH 4.3, type I fibers stain darkly, IIA and B fibers both stain lightly, and IIC fibers are of intermediate staining intensity.

Similar fiber diameters have been found for the EDL and SOL of rats of comparable age (19). Comparable total fiber numbers for these two muscles, based on analysis of mid-longitudinal transverse sections, have been reported (13,14). A transverse section at the mid-belly of the SOL muscle, in the age group studied, should include all fibers (25). However, a technique using nitric acid fixation has been recommended for determining total fiber number (26,27). With that technique, the average total fiber number in the left SOL and EDL was 2,926 and 5,473, respectively (26).

In conclusion, it was found that exposure to pharmacological doses of DEX for several days during the perinatal period was associated with growth inhibition in rats. The development of the SOL muscle seemed more susceptible than the EDL to DEX. The administration of DEX during the perinatal period needs to be considered cautiously in light of these findings.

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