THE OCCURRENCE OF MYOSIN LIGHT CHAIN 1 SLOW IN BOVINE MUSCLES

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SUMMARY

Mammalian slow muscles are thought to be responsible for posture, but certain slow muscles, e.g. bovine masseter, are highly active. These muscles contain myosin light chain 1 slow, a protein absent from muscles with a clear postural role. We propose that this protein is associated with movement.

INTRODUCTION

Histochemical and electromyographical studies (e.g. Edstrom and Kugelberg 1968; Smith et al. 1977) point to a division of labour between muscle fibres that is fundamental to vertebrate movement. Slow-contracting fibres, and muscles dominated by this type, are responsible for posture. Fast-contracting fibres and muscles are recruited as locomotion begins and speed increases (Armstrong 1981). Slow muscles have evolved for efficiency of isometric tension while fast muscles have evolved for efficiency of doing external work (Davies et al. 1970).

Although Armstrong's model is widely accepted, at least one mammalian muscle - bovine masseter - that comprises solely slow fibres (Young and Davey 1981; Talmant et al. 1986) is not postural. Masseter is rhythmically active in chewing and helps pump blood back to the heart when the animal's head is lowered. Young (1982) noted that all masseter fibres contained myosin light chain 1 slow (LC1 slow), whereas in diaphragm, only some slow fibres contained this protein. This difference could be related to function. By comparing the light chain's occurrence in muscles of known function, we hoped to assign a role to it.

MATERIALS AND METHODS

LC1 slow was purified from bovine masseter by conventional methods. Antibody was raised in chickens, and recovered in egg yolk (Polson et al. 1980). Antibody specificity was tested by immunoblotting (Towbin et al. 1979).

Samples of bovine and ovine muscles in rigor were cross-cryosectioned (8 m). Of a serial pair, one transverse section was stained for myofibrillar adenosine triphophatase (ATPase) activity (Guth and Samaha 1969). The other was stained with antibody then further stained with fluorescein-labelled secondary antibody. The occurrence of LC1 slow was gauged by comparison of serial micrographs. Fibre type occurrences were measured by a transect method.

RESULTS

Immunoblotting showed that immunoglobulin from treated chickens bound strongly to only one protein, LC1 slow, from masseter fibres. LC1 fast, from cutaneus, also bound antibody but much less avidly. There was no reaction with other light chains, slow or fast. Preimmune and non-immune preparations showed no specific fluorescence. Specific fluorescence could only be demonstrated in rigor tissue. Preliminary work with several animals showed that, as expected, only some slow fibres of the diaphragm contained LC1 slow, while all masseter fibres contained it. With rare exceptions fast fibres were devoid of it. The antibody bound to some ovine slow fibres.

Table 1. Muscles with high or low LC1 slow content in slow fibres

Muscle	Locality	Probable function	Approx. slow fibre content (%)
High LC1 slow content			
Masseter	Jaw	Chewing	100
Temporalis	Jaw	Chewing	100
Pterygoid	Jaw	Chewing	99
Digastricus	Jaw	Chewing	80
Sternomandibularis	Ventral neck	Head movement	35
Psoas major	Sublumbar	Hip flexion	45
Iliacus	Sublumbar	Hip flexion	65
Sartorius	Medial thigh	Limb movement	35
Adductor (upper)	Medial thigh	Limb movement	25
Gluteus medius	Lat. hip, thigh	Locomotion	20
Extens. digitorum longus	Ant. lower leg	Digit extension/ ankle flexion	35
Extens. digitalis lateralis	Lat. lower leg		20
Peroneus longus	Lat. lower leg	Ankle movement	40
Gastrocnemius (lat., med.)	Post. lower leg	Locomotion	30
Low LC1 slow content			
Gemellus	Deep hip	Movements about hip	40
Quadratus femoris	Deep med. thigh		95
Gluteus profundus	Deep lat. thigh		60
Rectus abdominis	Ventral abdomen	Visceral compression and back arching	40
Transversus abdominis			25
Obliquus abdom. internus			35
Flexor digitorum longus	Posterior lower leg	Digit flexion/ ankle extension	2
Flexor digiti I longus			70
Flexor digitorum superfic.			40
Rectus superior	Extraocular eye	Eyeball posture	10

Bovine leg muscles were also examined, as a group, for fibre type occurrence and fluorescence (Fig. 1). Soleus was the slowest (84% slow) followed by one head of the deep digital flexor (flexor digiti I longus, 73%). By contrast,

Table 2. Muscle fibre characteristics in ovine leg muscles

Muscle	Approx. slow fibre occurrence (%)	Fluorescence level in slow fibres
Sartorius	40	**
Vastus medialis	35	*
Rectus femoris	30	**
Vastus lateralis	30	***
Vastus intermedius	95	--

+ The highest fluorescence level is denoted by ***, while -- denotes no fluorescence.

In many bovine muscles, fluorescence due to LC1 slow was variable. In other muscles, however, all slow fibres contained the protein or all were nearly devoid of it (Table 1).

Bovine leg muscles were also examined, as a group, for fibre type occurrence and fluorescence (Fig.1). Soleus was the slowest (84% slow) followed by one head of the deep digital flexor (flexor digiti I longus, 73%). By contrast, the other two heads were among the fastest: tibialis caudalis, 18%, and flexor digitorum longus, 2%. Other posterior muscles were more mixed. Tibialis cranialis was the slowest anterior muscle. Muscles with the least-fluorescent slow fibres were the digital flexors, and the most fluorescent were the digital extensors, peroneus longus, and the gastrocnemii (Fig.1). The group comprising the gastrocnemii and the superficial digital flexor had the smallest fibres.

Of the upper ovine leg muscles the slowest was vastus intermedius (Table 2). Its slow fibres were non-fluorescent. The four other muscles had more fast fibres. The slow fibres in these muscles were variably fluorescent.

DISCUSSION

The cross-reactivity of the antibody with LC1 fast was not a serious problem, because in cryosections fast fibres did not bind significant antibody.

The slow fibres of bovine muscles with some probable postural function had a low LC1 content (Table 1): Gemellus, quadratus femoris and gluteus profundus are all deep muscles about the hip. The hip joint is stabilized by these muscles rather than ligaments, so they might be expected to have a postural role in addition to the locomotory role evidenced by their fast fibre content. Slow fibres in abdominal muscles might have a role in visceral compression and back arching, both postural actions. Since the fast fibres of extraocular muscle are specialized for fast contraction (Sartore et al. 1987), it is likely that the multiply-innervated slow fibres (Harker 1972) of rectus superior maintain "posture". Slow fibres of the deep digital flexor (three heads) and the superficial flexor (Fig.2) were low in LC1 slow (Fig.1; Table 1). The anatomy of the bovine digital muscles suggests some antigravity role for the flexors (Fig.2). If they did not posturally maintain tension about the joints, the leg would collapse. By contrast, the three digital extensors were high in LC1 slow, especially extensor digitorum longus, and lateralis. The precise role of slow fibres in the extensors is not known, but need not be postural (Fig.2).

The limited results from one ovine leg confirm the findings of several researchers (e.g., Ariano et al. 1973), that vastus intermedius is a slow muscle. It is generally agreed that its role is postural to prevent the leg collapsing at the knee joint. Although the specificity of the antibody was not tested against ovine proteins, the fluorescence data (Table 2) strongly suggests that the antibody also recognizes the ovine equivalent of LC1 slow and that vastus intermedius lacks ovine LC1 slow.

In contrast to the postural muscles, the highly active chewing muscles are all rich in slow fibres that contain LC1 slow (Table 1). Indeed, Sartore et al. (1987) confirmed that LC1 slow was present in bovine masseter, a basic tenet of the present paper. We propose that LC1 slow is associated with movement. Movement, as opposed to posture, is generally attributed to more superficial muscles (Armstrong 1981). Sternomandibularis, sartorius and gastrocnemius are superficial muscles, and the last is certainly involved in locomotion (Gambaryan 1974). These three muscles contain the light chain. Although the deeper muscles, psoas major, iliacus, adductor and gluteus medius all contain the light chain, a clear role for their slow fibres is not obvious.

Although many references point to difference between slow fibres in various muscles (see e.g. Carraro et al. 1981; Mabuchi et al. 1984), no particular physiological function has been proposed for any myosin light chain until now.

The bovine deep digital flexor comprises three heads that combine to a common tendon that flexes the digits (Fig. 2). The differing slow fibre composition (Fig.1) is a striking display of division of labour within a muscle, consistent with Armstrong's model of locomotion. The fast fibres of the deep digital flexor (Figs.1, 2) are presumably involved in locomotion. In bovine, a species that walks on its toe nails, toe flexion would propel the animal forward. However, division of labour did not extend to the superficial muscle group comprising the medial and lateral gastrocnemii and the superficial digital flexor. This may relate to the reduced role of soleus in this species and in dog (Armstrong et al. 1982).

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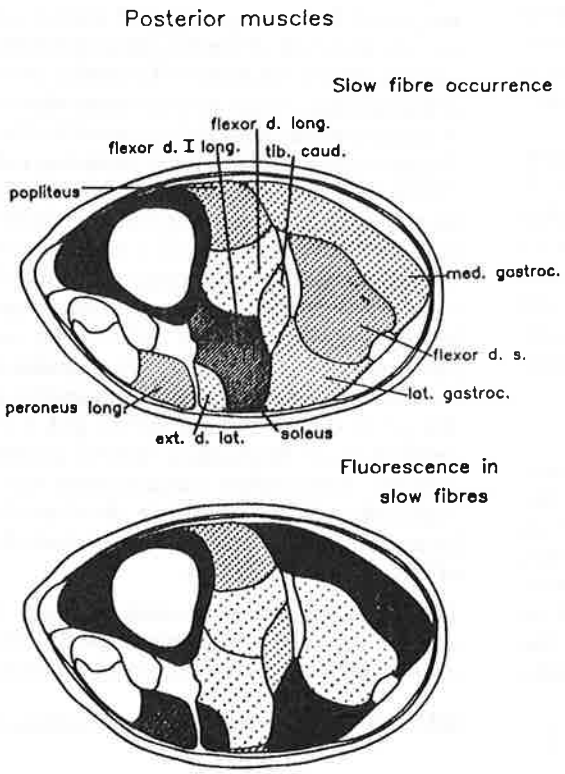

Fig. 1. Bovine lower leg muscles. Denser shading means more slow fibres and more fluorescence in them. Fluorescence reflects LC1 slow content. The tibia is blacked.

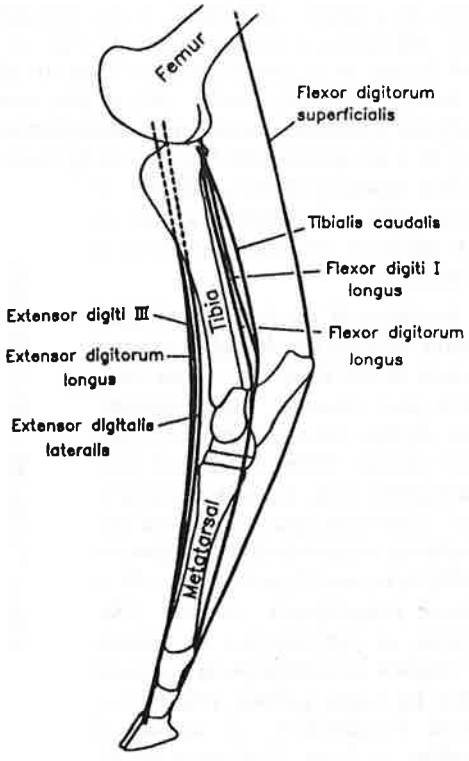

Fig. 2. Schematic view of digit flexors and extensors originating near the bovine stifle.