INTRODUCTION

Whether intrafusal and extrafusal fibres arise from a common progenitor in developing rat skeletal muscle is unresolved. Pedrosa & Thornell (1990) proposed that the primary myotubes which differentiate into the bag₂ intrafusal fibre form from fusion of myogenic cells committed to differentiate into intrafusal fibres only. They further suggested that the precursors of bag₂ fibres are capable of attracting sensory innervation to the developing muscle spindles, and that all subsequent generation types of intrafusal fibre form by a similar process (Pedrosa & Thornell, 1990). In contrast, Kucera & Walro (1990) proposed that both intrafusal and extrafusal fibres originate from several pools of bipotential myotubes, and that the interaction between primary afferents and bipotential myotubes mediates transformation of these myotubes into the different types of intrafusal fibres.

The present study reports that an avian monoclonal antibody (mAb), S46, binds to a myosin heavy chain (MHC) isoform expressed by subpopulation of slow primary myotubes in rats. This population of myotubes, the oldest myotubes in hindlimb muscles, is bipotential in that it consists of precursors of both bag₂ intrafusal and type 1 extrafusal fibres.

MATERIALS AND METHODS

Animals

Fetal, neonatal and adult (60 day) offspring of ten timed-pregnant Sprague-Dawley rats were a source of muscles. The ages of rats were determined either from the day of impregnation (embryonic day 0 or EO) or the date of birth (postnatal day 0 or P0). Two rats each were examined at 24 h intervals from day E15 to E21, and at P0, P7 and P60.
Tissue processing

Rats were anaesthetised with sodium pentobarbital (50 mg/kg i.p.). Entire right lower legs (crura) of prenatal or early postnatal rats or individual leg muscles of adult rats were removed and frozen in isopentane. Midportions of the crural muscles were cut transversely into serial 8-μm sections in a cryostat. Sets of serial sections were reacted with a panel of four monoclonal antibodies: WBMHC-s, specific for slow-twitch MHC; MY32, specific for neonatal/adult MHC; ALD19, specific for slow-tonic MHC; and S46, specific for SM1 and SM2 (Page, Miller, DiMario, Hager, Moser & Stockdale, 1992). Binding of the primary antibodies was demonstrated using the avidin-biotin-complex (ABC) peroxidase method (Kucera, Walro & Gorza, 1992).

Identification of muscles and spindles

Although all crural muscles were examined, particular attention was given to the tibialis anterior (TA), a muscle predominantly composed of type 2 extrafusal fibres, and the soleus (SOL), a muscle predominantly composed of type 1 fibres. Myotubes were classified as primary or secondary based on size and chronology of development, and as slow, fast or mixed (slow/fast) based on their patterns of binding for WBMHC-s and MY32 (Kucera & Walro, 1990). Spindles were identified as encapsulations of small-diameter muscle fibres that contained one or two fibres reactive to the slow-tonic mAb, ALD19 (Pedrosa & Thornell 1990). Intrafusal fibres were classified as nuclear bag2, nuclear bag1 and nuclear chain fibres on the basis of relative fibre size and patterns of MHC expression (Kucera & Walro, 1990). Regions of intrafusal fibres in P60 rats were classified as A (equatorial and juxta-equatorial), B (polar encapsulated) or C (extracapsular) based on established morphological criteria (Kucera, Dorovini-Zis & Engel, 1978).

RESULTS

Early in development at E14-16, the inner slow (red) and outer fast (white) regions characteristic of the mature TA muscle could not be distinguished by binding of WBMHC-s and MY32 because the newly-formed TA muscle contained a homogeneous population of primary myotubes which expressed both slow-twitch and neonatal/fast MHC isoforms. However, these two regions could be identified by differences in S46-binding as early as E16. Myotubes in the future slow region were S46-reactive, whereas myotubes in the future fast region did not bind S46. The two regions of the TA were first differentiable using WBMHC-s and MY32 on E17-18 when the primary myotubes in the outer region of the muscle began to lose their reactivity to the slow-twitch mAb, whereas the primary myotubes of the inner zone began to lose their reactivity to the neonatal/fast mAb. All primary myotubes bound S46 on E14 but by E18, the end of primary myogenesis, myotubes reactive to S46 were located only in the inner axial (future red) region of the TA muscle. Primary myotubes in the outer (future white) region of the TA, especially the lateral part of the TA that overlies the EDL muscle, did not bind S46. A band of myotubes moderately reactive to S46 separated the strongly reactive myotubes of the inner region from the unreactive myotubes of the outer region. No secondary myotubes bound S46 in the TA. Similar patterns of reactivity to mAb S46 were observed in other predominantly fast muscles.

Unlike the TA, the SOL muscle showed no distinct fibre regions at any age. All primary myotubes of the SOL bound the slow-twitch mAb WBMHC-s throughout fetal development. The predominantly slow SOL was the only muscle of the crus in which all primary myotubes, regardless of their location in the muscle, bound mAb S46. In addition,