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Prostaglandin synthetase and prostacyclin synthetase in mature rat skeletal muscles: immunohistochemical localisation to arterioles, tendons and connective tissues

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INTRODUCTION

Prostaglandins have been implicated in the control of various aspects of skeletal muscle homeostasis. The vasodilators, PGE_2 and PGI_2 (prostacyclin), affect intramuscular blood flow (Faber, Harris & Joshua, 1982; Koller & Kaley, 1990*a*, *b*), whereas $PGF_{2\alpha}$ has been suggested to mediate hormonal and tension-induced changes in protein synthesis (Smith, Palmer & Reeds, 1983; Reeds & Palmer, 1984; Reeds *et al.* 1985; Vandenburgh, Hatfaludy, Sohar & Shansky, 1990). E series prostaglandins may also increase the sensitivity of the muscle to insulin (Leighton *et al.* 1985; Nesher, Karl & Kipnis, 1985). Prostaglandin synthetase (PG SYN) inhibitors can cause arteriolar constriction (Faber *et al.* 1982) and insulin resistance (Dietze, Wicklmayr, Bottger & Mayer, 1978), decrease induced protein synthesis (Smith, Palmer & Reeds, 1983), counteract some effects of hypertrophic stimuli (Templeton, Padalino & Moss, 1986; McMillan, Reeds, Lobley & Palmer, 1987) and cause myofibrillar disruption in immature muscles (McLennan, 1985, 1987).

Isolated mature skeletal muscles, and extracts of them, produce appreciable quantities of PGI_2 and smaller amounts of $PGF_{2\alpha}$ and PGE_2 (Rodemann & Goldberg, 1982; Nowak *et al.* 1983, Kerry, 1985), but the cellular sources of these prostaglandins are unknown. We have sought to determine the cellular origin of prostaglandins produced within muscle by immunohistochemical localisation of two enzymes, PG SYN and prostacyclin synthetase (PGI SYN). PG SYN should be present in cells that produce PGE_2, $PGF_{2\alpha}$ or PGI_2, whereas PGI SYN is specific to cells producing PGI_2.

MATERIALS AND METHODS

Animals

Male and female Wistar rats weighing 250–350 g were obtained from the University of Otago breeding facility. They were maintained under a 12 h light/dark regimen and fed *ad libitum* unless otherwise stated.

Treatment of rats with inhibitors of prostaglandin synthetase

Six rats were anaesthetised with pentobarbitone sodium (Nembutal, Abbott) and given an i.p. injection of 150 mg of aspirin in 1 ml of ethanol. The rats did not regain consciousness before being killed 1 h later by cervical dislocation. Two rats were given a vehicle injection as a control.

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Four rats were lightly anaesthetised with ether and 1 ml of an aqueous suspension of 150 mg of aspirin and 2% tragacanth injected into each of their stomachs via a tube passed through their mouths. The rats were killed at various times up to 4 h after treatment. Another 4 rats were treated daily with 50 mg of orally administered fenbufen for 5 days and killed by cervical dislocation 2 h after the 5th dose.

Other treatments of rats

A group of 4 rats were starved overnight. Two of these rats were anaesthetised with Nembutal and a cannula inserted into their jugular veins. 300 mU of human recombinant insulin were infused through the cannula at a rate of approximately 15–20 mU/min and the rats then killed by cervical dislocation.

Soleus muscles from another group of rats were dissected and incubated at 37 °C for 1 h either in Tyrode's solution, Tyrode's solution including 200 μunit of insulin per ml or Tyrode's solution in which 100 mM of NaCl had been replaced with KCl. All solutions were bubbled with 95% air-5% CO_2.

Tissue preparation

Normal and treated rats were killed by cervical dislocation and their soleus or tibialis anterior muscles rapidly removed. In a few instances the cremaster muscles were also removed. In 2 of the rats this procedure was varied. These rats were anaesthetised with ether and their soleus muscles removed while their hearts were still beating. The rats were then killed by cervical dislocation.

All muscles were immediately frozen in melting isopentane and stored in a liquid nitrogen refrigerator until required. Sections of 5–6 μm were cut on a cryostat, picked up onto gelatin-coated slides and dried in front of a fan for 20–30 min.

Immunohistochemistry

PG SYN and PGI SYN were localised by using mouse IgG monoclonal antibodies, anti-PG SYN [cyo-1] and anti-PGI SYN [isn-1] (Oxford Biomedical Research, Inc; Smith, DeWitt & Allen, 1983). The detection of PG SYN by cyo-1 is affected by fixation, although weak fixation with paraformaldehyde can be used (Smith & Rollins, 1982). The sections were thus not usually fixed, although the immunoreactivity observed with anti-PGI SYN was unaffected when the sections were incubated for 5 min with 4% paraformaldehyde followed by three 5 min washes in 0·1 M glycine in 0·1 M sodium phosphate buffer, pH 7·2 (PB). Fixation for longer periods, or perfusion fixation, reduced the observed immunoreactivity.

Tissue sections were washed in PB and nonspecific binding sites were blocked by incubating the sections in a 5% solution of heat-inactivated goat serum in PB at room temperature. After 20 min, the goat serum was replaced with one of the primary antibodies diluted in a solution containing 1% bovine serum albumin and 0·1% Tween 20 in PB (diluting buffer). Anti-PG SYN and anti-PGI SYN were used at a final concentration of 4·5 and 2 μg IgG/ml respectively. The sections were incubated with the primary antibody at 4 °C overnight in a humid environment and then washed 3 times in a washing buffer that consisted of 1% NaCl, 1% nonfat dried milk powder, 0·2% Tween 20 in PB. The sections were then incubated at room temperature, sequentially, in biotinylated-antimouse IgG (Amersham RPN.1001) diluted 1:300 in diluting buffer for 1 h, 3 times in washing buffer for 15 min per wash, 0·3% H_2O_2 in methanol for 10 min, twice in PB for 5 min per wash, streptavidin-horseradish peroxidase (Amersham RPN.1231) diluted 1:300 in diluting buffer for 1 h, washing buffer for 5 min and twice with PB for 5 min per wash. The immunoreactivity was

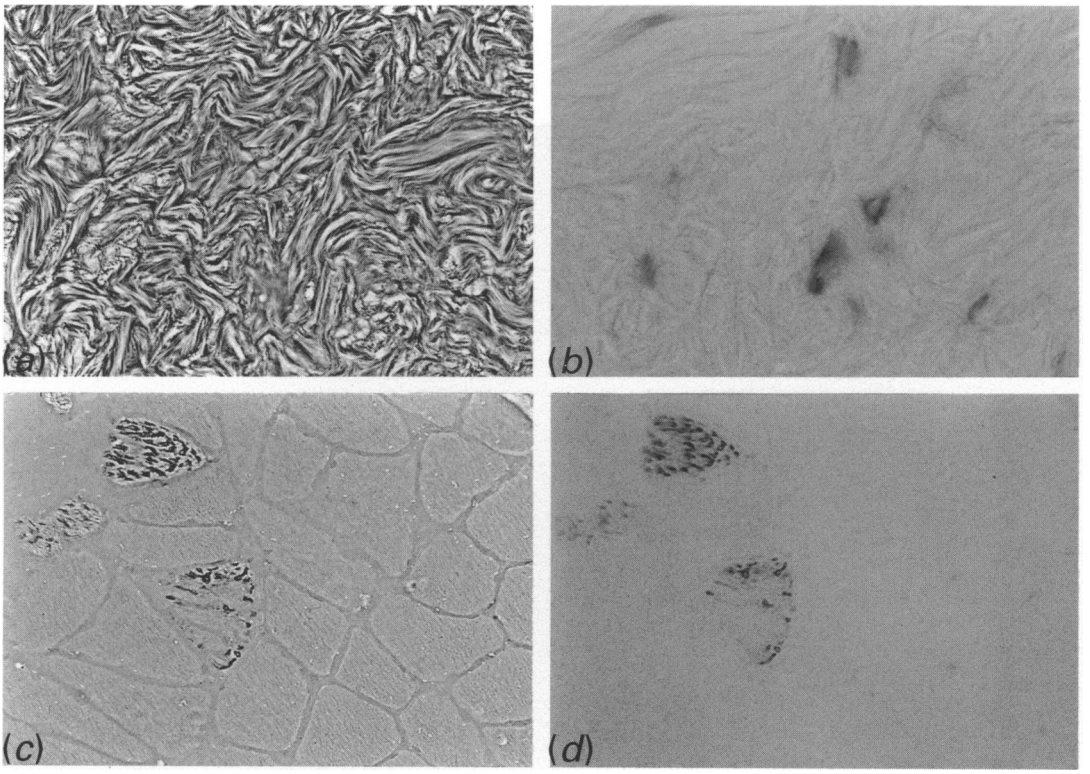

Fig. 1. Photomicrographs of sections of mature soleus muscles that have been immunolabelled with antiprostacyclin synthetase (PGI SYN). (*a*, *b*) Longitudinal section of a tendon; (*c*, *d*) cross-section through muscle. The stained profiles are tendons near the myotendinous junction of individual fibres. (*a*) and (*c*) are phase contrast images corresponding to the bright field images, (*b*) and (*d*). Magnification (*a*), (*b*) ×125, (*c*), (*d*) ×250.

developed by incubating the sections for up to 10 min in PB containing 0·05% diaminobenzidine (Sigma), 0·01% H_2O_2 and 0·02% nickel ammonium sulphate. The reaction was stopped by washing in water and the sections were then dehydrated in ethanol, cleared in xylene and mounted under coverslips with Permount. The sections were not counterstained. The cells stained by the antibodies were identified using phase contrast and also by examining adjacent sections that had been stained with either haematoxylin and eosin or the fast green modification of van Gieson's stain.

Nonspecific staining was controlled for by replacing the primary antibody with an antibody to an antigen which is not present in the tissues being studied (antihuman macrophage, Dakopatts M814).

RESULTS

Prostacyclin synthetase

The major site of anti-PGI SYN immunoreactivity was the tendon. Stain was observed around all nuclei within the tendon (Figs 1 *a*, *b*) and was particularly intense close to the myotendinous junction (Fig. 1 *c*, *d*). Weak cytoplasmic staining was observed when the perinuclear stain was intense, but it is unclear whether this resulted from diffusion of the reaction product or the presence of cytoplasmic enzyme. Weaker

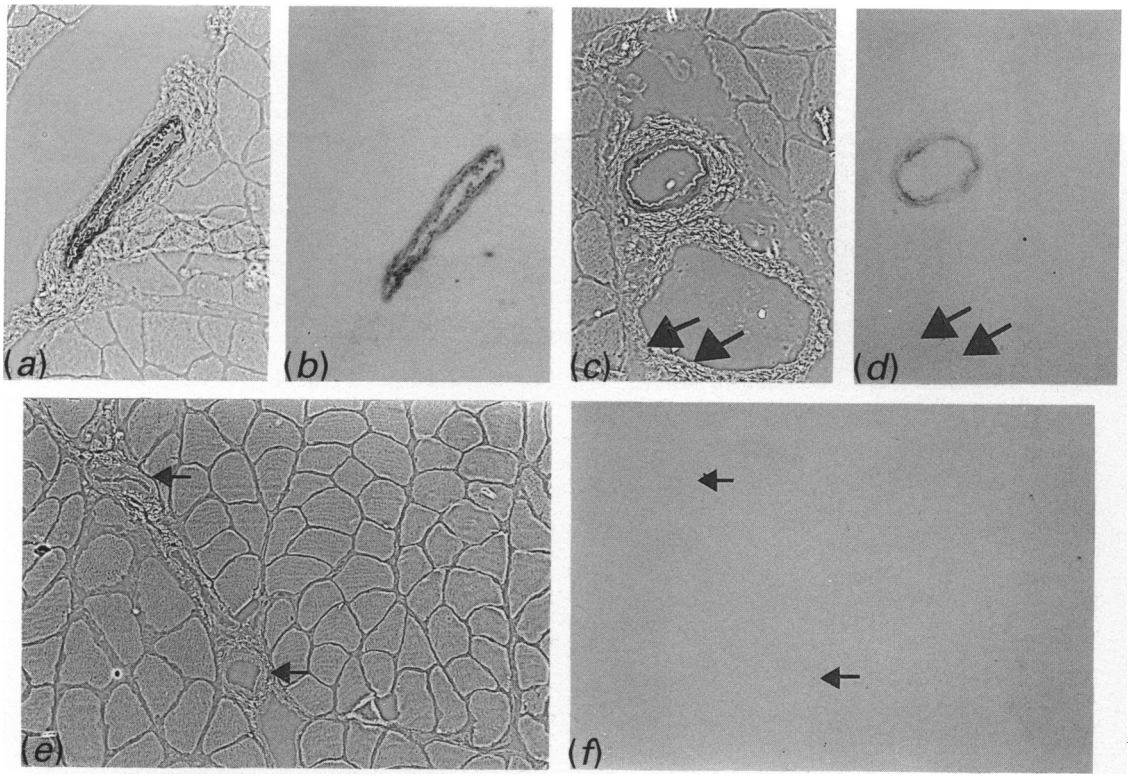

Fig. 2. Photomicrographs of sections of mature muscles that have been immunolabelled with antiprostacyclin synthetase (PGI SYN). (*a–d*) Cross-section of a tibialis anterior muscle showing a transverse arteriole (*a*, *b*) and a longitudinal arteriole (*c*, *d*) with PGI SYN immunoreactivity. Note that the venule (larger arrows) is not immunoreactive. (*e*, *f*) Cross-section of a soleus muscle. Note that the arterioles (smaller arrows) are not immunoreactive. (*a*), (*c*) and (*e*) are phase contrast images corresponding to the bright field images, (*b*), (*d*) and (*f*). Magnification ×63.

immunoreactivity was also observed in the fascia covering the proximal superficial surface of the soleus and to a lesser extent in the epimysium. The peri- and endomysium, the muscle fibres and macrophages were not stained.

The arterial supply to all muscles and many of the arterioles within the tibialis anterior and cremaster muscles were immunopositive (Fig. 2*a–d*). All other blood vessels, including the arterioles within the soleus, did not bind detectable levels of anti-PGI SYN (Fig. 2*e*, *f*). In the stained arterioles, the most intense immunoreactivity was localised around the myonuclei of the tunica media, with lesser amounts in the cytoplasm of the smooth muscle cells. At high magnification, thin lines of immunoreactivity could also be observed on the luminal side of the elastic lamina. We interpreted this to be staining of the plasma membrane of the epithelium. The tunica adventia and elastic lamina were not stained.

Prostaglandin synthetase

A prolonged DAB incubation was required before any PG SYN immunoreactivity was detected in the soleus, tibialis anterior or cremaster muscles of normal rats. Even after such an incubation, only very weak immunoreactivity was observed around a few fibres (data not shown).

Prostaglandin synthetase inhibitors

PG SYN protein and mRNA is very rapidly turned over. Inhibitors of PG SYN, such as aspirin or fenbufen, stabilise the enzyme. Rats were therefore treated with aspirin before their death in an endeavour to increase the amount of enzyme present. Some of the cells in the muscles of rats treated with aspirin were PG SYN positive. All the structures which were PGI SYN positive were also PG SYN positive; weak cytoplasmic immunoreactivity was observed in the tendons and fascia of all the muscles studied and the arterioles of the fast muscles (Figs 3*a*, *b*). The arterioles of the soleus were never as immunopositive as those of the tibialis anterior, although the epithelium of some of the soleus arterioles appeared to contain trace amounts of PG SYN.

In addition, there were small and variable numbers of fibres whose periphery was strongly stained with the anti-PG SYN antibody, but were PGI SYN immunonegative (Fig. 3*c–e*). This immunoreactivity appeared to be associated with mononucleated cells rather than the fibre plasmalemma or the sarcoplasmic reticulum underlying it. For instance, a gap is evident between the immunopositive cell in Figure 3*e* and the plasmalemma of its associated fibre (see arrows). In some instances, the entire circumference of a fibre was surrounded by immunopositive cells (see Fig. 3*d*), whereas in other cases only a single immunopositive cell was seen surrounding a portion of a fibre. The immunopositive cells were more common in sections where myotendinous junctions were evident and the endomysium adjacent to the immunopositive cells sometimes also contained immunonegative cells (Fig. 3*e*).

The effect of oral and intraperitoneal administration of aspirin was evident within 30 min, maximal after 1 h and no longer evident after 4 h. In one experiment, rats were chronically treated with an inhibitor to see if a greater enhancement of immunoreactivity could be achieved. Fenbufen was used, instead of aspirin, because of its lower toxicity. The muscles of rats treated in this way did not contain significant PG SYN immunoreactivity.

The preparation of PG SYN used to generate the anti-PG SYN antibody contained a PG SYN inhibitor as inhibitors were used to stabilise PG SYN during its purification. It was thus possible that the anti-PG SYN antibody may preferentially recognise the enzyme with an inhibitor bound to it. This appears not to be the case as preincubation of sections of normal muscle with aspirin did not cause a detectable increase in immunoreactivity.

PGI SYN immunoreactivity was not qualitatively changed by treatment of the rats with PG SYN inhibitors.

Other treatments

Rats, or their muscles, were treated in a variety of ways known to affect the rate of their prostaglandin production (see below). PG SYN is a secondary regulatory site of prostaglandin production and rapidly turned over. It was reasoned that these treatments could potentially increase PG SYN immunoreactivity by changing the amount or conformation of the enzyme.

In 1 of 4 pregnant rats, PG SYN immunoreactivity was observed which resembled that seen in the muscles of aspirin-treated rats. The muscles of the other 3 pregnant rats were immunonegative. The following treatments of rats did not affect either PG SYN or PGI SYN immunoreactivity: (1) starvation overnight; (2) i.v. infusion of insulin to starved rats; (3) ether or Nembutal anaesthesia; and (4) removal of the muscle from anaesthetised rats so that the muscle's blood supply was intact until

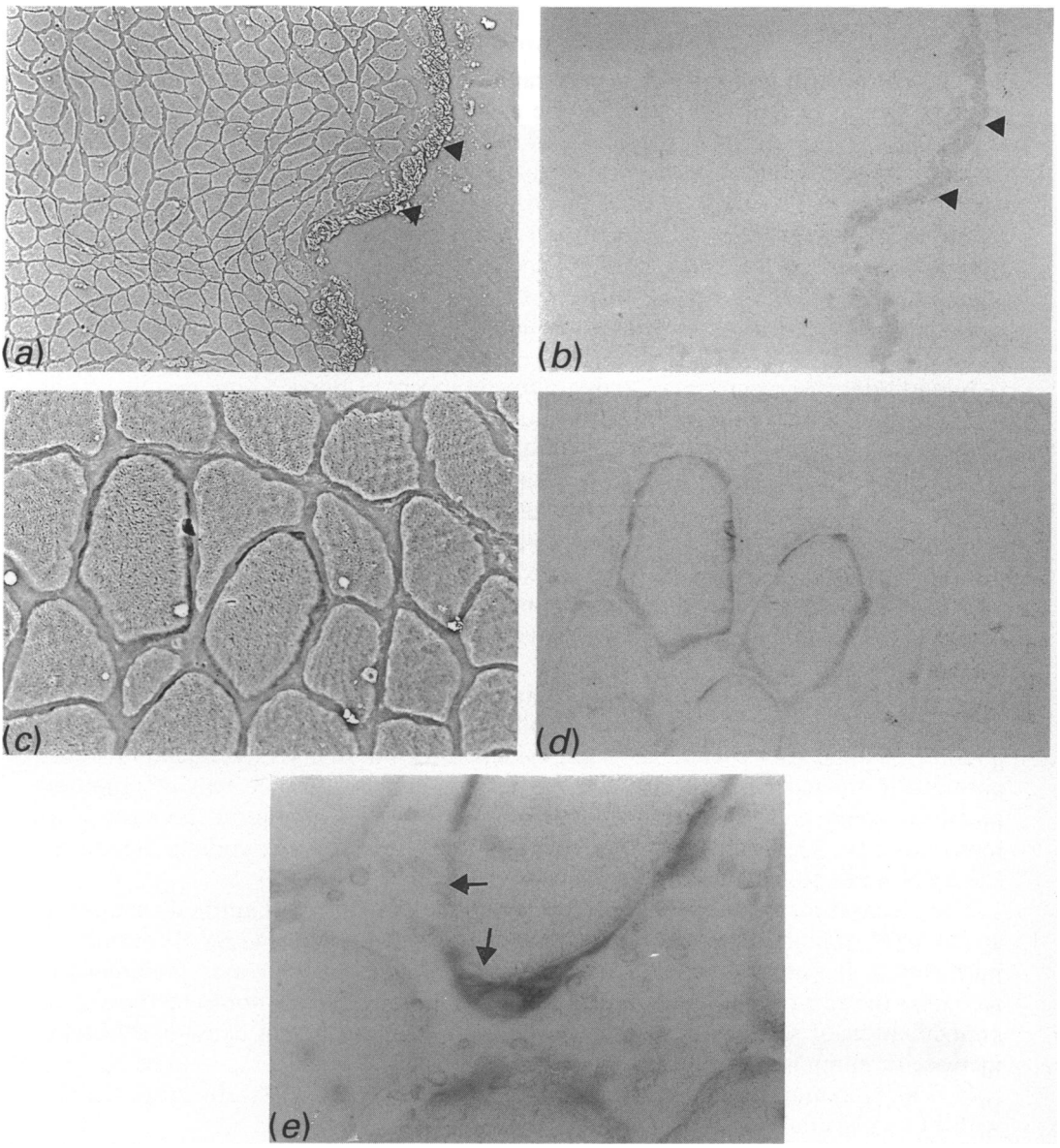

Fig. 3. Photomicrographs of sections of mature soleus muscles from rats which had been pretreated with aspirin. The sections were immunolabelled with antiprostaglandin synthetase (PG SYN). (*a*, *b*) Cross-section through muscle; the arrowheads point to the fibrous fascia, which is weakly immunopositive. (*c*–*e*) Cross-section through muscle; note that the fibres and their plasmalemma are not immunoreactive. The arrows in (*e*) point to the plasmalemma. (*a*) and (*c*) are phase contrast images corresponding to the bright field images, (*b*) and (*d*). Magnification ×63 (*a*, *b*), ×250 (*c*, *d*) and ×630 (*e*).

within seconds of its being frozen. Similarly, PG SYN or PGI SYN immunoreactivity was not effected by the following treatments of soleus muscles: (1) in vitro incubation in Tyrode's solution; (2) in vitro incubation in Tyrode's solution containing insulin; (3) depolarization in vitro with K^+; (5) incubation in vitro in either a stretched or flaccid state; and (6) freezing of the muscle in either a stretched or flaccid state.

DISCUSSION

Prostaglandins and blood flow

Increases in the rate of blood flow through a muscle's microcirculation elicits arteriolar dilation. This had been postulated to occur as a consequence of endothelial release of PGE_2 and PGI_2 in response to wall shear stress (Koller & Kaley, 1990*a*). The results obtained in this study are broadly consistent with this hypothesis, but also raise 2 issues: (1) the importance of the endothelium and (2) the universality of the mechanism.

Within the cremaster and tibialis anterior muscles, arterioles were stained by both antibodies, indicating that the arteriole system of these fast muscles has the synthetic capacity to produce PGI_2 and possibly other prostaglandins. In contrast, the microcirculation of the soleus was not stained by the anti-PGI SYN. Thus the arterioles of the soleus appear to lack the necessary enzymes to produce PGI_2 in response to changes in blood flow. It is possible, however, that the microcirculation of the soleus could be affected by arterial-produced PGI_2 as the arteries leading to the soleus muscles were PGI SYN immunoreactive. In this respect, the soleus is similar to the heart, where the endothelial cells of the major arteries have a greater capacity to produce PGI_2 than those of the microcirculation (Smith, 1986). Coronary microvessels produce PGE_2 in much greater quantities than PGI_2 (Smith, 1986) and this possibly could also be true of the soleus microcirculation. Trace amounts of PG SYN immunoreactivity were sometimes observed in the arterioles of the soleus muscles from aspirin-treated rats, which is consistent with their having the capacity to produce a prostaglandin other than PGI_2. A more definitive answer to the question of whether arterioles in fast and slow muscles produce PGE_2 may be gained from the immunolocalization of PGH-PGE isomerase, an enzyme which is unique to the biosynthetic pathway of PGE.

The link between an increased rate of blood flow and vasodilation of a muscle's arterioles has been suggested to be endothelial-released prostaglandins (Koller & Kaley, 1990). Flow-induced vasodilation does not occur in the presence of a dye which inhibits the function of the endothelium (Koller & Kaley, 1990*b*) and cultured arterial endothelia release prostacyclin in response to stress (Frangos, Eskin, McIntire & Ives, 1985; but see also Ager *et al.* 1982). However, on the basis of the immunohistochemistry, the tunica media appears to have a much greater capacity to produce prostacyclin than the endothelium. Thus the possibility that the smooth muscle fibres of the tunica media release prostacyclin in response to a signal from the endothelium needs to be considered. Alternatively, the prostacyclin produced within the tunica media may have a function unrelated to flow-induced vasodilation.

Prostaglandins and tendons

A large proportion of the immunoreactivity observed with the anti-PGI SYN antibody was associated with the tendon and epimysium. The tendinous part of the myotendinous junction was particularly intensely stained. Thus the vast majority of muscle PGI_2 is probably derived from the tendon and myotendinous junctions. The location of PGI SYN in the immunopositive cells was predominantly perinuclear, which is consistent with its localisation in other tissues. Both PG SYN and PGI SYN are integral membrane proteins which are most abundant in the endoplasmic reticulum and nuclear and plasma membranes (Smith *et al.* 1983; Needleman *et al.* 1986; Smith, 1989).

The physiological significance of the tendon being a major source of prostacyclin, and possibly other prostaglandins, is at present purely a matter of conjecture. Drugs which reduce prostaglandin production are commonly used in the treatment of overuse injuries, such as tendonitis (Sundquist, Forskahl & Kvist, 1987; Doherty, 1989). This raises the intriguing possibility that prostaglandins are involved either in the repair of the tendon or its adaptation to forces being imposed upon it.

Muscle fibres, endomysial cells and prostaglandin production

The muscle fibres did not contain detectable levels of either of the antigens being studied. PG SYN immunoreactivity was associated with the circumference of a few fibres (Fig. 3*c–e*) but when these fibres were examined at high magnification the immunoreactivity appeared to be located within a closely associated cell rather than the fibre itself. The identity of these immunopositive cells is unclear. Their scarcity indicates that they are not unactivated macrophages or satellite cells or typical fibroblasts. They may be a subpopulation of fibroblasts associated with the endomysial sheath as it fuses with the tendon. The morphology of the nuclei of the positive cells is characteristic of endomysial fibroblasts and they were frequently observed in regions of muscle where myotendinous junctions were present.

Alternatively, it could be argued that the presence of other cells in the immediate vicinity of some of the immunopositive cells indicates that a localised regenerative response is occurring. If this is the case, then the immunopositive cells are probably either activated macrophages or perhaps even activated satellite cells. However, we consider this possibility less likely as the fibres associated with the immunopositive cells appeared to be normal and similar immunopositive cells are very rarely observed in experimentally damaged muscles (unpublished observations).

Prostaglandins and the muscle response to insulin and other stimuli

The absence of immunoreactivity in the muscle fibres themselves is in contrast to a growing body of evidence which indicates that muscle fibres are affected by prostaglandins. Some responses of muscle fibres to hypertrophic stimuli (Templeton *et al.* 1986; McMillan *et al.* 1987), stretching (Smith *et al.* 1983; Vandenburgh *et al.* 1990), glucocorticoids (Reeds & Palmer, 1984) and insulin (Reeds *et al.* 1985) appear to be mediated via prostaglandins. If this is the case, then either (1) muscle fibres contain an undetected source of prostaglandin production (see below), (2) the muscle fibres are directly or indirectly influenced by prostaglandins produced in their arterioles, tendons or endomysium, or (3) PG SYN may only be expressed in fibres in response to certain stimuli. With respect to the last possibility, the amount of PG SYN in some cell types is known to be inducible by appropriate stimuli (Smith, 1989; Fu *et al.* 1990). Whether or not such a mechanism operates in skeletal muscle is unknown. Exposure of muscle to acute stimuli, such as in vivo infusion of insulin or in vitro K^+ depolarisation, did not result in detectable immunoreactivity in the muscle fibres. It remains to be determined, however, if chronic stimuli, such as hypertrophic influences, can induce PG SYN in skeletal muscle fibres.

As noted above, the presence of an undetected source of PG SYN in muscle fibres would explain various observations. This could occur if a part of muscle prostaglandin production was due to a form of PG SYN that is not recognised by the monoclonal antibody used in this study. Evidence for the existence of various forms of PG SYN is at present very sketchy. However, this possibility gains some credence from the recent observation that pulmonary epithelial cells produce PG SYN mRNA and an

mRNA species which is homologous to PG SYN and a correlate of the rate of PG production by these cells (Rosen, Birkenmeier, Raz & Holtzman, 1989).

Aspirin and PG synthetase immunoreactivity

The muscles of untreated rats contained only trace levels of anti-PG SYN immunoreactivity. The absence of significant PG-SYN immunoreactivity in skeletal muscle is in agreement with previous investigations using rabbit and bovine muscles (Smith, Palmer and Reeds, 1983; Yoshimoto *et al.* 1986), but is at variance with biochemical and physiological studies which clearly demonstrate that skeletal muscles produce and respond to prostaglandins (see above). Rats were therefore treated in a variety of ways in an endeavour to unmask the putative sites of PG production. Administration of aspirin to rats resulted in detectable immunoreactivity. A number of hypotheses could account for these observations.

PGH synthetase mRNA and protein both have very short half lives (Fagan & Goldberg, 1986; Raz, Wyche, Siegel & Needleman, 1988), with protein degradation linked to autocatalytic destruction of the enzyme (Needleman *et al.* 1986). Inhibitors could increase the amount of protein present either by stabilising the enzyme or by some feedback mechanism to increase transcription, translation or posttranscriptional modification of PGH synthetase. It is unlikely that increased immunoreactivity was due to the inhibitors changing the conformation of the enzyme as aspirin applied directly to sectioned muscle was without effect. Irrespective of the mechanism of increased immunoreactivity, aspirin pretreatment may be useful for the identification of the cellular origins of prostaglandins in other tissues.

SUMMARY

Mature skeletal muscles produce appreciable quantities of prostacyclin (PGI_2) and smaller amounts of $PGF_{2\alpha}$ and PGE_2, but the sources of these prostaglandins within skeletal muscle are unknown. Monoclonal antibodies to prostaglandin synthetase and prostacyclin synthetase were used to determine which muscle cells produce prostaglandins. The antibody to prostacyclin synthetase stained the tendon, fascia, epimysium and the arteries leading to the muscles. The endothelia of arterioles were also stained in the tibialis anterior and cremaster but not in the soleus muscles. Only trace levels of immunoreactivity were observed with the antibody to prostaglandin synthetase in normal muscles. However, immunoreactivity was observed in the muscles of rats that had been pretreated with aspirin, a drug that inhibits and stabilises prostaglandin synthetase. In muscles of the aspirin-treated rats, all cell types that were stained by the antiprostacyclin synthetase also reacted weakly with the antibody to prostaglandin synthetase. In addition, some cells in the endomysium were strongly stained with the antiprostaglandin synthetase but not with the antiprostacyclin synthetase. We conclude that (1) at least one aspect of the regulation of blood flow in the microcirculation of slow muscles is different from that of fast muscles, (2) that the tendon and connective tissue is the major source of PGI_2 in mature skeletal muscles, and (3) that the prostaglandin-dependent effects of insulin and some other stimuli on skeletal muscle may be mediated by the muscle's arterioles or connective tissue.

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REFERENCES

AGER, A., GORDON, J. L., MONCADA, S., PEARSON, J. D., SALMON, J. A. & TREVETHICK, M. A. (1982). Effects of isolation and culture on prostaglandin synthesis by porcine aortic endothelial and smooth muscle cells. *Journal of Cellular Physiology* **110**, 9–16.

DIETZE, G., WICKLMAYR, N., BOTTGER, I. & MAYER, L. (1978). Insulin action on glucose uptake into skeletal muscle. Inhibition of endogenous biosynthesis of prostaglandins. *FEBS Letters* **92**, 294–298.

DOHERTY, M. (1989). Soft tissue injections in the surgery. *Practitioner* **233**, 1305.

FABER, J. E., HARRIS, P. D. & JOSHUA, I. G. (1982). Microvascular response to blockade of prostaglandin synthesis in rat skeletal muscle. *American Journal of Physiology* **243**, H51–H60.

FAGAN, J. M. & GOLDBERG, A. L. (1986). Inhibitors of protein and RNA synthesis cause a rapid block in prostaglandin production at the prostaglandin synthase step. *Proceedings of the National Academy of Sciences of the USA* **83**, 2771–2775.

FRANGOS, J. A., ESKIN, S. G., MCINTIRE, L. V. & IVES, C. L. (1985). Flow effects on prostacyclin production by cultured human endothelial cells. *Science* **227**, 1477–1479.

FU, J.-Y., MASFERRER, J. L., SEIBERT, K., RAZ, A. & NEEDLEMAN, P. (1990). The induction and suppression of prostaglandin H_2 synthase (Cyclooxygenase) in human monocytes. *Journal of Biological Chemistry* **265**, 16737–16740.

KERRY, P. J. (1985). 6-oxo-prostaglandin $F_{1\alpha}$ production by guinea-pig skeletal muscle in vitro: the effects of oestradiol and progesterone. *Prostaglandins, Leukotrienes and Medicine* **17**, 283–289.

KOLLER, A. & KALEY, G. (1990*a*). Prostaglandins mediate arteriolar dilation to increased blood flow velocity in skeletal muscle microcirculation. *Circulation Research* **67**, 529–534.

KOLLER, A. & KALEY, G. (1990*b*). Endothelium regulates skeletal muscle microcirculation by a blood flow velocity-sensing mechanism. *American Journal of Physiology* **258**, H916–H920.

LEIGHTON, B., BUDOHOSKI, L., LOZEMAN, F. J., CHALLIS, R. A. J. & NEWSHOLME, E. A. (1985). The effect of prostaglandins E_1, E_2 and $F_{2\alpha}$ and indomethacin on the sensitivity of glycolysis and glycogen synthesis to insulin in stripped soleus muscles of the rat. *Biochemical Journal* **227**, 337–340.

MCLENNAN, I. S. (1985). Inhibition of prostaglandin synthesis produces a muscular dystrophy-like myopathy. *Experimental Neurology* **89**, 616–621.

MCLENNAN, I. S. (1987). Characterization of a prostaglandin dysfunction myopathy. *Muscle & Nerve* **10**, 801–809.

MCMILLAN, D. N., REEDS, P. J., LOBLEY, G. E. & PALMER, R. M. (1987). Changes in protein turnover in hypertrophying plantaris muscles of rats: effect of fenbufen – an inhibitor of prostaglandin synthesis. *Prostaglandins* **34**, 841–852.

NEEDLEMAN, P., TURK, J., JAKSCHIK, B. A., MORRISON, A. R. & LEFKOWITH, J. B. (1986). Arachidonic acid metabolism. *Annual Reviews of Biochemistry* **55**, 69–102.

NESHER, R., KARL, I. E. & KIPNIS, D. M. (1985). Dissociation of effects of insulin and contraction on glucose transport in rat epitrochlearis muscle. *American Journal of Physiology* **249**, C226–C232.

NOWAK, J., BOHMAN, S., ALSTER, P., BERLIN, T., CRONSTRAND, R. & SONNENFELD, T. (1983). Biosynthesis of prostaglandins in microsomes of human skeletal muscle and kidney. *Prostaglandins, Leukotrienes and Medicine* **11**, 269–279.

RAZ, A., WYCHE, A., SIEGEL, N. & NEEDLEMAN, P. (1988). Regulation of fibroblast cyclooxygenase synthesis by interleukin-1. *Journal of Biological Chemistry* **263**, 3022–3028.

REEDS, P. J. & PALMER, R. M. (1984). Changes in prostaglandin release associated with inhibition of muscle protein synthesis by dexamethasone. *Biochemical Journal* **219**, 953–957.

REEDS, P. J., HAY, S. M., GLENNIE, R. T., MACKIE, W. S. & GARLICK, P. J. (1985). The effect of indomethacin on the stimulation of protein synthesis by insulin in young post-absorptive rats. *Biochemical Journal* **227**, 255–261.

RODEMANN, H. P. & GOLDBERG, A. L. (1982). Arachidonic acid, prostaglandin E_2 and $F_{2\alpha}$ influence rates of protein turnover in skeletal and cardiac muscle. *Journal of Biological Chemistry* **257**, 1632–1638.

ROSEN, G. D., BIRKENMEIER, T. M., RAZ, A. & HOLTZMAN, M. J. (1989). Identification of cyclooxygenase-related gene and its potential role in prostaglandin formation. *Biochemical and Biophysical Research Communications* **164**, 1358–1365.

SMITH, R. H., PALMER, R. M. & REEDS, P. J. (1983). Protein synthesis in isolated rabbit forelimb muscles. The possible role of metabolites of arachidonic acid in the response to intermittent stretching. *Biochemical Journal* **214**, 153–161.

SMITH, W. L. (1986). Prostaglandin biosynthesis and its compartmentation in vascular smooth muscle and endothelial cells. *Annual Review of Physiology* **48**, 251–262.

SMITH, W. L. (1989). The eicosanoids and their biochemical mechanisms of action. *Biochemical Journal* **259**, 315–324.

SMITH, W. L. & ROLLINS, T. E. (1982). Anti-PGH synthase antibodies and use in immunocytochemistry. *Methods in Enzymology* **258**, 213–222.

SMITH, W. L., DEWITT, D. L. & ALLEN, M. L. (1983). Bimodal distribution of the prostaglandin I_2 synthase antigen in smooth muscle cells. *Journal of Biological Chemistry* **258**, 5922–5926.

SUNDQUIST, H., FORSKAHL, B. & KVIST, M. (1987). A promising novel therapy for achilles peritendinitis:

double-blind comparison of glycosaminoglycan polysulfate and high dose indomethacin. *International Journal of Sport Medicine* **8**, 298–303.

TEMPLETON, G. H., PADALINO, M. & MOSS, R. (1986). Influences of inactivity and indomethacin on soleus phosphatidylethanolamine and size. *Prostaglandins* **31**, 545–559.

VANDENBURGH, H. H., HATFALUDY, S., SOHAR, I. & SHANSKY, J. (1990). Stretch-induced prostaglandins and protein turnover in cultured skeletal muscle. *American Journal of Physiology* **259**, C232–C240.

YOSHIMOTO, T., MAGATA, K., EHARA, H., MIZUNO, K. & YAMAMOTO, S. (1986). Regional distribution of prostaglandin endoperoxidase synthase studied by enzyme-linked immunoassay using monoclonal antibodies. *Biochimica et biophysica acta* **877**, 141–150.