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FATIGUE AND CONTRACTILE RESPONSES OF RAT HINDLIMB MUSCLES FOLLOWING EXCESSIVE DEXAMETHASONE ADMINISTRATION

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Summary : Muscle weight, protein content and contractile performormance (tetanic tension, fatigue and recovery) of extensor digitorum longus and soleus were investigated in rat following systemic administration of Dexamethasone (DX), 5 mg/kg/day for ten days. These animals showed marked reduction in food intake during the course of DX treatment. As a control, a group of food restricted (FR) rats receiving equal amount of food consumed by the DX treated rats was also studied along with the saline control group, to differentiate the effect of DX on muscle from that of dietary deficiency. There was a greater degree of atrophy (reduced muscle mass and protein content) of extensor digitorum longus in DX treated rats as compared to that of the FR rats. *In-situ* isometric tetanic tension per gram of muscle and per unit weight of protein was similar in both the muscles in the DX treated and the FR rats. There was increased fatiguability with reduced post fatigue recovery in both the muscles of DX treated rats as compared to the FR rats. The results indicate that besides atrophy of fast twitch muscles, DX increases the fatiguability and decreases the postfatigue recovery in both fast and slow muscles.

Key words : dexamethasone muscular atrophy fatigue postfatigue recovery

INTRODUCTION

Excessive administration of glucocorticoid is known to produce skeletal muscular atrophy (loss of muscle mass and protein) in both human (1, 2) and experimental animals (3, 4, 5). Atrophy is reported to occur in rat hindlimb muscles as early as 7 days of treatment with 5mg/kg/day of a synthetic glucocorticoid, Dexamethasone (6). Several biochemical and histological changes including reduced oxidative capacity (6) and mitochondrial structural abnormality (3, 4) have been noted in muscles after glucocorticoid induced atrophy. Precise influence of these biochemical and histological alterations on repeated contractile performance of different type of muscles is yet to be known. It is also not clear whether the changes in contractile properties (7) are contributed by the reduced food intake which accompanies high dose of glucocorticoid treatment (8). Therefore, the present study was aimed to investigate the contractile performance of atrophied extensor digitorum longus (EDL), which is preponderant with fast twitch fibres, and soleus (SOL), predominantly containing slow twitch fibres, by recording peak tetanic tension, fatiguability and post fatigue recovery following ten days of systemic administration of Dexamethasone (DX), 5 mg/kg/day in rat and also to compare the results with those in another group of rats having similar nutritional status, but without the DX.

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MATERIAL AND METHODS

Female rats of Charles Foster strain, weighing 150±2 g were used in the present study. All the rats were kept separately in their individual cages and the animals were studied in three groups. One of the groups was injected Dexamethasone sodium phosphate (Decadron, MSD) 5 mg/kg/day i.m. in forelimb for ten days and the second group received same volume of normal saline to serve as saline control (SC). The third group was subjected to food restriction (FR); the amount of food dispensed to this group was similar to that of previous days' consumption of Dexamethasone treated (DXT) rats. All injections were made under aseptic precaution using sterile pyrogen free solutions. DXT and SC rats had free access to food pellets (Hindustan Lever, India) and tap water, whereas FR rats received only water ad libitum, the food supply was monitered as mentioned above.

After ten days of treatment (on the 11th day) EDL and SOL muscles were exposed in the left hindlimb, under urethane anaesthesia (1 g/kg, i.p.) to record in-situ isometric tetanic contractions following the procedures of Close (9), Buller et al (10) and Deshpande et al (11). Muscles were exposed alongwith their innervating nerves, without damaging the blood vessels supplying these muscles. Skin flaps on either side of the muscles were raised to make a pool for warm liquid paraffin (35±1°C). The distal end of the tendon was severed and fastened to an isometric force transducer (ET-2, Encardiorite, India) with the help of 3-0 silk thread. Muscles were stimulated through their nerves by bipolar silver chlorided electrodes using square wave pulses of 0.2 msec duration and supramaximal strength (5 time threshold) from a stimulator (model S-88, Grass Instruments, USA). Muscle length was adjusted to obtain maximum twitch amplitude and contractions were recorded on chart recorder (Encardiorite, India). In the beginning of the recording, five isometric tetanic contractions were obtained with 2 0 Hz pulse frequency for 500 msec, with the interval of 10 seconds in between the contractions and the average of these five tetanic tensions was considered as peak tetanic tension

Fatigue in the present study has been defined as the impairment of contractile function that develops in muscle during repeated stimulation. The percentile decrease in tetanic tension following repeated tetanic stimulation was used as "fatigue index". Fatiguability was recorded by stimulating the muscles (EDL and SOL) with 100 Hz pulse frequency, 250 msec train duration and 60/min train rate. In case of EDL, the fatigue index was noted at 25, 50, 75 and 100 sec of stimulation, by calculating percentage decrease in tension considering the tension at the beginning of stimulation as 100%. Fatigue index in EDL was also expressed as time (sec) required to reduce 50% of its initial tension. Because, the SOL is composed of slow oxidative fibres in greater proportion (12) and is relatively resistant to fatigue (9), tension reduction to 50% could not be achieved in SC rats even after stimulation for 60 min. Therefore, the stimulation was continued for 30 min and fatigue index was expressed only as percentile reduction in tension at 10, 20 and 30 min of stimulation.

Recovery after fatigue was determined by recording contractions after two min of rest following 50% reduction in tension in EDL and 30 min of stimulation in case of SOL. Recovery was expressed as percentage of tension at the beginning of stimulation procedure for recording fatiguability.

After the recording was over, the muscles were excised to record the muscle weight and to estimate the protein content (13).

Statistical analysis : All the values were expressed as mean±SD. Absolute values were compared, using Student's unpaired 't' test, to evaluate statistical significance. P value<0.05 was accepted as significant.

RESULTS

Food intake and growth: Ten days of treatment with DX caused reduction in weight in animals by an average of 23% from their initial values and similar weight loss was also observed in FR rats. SC group gained 6% of their initial body weight in ten days. Daily food intake was 8 5±0.9 g in SC rats whereas in DXT and FR rats it was 3.3±1.2 g. Significant ($P<0.001$) loss of muscle weight and protein content was observed in DXT and FR rats as compared to SC, in both EDL and SOL muscles and the loss was most severe in EDL of DXT group. On the other hand in SOL, these parameters were similar in DXT and FR groups (Table. I).

Tetanic tension and fatiguability: The peak tetanic tension was significantly ($P<0.001$) less in DXT and FR groups as compared to the SC group (Table I). The tension developed was significantly low in EDL of DXT rats as compared to FR, while in SOL the tension was similar ($P>0.05$) in these groups. There was a decreased tension per gram of muscles in both the muscles of DXT and FR rats as compared to SC group. However, there was no significant difference between DXT and FR rats. Tetanic tension per 100 mg of protein, in EDL, was significantly low in DXT and FR groups as compared to that in SC group. The time taken for 50% reduction in tension in EDL was least in DXT (87.5±8.6 sec), it was followed by FR (123.7±11.8 sec) and SC (150±6.5 sec) groups. The rate of

TABLE I : Dexamethasone induced changes in muscle weight, protein content and peak tetanic tension in EDL and SOL as compared to food restricted control rats. Values are mean±SD. Figures in parentheses represent the 'n' of animals.

	EDL		*SOL*	
	DXT (12)	*FR (8)*	*DXT (12)*	*FR (8)*
Muscle weight (mg)	47.5±5.2*	61.8±4.5	54.4±4.2	55.0±5.4
% of SC	63	82	79	80
Total protein (mg)	9.1±1.4*	12.8±0.9	10.9±1.1	11.4±1.1
% of SC	50	71	71	74
Peak tetanic tension (g)	58.6±9.6*	78.4±7.2	67.0±5.6	69.3±5.4
% of SC	45	60	67	70
Tetanic tension kg/g of muscle	1.23±0.09	1.27±0.11	1.23±0.10	1.26±0.14
% of SC	70	72	84	87
Tetanic tension g/100 mg of protein	644±52	613±53	614±37	608±31
% of SC	88	84	96	95

* Significantly lower than FR, $P<0.001$

All the values except tetanic tension/100 mg of protein in SOL of DXT and FR, are significantly low as compared to SC group (n=10).

reduction in tension in DXT was significantly different from FR after 100 sec of stimulation (Fig. 1).

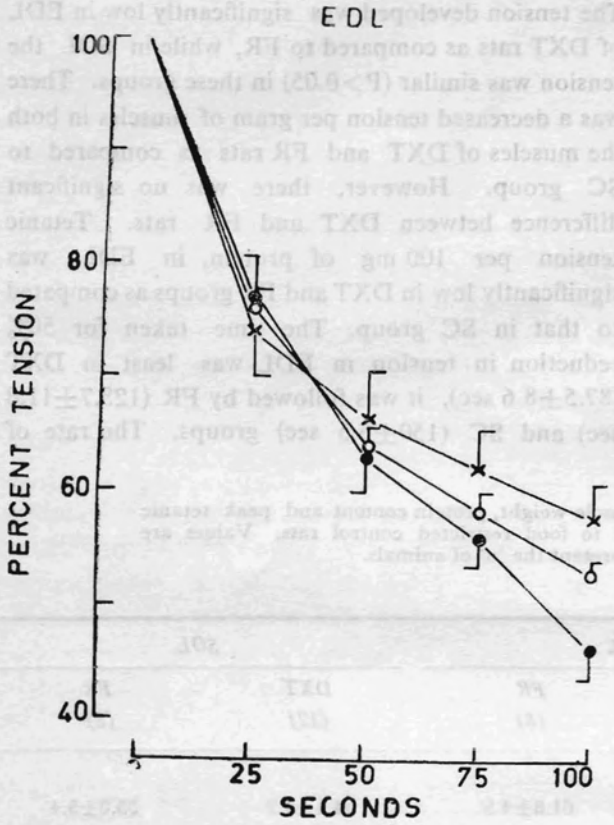

Fig. 1 : Rate of reduction in tetanic tension upto 100 sec of stimulation in DXT (●——●), FR (o——o) and SC(x——x) rats. Each point represents Mean±SD from 5 different observations and the values are normalised with the tetanic tension at the onset of the stimulation procedure for fatiguability study. Values in DXT group are significantly different (P<0.001) from those in SC group after 75 sec onwards. FR group is different (P<0.05) from SC only at 100 sec.

The reduction in tension of SOL after 30 min of stimulation was maximum in DXT group (34%). It was significantly (P<0.001) more than the FR group (25%), which in turn was higher (P<0.001) than that of SC (15%) rats (Fig. 2).

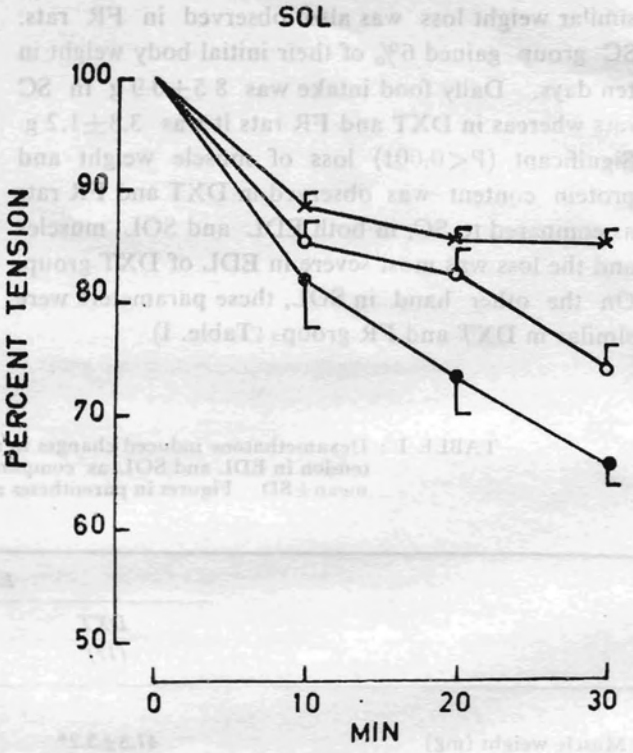

Fig. 2 : Percentage reduction in tension in SOL during 30 min of stimulation. Each point represents Mean±SD from 6 different experiments. The decrease in tension in DXT group was significant (P<0.001) after 10 min in SC and 20 min in FR groups. The difference between FR and SC groups is significant (P<0 001) only at 30 min. The symbols used for different groups are same as in Fig. 1.

Post fatigue recovery : The recovery after fatigue was 97%, 91% and 72% in SC, FR and the DXT groups respectively in case of EDL (Fig. 3). SOL recovered 10[illegible]% in SC, 98% in FR and 88% in DXT rats. Recovery in both the muscles was not significantly different (P>0.05) in SC and FR groups, whereas the recovery in DXT rats was significantly

low ($P<0.001$) as compared to FR and SC groups, in both the muscles (Fig. 3).

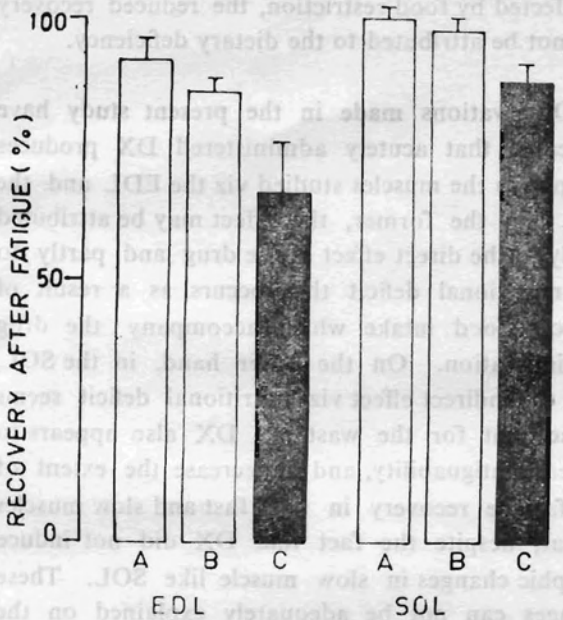

Fig. 3 : Decreased post fatigue recovery in the muscles after dexamethasone treatment. Histograms A, B and C represent Mean±SD values in SC, FR and DXT rats respectively for both EDL and SOL. The tetanic tension at the beginning of the stimulation procedure for fatiguability recording is taken as 100% and the recovery is expressed as percentage Values in DXT (filled bars) are significantly ($P<0.001$) lower as compared to the FR group.

DISCUSSION

Our present results confirmed the earlier observations (6, 14) that excessive administration of DX in experimental animals leads to skeletal muscular atrophy. However, we observed that animals developed considerable anorexia (food intake 30% of SC) during the course of DX treatment. Therefore, the influence of dietary deficiency alone on muscular atrophy and contractile performance was examined by analysing the results in FR rats which served as experimental controls for DXT rats.

The results indicate that dietary deficiency itself can lead to the marked muscular atrophy in both fast and slow muscles, which has also been reported earlier (15). Thus, it appears that the muscuarl atrophy observed in DXT rats may be the effect of low dietary intake. But on critical examination of the results, it became apparent that as compared to SOL, atrophy in EDL of DXT rats was more pronounced than that observed in FR group. This finding strongly suggests that, apart from the associated dietary deficiency the atrophy of fast muscle is largely resulted from the action of DX on muscle, whereas that in slow muscle like SOL is primarily due to dietary deficiency.

That the fast muscles are more susceptible to DX action is also supported from the changes in peak tetanic tension, which is a measure of contractile force generating ability of a muscle. However, when the peak tetanic tension was expressed as per unit mass of muscle or unit quantity of protein, in EDL, there was no difference in DXT and FR group (Table 1), indicating that the low tetanic tension was due to the greater loss of muscle mass or contractile protein, which is known to decrease proportionately with total protein in glucocorticoid induced muscular atrophy (16). The decreased tetanic tension per unit weight of protein reflects some qualitative changes in contractile protein to reduce the tension generating ability of protein which has already been reported to occur in EDL of rat following dietary deficiency (11). Similar observation was also made in the present study following DX treatment and after food restriction; therefore, it is difficult to ascribe the qualitative changes is contractile protein to the DX treatment alone.

Fatiguability, as assessed by the ability to maintain tension during the regimen of stimulation, showed that 50% tension reduction in EDL occurred in 150 sec and 15% reduction in tension in SOL after 30 min of stimulation, in saline control (SC) rats; these values are comparable to those obtained by others in control animals (17, 18). The tension reduction in EDL was faster in DXT (50% in 87 sec.) than FR (50% in 124 sec) indicating that DX has a role in

the increased fatiguability of this muscle. It was also observed that fall of tension in EDL of DXT rats was more steep after 75 sec of stimulation (Fig. 1). This perhaps, is resulted from the limited storage and rapid depletion of energy sources, or inability to utilise the energy substrates required for muscular contractions in the latter phase of stimulation. The faster rate of reduction of tension in SOL of DXT rats as compared to FR group (Fig. 2) indicates that the non-nutritional factor like DX is also involved in the enhancement of fatigue in SOL of DXT rats. Here it is surprising to note that, inspite of the similar degree of atrophy observed in SOL of FR and DXT rats, the fatiguability was more in DXT group, which depicts that the degree of atrophy of a muscle may not always be the measure of fatiguability.

The enhanced fatigue in DXT rats may be partly due to the changes in mitochondria operated oxidative system, which is necessary for the energy supply and to resist fatigue. Mitochondrial structural abnornality (3, 4) and deficit in oxidative capacity (6) as observed in both fast and slow muscles following glucocorticoid treatment may be responsible to certain extent for the greater fatiguability observed in both the muscles in DXT rats. Moreover, the nutritional deficiency may have also contributed to accelerate the fatigue in muscles of DXT rats because, FR rats showed increased fatiguability towards the end of the stimulation programme when compared to SC group (fig. 1 & 2).

Reduced capacity to recover from fatigue in both the muscles of DXT rats as compared to FR and SC (Fig. 3) indicates reduced regenerative power of energy sources. Further, since the recovery remained unaffected by food restriction, the reduced recovery can not be attributed to the dietary deficiency.

Observations made in the present study have indicated that acutely administered DX produces atrophy in the muscles studied viz the EDL and the SOL. In the former, the effect may be attributed partly to the direct effect of the drug and partly to the nutritional deficit that occurs as a result of reduced food intake which accompany the drug administration. On the other hand, in the SOL, only the indirect effect viz nutritional deficit seems to account for the wasting. DX also appears to increase fatiguability, and to decrease the extent of postfatigue recovery in both fast and slow muscles of rat, despite the fact that DX did not induce atrophic changes in slow muscle like SOL. These changes can not be adequately explained on the basis of present knowledge regarding the alterations of metabolism in muscles following glucocorticoid treatment. Therefore, an investigation into the metabolism of energy resources and ATP turnover during and after prolonged activity in glucocorticoid induced atrophied muscles, would be promising to explain the observed changes on fatiguability and recovery in the present study.

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