The influence of changes in tension on protein synthesis and prostaglandin release in isolated rabbit muscles

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Intermittent stretching of isolated rabbit muscles increased the rate of protein synthesis by 70% and prostaglandin- $F_{2\alpha}$ release by 105%. Both effects of intermittent stretching were blocked by indomethacin and meclofenamate; the absence of Ca^{2+} together with added EGTA also inhibited both processes. The residual influence of intermittent stretching on protein synthesis was closely correlated with prostaglandin- $F_{2\alpha}$ release, which may be the link between mechanical activity and protein synthesis in skeletal muscle.

When isolated forelimb digit extensor muscles of rabbits were subjected to intermittent increases in tension, the rate of protein synthesis was significantly increased (Palmer *et al.*, 1981; Smith *et al.*, 1983*a,b*). The effects on protein synthesis of additions of $PGF_{2\alpha}$ or arachidonic acid, and the inhibitors of prostaglandin synthesis, indomethacin and meclofenamic acid, suggested that changes in tension applied *in vitro* affected protein synthesis via increases in $PGF_{2\alpha}$ concentration owing to an activation of membrane phospholipase A_2 . Rodemann & Goldberg (1982) have also reported that $PGF_{2\alpha}$ stimulates protein synthesis in skeletal muscle and that PGE_2 stimulates protein degradation.

Although the results of experiments based on manipulating prostaglandin synthesis suggested that prostaglandins might mediate changes in protein synthesis induced by tension, no systematic measurements were made of prostaglandin release, although $PGF_{2\alpha}$ is known to be produced in skeletal muscle (Nowak *et al.*, 1981; Rodemann & Goldberg, 1982; Smith *et al.*, 1983*a*). The results in the present paper demonstrate the close association between the release of $PGF_{2\alpha}$ from muscle and the rate of protein synthesis under several different conditions of incubation.

Materials and methods

The composition of the incubation medium and the conditions for dissecting and incubating the muscles have been described fully in previous papers

(Reeds *et al.*, 1980; Palmer *et al.*, 1981; Smith *et al.*, 1983*b*). In brief, two muscles (extensor quarti digitorum proprius and extensor carpi ulnaris) were dissected intact from each forelimb of rabbits weighing between 800 and 1200 g. The muscles were incubated initially for 90 min either under a constant tension of 10 g or with intermittent increases in tension equivalent to 8 g for 100 ms every 3 s. After 90 min the medium was removed for analysis of $PGF_{2\alpha}$ content, and the incubation was continued for a further 30 min under identical conditions but in a medium containing L-[2,6- 3H]phenylalanine (Amersham International, Amersham, Bucks., U.K.) at a concentration of 2.5 mM (final sp. radioactivity 1500 d.p.m./nmol). Protein-synthesis rates are maintained linearly in this preparation for at least 6 h (Palmer *et al.*, 1981).

As in earlier experiments, the residual influence of intermittent stretching was examined by incubating the muscles with intermittent stretching for 30 min, followed by a further 40 min of incubation under constant tension, during which time $PGF_{2\alpha}$ release into the medium was measured every 10 min.

The concentration of $PGF_{2\alpha}$ in the medium was assayed by a radioimmunoassay with reagents and antisera obtained from Travenol Laboratories (Thetford, Norfolk, U.K.). The antiserum used in this assay was stated to cross-react 28% with $PGF_{1\alpha}$.

Rates of protein synthesis were determined from the rate of incorporation of labelled phenylalanine. Muscles were removed from the medium, washed briefly in ice-cold NaCl (9 g/l), blotted dry, weighed and homogenized in 3 ml of 0.5 M- $HClO_4$ (Reeds *et al.*, 1980). After purification (Palmer *et al.*, 1981), the protein was hydrolysed in 5.6 M-HCl and

Abbreviation used: PG, prostaglandin.

analysed for phenylalanine and radioactivity (Smith *et al.*, 1983b). Phenylalanine was measured on a Locarte automatic amino acid analyser (Locarte Co., London W.12, U.K.) by elution from Locarte LA49 resin with 0.2M-sodium citrate buffer, pH4.27, at 50°C. Radioactivity was measured in a Beckman liquid-scintillation counter by using Lumax/xylene (1:1, v/v) scintillation fluid (LKB, Croydon, Surrey, U.K.). Preliminary measurements demonstrated that protein-bound radioactivity was located exclusively in phenylalanine. The rate of protein synthesis was calculated as described by Smith *et al.* (1983b).

Results

Effects of intermittent stretching, Ca²⁺ and inhibitors of prostaglandin synthesis on protein synthesis and PGF_{2α} release

In two series of experiments, muscles were incubated under a constant tension or with intermittent stretching in either the absence or the presence of indomethacin (50 μM) or meclofenamic acid (100 μM). In a third series of experiments,

muscles were incubated in normal medium or in a medium containing no Ca²⁺ but with the addition of 50 μM-EGTA. The results of these experiments are shown in Table 1. Intermittent stretching increased the rate of protein synthesis by 70% ($P < 0.02$) and the rate of PGF_{2α} release by 105% ($P < 0.01$). The addition of indomethacin or meclofenamate or the omission of Ca²⁺ decreased or completely abolished the effects of intermittent stretching on protein synthesis and PGF_{2α} release.

Residual influence of intermittent stretching

It has previously been demonstrated (Smith *et al.*, 1983b) that there was an effect of intermittent stretching on rates of protein synthesis, which persisted for up to 20 min after intermittent stretching had ended. The results in Table 2 demonstrate that the release of PGF_{2α} was higher in muscles being intermittently stretched than in those being held under a constant tension. Once intermittent stretching was ended, the release of PGF_{2α} decreased. During the first 10 min after intermittent stretching, the PGF_{2α} released was 88% of that with intermittently stretched muscles, and there was a progressive fall, so that during the final 10 min

Table 1. *Influence of indomethacin, meclofenamic acid and the omission of Ca²⁺ on protein synthesis and PGF_{2α} release*

Muscles were incubated under constant tension or with intermittent stretching for 90 min. The medium contained either indomethacin (50 μM) or meclofenamic acid (100 μM), or Ca²⁺ was omitted and EGTA (50 μM) was added. The medium was removed and assayed for PGF_{2α}, and protein synthesis was measured for a further 30 min as described in the Materials and methods section. Values are means ± S.E.M. for the numbers of experiments in parentheses. Effect of intermittent stretching significantly different from corresponding constant tension by paired statistics: * $P < 0.05$, ** $P < 0.01$; effect of inhibitors significantly different from corresponding value with no additions: † $P < 0.05$, †† $P < 0.01$.

Addition	Constant tension		Intermittent stretching	
	Protein synthesis (%/day)	PGF _{2α} (pg/90 min per mg of muscle)	Protein synthesis (%/day)	PGF _{2α} (pg/90 min per mg of muscle)
None	2.29 ± 0.32	9.96 ± 0.94	3.99 ± 0.48*	22.1 ± 1.70 (5)**
EGTA (Ca ²⁺ free medium)	1.99 ± 0.32	7.10 ± 0.39†	2.93 ± 0.30†	15.8 ± 0.90 (5)††
None	2.25 ± 0.36	10.12 ± 1.48	3.38 ± 0.49*	22.6 ± 2.32 (4)**
Indomethacin	2.14 ± 0.21	9.7 ± 0.62	2.05 ± 0.38†	9.6 ± 0.45 (4)††
None	2.22 ± 0.31	8.22 ± 0.31	3.04 ± 0.12*	16.8 ± 0.36 (4)**
Meclofenamic acid	1.56 ± 0.18	7.06 ± 0.46†	1.48 ± 0.20††	6.4 ± 0.34 (4)††

Table 2. *Residual effect on PGF_{2α} release after withdrawal of the mechanical stimulus*

Muscles were incubated for 30 min with intermittent stretching (IS) or under a constant tension (CT), and then for a further 40 min during which some of the muscles initially subjected to intermittent stretching were incubated under a constant tension. During this final 40 min period the medium was removed for PGF_{2α} assay and replaced with fresh medium every 10 min.

Treatment		PGF _{2α} release (pg/mg per 10 min)			
0–30 min	30–70 min	30–40 min	40–50 min	50–60 min	60–70 min
CT	CT (n = 2)	2.9	1.6	1.7	1.8
IS	IS (n = 2)	4.2	3.6	3.9	3.1
IS	CT (n = 4)	3.7	2.9	1.5	1.5

period (60–70 min) $\text{PGF}_{2\alpha}$ release was 40% of the initial value. Thus the residual influence of intermittent stretching on $\text{PGF}_{2\alpha}$ release was lost after about 20 min of incubation and was closely correlated with the change in protein synthesis.

Discussion

Exercise increases the release of prostaglandins from muscle (Young & Sparks, 1980), and the present results support our previous proposition (Smith *et al.*, 1983b) that the stimulation of protein synthesis by intermittent changes in tension *in vitro* involves an increase in prostaglandin synthesis. Thus intermittent stretching increased both protein synthesis and $\text{PGF}_{2\alpha}$ release, and the reversal of the effects of intermittent stretching by the addition of indomethacin and meclofenamate was associated with a decrease in $\text{PGF}_{2\alpha}$ release to the rates that were observed in control muscles. Incubation in a Ca^{2+} -free medium containing EGTA, which produced only a partial reversal of the effect of intermittent stretching on protein synthesis (Table 1, and Smith *et al.*, 1983b), also had a smaller effect on $\text{PGF}_{2\alpha}$ release than that produced by the pharmacological blocking agents. Furthermore, although the addition of meclofenamate or the omission of Ca^{2+} from the medium bathing control muscles was associated with a small decrease in both protein synthesis and $\text{PGF}_{2\alpha}$ release, the addition of indomethacin to control muscles affected neither process. Overall, there was a highly significant correlation ($P < 0.0001$) between the rate of protein synthesis and the release of $\text{PGF}_{2\alpha}$.

The intracellular concentration of $\text{PGF}_{2\alpha}$ after 90 min of incubation, although detectable, proved too low to measure accurately, whereas the extracellular concentration after the same period of incubation attained values of between 0.05 (control) and $0.1 \mu\text{M}$. These observations are consistent with the generally accepted view that the prostaglandins are neither stored nor concentrated by cells, but are rapidly released into the extracellular phase (Samuelsson *et al.*, 1978; Irvine, 1982). The results in Table 2 also demonstrate the rapidity with which the release (and by implication the synthesis) of $\text{PGF}_{2\alpha}$ may be affected by altering the conditions of incubation. Unfortunately the high concentration of [^3H]phenylalanine required to measure protein synthesis interfered with the PG assay and precluded the simultaneous measurement of $\text{PGF}_{2\alpha}$ release and protein synthesis in these experiments. Nevertheless there is a marked similarity between the time course of $\text{PGF}_{2\alpha}$ release (Table 2) and that of protein synthesis measured under similar conditions (Smith *et al.*, 1983b) after intermittent stretching had ceased, another example of the close association of these two processes.

A causal link between PG metabolism and protein synthesis is suggested by the observation that the addition of arachidonic acid ($0.2 \mu\text{M}$) resulted in a significant increase in rates of protein synthesis in muscles incubated under a constant tension, an effect that was blocked by indomethacin (Smith *et al.*, 1983b). In addition, of the prostaglandins added, only $\text{PGF}_{2\alpha}$ produced a similar response, and only at a higher extracellular concentration ($2.8 \mu\text{M}$). If, as appears likely, prostaglandins are rapidly excluded from the cell while arachidonic acid is taken up, the requirement for a higher concentration of extracellular prostaglandins in order to stimulate protein synthesis is not unexpected.

The primary point of control of PG synthesis may be the release, from membrane-bound phospholipids (phosphatidylcholine and phosphatidylinositol), of precursor arachidonic acid (Hassid, 1982; Irvine, 1982). Two possible ways by which changes in tension during intermittent stretching may alter the activity of phospholipase A_2 are by altering the spatial relationship between the enzyme and other components of the cell membrane (discussed by Vogt, 1978; Van den Bosch, 1980) and by releasing Ca^{2+} , a cofactor for phospholipase A_2 from the sarcoplasmic reticulum (Brenner, 1979). Whatever the precise mechanism for this activation, the present results, and those published previously (Smith *et al.*, 1983b), suggest that the stimulation of PG synthesis acts as a link between changes in the plasma membrane and changes at the ribosomal level. The present experiments are specifically related to the effects of tension on protein synthesis; however, other extracellular factors that stimulate muscle protein synthesis, for example leucocyte pyrogen (Baracos *et al.*, 1983), may exert a similar influence. Changes in PG synthesis may thus be a general mechanism whereby protein turnover in skeletal muscle is influenced by the nature of the extracellular environment.

References

- Baracos, V., Rosemann, H. P., Dinarello, C. A. & Goldberg, A. L. (1983) *N. Engl. J. Med.* **308**, 553–558
- Brenner, B. (1979) *Basic Res. Cardiol.* **74**, 177–202
- Hassid, A. (1982) *Am. J. Physiol.* **243**, C205–C211
- Irvine, R. F. (1982) *Biochem. J.* **204**, 3–16
- Nowak, J., Bohman, S.-O., Berlin, T. & Sonnenfeld, T. (1981) *Acta Physiol. Scand.* **113**, 557–559
- Palmer, R. M., Reeds, P. J., Loble, G. E. & Smith, R. H. (1981) *Biochem. J.* **198**, 491–498
- Reeds, P. J., Palmer, R. M. & Smith, R. H. (1980) *Int. J. Biochem.* **11**, 7–14
- Rodemann, H. P. & Goldberg, A. L. (1982) *J. Biol. Chem.* **257**, 1632–1638

- Samuelsson, B., Goldyne, M., Grandström, E., Hamberg, M., Hammarström, S. & Malmsten, C. (1978) *Annu. Rev. Biochem.* **47**, 977–1029
- Smith, R. H., Palmer, R. M. & Reeds, P. J. (1983a) *Biochem. Soc. Trans.* **11**, 182–183
- Smith, R. H., Palmer, R. M. & Reeds, P. J. (1983b) *Biochem. J.* **214**, 153–161
- Van den Bosch, H. (1980) *Biochim. Biophys. Acta* **604**, 191–246
- Vogt, W. (1978) *Adv. Prostaglandin Throboxane Res.* **3**, 89–95
- Young, E. W. & Sparks, H. V. (1980) *Am. J. Physiol.* **238**, H191–H195