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Prostaglandin E_2 does not regulate total or myofibrillar protein breakdown in incubated skeletal muscle from normal or septic rats

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The role of prostaglandins in the regulation of muscle protein breakdown is controversial. We examined the influence of arachidonic acid (5 μM), prostaglandin E_2 (PGE_2) (2.8 μM) and the prostaglandin-synthesis inhibitor indomethacin (3 μM) on total and myofibrillar protein breakdown in rat extensor digitorum longus and soleus muscles incubated under different conditions *in vitro*. In other experiments, the effects of indomethacin, administered *in vivo* to septic rats (3 mg/kg, injected subcutaneously twice after induction of sepsis by caecal ligation and puncture) on plasma levels and muscle release of PGE_2 and on total and myofibrillar protein breakdown rates were determined. Total and myofibrillar proteolysis was assessed by measuring production by incubated muscles of tyrosine and 3-methylhistidine respectively. Arachidonic acid or PGE_2 added during incubation of muscles from normal rats did not affect total or myofibrillar protein degradation under a variety of different conditions *in vitro*. Indomethacin inhibited muscle PGE_2 production by incubated muscles from septic rats, but did not lower proteolytic rates. Adminstration *in vivo* of indomethacin did not affect total or myofibrillar muscle protein breakdown, despite effective plasma levels of indomethacin with decreased plasma PGE_2 levels and inhibition of muscle PGE_2 release. The present results suggest that protein breakdown in skeletal muscle of normal or septic rats is not regulated by PGE_2 or other prostaglandins.

INTRODUCTION

Prostaglandin E_2 (PGE_2) has been proposed to be an important regulator of muscle protein breakdown. Rodemann & Goldberg [1] reported that addition either of arachidonic acid, the common precursor of prostaglandins, or of PGE_2 to incubated rat skeletal muscle resulted in increased protein degradation. In other experiments in the same laboratory, Baracos *et al.* [2] noted that the prostaglandin-synthesis inhibitor indomethacin blocked the stimulation of both PGE_2 production and protein breakdown induced by leucocytic pyrogen. In more recent experiments, Tian & Baracos [3] found that administration of the prostaglandin synthesis inhibitor naproxen to *Escherichia coli*-infected animals decreased PGE_2 production and net protein degradation in skeletal muscle. From these observations it was proposed that cyclo-oxygenase inhibitors may be useful in the treatment of negative nitrogen balance during fever and infection [1–3].

The role of PGE_2 in muscle proteolysis, however, is controversial, and conflicting results have been reported. Thus Barnett & Ellis [4] were unable to confirm increased protein degradation when arachidonic acid or PGE_2 was added to incubated rat or mouse muscles. In other studies, indomethacin effectively blocked PGE_2 production in muscle from burned rats, without affecting the elevated protein breakdown rates [5,6]. In recent experiments in our laboratory, administration of indomethacin *in vivo* or addition of the substance to incubated muscles from septic rats inhibited PGE_2 production, but did not decrease muscle proteolysis [7,8].

In previous studies of the possible role of PGE_2 in the regulation of protein turnover, protein breakdown rate was assessed as release of tyrosine by incubated muscles. Although tyrosine release adequately reflects total protein degradation, it does not distinguish between the breakdown of total and myofibrillar proteins. This distinction is important, because sepsis mainly stimulates myofibrillar protein breakdown, measured as release of 3-methylhistidine (3-MH) [9]. Previous studies demonstrated that non-myofibrillar and myofibrillar protein breakdown rates in skeletal muscle are regulated independently, and that changes in myofibrillar degradation can be undetected if only total proteolysis is measured [10,11]. Thus, in studies designed to examine the role of PGE_2 in muscle proteolysis during sepsis and other catabolic conditions, measurement of myofibrillar protein breakdown is of particular importance. The influence of prostaglandins on myofibrillar protein breakdown is not known, either in normal or in septic muscle.

In the present study, the effect of prostaglandins on total and myofibrillar protein breakdown was examined by adding arachidonic acid or PGE_2 to incubated muscles from normal untreated rats. In other experiments, we tested the hypothesis that inhibition of PGE_2 production by indomethacin decreases protein breakdown in muscle from septic rats.

MATERIALS AND METHODS

Three series of experiments were performed. In the first series of experiments, the effects *in vitro* of arachidonic acid (5.0 μM) or PGE_2 (2.8 μM) on total and myofibrillar protein breakdown were tested in incubated muscles from normal fed male Sprague–Dawley rats (40–60 g).

In the second series of experiments, the effects *in vitro* of the cyclo-oxygenase inhibitor indomethacin (3 μM) on total and myofibrillar protein breakdown rates were tested in incubated muscles from sham-operated or septic rats. Sepsis was induced by caecal ligation and puncture (CLP) in male Sprague–Dawley rats (40–60 g) as described previously [12]. Control rats were sham-operated. All animals were resuscitated with 5 ml of normal saline/100 g body wt. injected subcutaneously in the back at the time of the operative procedure, and were fasted with free access to water until muscles were harvested (16 h after sham-operation or CLP).

In the third series of experiments, CLP was performed as described above and one group of rats was treated with indo-

Abbreviations used: PGE_2, prostaglandin E_2; 3-MH, 3-methylhistidine; CLP, caecal ligation and puncture; EDL, extensor digitorum longus; SOL, soleus.

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methacin (3 mg/kg dissolved in 1 ml of 0.1 M-$NaHCO_3$/kg body wt.), injected subcutaneously at the time of CLP immediately after closure of the abdomen and again after 3 h, and another group of rats received corresponding volumes of $NaHCO_3$. Plasma levels of indomethacin and PGE_2 were determined 8 and 16 h after CLP. PGE_2 release and protein breakdown rates in incubated muscles were measured 16 h after CLP.

The time point for metabolic studies was chosen on the basis of previous experiments in which we found that muscle protein breakdown, in particular myofibrillar protein degradation, was increased 16 h after CLP in rats [9,12]. Arachidonic acid, PGE_2 and indomethacin were purchased from Sigma Chemical Co., St. Louis, MO, U.S.A.

Muscle incubations

Extensor digitorum longus (EDL) and soleus (SOL) muscles were dissected with intact tendons with the rats under diethyl ether anaesthesia. EDL is a white fast-twitch muscle, and SOL is a red slow-twitch muscle [13]. Both muscles were studied in most of the experiments, because regulation of protein turnover by different substances and conditions may be different in red and white muscle [14]. Unless stated otherwise, the muscles were fixed at approximate resting length to stainless-steel supports, weighed and preincubated for 30 min in 3 ml of oxygenated (O_2/CO_2, 19:1) Krebs–Henseleit [14a] bicarbonate buffer (pH 7.4) with 10 mM-glucose. The incubations were performed in a shaking water bath at 37 °C unless stated otherwise. After preincubation, muscles were gently blotted and transferred to 3 ml of fresh medium of the same composition as described above with the addition of 0.5 mM-cycloheximide and incubated for 2 h. In experiments in which the effects *in vitro* of arachidonic acid, PGE_2 or indomethacin were studied, the substance was added to the incubation medium at the onset of the 2 h incubation in 30 μl of 50% (v/v) ethanol, and the same volume of ethanol was added to the medium of the contralateral muscle. In some experiments, the incubation medium was supplemented with 10 mM-glucose, 0.1 unit of insulin/ml, 0.85 mM-leucine, 0.5 mM-isoleucine and 1.0 mM-valine, as described by Fulks *et al.* [15]. When protein synthesis was studied, and in some experiments when protein breakdown was measured, cycloheximide was omitted from the incubation medium.

Protein synthesis and breakdown

For the study of protein synthesis, the incubation medium contained [^{14}C]phenylalanine (0.05 μCi/ml; 0.5 mM), and the amount of phenylalanine incorporated into trichloroacetic acid-precipitated proteins was determined as described in detail elsewhere [16].

Total and myofibrillar protein breakdown rates were determined by measuring free tyrosine and 3-MH respectively. When paired muscles were used, the total amount of free tyrosine and 3-MH in muscle and incubation medium at the end of the 2 h incubation was calculated. Because the muscles were preincubated under identical conditions, tissue levels of the amino acids at the start of the 2 h incubation were presumed to be the same in the paired muscles. Consequently, any differences in total amount of tyrosine and 3-MH in muscle and medium at the end of incubation should reflect differences in protein breakdown rates between the paired muscles. In experiments in which indomethacin was administered *in vivo*, protein breakdown rates in muscles from different groups of rats were studied. In these experiments, tissue levels of tyrosine and 3-MH were determined at both the start and the end of incubation, and actual breakdown rates were determined from release of the amino acids into the incubation medium, corrected for changes in tissue levels during incubation as described in detail elsewhere [17]. Tyrosine and 3-MH in incubation medium and muscle extracts were determined by h.p.l.c. as reported previously [9,17].

PGE_2 and indomethacin

PGE_2 production by incubated muscles was determined by measuring PGE_2 released into incubation medium by radioimmunoassay as described previously [1]. The same assay was used for measurement of plasma PGE_2. Indomethacin was determined in incubation medium or plasma by h.p.l.c. as described by Bernstein & Evans [18].

Statistics

Results are presented as means ± S.E.M. Student's *t* test for paired or unpaired differences was used for statistical comparisons.

RESULTS

Arachidonic acid (5.0 μM) or PGE_2 (2.8 μM) added to incubated EDL or SOL muscles from normal rats did not affect total or myofibrillar protein breakdown rates (Table 1). One reason why the substances did not influence protein breakdown in the muscles could be that the concentration of the drugs in the incubation medium was much lower than expected, owing to either degradation or poor solubility. Control experiments were therefore performed in which PGE_2 levels were determined in medium after 2 h of incubation of EDL muscles to which PGE_2 (2.8 μM) had been added. The concentration was 2.71 ± 0.20 μM ($n = 3$), suggesting that the substance was relatively stable during the incubation.

Rodemann & Goldberg [1] reported that the addition of arachidonic acid to muscles incubated at 33 °C resulted in a greater increase in proteolysis than at 37 °C. We therefore also tested the effects of arachidonic acid and PGE_2 in EDL and SOL muscles incubated at 33 °C. Again, however, neither total nor myofibrillar protein breakdown rates were affected by the substances (Table 1). The apparently higher protein breakdown rates, in particular total protein breakdown rates, noted in muscles incubated at 33 °C probably reflect day-to-day variation in protein turnover rates.

Another reason why the present results differ from those reported previously may be that different conditions *in vitro* were used. In order to duplicate the conditions used by Rodemann & Goldberg [1], we incubated flaccid SOL muscles (i.e. not tied to supports) in medium supplemented with insulin (0.1 unit/ml) and branched-chain amino acids at 5 times normal plasma concentrations. This contrasts with most of the other experiments in the present study, in which muscles were incubated at resting length in an unsupplemented medium. Because the presence of a relatively high (mM) concentration of other amino acids in the incubation medium interferes with the 3-MH assay (P.-O. Hasselgren, J. H. James & J. E. Fischer, unpublished work), only total protein breakdown could be assessed when the supplemented medium was used. Arachidonic acid (5.0 μM) or PGE_2 (2.8 μM) did not affect protein degradation in this preparation *in vitro*. The total amount of free tyrosine at the end of incubation was 302 ± 30 and 307 ± 25 nmol/g when seven paired SOL muscles were incubated without or with arachidonic acid respectively (not significant). The corresponding values when muscles were incubated in the absence or presence of PGE_2 were 258 ± 15 and 280 ± 21 nmol/g ($n = 7$ paired muscles; not significant).

We next studied the effect of indomethacin, added at a concentration of 3 μM to incubated EDL muscles from sham-operated or septic rats. Both total and myofibrillar protein breakdown rates were unaffected by the prostaglandin synthesis

Table 1. Effect of arachidonic acid (5.0 μM) or PGE_2 (2.8 μM) on total and myofibrillar protein breakdown in incubated EDL and SOL muscles from normal untreated rats

Paired EDL and SOL muscles from normal untreated rats were preincubated for 30 min as described in the Materials and methods section. After preincubation, muscles were incubated for 2 h with or without the substances indicated in the Table. After incubation, total amounts (nmol/g) of tyrosine and 3-MH in medium and tissue were determined. Incubations were performed at 37 °C or 33 °C as indicated: (−) indicates muscle incubated without addition of arachidonic acid or PGE_2, but with addition of 30 μl of solvent (50% ethanol); (+) indicates contralateral muscle incubated with arachidonic acid or PGE_2; no difference between (−) and (+) was significant. A minimum of seven paired muscles was used in each experiment.

	Temp.	Muscle	Arachidonic acid (−)	Arachidonic acid (+)	PGE_2 (−)	PGE_2 (+)
Tyrosine	37 °C	EDL	144±11	137±5	164±15	142±10
		SOL	203±11	187±10	178±17	180±13
3-MH	37 °C	EDL	4.07±0.21	4.17±0.24	4.14±0.17	3.89±0.18
		SOL	4.96±0.44	4.82±0.57	5.64±0.24	5.42±0.31
Tyrosine	33 °C	EDL	220±8	220±7	228±10	234±4
		SOL	297±9	277±8	286±11	288±5
3-MH	33 °C	EDL	5.37±0.30	5.57±0.45	4.99±0.53	5.34±0.29
		SOL	6.42±0.31	5.74±0.33	6.35±0.38	7.20±0.41

Table 2. Effect of indomethacin (3 μM) on total and myofibrillar protein breakdown in incubated EDL muscles from sham-operated control rats or septic rats

Paired EDL muscles, harvested from rats 16 h after sham-operation (control muscles) or CLP (septic muscles), were preincubated for 30 min and then incubated for 2 h in the absence (−) or presence (+) of indomethacin. Total amounts (nmol/g) of tyrosine and 3-MH in muscle and medium were determined after the 2 h incubation; no difference between (−) and (+) was significant. Results are from seven paired muscles in each group.

	Control muscles (−)	Control muscles (+)	Septic muscles (−)	Septic muscles (+)
Tyrosine	307±15	305±17	557±40	568±40
3-MH	5.30±0.24	5.26±0.36	11.27±0.99	10.59±0.56

Table 3. Effect of indomethacin (3 μM) on protein synthesis and total and myofibrillar protein breakdown in incubated EDL muscles from septic rats

Paired EDL muscles from septic rats (16 h after CLP) were preincubated for 30 min and then incubated for 2 h in the absence (−) or presence (+) of indomethacin. Cycloheximide was omitted from the incubation medium. At the end of the 2 h incubation, the amount of [^{14}C]phenylalanine incorporated into trichloroacetic acid-precipitated proteins was determined. Total and myofibrillar protein breakdown rates were assessed as release of tyrosine and 3-MH respectively into the incubation medium; no difference between (−) and (+) was significant. Results are from seven paired muscles.

	Amino acid incorporated or released (nmol/2 h per g)	
Indomethacin...	(−)	(+)
Phenylalanine	148±9	150±10
Tyrosine	411±25	405±12
3-MH	2.97±0.22	2.84±0.09

inhibitor (Table 2). Measurement of indomethacin in the incubation medium of EDL muscles ($n = 7$) from septic rats showed that the concentration was 2.21±0.11 μM immediately after addition of the substance and 2.10±0.18 μM at the end of the 2 h incubation, which was approx. 70% of the expected concentration. This may reflect incomplete solubility of indomethacin. However, the indomethacin concentration achieved was high enough to decrease PGE_2 production by incubated septic EDL muscles ($n = 7$ paired muscles) from 46.0±8.9 to 19.3±3.7 ng/2 h per g (−58%; $P < 0.01$), similar to a previous report [7].

Cycloheximide (0.5 mM) was added to the incubation medium in the present study to prevent re-incorporation of amino acids after their release during proteolysis. Because cycloheximide can inhibit muscle PGE_2 production [6], it may be argued that the effect of a PGE_2-synthesis inhibitor cannot be detected when it is added to a medium containing cycloheximide. We therefore also tested the effect of indomethacin (3 μM) on amino acid release into incubation medium from seven paired septic EDL muscles incubated without cycloheximide. Protein synthesis was measured by determining incorporation of [^{14}C]phenylalanine into protein in the same muscles. Indomethacin did not affect tyrosine or 3-MH release or protein synthesis (Table 3), suggesting that total or myofibrillar protein breakdown was not affected by indomethacin in the absence of cycloheximide. Protein synthesis was determined in this experiment, since unaltered release of amino acids in the absence of cycloheximide could be consistent with a simultaneous inhibition of both protein breakdown and synthesis. Since the muscles were used for determination of protein synthesis, tissue levels of free tyrosine and 3-MH were not measured, but protein breakdown rates were assessed from the release into incubation medium only. This explains why the amounts of tyrosine and 3-MH reported in this experiment were lower than in the preceding experiments using septic muscles (compare Tables 3 and 2).

When indomethacin (3 mg/kg) was administered *in vivo* to septic rats at the time of CLP and again after 3 h, plasma concentrations of the substance were 30.5±3.2 μM and 21.5±3.8 μM at 8 h ($n = 5$) and 16 h ($n = 5$) after CLP respectively. These plasma concentrations are above therapeutic levels reported in man [19]. The plasma level of PGE_2 in control rats 16 h after sham-operation ($n = 8$) was 138±45 pg/ml. In septic animals, plasma PGE_2 levels were increased more than 2-fold as early as 8 h after CLP (Table 4). In septic animals treated with indomethacin, PGE_2 was not detectable in plasma 8 h or 16 h after CLP. Release of PGE_2 by incubated muscles 16 h after

Table 4. Effect of indomethacin, administered to septic rats, on plasma PGE_2 levels and muscle release of PGE_2, tyrosine and 3-MH

Plasma PGE_2 levels were determined 8 h and 16 h after CLP in rats treated with indomethacin (3 mg/kg) at the time of CLP and again after 3 h or treated with corresponding volumes of vehicle. EDL muscles were harvested from rats 16 h after CLP and incubated for determination of PGE_2 production or total (i.e. tyrosine release) and myofibrillar (i.e. 3-MH release) protein breakdown rates, as described in the Materials and methods section. N.S., not significant; N.D., not detectable; $n = 7$ or 8 for each mean value.

	Plasma PGE_2 (pg/ml)		Muscle release of:		
	8 h	16 h	PGE_2 (ng/2 h per g)	Tyrosine (nmol/2 h per g)	3-MH (nmol/2 h per g)
CLP + vehicle	315 ± 44	311 ± 110	46.6 ± 4.6	500 ± 19	6.87 ± 0.59
CLP + indomethacin	N.D.	N.D.	31.7 ± 2.6	497 ± 65	7.47 ± 1.62
			$P < 0.01$	N.S.	N.S.

sham-operation ($n = 8$) was 29.2 ± 2.8 ng/2 h per g, and was approx. 60% higher during sepsis (Table 4). This increase was abolished by treatment with indomethacin. Despite non-detectable plasma PGE_2 levels and normalized muscle PGE_2 release, total and myofibrillar protein breakdown rates in septic rats were unaffected by indomethacin treatment (Table 4).

DISCUSSION

In the present study, we found no evidence for prostaglandin regulation of total or myofibrillar protein breakdown in skeletal muscle. The results of unaltered total proteolysis, i.e. tyrosine release, after addition of arachidonic acid or PGE_2 to incubated normal muscle or after inhibition of PGE_2 production by indomethacin, differ from previous reports from Goldberg's & Baracos' laboratories [1–3], but are in line with several other studies [4–8]. The unresponsiveness of myofibrillar protein breakdown to PGE_2 and indomethacin is a new finding which further supports the concept that cyclo-oxygenase inhibitors may not be useful in the treatment of muscle catabolism in sepsis, since this condition mainly stimulates myofibrillar protein degradation [9].

It is unclear why we and others [4–8] have not been able to confirm a role of prostaglandins in muscle proteolysis. Several experiments were performed in the present study to exclude the possibility that the discrepancy between our results and those reported by Rodemann & Goldberg [1] was caused by differences in experimental conditions. However, although our variations in experimental conditions matched those of Rodemann & Goldberg [1], protein breakdown rates remained unchanged when PGE_2 or arachidonic acid was added to muscles at different temperatures, when muscles were incubated flaccid rather than stretched, and when the incubation medium was supplemented with insulin and branched-chain amino acids. Our results also demonstrated that the lack of effect of PGE_2 could not be ascribed to breakdown of the substance during incubation, since it was measured and found to be present in adequate concentrations. Similarly, Barnett & Ellis [4] found that the biological activity of PGE_2 (2.8 μM) was retained over a 2 h incubation period, without influencing muscle protein degradation. In the same study, the effects of PGE_2 and arachidonic acid were tested under different experimental conditions, e.g. in muscles harvested from anaesthetized or unanaesthetized animals, in muscles incubated in media of different composition, in tissue incubated stretched or flaccid, and in muscles from both rats and mice. Under none of these conditions did PGE_2 stimulate muscle proteolysis, measured as release of tyrosine [4].

In a recent report, Tian & Baracos [3] discussed several possible reasons why no evidence of PGE_2-regulated muscle proteolysis was observed in previous studies from our laboratory [7,8]. First, they questioned if indomethacin was solubilized at an effective concentration in our previous experiments. The results of the present study suggest that, even if indomethacin was not completely dissolved in the incubation medium, the concentration of the substance was sufficient to inhibit PGE_2 production effectively. An even higher concentration of indomethacin (6 μM) was added to incubated muscles from septic rats in a previous study [8], but protein breakdown was still unaffected. Thus it is not likely that the lack of effect of indomethacin on muscle proteolysis found in the present report and in previous studies from our [7,8] and other [4–6] laboratories reflects ineffective concentrations of the substance. Comparisons with previous studies are complicated by the fact that neither Rodemann & Goldberg [1] nor Baracos *et al.* [2] reported how they dissolved indomethacin or what concentrations *in vitro* they achieved.

Secondly, Tian & Baracos [3] suggested that a 2 h incubation period may be too short for indomethacin to influence protein turnover. This explanation seems inconsistent with reports by Goldberg *et al.* [1,2,20] that indomethacin inhibited muscle PGE_2 production and protein breakdown during a 2 h incubation. Furthermore, when muscles were incubated for up to 6 h in the presence of 3 μM-indomethacin, protein breakdown rates remained unaffected in muscle from both septic and control rats [8].

Third, it was suggested [3] that the effect of indomethacin on PGE_2 production and protein breakdown may be masked when muscles are incubated in the presence of cycloheximide, a substance which by itself can block prostaglandin synthesis [6]. The present results showed, however, that indomethacin inhibited PGE_2 production by approx. 60% when muscles were incubated in the presence of 0.5 mM-cycloheximide, similar to a previous report from our laboratory [7]. Clark *et al.* [5] reported an approx. 85% inhibition of PGE_2 production by indomethacin (3 μM) when muscles from burned rats were incubated in the presence of cycloheximide, but found no effect on protein degradation. They reported that similar conclusions were reached from experiments in which cycloheximide was omitted from the incubation media [5]. In the present study as well, protein breakdown rates were not affected by indomethacin when muscles were incubated without cycloheximide.

In the previous reports by Rodemann & Goldberg [1] and Baracos *et al.* [2], two different techniques were used to measure muscle protein breakdown rates. In one technique, muscles were incubated in the presence of 0.5 mM-cycloheximide and tyrosine released into the incubation medium was used as a measure of protein breakdown. In the alternative technique, muscles were incubated without cycloheximide, so that both protein synthesis and net protein degradation could be determined as described by Tischler *et al.* [21]. Unfortunately, it was not obvious in the previous reports [1,2] which technique was used when the effect of indomethacin was tested, and it is therefore unclear whether

the inhibition of protein breakdown by the prostaglandin-synthesis inhibitor was noted only in the absence of cycloheximide.

An additional reason why we found no effect of indomethacin on protein turnover rates in the present study may be that muscles were incubated in a medium lacking insulin and amino acids. It could be argued that PGE_2 production by muscles under these conditions is too high to be regulated by indomethacin. However, since PGE_2 production rates in muscles from control rats were similar to those in several previous reports [2,3, 5,7,20,22], it is not likely that our conditions *in vitro* affected muscle PGE_2 metabolism. It is also unlikely that our conditions *in vitro* could explain the lack of effect of PGE_2 or arachidonic acid on muscle protein breakdown, because muscles remained unresponsive to the substances also when incubated in the presence of insulin and amino acids. In a recent study [3], PGE_2 production by incubated epitrochlearis muscle from control and infected rats was almost identical with the present results in muscles from control and septic animals. Moreover, treatment of infected rats with naproxen decreased PGE_2 production to control values [3], similar to the current results in indomethacin-treated septic rats. Thus it is not likely that the lack of evidence for PGE_2-regulated muscle proteolysis in the present study was due to impairment of muscle PGE_2 metabolism induced by factors specific for the methods used here.

Since, in a previous report from this laboratory [7], plasma levels of indomethacin were not reported when rats were treated with the substance *in vivo*, results from those experiments as well were questioned by Tian & Baracos [3]. In the present study we therefore repeated those experiments, using an identical treatment protocol, and extended the previous study by including measurements of plasma levels of indomethacin and PGE_2 and by measuring muscle PGE_2 production and myofibrillar protein breakdown rates. Results showed that effective plasma levels of indomethacin were indeed achieved and maintained during the length of the experiment, resulting in decrease in muscle PGE_2 production to control levels and non-detectable plasma levels of PGE_2. In spite of this, total or myofibrillar protein breakdown rates were not affected. It should be pointed out that in these experiments the first dose of indomethacin was given at the time when CLP was performed. Thus it is likely that effective plasma levels of indomethacin were present when animals started to become septic. The data clearly demonstrated that the plasma concentration of PGE_2 was effectively decreased during the major part of the experiment. This would argue against the concept that high proteolytic rates, despite indomethacin treatment, reflected a role of PGE_2 only for the initiation of protein breakdown. This interpretation is further supported by a previous study in which pretreatment of rats with indomethacin 45 min before CLP failed to normalize protein degradation rates [7].

We cannot offer a definitive explanation for the differences between the present results and those reported from Goldberg's and Baracos' laboratories [1–3]. However, certain pertinent points deserve mention. First, a lack of evidence for regulation of muscle proteolysis by PGE_2 has been reported by several other authors [4–6,23,24]. Second, the role of PGE_2 in muscle protein breakdown does not seem to be consistent even in experiments performed in the same laboratory. Thus, although Rodemann *et al.* [20] reported that stimulation of muscle proteolysis by Ca^{2+} was mediated by PGE_2, those results could not be reproduced by Baracos *et al.* [24]. In other experiments, Fagan & Goldberg [25] found that treatment of rats with indomethacin after endotoxin injection did not affect the increased muscle proteolysis, despite inhibition of PGE_2 production. This contrasts with the study by Tian & Baracos [3], in which treatment with a cyclo-oxygenase inhibitor 24 h after the infection improved muscle nitrogen balance. Baracos and co-workers [26] reported previously that protein turnover rates in cultured isolated muscle cells were not influenced by arachidonic acid or prostaglandins, and in the same study pointed out that previous findings in incubated muscles are not always consistent.

Furthermore, a simultaneous inhibition of PGE_2 production and protein breakdown by cyclo-oxygenase inhibitors does not necessarily imply a direct cause–effect relationship. Thus the decreased net protein degradation in incubated muscles from naproxen-treated *E. coli*-infected animals, reported recently by Tian & Baracos [3], could have been the result of some property of naproxen other than inhibition of PGE_2 synthesis. In previous studies, indomethacin improved the haemodynamic status during endotoxin shock in dogs [27]. If this effect is shared by naproxen as well, the decreased muscle protein breakdown reported by Tian & Baracos [3] may have been caused by improved tissue perfusion, rather than by the inhibited PGE_2 production itself. Another secondary effect of naproxen which could have explained improved muscle nitrogen balance is inhibition of fever, because elevated temperature in itself stimulates muscle proteolysis [28], in particular in muscle from septic animals [29]. Interpretation of studies using naproxen is further complicated by the fact that, in addition to its effect on prostaglandin synthesis, the substance may act by inhibiting lysosomal enzymes and neutral proteases or by stabilizing certain membrane proteins [30].

In addition to the studies by Goldberg & Baracos [1–3,20], a report by Ruff & Secrist [31] seems to support a role of prostaglandins in muscle protein breakdown. In that study, infection of rats with live *Streptococcus pneumoniae* resulted in decreased muscle mass, decreased twitch and tetanic tension, and increased muscle cathepsin B activity. Because these changes were prevented by indomethacin, it was suggested that muscle breakdown was mediated by prostaglandins. Interpretation of the results in that study, however, is difficult for several reasons. First, protein synthesis or degradation rates were not measured, and no data on muscle protein content were given, and it is therefore not known if muscle protein metabolism was affected by the septic model and/or by the indomethacin treatment. Second, indomethacin, in addition to inhibiting prostaglandin synthesis, decreased muscle cathepsin B activity, and, although it was implied that the decreased activity of the proteolytic enzyme was secondary to decreased prostaglandin synthesis, no evidence for that assumption was presented. A direct inhibition of capthepsin B activity by indomethacin could explain the prevention of muscle breakdown. In fact, the study demonstrated that treatment with leupeptin, which is a lysosomal protease inhibitor, gave rise to results almost identical with those of treatment with indomethacin.

In conclusion, the present study found no evidence that total or myofibrillar protein breakdown in normal or septic skeletal muscle is regulated by PGE_2 when experiments were done under a variety of conditions. These findings agree with the majority of studies published by other laboratories. Since previous reports are inconsistent, however, further experiments may be required to determine more definitively the role of prostaglandins in muscle proteolysis. Such studies are important, because muscle catabolism is a prominent metabolic response in septic and other critically ill patients.

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