Prostaglandin production in myotube cultures

Influence on protein turnover

Mary Ann McELLIGOTT,*§ Lee-Yuh CHAUNG,* Vickie BARACOS† and Eric A. GULVE‡||

*Merck Sharp & Dohme Research Laboratories, Department of Animal Physiology, P.O. Box 2000, Rahway, NJ 07065,

U.S.A., †Department of Animal Science, University of Alberta, Edmonton, Alberta, Canada T6G 2P5, and

‡Department of Physiology and Biophysics, Harvard Medical School, 25 Shattuck Street, Boston, MA 02115, U.S.A.

The production of prostaglandins (PG) E_2 and $F_{2\alpha}$ and their possible role in regulation of protein turnover in cultured skeletal-muscle cells were examined. Primary chick myoblasts and myotubes, and L8 myotubes, produced PGE_2 and $PGF_{2\alpha}$ from endogenous arachidonic acid. PG production by all three cell types was increased manyfold by the addition of exogenous arachidonic acid. Arachidonate-stimulated PG production was inhibited by the addition of indomethacin (0.1 mM). When L8 and chick myotubes were treated with PGE_2 , $PGF_{2\alpha}$, arachidonic acid (0.01 mM) or indomethacin (0.1 mM), no significant alterations in rates of protein synthesis or degradation were observed. Rates of protein synthesis and degradation in these cells were responsive to the addition of 10% fetal-bovine serum under identical experimental conditions. Thus, in contrast with incubated adult skeletal muscle, it appears that the production of prostaglandin metabolites from arachidonic acid is unrelated to regulation of protein turnover in cultured muscle cells.

Recent work has focused on the possible role of prostaglandins (PG) in the regulation of intracellular protein turnover in skeletal muscle in various physiological and pathological states [1–5]. Prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) directly stimulates protein synthesis in incubated skeletal muscle, but does not affect protein degradation [1,3,5], whereas prostaglandin E_2 (PGE_2) enhances rates of protein degradation without affecting protein synthesis [1,4,6]. These incubated muscle preparations also synthesize and release both PGE_2 and $PGF_{2\alpha}$, and this process is stimulated by the addition of arachidonic acid [1,2,4,6,7].

The physiological significance of the role of prostaglandins in the regulation of protein turnover has also begun to be explored. $PGF_{2\alpha}$ appears to be involved in the stimulation of protein synthesis by insulin in muscle from fasted animals [5], and to mediate the stretch-induced increase in protein synthesis in muscle [2,3]. The repletion of muscle protein after disuse atrophy also appears to be a $PGF_{2\alpha}$ -dependent event [8]. PGs are also reported to be involved in the catabolic effect of dexamethasone [2], and the enhanced protein degradation in muscle from rats with endotoxin-induced fever or bacterial infection is associated with an increase in PGE_2 [4,9].

We sought to define further the involvement of PGs in the regulation of muscle protein turnover. Incubated whole-muscle preparations used in previous studies are capable of producing prostaglandins, although it is not established that the mature muscle cells are the site of PG production. Although most of the cells in muscle tissue are skeletal myocytes, the tissue is composed of many other cell types, including endothelial, smooth-muscle and connective-tissue cells [10], which could contribute a disproportionate amount to PG production. We have used a cultured muscle-cell system to circumvent the

heterogeneous nature of whole muscle, in order to examine whether muscle cells are the specific site of PG production. We assessed the capability of both chick primary myoblast and myotube cultures and the L8 line of muscle cells to produce $PGF_{2\alpha}$ and PGE_2 . The effects of $PGF_{2\alpha}$, PGE_2 and arachidonic acid on rates of protein turnover were also determined in the culture system. We provide here the first evidence that differentiated myotubes synthesize PGs, but that alterations in concentrations of PGs do not appear to influence rates of protein turnover in myotubes or L8 muscle cells.

EXPERIMENTAL

Tissue culture

L8 muscle cells (kindly given by Dr. F. J. Roisen) were grown in Dulbecco's Modified Eagle Medium (DMEM) containing 10% (v/v) fetal-bovine serum (FBS), penicillin/streptomycin (final concns. 100 units/ml and 100 µg/ml respectively) and nystatin (100 units/ml; all from Grand Island Biological Co., Grand Island, NY, U.S.A.) in a humidified atmosphere of CO_2 /air (1:19) at 37 °C. Cells were plated in multiwell plates at a density of 3×10^4 cells/cm². Primary chick muscle cultures were prepared from White Leghorn embryos as described previously [11]. Myoblast cultures were used 1 day after plating; myotube cultures were used after at least 5 days in culture, when cells were fully fused.

Protein synthesis

Protein synthesis was measured as described previously [12]. Briefly, cells were rinsed with Hanks' balanced salt solution and incubated for the indicated time period in the presence or absence of the compound to be tested in DMEM (containing an additional 2 mM unlabelled

Abbreviations used: PG, prostaglandin; DMEM, Dulbecco's Modified Eagle Medium; FBS, fetal-bovine serum.

§ To whom reprint requests should be sent. Present address: Roberts Pharmaceutical Corp., Meridian Center III, 6 Industrial Way West, Eatontown, NJ 07724, U.S.A.

|| Present address: Department of Medicine, Washington University School of Medicine, 4566 Scott Avenue, Box 8113, St. Louis, MO 63110, U.S.A.

tyrosine) either alone or supplemented with 1 % FBS. [^3H]-Tyrosine (final concn. $3 \mu\text{Ci}/\text{ml}$; New England Nuclear, Boston, MA, U.S.A.) was added during the final 1 h. The label was washed from the cells by rinsing three times with ice-cold Hanks' balanced salt solution. The 20 %-trichloroacetic acid-precipitable radioactivity was determined. Protein was measured in a small sample by a fluorimetric assay [13]. Protein synthesis was expressed as d.p.m. of [^3H]tyrosine/h per μg of protein or per well.

Protein degradation

Cellular protein was labelled as previously described [14] with [^3H]tyrosine final concn. ($0.2 \mu\text{Ci}/\text{ml}$; New England Nuclear) for the time period indicated. The cultures were rinsed with Hanks' balanced salt solution to remove free label and incubated in DMEM or DMEM plus 1 % FBS, with or without the test compounds. The incubation media contained an additional 2 mM unlabelled tyrosine to prevent re-utilization of the radiolabelled tyrosine (E. A. Gulve & J. F. Dice, unpublished work). The acid-soluble radioactivity was determined at the indicated time, and the radioactivity remaining in the monolayer was determined at the termination of the experiment and the percentage protein degraded during that time period was calculated as previously described [14].

Prostaglandin determination

The cells were incubated for 4 h in DMEM and the indicated test compound. The medium was collected from three wells, centrifuged at $9600 g_{\text{max}}$ for 1 min in a Microfuge to remove cells and cellular debris, and frozen at -80°C until assay. Duplicates of each sample were tested. $\text{PGF}_{2\alpha}$ was determined with a radioimmunoassay kit (Clinical Assay). Owing to the small amounts of PGs produced under basal conditions, we performed the $\text{PGF}_{2\alpha}$ assay with reagents which were 5 times more concentrated, to allow the use of a larger sample size. The appropriate control experiments indicated that this modification did not affect assay accuracy. PGE_2 was measured by radioimmunoassay as previously described [1]. Standards were prepared in phosphate buffer, and linear standard curves were obtained over the range 2–800 pg/sample for PGE_2 . Standard curves run in the presence of DMEM were identical with those obtained with standards in phosphate buffer, indicating that DMEM did not affect the PG assay.

RESULTS

Prostaglandin production

Primary chick myotubes synthesized both PGE_2 and $\text{PGF}_{2\alpha}$ (Fig. 1, Table 1). Production of $\text{PGF}_{2\alpha}$ and PGE_2 in chick primary myotubes was stimulated as early as 1–2 h after the addition of $10 \mu\text{M}$ -arachidonic acid (Fig. 1). The response levelled off and declined after 4 h. Thus the prostaglandin production measured at 4 h in Table 1 was within the range of the curve at which the PG production was increasing. Basal production was less than $1 \text{ pg}/\mu\text{g}$ of protein in 4 h (Table 1). The addition of arachidonic acid ($1 \mu\text{M}$) to these cultures stimulated PG production, and at $10 \mu\text{M}$ greatly increased the formation of both PGE_2 and $\text{PGF}_{2\alpha}$ from the myotubes. Indo-

Fig. 1. Time course of arachidonic acid stimulation of PGE_2 (●) and $\text{PGF}_{2\alpha}$ (○) production

Primary chick myotubes were incubated in DMEM alone (continuous lines) or in DMEM containing $10 \mu\text{M}$ -arachidonic acid (dotted lines). At the indicated time, the media were collected from each well ($n = 3$) for PG determination. PGs produced are expressed as $\text{pg}/\mu\text{g}$ of protein. Data points are means $\pm$ S.E.M.. Error bars not shown are included within the data point.

methacin (0.1 mM) inhibited PG production, owing to the addition of exogenous arachidonic acid.

Primary cultures of chick myoblasts produced both $\text{PGF}_{2\alpha}$ and PGE_2 (Table 1). Cells at this stage produced about the same quantities of PGs, on the basis of cellular protein, as the cells after fusion. Arachidonic acid ($10 \mu\text{M}$) significantly stimulated PGE_2 production and the formation of $\text{PGF}_{2\alpha}$. This effect was decreased by indomethacin.

Rat L8 myotubes also had the capacity to produce both $\text{PGF}_{2\alpha}$ and PGE_2 under basal conditions. The addition of arachidonic acid to the incubation media resulted in a large significant stimulation of the production of both PGs in the medium. Both basal and arachidonic acid-stimulated PG production were indomethacin-sensitive.

Protein synthesis

Protein synthesis in cultured myotubes was stimulated by the addition of FBS (Table 2), as it is in many other cell types in culture. To determine whether arachidonic acid or its metabolites had a direct effect on muscle cells in culture, we looked at the response of cells incubated in DMEM in which rates of protein synthesis were basal, and also in cells incubated in 1 % FBS, in which protein synthesis was slightly enhanced. The cells were in positive nitrogen balance, and protein accumulation increased by 25–54 % in L8 and chick primary myotubes incubated in the presence of 1 % FBS for 2 days. The latter condition was examined in case PGs do not directly stimulate basal rates of protein synthesis, but do enhance already stimulated rates, e.g. by interacting with factors present in serum. Table 3 showed that rates of protein synthesis in the presence or absence of 1 % FBS were unaltered by arachidonic acid, or its metabolites $\text{PGF}_{2\alpha}$ or PGE_2 . Indomethacin also did not affect rates of protein synthesis.

Table 1. Production of PGE₂ and PGF_{2α} by muscle cells

Cells were incubated with the indicated additions for 4 h and the PG released into the media determined. Abbreviations: AA, arachidonic acid; Indo, indomethacin; ND, not detectable (samples contained less than 4 pg/ml). Results are means $\pm$ S.E.M. ($n = 3$). Significance: ^a $P < 0.05$, ^b $P < 0.01$, ^c $P < 0.001$ versus no additions; ^a $P < 0.05$, ^b $P < 0.01$, ^c $P < 0.001$ versus appropriate control with no indomethacin.

Additions to DMEM	PGE ₂ produced (pg/ μ g of protein)		
	Chick myoblasts	Chick myotubes	L8 myotubes
—	0.081 $\pm$ 0.001	0.01 $\pm$.01	0.34 $\pm$ 0.04
AA (1 μ M)	0.4 $\pm$ 0.1 ^b	0.15 $\pm$ 0.02 ^b	29.7 $\pm$ 7.3 ^a
AA (10 μ M)	114.2 $\pm$ 12.6 ^c	50.0 $\pm$ 1.7 ^c	56.1 $\pm$ 14.9 ^a
Indo (0.1 mM)	ND	ND	ND
AA (1 μ M)/Indo (0.1 mM)	ND	ND	ND
AA (10 μ M)/Indo (0.1 mM)	ND	ND	ND

	PGF _{2α} produced (pg/ μ g of protein)		
	Chick myoblasts	Chick myotubes	L8 myotubes
—	0.2 $\pm$ 0.1	0.6 $\pm$ 0.3	2.9 $\pm$ 0.4
AA (1 μ M)	6.0 $\pm$ 0.2 ^c	12.2 $\pm$ 1.2 ^c	62.0 $\pm$ 6.5 ^c
AA (10 μ M)	23.0 $\pm$ 4.1 ^b	38.8 $\pm$ 3.4 ^c	288.6 $\pm$ 31.7 ^c
Indo (0.1 mM)	0.4 $\pm$ 0.5	1.6 $\pm$ 0.6	0.5 $\pm$ 0.1 ^b
AA (1 μ M)/Indo (0.1 mM)	3.0 $\pm$ 0.2 ^f	1.2 $\pm$ 0.4 ^f	4.5 $\pm$ 0.1 ^f
AA (10 μ M)/Indo (0.1 mM)	6.7 $\pm$ 0.6 ^d	8.4 $\pm$ 3.6 ^e	117.0 $\pm$ 7.3 ^e

Table 2. Effect of FBS on protein turnover in muscle cells

Cells were incubated in DMEM with or without FBS at the indicated concentration. Protein synthesis and protein degradation were measured as described in the text. The percentage increase in protein synthesis (after 6.5 h in serum) above the value in DMEM alone is presented. The percentage decrease in protein degradation in the presence of serum compared with DMEM alone is presented. Cells were labelled for 1 day and the protein degraded in 19 h was determined. Significance versus DMEM with no serum, determined by Students' *t* test: ^a $P < 0.05$; ^b $P < 0.01$; ^c $P < 0.001$.

FBS	Increase in protein synthesis (%)		Decrease in protein degradation (%)	
	Chick myotubes	L8 myotubes	Chick myotubes	L8 myotubes
1 %	17.9	21.7 ^b	4.1	4.3
10 %	40.4 ^a	36.8 ^c	24.1 ^c	19.2 ^b

Protein degradation

Serum contains factors which inhibit protein degradation in primary muscle-cell cultures [14] and L8 myotubes (Table 2). FBS (10 %) significantly inhibited proteolysis in these experiments; at 1 % it retarded proteolysis slightly, but the concentration was not optimal. Rates of protein degradation both in chick primary myotubes and in L8 muscle cultures were not affected by arachidonic acid, PGF_{2α}, PGE₂ or indomethacin in the presence or absence of 1 % FBS (Table 4).

DISCUSSION

Our data demonstrate for the first time that differentiated cultured muscle cells produce prostaglandins. However, the stimulation of PG production in cultured muscle cells, unlike in incubated muscle preparations [1–6], did not appear to influence rates of protein turnover. PG production occurred under basal conditions in these muscle cells. Primary chick myotubes, myoblasts and L8 rat myotubes produced less than 3 pg of both PGE₂ and PGF_{2α}/4 h per μ g of cellular protein. Since muscle and other tissues do not store PGs, release into the extracellular medium was a valid measure of production [15]. Thus these muscle cells in culture contained the full complement of enzymes involved in the synthesis of PGs. It is possible that an effect in primary cell cultures could be due to contaminating fibroblasts. Fibroblast contamination in our preparation of muscle cultures is negligible, however [11]. The fact that the L8 myotubes (a continuous line of pure muscle cells) produced similar amounts of PGs to the primary cultures confirms that muscle cells are a site of PG production. Interestingly, the differentiated myotubes appeared to produce prostaglandins in much larger quantities than do incubated muscle preparations [1]. This large capacity of the cultured muscle cells to produce PGs suggests that the predominant site of PG production in incubated muscle preparations was most likely the muscle cells.

Addition of arachidonic acid, the precursor of PG synthesis, resulted in a manyfold stimulation of the production of both PGF_{2α} and PGE₂ in cultured myotubes, revealing that these cells have substantial abilities to modulate their PG output. The cyclooxygenase inhibitor indomethacin (0.1 mM) lowered the production of both PGs from arachidonic acid under

Table 3. Effect of PGs on protein synthesis

Chick myotubes were incubated for 6 h and L8 cells for 6.5 h with the test compounds; 10 μ M-PG is equivalent to adding 3.5×10^{-6} pg per well. Key: (+), 1% FBS; (–), DMEM. Results are means $\pm$ S.E.M. ($n = 4$). Significance: * $P < 0.05$ versus DMEM.

Addition to DMEM	Protein synthesis (d.p.m./h per μ g of protein)			
	Chick myotubes		L8 myotubes	
	(–)	(+)	(–)	(+)
—	11.3 $\pm$ 1.6	12.6 $\pm$ 2.1	14.7 $\pm$ 0.7	19.3 $\pm$ 1.6*
PGF _{2α} (10 μ M)	10.5 $\pm$ 2.3	12.8 $\pm$ 0.6	16.3 $\pm$ 0.5	18.5 $\pm$ 0.7
PGE ₂ (10 μ M)	11.1 $\pm$ 0.7	11.4 $\pm$ 1.0	16.5 $\pm$ 0.8	17.5 $\pm$ 0.4
Arachidonic acid (10 μ M)	12.0 $\pm$ 2.1	13.0 $\pm$ 1.0	16.9 $\pm$ 0.7	18.4 $\pm$ 1.2
Indomethacin (0.1 mM)	10.5 $\pm$ 1.1	9.7 $\pm$ 1.0	15.2 $\pm$ 1.2	21.2 $\pm$ 1.1*

Table 4. Effect of PGs on protein degradation

In separate experiments, cells were labelled for 1 day, then chick myotubes were incubated for 19 h and L8 myotubes for 18 h in the presence of the test compound, and percentage of total radioactive material degraded during this period was determined; 10 μ M-PG is equivalent to adding 3.5×10^{-6} pg per well. Results are means $\pm$ S.E.M. ($n = 4$). Key: (–), DMEM; (+), 1% FBS.

Additions to DMEM	Protein degradation (% of radioactivity degraded)			
	Chick myotubes		L8 myotubes	
	(–)	(+)	(–)	(+)
—	50.4 $\pm$ 1.7	45.3 $\pm$ 1.4	45.0 $\pm$ 5.7	41.6 $\pm$ 2.3
PGF _{2α} (10 μ M)	52.6 $\pm$ 4.1	47.2 $\pm$ 3.6	44.0 $\pm$ 1.0	44.9 $\pm$ 4.6
PGE ₂ (10 μ M)	54.2 $\pm$ 1.5	49.9 $\pm$ 1.7	44.6 $\pm$ 3.6	43.2 $\pm$ 2.1
Arachidonic acid (10 μ M)	53.3 $\pm$ 3.3	48.3 $\pm$ 2.1	43.4 $\pm$ 2.2	40.1 $\pm$ 3.3
Indomethacin (0.1 mM)	48.7 $\pm$ 2.1	47.5 $\pm$ 6.5	40.9 $\pm$ 0.6	39.4 $\pm$ 2.1

these experimental conditions. The ability of both L8 and primary cultures to produce PGs, as well as to respond to arachidonic acid by increasing production, suggests that the arachidonic acid metabolism cascade in L8 myotubes is normal.

Rates of protein turnover in these cultured muscle cells were not affected by either PGE₂ or PGF_{2 α} , even in the presence of large exogenous amounts of PGs which were 3500–35000 times the basal amount produced by these cells. The influence of PGs on protein turnover was examined in two different states: a positive growth condition, in the presence of serum, with enhanced rates of protein synthesis and decreased rates of protein degradation; and in step-down conditions, after withdrawal of FBS, so that rates of protein synthesis were decreased to basal values and proteolysis was enhanced as a result of activation of lysosomal pathways [16,17]. Sub-optimal amounts of FBS (1%) were used in the positive-growth studies, since a maximal concentration of serum might obscure effects of these compounds. Tables 3 and 4 show that, under both of these conditions, rates of protein synthesis and degradation were equivalent in the presence or absence of PGs, arachidonic acid or indomethacin.

It is interesting to discover that arachidonic acid and

its metabolites affect protein turnover in one model muscle system (incubated muscle), but not in another (cultured muscle cells), even though the arachidonic acid cascade is functional in both. Upon close examination, however, the findings in incubated muscle studies are not always consistent. PGs appear to control rates of degradation under some conditions, but not others. A PGE₂-mediated mechanism was implicated in the stimulation of muscle proteolysis during endotoxin-induced fever and bacterial infection [4,9], and the enhanced breakdown occurring in incubated muscle from these treated rats was blocked by indomethacin. However, rates of protein degradation in burned muscle were significantly elevated, but were not associated with an increase in PGE₂ release [18]. Indomethacin at a concentration sufficient to block PG production in those muscles did not affect protein breakdown [18]. *Pseudomonas aeruginosa* experimental sepsis is characterized by a large increase in PGE₂ production, but only a slight elevation in protein breakdown [19]. Barnett & Ellis [20] recently reported that mouse and rat muscle incubated under a wide variety of conditions did not respond to bioactive PGE₂.

A definitive role for PGF_{2 α} in modulating rates of protein synthesis in incubated muscle is also lacking.

PGF_{2α} stimulates protein synthesis by 35% in the diaphragm but not the soleus muscle [1], whereas arachidonic acid in these same experiments significantly stimulates protein synthesis in the soleus but not in the diaphragm muscle. Insulin stimulates protein synthesis and PGF_{2α} production in gastrocnemius, plantaris and soleus muscles of fasted rats, but indomethacin only blocks this effect in the first two muscles but not in the last [21]. PGF_{2α} appears to be involved in the stretch-induced increase in protein synthesis in skeletal muscle [3], since the effects were blocked by inhibitors of prostaglandin synthesis, but does not play a role in the increase in protein synthesis owing to stretch of the ventricular wall [22]. These different responses in the incubated-muscle experiments suggest that the role of PGs in regulating protein turnover may be quite complex.

Reasons for the lack of an effect of PGs on protein turnover in muscle cell cultures remain to be determined. Possibly additional cell types present in a whole muscle are necessary for regulation of protein turnover. PG production in the muscle cells may stimulate the production of local factors in neighbouring cells, which may regulate the responses of muscle cells in a paracrine manner. The absence of other cell types in cultured muscle cells may explain the presence of a well-developed eicosanoid cascade but a short-circuited activation of protein turnover. Alternatively, muscle cells in culture, although differentiated, do not express all characteristics of mature muscle *in vivo*, e.g. the adult isoenzyme of myosin is not synthesized in these embryonic cells [23]. The mechanisms for PG modulation of protein turnover may be characteristic of adult muscle but lacking in cultured muscle cells.

Whether the role of prostaglandins in the regulation of rates of protein turnover is of physiological significance, or whether their presence is crucial to some other physiological mechanism, remains to be determined. In view of the large capacity of differentiated muscle cells to modulate rates of PG production, it appears likely that there is a function for the arachidonic acid cascade in muscle.

We thank Dr. Yi-Tien Yang for critical reading of the manuscript. Mr. Albert Barreto for preparation of muscle cell cultures, Mr. Allan Mak for technical assistance and Ms. Karen G. Rosa for preparation of the manuscript.

REFERENCES

1. Rodemann, H. P. & Goldberg, A. L. (1982) *J. Biol. Chem.* **257**, 1632–1638
2. Reeds, P. J. & Palmer, R. M. (1984) *Biochem. J.* **219**, 953–957
3. Smith, R. H., Palmer, R. M. & Reeds, P. J. (1983) *Biochem. J.* **214**, 153–161
4. Baracos, V., Rodemann, H. P., Dinarello, C. A. & Goldberg, A. L. (1983) *New Engl. J. Med.* **308**, 553–558
5. Reeds, P. J. & Palmer, R. M. (1983) *Biochem. Biophys. Res. Commun.* **116**, 1084–1090
6. Goldberg, A. L., Baracos, V., Rodemann, P., Waxman, L. & Dinarello, C. (1984) *Fed. Proc. Fed. Am. Soc. Exp. Biol.* **43**, 1301–1306
7. Nowak, J., Bohman, S.-O., Alster, P., Berlin, T. Cronstrand, R. & Sonnenfeld, T. (1983) *Prostaglandins Leukotrienes Med.* **11**, 269–279
8. Templeton, G. H., Padalino, M. & Moss, M. (1986) *Prostaglandins* **31**, 545–559
9. Fagan, J. M. & Goldberg, A. L. (1985) in *The Physiological, Metabolic and Immunologic Actions of Interleukin-1* (Kluger, M. J., Oppenheim, J. J. & Powanda, M. C., eds.), pp. 201–210, Alan R. Liss, New York
10. Enesco, M. & Puddy, D. (1964) *Am. J. Anat.* **114**, 235–244
11. McElligott, M. A., Roisen, F. J., Keaton, K. S., Trimer, D. L., Li, Q.-S., St. John A. C., Yorke, G. & Bird, J. W. C. (1981) *Acta Biol. Med. Germ.* **40**, 1333–1347
12. McElligott, M. A. & Dice, J. F. (1983) *Biochem. J.* **216**, 559–566
13. Bohlen, P., Stein, S., Dairman, W. & Udenfriend, S. (1973) *Arch. Biochem. Biophys.* **155**, 213–220
14. McElligott, M. A. & Dice, J. F. (1983) *Exp. Cell Res.* **150**, 442–451
15. Palmer, R. M., Reeds, P. J., Atkinson, T. & Smith, R. H. (1983) *Biochem. J.* **214**, 1011–1014
16. McElligott, M. A., Miao, P. & Dice, J. F. (1985) *J. Biol. Chem.* **260**, 11986–11993
17. Janeczko, R. A., Carriere, R. M. & Etlinger, J. D. (1985) *J. Biol. Chem.* **260**, 7051–7058
18. Odessey, R. (1985) *Metab. Clin. Exp.* **34**, 616–620
19. Turisky, J. & Loegering, D. J. (1985) *Biochim. Biophys. Acta* **840**, 137–140
20. Barnett, J. G. & Ellis, S. (1987) *Muscle Nerve* **10**, 556–559
21. Reeds, P. J., Jay, S. M., Glennie, R. T., Mackie, W. S. & Garlick, P. J. (1985) *Biochem. J.* **227**, 255–261
22. Smith, D. M. & Sugden, P. H. (1987) *Biochem. J.* **243**, 473–479
23. Whalen, R. G., Schwartz, K., Bouveret, P., Sell, S. M. & Gros, F. (1979) *Proc. Natl. Acad. Sci. U.S.A.* **76**, 5197–5201

Received 9 February 1988; accepted 22 March 1988