Effects of glucocorticoid treatment on excitation-contraction coupling

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LASZEWSKI, BARBARA, AND ROBERT L. RUFF. *Effects of glucocorticoid treatment on excitation-contraction coupling.* Am. J. Physiol. 248 (Endocrinol. Metab. 11): E363–E369, 1985.— We studied the myofibrillar calcium affinity and mechanical threshold of single muscle fibers from the extensor digitorum longus (EDL) and soleus muscles of male Sprague-Dawley rats treated with daily intramuscular injections of dexamethasone, 1.5 mg/kg, for 14 days. The strength-duration and pCa-tension relationships of EDL fibers were not altered by glucocorticoid treatment. However, in soleus fibers the mechanical threshold was increased and the myofibrillar calcium sensitivity was reduced by dexamethasone treatment. Glucocorticoid treatment did not change the maximum tension per cross-sectional area of single-skinned EDL or soleus fibers. The glucocorticoid-induced atrophy and impaired force-generating capacity of fast-twitch muscle are probably not caused by impaired excitation-contraction coupling.

rat; steroid myopathy; mammalian muscle; skinned fibers; strength duration; mechanical threshold

PROLONGED GLUCOCORTICOID treatment produces severe muscle wasting and weakness primarily involving fast-twitch muscles (10). The mechanism by which glucocorticoids produce muscle weakness is not yet known. Neuromuscular transmission (11) and surface membrane excitability (25, 26) are not impaired by glucocorticoid treatment. However, the weakness could reflect alterations in the contractile proteins or excitation-contraction coupling. Shoji et al. (27) suggested that the catabolic effects of glucocorticoids could impair the function of the sarcoplasmic reticulum. These investigators reported that isolated sarcoplasmic reticulum fragments from fast-twitch muscle of rabbits treated with triamcinolone (10 mg/kg for 14 days) and from the muscle of one patient with steroid myopathy (drug not indicated) had decreased calcium accumulation capacity and diminished calcium-stimulated ATPase activity. In contrast, Peter et al. (22) found that triamcinolone treatment (5 mg/kg for 10 days) did not alter the calcium accumulation capacity, calcium-stimulated ATPase activity, or calcium affinity of rat muscle fragmented sarcoplasmic reticulum. Shoji et al. (27) suggested that the animals studied by Peter et al. (22) were not treated long enough to develop changes in excitation-contraction coupling and weakness.

We studied the effects of glucocorticoid treatment on excitation-contraction coupling and force development by studying the myofibrillar calcium affinity and mechanical threshold of fast- and slow-twitch rat muscle fibers.

METHODS

Treatment of animals. Male Sprague-Dawley rats weighing 290–310 g received daily intramuscular injections of dexamethasone 1.5 mg/kg or an equivalent volume of physiological saline for 14 days. The techniques for injection, killing of the animals, and removal of the muscles were previously described (25). In our laboratory this dose and duration of treatment was associated with a 20% reduction in muscle weight and 17% reduction in the tetanic tension of the fast-twitch extensor digitorum longus (EDL) muscle (25) but not with diminished food intake (25), impaired sarcolemmal excitability (25, 26), or reduced animal activity (R. L. Ruff, unpublished data).

Myofibrillar calcium sensitivity. The fast-twitch EDL and slow-twitch soleus muscles were chemically skinned according to the technique of Wood et al. (33). The muscles were kept in refrigerated skinning solution for 1 to 2 days.

To study calcium sensitivity, a 4- to 5-mm portion of a single-skinned fiber was dissected free in silicone oil (Dow Corning 200, viscosity 10 centistokes, Midland, MI), and each end was wrapped around a fine stainless steel hook that had been slightly abraided to prevent fiber slippage (1). One hook was connected to a Hellam-Pododsky type tension transducer (14). The other hook was attached to a micrometer drive so that fiber length could be adjusted. The fiber was then lowered into a well containing relaxing solution (pCa 8). The sarcomere length was measured, based on the diffraction pattern produced by a 5 mW He/Ne laser (Aerotech, Pittsburgh, PA) beam directed perpendicular to the long axis of the fiber and set at 2.7–2.8 μm. This length was chosen because it is an optimal length for tension generation (3, 30). In some experiments the laser was not available and the fiber length was set at the length where passive tension began to increase steeply as the fiber was slowly stretched. In 17 EDL or soleus fibers from dexamethasone-treated or control rats the increase in passive tension occurred at a sarcomere spacing of 2.7–2.8 μm. The fiber was viewed through a dissecting microscope and the fiber diameter was measured by an ocular micrometer

with 2 μm resolution. The fiber was then immersed in bathing solutions with progressively increasing calcium concentrations (pCa 7–pCa 4) to determine the pCa-tension relationship. The wells containing the bathing solutions were covered with a thin layer of silicone oil to prevent evaporation. Several measurements of the pCa-tension relationship were made for each fiber. The values from the second and later runs were averaged to yield a pCa-tension relationship for a given fiber. The skinned fibers were studied at 23 ± 1°C.

The composition of the relaxing and bathing solutions were: 138 mM K; 15 mM creatine phosphate; 20 U/ml creatine kinase; 5 mM Mg ATP; 20 mM EGTA; >15 mM morpholinopropane sulfonic acid (MOPS); 0.1 or 1 mM free Mg; and free Ca from 10 nM to 0.1 mM. The ionic strength was set at 210 mM by varying the amount of the pH buffer. The composition of the skinning solution was (in mM): 138 K; 15 creatine phosphate; 5 Mg ATP; 2.5 free Mg; 5 EGTA; and 9.8 MOPS. The ionic strength was 189 mM; and the free calcium concentration was <10 nM. For both the bathing and skinning solutions the major anion was propionate, and pH was adjusted to 7.0. The free calcium concentration of the solutions was calculated by using constants for complex ion formation (7, 13, 17, 21) with total calcium verified by atomic absorption spectroscopy. The binding constants of EGTA with hydronium ions (7) and calcium and magnesium ions (13) were adjusted for the ionic strength. The pH was converted to hydronium ion concentration using the relationship of the activity coefficient of HCl to ionic strength and temperature as described by Khoo et al. (16). The stock solutions were assayed according to the following procedures. The concentration of cations in stock solutions were measured by atomic absorption spectroscopy. The concentration of the K_2 EGTA stock solution was determined by titration of the stock solution with $CaCl_2$ using a calcium-sensitive electrode to measure the unbound calcium and atomic absorption spectroscopy to determine the total calcium content. By assaying the EGTA stock solution we avoided the uncertainty associated the variable purity of EGTA from commercial suppliers (20). The ATP concentration was determined by measuring the adenosine content of the stock solution with ultraviolet spectrophotometry and then determining the portion of adenosine that is ATP by thin-layer chromatography. The solutions were mixed in batch from stock solutions and kept frozen until used. The bathing solutions came from the same batch. Creatine kinase was added to the bathing solutions at the time of the experiment.

Mechanical threshold. The EDL and soleus were removed from the uninjected hindlimb and pinned at rest length in a chamber that was perfused at 37°C with oxygenated Tyrode solution (25) containing 1 μM tetrodotoxin. The surface connective tissue was dissected free and the muscle was viewed through a dissecting microscope (×100). A two-microelectrode voltage-clamp technique was used to measure the mechanical threshold (6). The microelectrode used for passing current was filled with 2 M potassium citrate and held inside a grounded aluminum tube with only a few millimeters of the pipette tip exposed. The voltage-recording electrode was filled with 3 M KCl. The fibers were studied on the lateral margins of the muscle where visualization was optimal. The electrodes were positioned within 50 μm of each other, and the membrane was clamped at −80 mV. The contraction threshold was determined by setting the pulse duration at 1, 10, and 100 ms and 1 s and increasing the pulse amplitude in millivolt steps until contraction was just observed and by setting the pulse amplitude to produce depolarizing steps of 100, 150, and 200 mV and increasing the pulse duration in 10 μs steps until contraction was observed. The following criteria were used to determine whether the data should be accepted. With only the voltage electrode in the cell, the membrane potential was recorded and the cell was discarded if the rest potential was more positive than −65 mV. With the cell under current clamp, the input resistance was measured and the cell was discarded if the input resistance was <300 kohm. With the cell voltage clamped and the voltage step optimized, a 0.8 ms depolarizing step of +100 mV was observed; if the step had not settled to within a few millivolts of command by 80 μs, the cell was discarded if there was a significant change in the holding current.

All values are expressed as means ± SE. The value of P for significance of the difference between means was determined by the two-tailed Student's t test.

RESULTS

Figures 1 and 2 show the contractile responses of representative skinned EDL and soleus fibers from dexamethasone-treated and control rats studied in bathing solutions with 1 mM free Mg. Both EDL fibers started to develop tension at ~pCa = 6 and had similar contractile responses to higher calcium concentrations. The calcium sensitivity of the EDL fibers from dexamethasone-treated rats was indistinguishable from that of control fibers. The calcium concentration corresponding to half-maximal tension and the Hill coefficients for EDL fibers were pCa = 5.63 ± 0.007 and h = 3.04 ± 0.12 for control rats and pCa = 5.64 ± 0.007 and h= 2.92 ± 0.13 for dexamethasone-treated rats. In contrast, the soleus fibers from dexamethasone-treated rats had diminished calcium sensitivity compared with control fibers. The control fiber in Fig. 2 had a tension threshold of ~pCa = 6.2, whereas the dexamethasone-treated fiber did not develop tension until pCa = 6. The calcium concentration corresponding to half-maximal tension and the Hill coefficients (h) were pCa = 5.88 ± 0.02 and h = 2.02 ± 0.18 for control fibers and pCa = 5.78 ± 0.02 ($P < 0.01$) and h = 2.08 ± 0.21 for steroid-treated soleus fibers. The soleus fibers from both control and dexamethasone-treated rats had tension oscillations when studied in bathing solutions with calcium concentrations that were just slightly above threshold. When oscillations were present, the average tension was used for the pCa-tension relationship. The force oscillations were similar to those previously noted in slow-twitch muscle fibers (29).

The pCa-tension curves in Figs. 1 and 2 were obtained by averaging the fraction of maximum tension generated

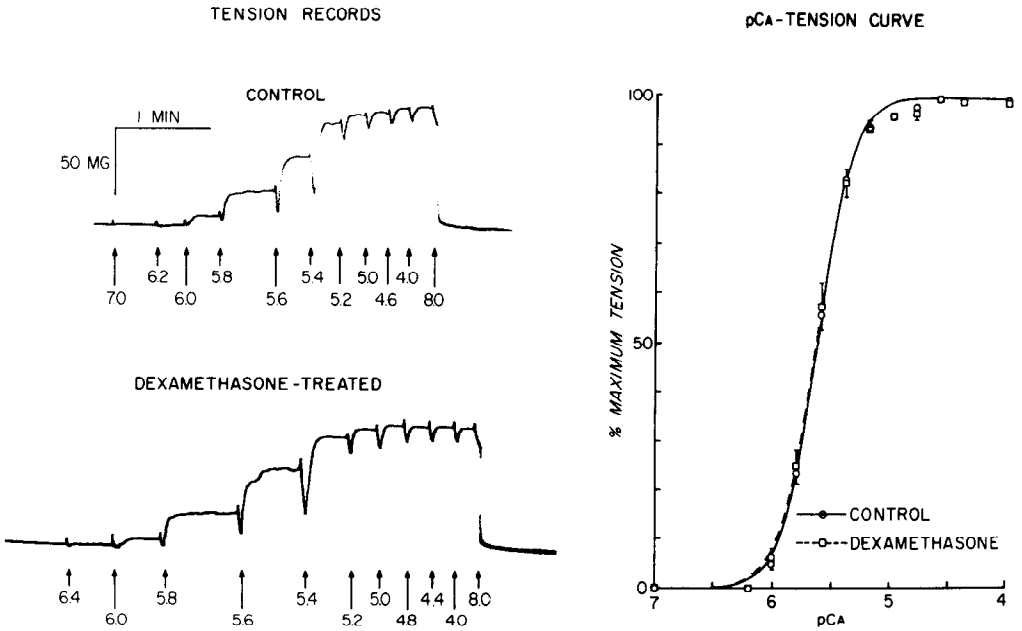

FIG. 1. pCA-tension relationships of dexamethasone-treated and control skinned extensor digitorum longus (EDL) fibers studied with 1 mM free Mg. *Left*: tension records of representative individual fibers. Values below *arrows* indicate pCa of bathing solutions. *Right*: pCA-tension curves obtained by averaging fraction of maximum tension generated at a given pCa for 12 fibers from control rats and 14 fibers from dexamethasone-treated rats. There were no significant differences between relative tensions generated by fibers from dexamethasone-treated and control rats for any pCa. *Smooth curves* are nonlinear least-square fits of the data to the Hill equation

$$P/P_{max} = (C/K)^h/[1 + (C/K)^h]$$

where C is free calcium concentration, P/P_{max} is normalized tension, K is calcium concentration producing half-maximal tension, and h is Hill coefficient.

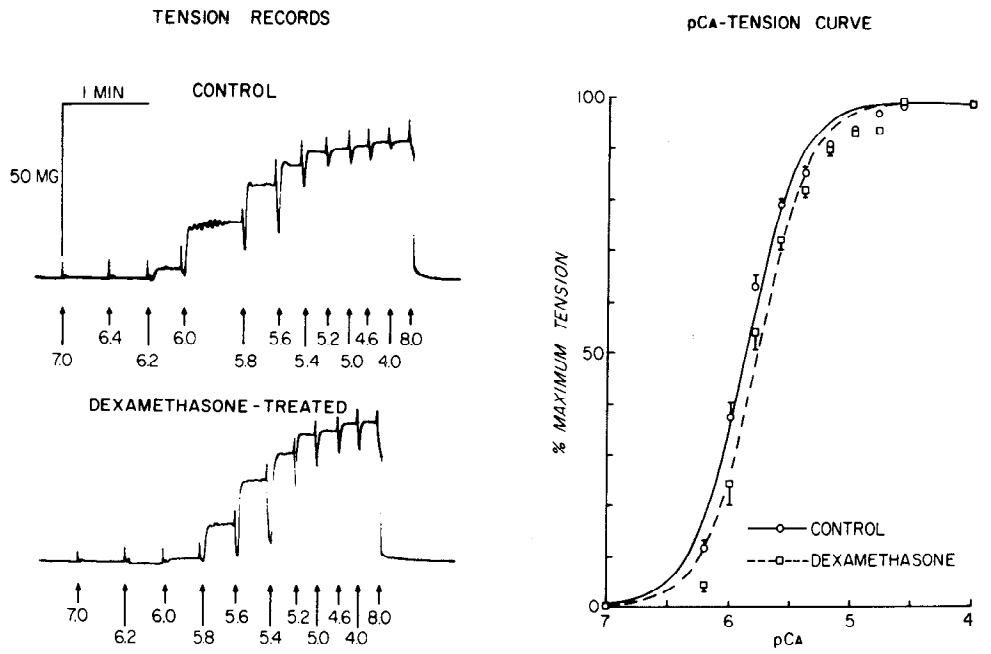

FIG. 2. pCa tension relationships of dexamethasone-treated and control skinned soleus fibers studied with 1 mM free Mg. *Left*: tension records of representative individual fibers. *Right*: averaged pCa-tension curves for 13 fibers from control rats and 15 fibers from dexamethasone-treated rats. Fibers from dexamethasone-treated rats generated less tension than fibers from control animals at pCa = 6.2 ($P < 0.01$), 6.0 ($P < 0.05$), 5.8 ($P < 0.05$), and 5.6 ($P < 0.01$). Other details are same as in Fig. 1.

at a given pCa for each group of fibers. Table 1 shows that similar values for pCa at half-maximal tension and the Hill coefficient were obtained when these values were calculated from the pCa-tension curves of the individual fibers.

When the skinned fibers were studied in bathing solutions with 0.1 mM free pMg, both EDL and soleus fibers had increased calcium sensitivities, and there was no difference between the calcium sensitivity of soleus fibers from control and dexamethasone-treated rats (Fig. 3). The values of the calcium concentration corresponding to half-maximal tension and the Hill coefficient obtained from the averaged pCa-tension curves from control and dexamethasone-treated fibers were: EDL pCa = 6.01 ± 0.003, h = 3.10 ± 0.06 (control) and pCa = 6.00 ± 0.005, h = 3.05 ± 0.09 (steroid treated); and soleus pCa = 6.31 ± 0.01, h = 2.37 ± 0.16 (control) and pCa = 6.30 ± 0.13, h = 2.30 ± 0.14 (steroid treated).

TABLE 1. *Calcium sensitivity of control and steroid-treated skinned muscle fibers based on analysis of individual fibers*

Fiber Type	pCa Producing Half-Maximal Tension	Hill Coefficient
Control EDL	5.63 ± 0.02 (12)	3.23 ± 0.18
Steroid EDL	5.62 ± 0.03 (14)	3.21 ± 0.08
Control soleus	5.88 ± 0.02 (13)	2.07 ± 0.06
Steroid soleus	5.79 ± 0.03* (15)	2.13 ± 0.13

Values are means ± SE; no. in parentheses equal no. of fibers. EDL, extensor digitorum longus. * $P < 0.001$.

Glucocorticoid treatment produced a 34% reduction in

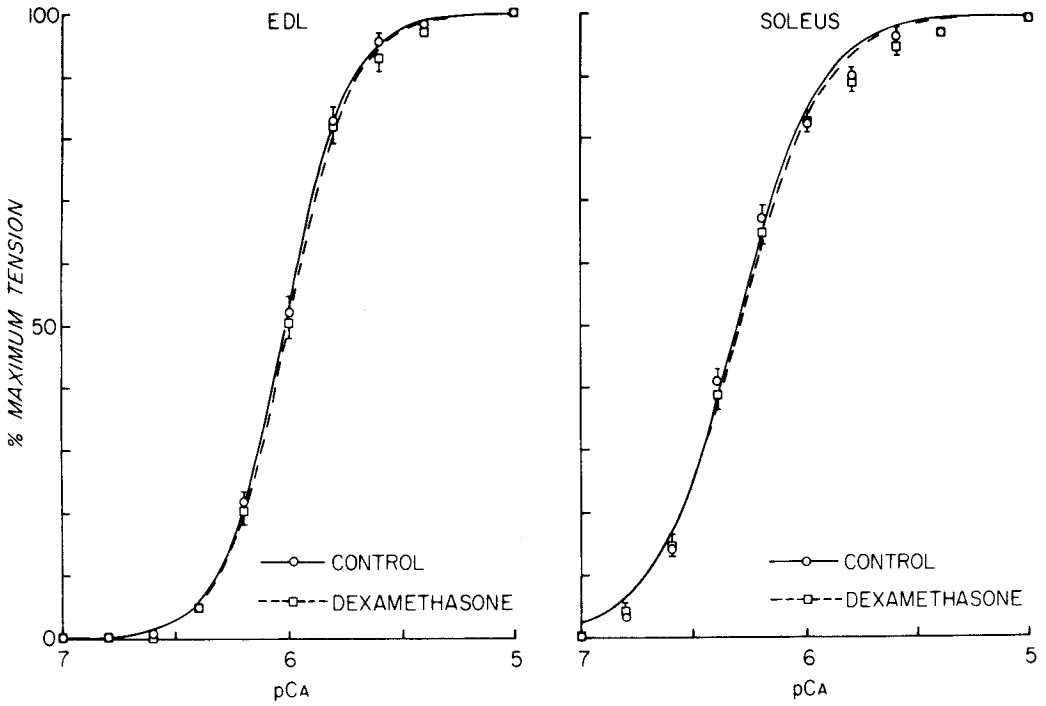

FIG. 3. pCa tension relationships of dexamethasone-treated and control skinned extensor digitorum longus (EDL) and soleus fibers studied with 0.1 mM free Mg. Each *curve* represents average of data from at least 6 fibers. There was no significant difference between relative tensions at any value of pCa for dexamethasone-treated and control fibers from EDL or soleus muscles. Other details are same as in Fig. 1.

the cross-sectional area of EDL fibers and reduced the maximum tension of EDL fibers by 22%. However, dexamethasone treatment did not produce atrophy or reduce the single-fiber tension of soleus fibers, and dexamethasone treatment did not alter the maximum tension per cross-sectional area for EDL or soleus fibers (Table 2).

The minimum stimulus duration of the strength-duration relationship primarily reflects the minimal calcium concentration necessary to stimulate the myofibrils, as a result of calcium release from the sarcoplasmic reticulum. This is because the voltage-dependent calcium release should be strongly activated producing calcium fluxes that are large compared with the sarcoplasmic reticulum pumping rate (19). In addition to calcium release and myofibrillar calcium sensitivity, the contraction threshold for long duration depolarizations is also influenced by the voltage seen by the voltage sensor in the transverse tubules, the uptake of calcium by the sarcoplasmic reticulum and mitochondria, and calcium buffering in the myoplasm (19). The strength-duration relationships of EDL fibers from dexamethasone-treated and control fibers were very similar (Fig. 4). The contractile thresholds for EDL fibers were -39.8 ± 0.4 mV ($n = 15$; control) and -40.1 ± 0.4 mV ($n = 17$; dexamethasone treated) for 1-s long depolarizations, and the minimum stimulus durations were 0.293 ± 0.013 ms (control) and 0.300 ± 0.014 ms (dexamethasone treated) for depolarizations to a membrane potential of 120 mV. The resting potentials were also similar at -82.9 ± 0.5 mV (steroid treated) and -82.8 ± 0.6 mV (control). The mean input resistance of the dexamethasone-treated fibers, 547 ± 20 kohm, was greater than that of control fibers, 443 ± 12 kohm ($P < 0.001$), which probably reflected glucocorticoid-induced atrophy (Table 2; 25, 26).

The mechanical thresholds for soleus fibers from glucocorticoid-treated rats were greater than the control values (Fig. 5). The contractile thresholds for soleus fibers were -59.2 ± 0.4 mV ($n = 17$; control) and -48.3 ± 0.4 mV ($n = 18$; dexamethasone treated, $P < 0.01$) for 1-s long depolarizations, and the minimum stimulus durations were 0.179 ± 0.012 ms (control) and 0.262 ± 0.012 ms (dexamethasone treated, $P < 0.01$). The resting potentials and input resistances of the fibers were similar (dexamethasone treated, -74.8 ± 0.5 mV and 678 ± 16 kohm; control, -74.5 ± 0.4 mV and 690 ± 15 kohm).

TABLE 2. *Maximum normalized tension of individual muscle fibers*

Fiber Type	Area, μm^2	Maximum Tension, mg	Normalized Tension, kg/cm^2
Control EDL	2,600 ± 200	52 ± 4	2.1 ± 0.2 (11)
Steroid EDL	1,700 ± 200†	41 ± 2*	2.6 ± 0.3 (11)
Control soleus	2,500 ± 300	40 ± 4	1.9 ± 0.2 (8)
Steroid soleus	2,000 ± 300	43 ± 5	2.4 ± 0.3 (11)

Values are means ± SE; no. in parentheses equal no. of fibers. EDL, extensor digitorum longus. * $P < 0.05$; † $P < 0.01$.

DISCUSSION

Dexamethasone treatment apparently did not alter excitation-contraction coupling in fast-twitch muscle. The similarity between the myofibrillar calcium sensitivity and strength-duration relationships of steroid-treated and control EDL fibers supports the conclusion of Peter et al. (22) that glucocorticoid treatment did not alter calcium release or uptake by the sarcoplasmic reticulum of fast-twitch muscle. There are several possible ways to reconcile the results of Shoji et al. (27) with our findings. Glucocorticoid effects on the sarcoplasmic reticulum of fast-twitch muscle may be species specific. Alternatively,

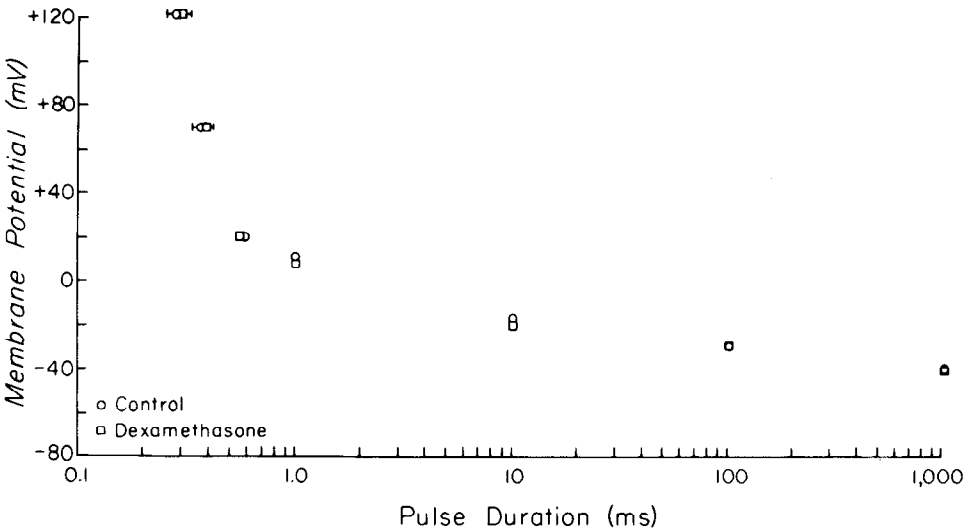

FIG. 4. Strength-duration curves for extensor digitorum longus (EDL) fibers from dexamethasone-treated (n = 17) and control (n = 15) rats. *Horizontal axis*: duration of voltage pulses that cause threshold contractions. *Vertical axis*: amplitude of voltage pulses that cause threshold contractions. Mechanical thresholds of fibers from dexamethasone-treated rats did not differ significantly from control values.

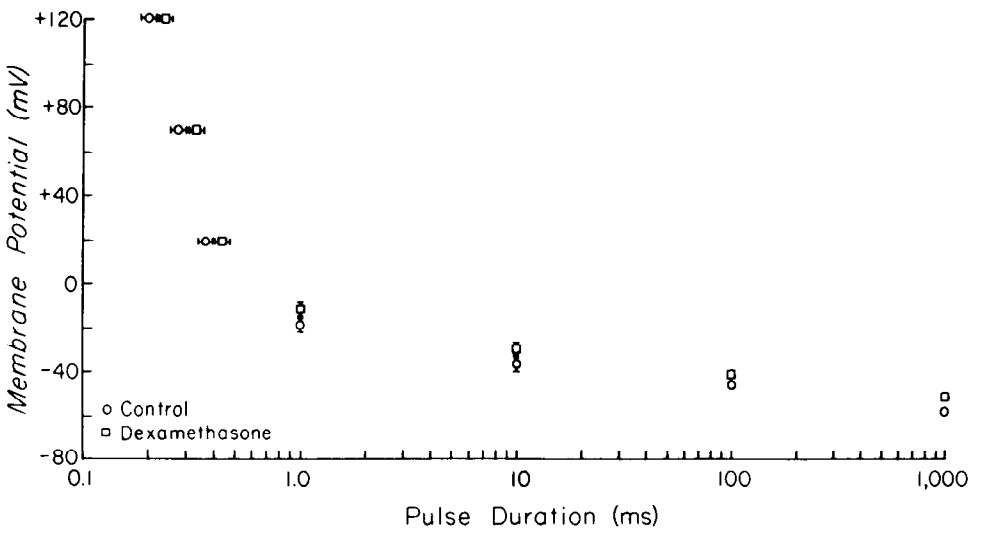

FIG. 5. Strength-duration curves for soleus fibers from dexamethasone-treated (n = 18) and control (n = 17) rats. All of the mechanical thresholds for fibers from dexamethasone-treated rats differed from control values ($P < 0.01$).

the steroid effect on calcium uptake may have been an artifact of preparation of the fragmented sarcoplasmic reticulum, due to contamination of the membrane fragments with steroid molecules that were released from their cytoplasmic receptors. Martinosi (18) reported that glucocorticoids could inhibit calcium uptake of fragmented sarcoplasmic reticulum prepared from rabbit muscle if the steroids were applied directly to the vesicles. Consequently, Shoji et al.'s (27) findings do not necessarily imply that glucocorticoids affect the sarcoplasmic reticulum as a result of altered cellular metabolism. It is unlikely that glucocorticoids produced weakness as a result of a direct action on the sarcoplasmic reticulum because such an effect should be manifested soon after treatment is started, and yet weakness usually takes many days to develop and then progresses slowly (8, 25). In addition, atrophy always precedes weakness (25). The temporal pattern of the development of weakness suggests that the impaired force-generating capacity is a consequence of altered cellular metabolism rather than a result of steroid incorporation into the sarcoplasmic reticulum membrane.

Glucocorticoid treatment increased the mechanical threshold of soleus fibers to both short and long duration depolarizations (Fig. 5) and decreased the calcium sensitivity of skinned fibers studied with pMg = 3. The most parsimonious explanation is that the change in the strength-duration relationship of soleus fibers is due to an elevation in the threshold calcium concentration for contraction. However, the strength-duration findings could also result from reduced calcium release by the sarcoplasmic reticulum.

Reducing the free magnesium from 1 to 0.1 mM increased the calcium sensitivity of both EDL and soleus fibers as previously noted (5, 28, 29). The mechanism by which magnesium alters the calcium sensitivity of skinned fibers is not clear. The calcium binding subunit of troponin, TnC, contains sites that bind calcium or magnesium (Ca-Mg sites), magnesium only, as well as calcium-specific sites (23). The role of the Ca-Mg sites and the magnesium-specific sites in regulating myofibrillar ATPase has not been resolved. Potter and Gergely (23) reported that only the calcium-specific sites regulate ATPase activity, whereas Solaro and Shiner (28) speculated that the magnesium-specific sites on TnC could modulate the ability of TnC to regulate ATPase activity. Magnesium also alters calcium binding to myosin (2); however, the role of calcium binding sites on myosin in

regulating cross-bridge turnover is unclear (24). Troponin and myosin in slow-twitch mammalian skeletal muscle are diffferent from the proteins found in fast-twitch muscle (15, 32) so that magnesium-mediated effects could depend on fiber type. In low magnesium bathing solutions the calcium sensitivity of glucocorticoid-treated and control fibers were similar, which suggests that steroid treatment might alter the ability of magnesium to reduce myofibrillar calcium sensitivity in soleus fibers.

The changes in excitation-contraction coupling of soleus muscle fibers produced by glucocorticoid treatment are probably not physiologically important in the genesis of steroid myopathy because this muscle is relatively resistant to steroid-induced reduction in force-generating capacity and atrophy (8, 9, 25, 31). Several studies reported that glucocorticoid treatment did not alter any time-related contractile properties of slow-twitch fibers (11, 25, 31). Gardiner and Edgerton (8) noted a slight prolongation of the time to peak twitch tension in soleus from triamcinolone-treated rats. However, a subsequent study from that laboratory did not demonstrate a change in the time course of the twitch of soleus muscles from steroid-treated rats (9).

The change in the myofibrillar calcium sensitivity and strength-duration relationship of soleus fibers from glucocorticoid-treated rats may be a result of an altered activity pattern of this muscle. The soleus motoneurons of cats treated with glucocorticoids show increased post-tetanic potentiation and develop high-frequency repetitive afterdischarges (12). Consequently, the soleus muscle fibers may be exposed to episodic high-frequency stimulation which is similar to that normally experienced by fast-twitch fibers (3). The changes in the myofibrillar calcium sensitivity and strength-duration relationship are both in the direction of making the behavior of soleus fibers from steroid-treated rats more like that characteristic of fast-twitch fibers.

Several studies noted that glucocorticoid treatment in the rat produced atrophy that was more severe than the reduction in force-generating capacity. Consequently, the twitch or tetanic tension per unit of muscle weight increased with glucocorticoid treatment (8, 9, 25). Gardiner et al. (9) suggested that the increase in the force per unit muscle mass associated with glucocorticoid treatment might be due to a change in the muscle geometry so that the angle of pull of the fibers was more acute or to an increase in the myofibrillar force-generating capacity. In this study, dexamethasone treatment produced significant atrophy and reduced the maximum tension generated by individual EDL, but not soleus, muscle fibers. Glucocorticoid treatment did not alter the maximal normalized tension of single-skinned EDL or soleus fibers. The values for the normalized forces were similar to those previously reported for skinned fibers (30) or whole muscle (4) from the rat.

In summary, the weakness associated with dexamethasone treatment in fast-twitch muscle is probably not due to impaired excitation-contraction coupling or altered myofibrillar force-generating capacity. The weakness may reflect loss of contractile protein due to net protein catabolism (10, 25).

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