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EFFECT OF GLUCOCORTICOIDS ON THE TURNOVER RATE OF ACTIN AND MYOSIN HEAVY AND LIGHT CHAINS ON DIFFERENT TYPES OF SKELETAL MUSCLE FIBRES

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Summary—The catabolic action of glucocorticoids on the molecular level of the two main muscular proteins, myosin and actin, was found to depend on the type of muscle fibres. The synthesis rate of actin and myosin heavy chain was decreased in all types of muscle fibres, and in myosin light chain only in the slow-twitch red fibres. The turnover rate of actin and myosin heavy chain was also found decreased in all types of muscle fibres. The myosin light chains turned over more rapidly in dexamethasone-treated than in the control rats in all types of muscle fibres except in the case of the slow-twitch red ones as was shown by single and double isotope methods. Dexamethasone treatment enhanced the urinary 3-methylhistidine excretion in rats by 60%.

INTRODUCTION

It has been observed that large doses of glucocorticoids, which cause muscle atrophy, lead to loss of strength, decrease the rate of myofibrillar as well as soluble proteins and DNA synthesis in the skeletal muscle [1, 2] at the translational level [3] and reduce amino acid uptake into this tissue [4]. It is generally assumed that glucocorticoids promote proteolysis in the muscle [1] and stimulate myofibrillar protein breakdown as has been shown by the enhanced excretion of 3-methylhistidine [5]. However, there are contradictory results concerning the proteolytic activity of glucocorticoids in the muscle [6, 7], and still less is known about the mechanism by which glucocorticoids promote the mobilization of amino acid from muscle proteins. The effects of glucocorticoids remain controversial as well. Much less is known about changes in the synthesis and degradation of actin, myosin heavy chain and light chain in the muscle by the action of glucocorticoids.

The present study was undertaken to examine the turnover rate of actin, myosin heavy and light chains in different types of skeletal muscle fibres following glucocorticoid administration.

EXPERIMENTAL

Sixteen to seventeen-week old male rats of the Wistar strain were maintained on a diet containing 12% protein, 28% carbohydrate and 9% lipids. Food and water were given *ad libitum*. The rats were assigned to control and dexamethasone-treated. Dexamethasone sodium phosphate (Galenika, Yugoslavia) was diluted to 200 μg/ml with 0.15 M NaCl and administered intraperitoneally in doses of 100 μg/100 g body weight during 10 days. The control animals received appropriate amounts of 0.15 M NaCl. The rats were weighed during the treatment period to monitor changes in growth.

For studies of the fast-twitch white and the fast-twitch red type of muscle fibres, the m. quadriceps femoris were cut out, freed of fat and connective tissue, and separated into a superficial white portion, which consisted almost entirely of white fibres, and a deep red portion, which consisted predominantly of fast-twitch red fibres [8]. Cytochromes aa_3 and myoglobin were used as markers of the fast-twitch white and fast-twitch red type muscle fibres. The white type fibres consisted of cytochromes aa_3 9.20 ± 0.80 nmol per g muscle and myoglobin 0.94 ± 0.10 mg per g muscle. The red type fibres consisted of 32.10 ± 2.70 nmol and 3.30 ± 0.28 per g muscle respectively. The soleus muscle, which consisted predominantly of slow-twitch red fibres [9], was used for studies on the slow-twitch red muscle and m. gastrocnemius was used for studies on the mixed muscles. The muscle samples were homogenized in a buffer containing (mM): KCl, 50; K_2HPO_4 10; EGTA, 1; $MgCl_2$, 1; dithiothreitol, 1; at pH 7.0. The homogenates were centrifuged at 1000 *g* for 10 min, and the supernatant was taken as a sarcoplasmic fraction. The crude myofibrillar pellet was rehomogenized in the same buffer with 0.1% Triton X-100 and centrifuged at 1.000 *g* for 10 min. The myofibrils were rehomogenized in 0.6 MKl, 8 mM EGTA, 20 mM Na HCO_3, 2 mM ATP, 1 mM dithiothreitol, 50 mM Tris at pH 7.0. The homogenate, which contained myofibrils from 1 g of muscle per 10 ml was kept at 0°C for 20 h to extract actomyosin. After that it was

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centrifuged at 18,000 *g* for 60 min. The supernatant was diluted 10-fold in 1.75 mM Tris, 0.1 mM dithiothreitol at pH 7.0 to precipitate actomyosin. The protein was then dissolved in 0.6 MKI, 0.1 M dithiothreitol and centrifuged at 18,000 *g* for 30 min. Actomyosin was precipitated and washed twice.

L-[4.5-^{3}H]Leucine (170 Ci/mmol) was infused intraperitoneally for 6 h, 250 μCi per 100 g body weight. Muscle samples were prepared for gel filtration and gel filtration of Sephacryl S-300 was provided as described by Schreurs *et al.* [10]. SDS electrophoresis was used to show the degree of purity, and for the purification of actin, myosin heavy chain (HC) and light chains (LC)—sum of LC_1, LC_2 and LC_3. SDS gel electrophoresis confirmed that better myosin LC fractions were obtained when actomyosin precipitate was subjected to gel filtration of Sephacryl S-300 and eluted as described by Schreurs *et al.* [10]. Actomyosin was analyzed for ^{3}H-radioactivity and protein, the ratio of which is the specific activity (Sp. act). The relative specific activity (RSA) of each fraction (actin, myosin HC and myosin LC) was calculated as the ratio of the specific activity of the fractions and the specific activity of actomyosin which characterizes the turnover rate of different actomyosin proteins.

In order to investigate the heterogensity of the turnover of actomyosin proteins, the double isotope method of Funabiki [11] was used. L-[U-^{14}C]Lysine (336 mCi/mmol) 25 μCi per day was discontinued after 5 days and L-[4.5-^{3}H]lysine (40 Ci/mmol), 250 μCi per day, was continued for 12 days. The relative turnover rate of the protein fraction was estimated from the ^{3}H/^{14}C ratios. If the proteins turned over at the same rate, the ^{3}H/^{14}C ratios would be the same. Protein with a more rapid turnover rate would have a greater ^{3}H/^{14}C ratio, SDS polyacrylamide gel electrophoresis was carried out in 10% acrylamide gels according to the technique of Porcio *et al.* [12]. The identified fractions were sliced and dissolved in hydrogen peroxide at 50°C overnight and radioactivity was determined.

The incorporation of ^{3}H or ^{14}C radioactivity into the total homogenate (sarcoplasmic proteins, actomyosin, myosin heavy chain, myosin light chain and actin) was determined with a 1211 Minibeta liquid scintillation counter.

Myoglobin was measured by a modification of Reunaforje [13] and cytochromes aa_3 were measured by the method of Schoelmeyer [14]. The protein content was determined either by the method of Lowry *et al.* [15] or by the biuret method [16].

Complete 24-h urine was collected, the urine samples were centrifuged in order to remove food particles and occasional faecal pellets. Since some N^1-methylhistidine was excreted in the *N*-acetylated form [17], the urine was hydrolyzed before measurement and heated in an equal volume of 4 M HCl in a boiling waterbath for 2 h. The hydrolyzed samples (2 ml) were loaded onto Dowex 50 W × 8 (1 × 4 cm), which was previously equilibrated with 0.2 M pyridine. Neutral and acidic amino acids were removed from the column by eluting it with 10 vol of 0.2 M pyridine. 3-Methyl-histidine was eluted from the column in the first 8 column vol of 1.0 M pyridine. Urinary 3-methylhistidine was determined with the aid of a Hitachi KLA-4 B amino acid analyzer. Urinary creatinine was determined by the Jaffe picrate reaction.

RESULTS

In dexamethasone-treated rats the rates of myofibrillar protein degradation *in vivo*, as measured by 3-methylhistidine excretion, reached the maximal level on the third day. Daily 3-methylhistidine excretion rates in the control group were 0.34 ± 0.04 μmol/mg of creatinine and in the dexamethasone-treated group 0.55 ± 0.07 μmol/mg of creatinine ($P < 0.05$). The ratio of incorporation of [^{3}H]leucine into sarcoplasmic proteins and actomyosin (cmp/cpm) in slow-twitch red muscle fibres in dexamethasone-treated rats was elevated by 65%, compared with that of the control rats (24.49 ± 2.60 and 14.86 ± 0.86; $P < 0.05$ respectively). In the fast-twitch red fibres this ratio in the dexamethasone-treated group was 17.51 ± 1.80, and in the control group 12.17 ± 0.92; $P < 0.05$, and in the fast-twitch white fibres 27.48 ± 2.40 and 21.75 ± 1.30 ($P < 0.05$ respectively). In the mixed muscles the ratio was 28.76 ± 2.40 in the dexamethasone-treated group, and 21.40 ± 1.50 in the control group ($P < 0.05$). These results showed that the incorporation of [^{3}H]leucine in dexamethasone treatment had decreased only in actomyosin but not in sarcoplasmic proteins. A comparison of changes in the ratio of myosin and actin concentrations in different types of muscles between the dexamethasone-treated group and the control group showed that this ratio had decreased in all the muscle types, especially in the fast-twitch white muscle fibres (23%). The myosin HC/LC concentration ratio in the dexamethasone-treated group was considerably lower in the slow-twitch red muscle fibres (21%). There were no significant differences in the ^{3}H/^{14}C ratio of actomyosin in different types of muscle fibres in the control group. But this ratio was significantly lower in the slow-twitch red fibre and the fast-twitch red fibres in the dexamethasone-treated group (16 and 29% respectively). As shown in Table 1 and Fig. 1 the relative turnover rate of actin had significantly decreased in all types of muscle fibres during dexamethasone treatment. The turnover rate of myosin HC was lower in the fast-twitch muscle fibres (see Fig. 1 and Table 1). A comparison of the myosin light chain turnover rate with the turnover rate of the myosin heavy chain and actin shows that the myosin light chain has a more rapid turnover rate in all types of muscle fibres. There was a more rapid turnover rate of the myosin light chain in the dexamethasone-

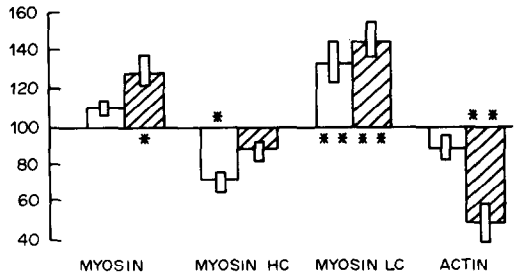

Fig. 1. Percentile changes in the turnover rate of actomyosin proteins in fast-twitch white fibres in the dexamethasone-treated group compared with the controls (100%) by two different methods: □—results obtained by single isotope method (RSA): ▨—results obtained by double isotope method ($^3H/^{14}C$). HC—myosin heavy chain; LC—myosin light chain (sum of LC_1, LC_2 and LC_3). Values are means ± SE, 6 animals per group, significantly different from the control group: *—$P < 0.05$; **—$P < 0.01$.

treated group, especially in the fast-twitch red and fast-twitch white fibres (see Fig. 1 and Table 1). A comparison of the turnover rate of actomyosin proteins by single and double isotope methods showed that there were practically no differences in the results obtained by these two methods (see Fig. 1).

The rate of the incorporation of [^{3}H]leucine into myosin, myosin HC and actin was significantly slower in all types of muscle fibres in the dexamethasone-treated rats. But there were no changes in the incorporation of [^{3}H]leucine into the myosin light chain in the dexamethasone-treated group in all types of muscle fibres, except in the slow-twitch red fibres (see Fig. 2).

DISCUSSION

It is well known that overproduction of adrenal steroids in Cushing's syndrome or due to large doses of glucocorticoids, leads to a marked reduction in the muscle mass, wasting of the muscle and loss of strength. The works of Goldberg [1] suggest that glucocorticoids stimulate muscle protein breakdown. A promoted breakdown of contractile protein, myosin and actin in the muscle during glucocorticoids administration was also shown by the enhanced excretion of 3-methylhistidine in rats [5]. Administration of large amounts of glucocorticoids increased the excretion of 3-methylhistidine in rats by 60%. Dexamethasone administration decreased the relative weight of the fast-twitch white fibres by 48% [18]. In these fibres the ratio of myosin and actin, and the turnover rats of the myosin heavy chain were decreased. It is not surprising that the slow-twitch and fast-twitch skeletal muscles differ in their response to hormones, but it is surprising that in the fast-twitch fibres large doses of glucocorticoids cause a more rapid turnover rate of the myosin light chain. On the other hand, the myosin heavy chain turnover was more significantly decreased in the fast-twitch muscle fibres than in the slow-twitch ones.

A comparison of the single [10] and double [11] isotope methods for the measurement of the turnover rate of myosin and actin showed that there were practically no differences in the obtained results. The double isotope method showed a somewhat more rapid turnover of the myosin heavy chain and a slower turnover rate of actin in the glucocorticoid-treated animals. Glucocorticoids reduced the incorporation of amino acids into muscle proteins. Our results showed a reduced incorporation rate of [^{3}H] or [^{14}C]leucine only into actomyosin, but not into sarcoplasmic proteins. This is not in accordance with the earlier suggestions where a decreased rate of myofibrillar as well as of soluble proteins synthesis has been registered in dexamethasone treatment [1].

In all types of muscle fibres a decreased incorporation of leucine into actin was observed. While a decrease in the synthesis rate of myosin was observed in all types of fibres, decrease in the myosin light chains was observed only in the slow-twitch red

Table 1. The effect of glucocorticoids on the turnover rate of actin, myosin heavy and light chains

Fiber types	Group	n	Actin	% Changes	Myosin HC	% Changes	Myosin LC	% Changes
Slow-twitch red	Control	3	0.69 ± 0.05		0.57 ± 0.06		2.98 ± 0.31	
	Dexamethasone-treated	3	0.58 ± 0.07	−16	0.51 ± 0.07	−10	3.03 ± 0.40	+2
Fast-twitch red	Control	6	0.69 ± 0.04		0.58 ± 0.05		2.87 ± 0.20	
	Dexamethasone-treated	6	0.49 ± 0.05 **	−29	0.36 ± 0.05 **	−38	5.89 ± 0.72 **	+105
Fast-twitch white	Control	6	0.84 ± 0.04		0.74 ± 0.05		2.08 ± 0.24	
	Dexamethasone-treated	6	0.71 ± 0.05	−15	0.56 ± 0.06 *	−24	2.79 ± 0.40	+34
Mixed	Control	6	0.75 ± 0.04		0.63 ± 0.05		2.62 ± 0.22	
	Dexamethasone-treated	6	0.58 ± 0.06 *	−23	0.30 ± 0.04 **	−52	3.92 ± 0.44	+50

Values are means ± SE.
n—the number of animals per group.
Significantly different from control group:
*—$P < 0.05$
**—$P < 0.01$

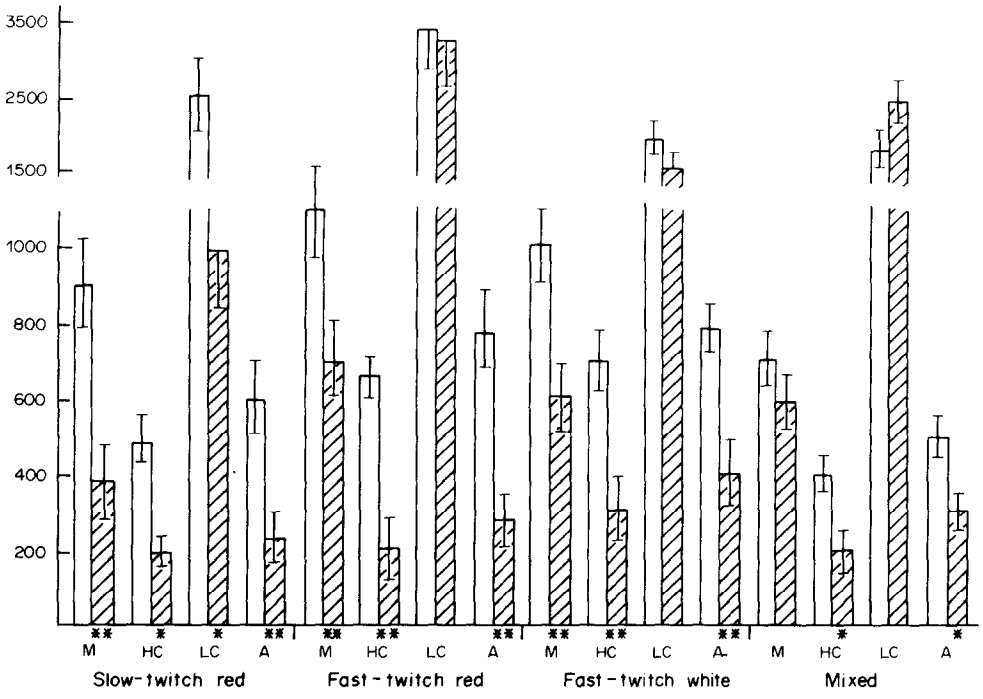

Fig. 2. Incorporation of [^{3}H]leucine (cpm/mg) into myosin and actin in different types of muscle fibres: □—control; ▨—dexamethasone treated; M—myosin; HC—myosin heavy chain; LC—myosin light chain (sum of LC_1, LC_2 and LC_3); A—actin. Values are means ± SE. Significantly different from the control group: *—$P < 0.05$; **—$P < 0.01$.

fibres. In both fast-twitch fibre types the myosin light chain synthesis rate had not significantly changed in the dexamethasone-treated rats.

One of the most reliable mechanisms through which glucocorticoids affect muscle protein synthesis is that of hormone-induced changes in the ribosomal structure and function. The muscle contains polysomes of different sizes which code for the synthesis of various myofibrillar proteins [19, 20]. The myosin heavy and light chains, which together constitute myosin, are translated independently by different monocistronic m RNA [21, 22]. The heavy chains of myosin are synthesized on polyribosomes containing 60–80 ribosomes by a 26 S mRNA; the light chains are coded for by another mRNA from polyribosomes containing 4–8 ribosomes [19, 21, 22]. Following the synthesis of different sub-units of myosin on the separate mRNA, the polypeptides are assembled to form the native myosin molecule [22].

It seems that glucocorticoids decrease the *in vivo* rate of incorporation of amino acid into the myosin heavy chain, and they likewise decrease the turnover of the myosin heavy chain. In dexamethasone-treated rats the myosin light chain synthesis was not affected by glucocorticoids, and the light chains turned over more rapidly than they did in the control rats in all types of muscle fibres, except in the slow-twitch red ones. In the slow-twitch red fibres the synthesis of the myosin light chains was inhibited and there were no significant changes in their turnover rate. It is possible that the slow-twitch muscle fibres are less sensitive to the action of glucocorticoids on the molecular level. The synthesis of actin was inhibited in all types of muscle fibres in the dexamethasone-treated animals and its turnover rate was slower than that in the control animals. The catabolic effect of glucocorticoids seems to be realized through the augmented alkaline myofibrillar protease activity [23]. On the other hand, it is possible that the myosin heavy chain and actin are more sensitive to the action of alkaline myofibrillar protease than the myosin light chains. As has been pointed out in our earlier papers, the weight reduction in different muscle types in the dexamethasone-treated animals is in full accordance with the augmentation of their myofibrillar protease activity [18, 23]. Although there occurred a significant weight reduction only in the fast-twitch white fibres [18], the intracellular catabolic effect of glucocorticoids was noted in the fast-twitch and slow-twitch red muscle fibres as well.

These studies indicate that the catabolic effect of glucocorticoids on the molecular level of the two main muscle structural proteins, myosin and actin, depend on the type of the muscle. The synthesis rate of actin and the myosin heavy chain was decreased in all types of muscle fibres, and that of the myosin light chain was decreased only in the slow-twitch red muscle fibres. The turnover rate of actin and the myosin heavy chain was decreased in all types of muscle fibres. A more rapid turnover rate of the myosin light chains was registered in the fast-twitch white and fast-twitch red muscle fibres.

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