Hormone-responsive myofibrillar protease activity in cultured rat myoblasts

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The effect of exposure to dexamethasone and serum-deprivation on myofibrillar protease activity was determined by following cleavage of [^{14}C]globin by isolated myofibrils obtained from rat skeletal muscle in culture. Dexamethasone [10^{-7}M] produced a 46% increase in protease activity, and serum-deprivation caused a 50% increase in activity over that of the enzyme in control cultures. The increases in proteolysis occurred concurrently with increased rate of overall protein degradation in these cells and were not associated with changes in cell viability. In cultured rat cardiac muscle cells dexamethasone failed to enhance myofibrillar protease activity, while serum-deprivation produced a 52% increase in the enzyme activity. Addition of insulin (50 mU/ml) to the cultures did not affect proteolysis or myofibrillar protease activity, but completely prevented the dexamethasone-induced increase of these activities. This effect of insulin suggests that the increase of muscle proteolysis in insulin-deficient diabetic animals reflects an enhanced response of the muscle to circulating glucocorticoids rather than a direct effect of insulin-deprivation on muscle proteolysis. Taken together, the present observations indicate that muscle cells in culture retain the ability to respond to catabolic stimuli by adaptive changes in the myofibrillar protease activity in a manner analogous to that of their parent tissue in the intact animal.

Cardiac muscle *Dexamethasone* *Myoblast* *Protease muscle* *Skeletal muscle*

1. INTRODUCTION

Protein degradation in skeletal muscle is regulated by a variety of factors including the nutritional state of the animal [1], availability of certain amino acids [2], insulin [3,4] and glucocorticoid hormones [4,5]. However, the relative importance of specific proteolytic enzymes in the nutritionally- and hormonally-controlled proteolysis is as yet uncertain [6]. We had focused our attention on the myofibrillar alkaline protease of skeletal and cardiac muscle [7,8] particularly because this activity appeared to be associated with the contractile proteins that are degraded. We found that this activity is adaptive and correlates with concomitant changes in muscle proteolysis (e.g., it increased following starvation) in diabetes and after glucocorticoid hormone treatment [7–10].

Using skeletal and cardiac muscle cells in culture we have attempted to establish whether the myofibril-bound alkaline protease is involved in the tissue-specific proteolytic response to glucocorticoids, serum-deprivation and insulin.

2. MATERIALS AND METHODS

Ham F-10 tissue culture medium, fetal bovine serum and horse serum were obtained from GIBCO (Grand Island NY). L[U-^{14}C]Phenylalanine in sterile, aqueous solution, 450 mCi/mmol, and $Na_2{}^{51}CrO_4$ in sterile isotonic solution, 250–500 mCi/mmol, were obtained from the Radiochemical Centre (Amersham). L-Phenylalanine was purchased from Sigma (St Louis MO). Dexamethasone (sodium phosphate) was obtained from Ikapharm (Tel Aviv) and insulin (Actrapid,

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monocomponent, 40 IU/ml) from Novo (Copenhagen).

Neonatal rats of the Hebrew University strain were used as the source of skeletal muscle and cardiac muscle primary culture cells.

The procedure for isolation and culture of myoblast-enriched skeletal and cardiac muscle cells was described in [11], and the cells grown for 5 days. These cultures were composed of over 90% functioning myoblasts and contained no mast cells by microscopical examination [11]. In some experiments 10^{-7} M dexamethasone sodium phosphate was added to the medium on the first day of growth and at every change of medium. When indicated, insulin, 50 mU/ml, was added twice daily for 5 days to the culture. On the fifth day of culture the medium was removed, the cells washed 3 times in medium containing the same amount of dexamethasone or insulin as during the growth period and incubated for an additional 48 h in the medium before harvesting. For the serum-deprivation experiments serum was omitted from the 48 h incubation medium.

The procedure of labeling the cells with radioactive phenylalanine and determination of the rate of proteolysis by release of label in presence of excess (chase) concentrations of non-labeled phenylalanine was previously described in detail [10]. Viability measured by ^{51}Cr retention was found to correlate with viability measured by the trypan blue dye exclusion method ([10] and Chaouat and Mayer, unpublished).

Myofibrillar protease activity of skeletal and cardiac muscle cells was assayed after the cells were grown in culture and incubated for 48 h with the various tested compounds as described above. The cells were harvested and washed 3 times in potassium phosphate buffer, 0.01 M (pH 7.7) containing 0.05 M KCl. For each determination 12×10^6 cells were used. The cells were disrupted by repeated freezing and thawing. The pellet was washed 3 times in the same buffer, the supernatant was discarded and the sediment containing the myofibrillar protease was resuspended in 1 ml of the buffer. Protease activity was determined according to [13] with the modification that ^{14}C-labeled globin was used instead of hemoglobin. The specific activity of the globin substrate was usually around 3×10^5 cpm/mg protein. The enzyme activity was expressed in μg globin degraded.mg protein^{-1}.h^{-1}. Protein was determined according to [14] using bovine albumin as standard. In different experiments different specific activities of the myofibrillar enzyme in control cells were noted. The cause of these fluctuations in enzyme activity is as yet undetermined.

In all experiments statistical analysis was performed by Student's t-test.

3. RESULTS

Table 1 shows that addition of dexamethasone to cultured myoblasts produced a significant increase in the specific activity of [^{14}C]globin-degrading alkaline protease associated with the myofibrillar fraction of skeletal muscle cells. However, dexamethasone had no effect on the same enzyme in cardiac muscle cells. Table 1 also shows that deprivation of serum from the medium for 48 h caused an increase in the alkaline myofibrillar protease activity of both skeletal and cardiac muscle cells in culture. Viability as assessed by ^{51}Cr retention was not affected under these conditions.

The effect of insulin on proteolysis and myofibrillar protease activities in cultured

Table 1

Effect of dexamethasone and serum-deprivation on myofibrillar protease activity of cultured skeletal and cardiac muscle cells

Muscle cells	Control	Dexamethasone	Serum deprivation
	μg globin.mg protein^{-1}.h^{-1}		
Skeletal	125 ± 11	183 ± 15[a]	189 ± 17[a]
Cardiac	126 ± 13	124 ± 12	192 ± 15[a]

[a] Significantly different from control value, $p < 0.01$

Myoblasts were obtained from either hind-thigh skeletal or cardiac muscle and grown in culture for 5 days in presence or absence of 10^{-7} M dexamethasone. Subsequently the cells were washed and incubated for 48 h in presence or absence of either 10^{-7} M dexamethasone or 20% serum. Myofibrillar protease activity was determined by rate of hydrolysis of [^{14}C]globin by myofibrils obtained from the cells after the incubation and is given in mean values ± SEM for 5 plates/group. Viability as assayed by retention of ^{51}Cr was between 87% and 91% in all experimental groups

Table 2

Effect of dexamethasone and insulin on cultured myoblasts

Addition	Proteolysis (%)	Myofibrillar protease activity (μg globin.mg protein^{-1}.h^{-1})	Viability (%)
None	29 ± 2	123 ± 17	86 ± 10
Dexamethasone	53 ± 6*	214 ± 15[a]	80 ± 3
Insulin	27 ± 3	123 ± 13	82 ± 6
Dexamethasone + insulin	28 ± 3	133 ± 10[b]	81 ± 3
		Cardiac muscle myoblasts	
None	35 ± 4	127 ± 8	89 ± 4
Dexamethasone	36 ± 6	138 ± 9	86 ± 7
Insulin	34 ± 2	130 ± 13	86 ± 3
Dexamethasone + insulin	32 ± 6	124 ± 9	87 ± 4

[a] Significantly different from control value, $p < 0.01$

[b] Significantly different from value with dexamethasone alone, $p < 0.01$

Skeletal or cardiac muscle myoblasts were grown in culture in presence or absence of either 10^{-7} M dexamethasone or 50 mU/ml insulin or both for 5 days and subsequently incubated for 48 h in presence or absence of the same compound(s) as in the growth medium to follow proteolysis and viability. Myofibrillar protease activity was determined in myofibrils obtained after the 48 h incubation period. Results are mean values ± SEM for 5 plates/group

myoblasts was determined alone and in combination with dexamethasone. Table 2 shows that insulin, added to the growth medium at 50 mU/ml daily throughout the period of culture did not affect proteolysis, myofibrillar protease activity or viability in skeletal muscle cells. However, insulin completely reversed the dexamethasone-induced enhancement of proteolysis and myofibrillar protease activity in these myoblasts. A 10-fold lower concentration of insulin (5 mU/ml) failed to have any effect on these parameters either when present alone or in combination with dexamethasone (not shown). Table 2 also shows that proteolysis and myofibrillar protease activity in cardiac muscle myoblasts were refractory to 50 mU/ml insulin, either when added alone or in combination with dexamethasone.

4. DISCUSSION

Proteolysis in cultured muscle cells is regulated by the same nutritional and hormonal factors which regulate proteolysis of skeletal and cardiac muscle cells in vivo. Administration of dexamethasone increased protein degradation in skeletal muscle in vivo [7,9] and in vitro [10,11], while cardiac muscle was refractory to the protein wasting effect of glucocorticoid hormones [10,11]. Serum deprivation caused an increase in cell protein degradation in cultured skeletal and cardiac muscle cells [10,11], and starvation of the intact animal enhanced protein catabolism in both tissues to a similar extent [8].

The present results indicate a correlation between overall proteolysis and specific activity of the myofibrillar protease. Specifically, the alkaline protease of cultured muscle cells, measured by rate of degradation of [^{14}C]globin by isolated myofibrils responds to dexamethasone and serum-deprivation by enhanced activity. The same activity in cultured heart cells responds only to serum-deprivation. The analogous reaction of intracellular proteolysis in intact cells and the isolated myofibrillar enzyme to catabolic conditions strongly suggests that the myofibril-bound protease is involved in the tissue-specific response of skeletal and cardiac muscles to catabolic stimuli.

Diabetes is another condition associated with skeletal muscle wasting and a marked increase in the myofibrillar protease activity. Treatment of diabetic animals with insulin is known to reverse the enhanced degradation of skeletal muscle proteins [3,8]. We find here that the enhancement of proteolysis and the increase in myofibrillar protease action induced by glucocorticoids are both reversed by insulin. Apparently, the myofibrillar

protease, by virtue of its adaptive responses to catabolic stimuli, mediates the enhanced degradation of muscle proteins occurring under conditions of muscle protein mobilization. Insulin under these conditions only acts on the induced but not on basal protein degradation and basal myofibrillar protease activity.

The role of the alkaline myofibrillar protease in regulation of intracellular proteolysis in skeletal muscle has been recently challenged by findings that rat peritoneal mast cells contain high activities of a chymotrypsin-like alkaline protease [17–19], and that the muscle enzyme has a structural and immunological similarity to a chymotrypsin-like mast cell enzyme [16,20,21]. Since mast cells are removed from the culture by the selective attachment and continuous washing of the myoblasts [11], the present finding that the myofibrillar protease is an intrinsic component of pure myoblasts in culture, and its responsiveness to mediators which induce muscle wasting and proteolysis, supports the contention that the enzyme is not a contaminant derived from mast cells. In another study (in preparation) the enzyme in culture was found to be refractory to the mast cell degranulator compound 48/80.

The response of the protease in muscle to glucocorticoids is likely to involve binding of the hormone to cytoplasmic receptor sites [22]. Specific glucocorticoid receptors are demonstrable in skeletal muscle and in cultured myoblasts [23,24]. Presence of glucocorticoid-specific binding activity in the cells, the effect of glucocorticoids on proteolysis and the effect of glucocorticoids on myofibrillar protease activity strongly suggest that the steroid-specific receptor system in myoblasts is involved in the proteolytic response of this target tissue to glucocorticoid hormone.

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