Expression of L-type calcium channels associated with postnatal development of skeletal muscle function in mouse

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Abstract

Several factors have an influence on the improvement of muscle activity and motor co-ordination of mammals during post-natal development. One of them is voltage sensitive L-type calcium channel function. In striated muscles of adult mammals these channels are located in T-tubule membranes thus linking the on-coming action potential to the molecular process of muscle contraction. The postnatal development of L-type calcium channels is therefore critical not only for contraction but also for all subsequent motor learning. We used high affinity enantiomeri of dihydropyridine labelled with a fluorophore in order to show the relative amount of L-type calcium channels by histofluorescence in tissue. We found by qualitative microscopical analysis that the amount of L-type calcium channels increased during the postnatal development in the mouse skeletal muscle (m. rectus femoris and m. gastrocnemius). We also noted variation between different fibre types in the increase of the amount of L-type calcium channels, as judged by the intensity of histofluorescence. We showed by histochemical staining and statistical analysis that the high density of L-type calcium channels in adult muscles is correlated with fast oxidative glycolytic fibre type of striated muscles rather than slow oxidative or fast glycolytic fibres. Based on this finding we propose that the development of L-type calcium channels can be considered as one of the factors determining the different physiological properties of fibre types.

Introduction

The movements of newborn mice are inaccurate and clumsy while those of adult mice are highly co-ordinated. The development of muscle co-ordination in mammals is a complex process influenced by many factors. Previous studies have shown that the structure and electrical properties of skeletal muscle change during the postnatal development as does the control of movement (Spector and Prives, 1977; Järvillehto and Rissanen, 1987).

Calcium takes part in a wide range of cellular functions, including contraction of skeletal muscle. There are different types of voltage-dependent calcium channels in adult skeletal muscle. The L-type calcium channels play two roles: to function (1) as the voltage controlled sensor for release of calcium and, (2) as an ionic pathway for calcium transport to filaments (Grabner et al., 1991). In triads, gate molecules control the calcium release from sarcoplasmatic reticulum (SR). Depolarization through T-tubule system mediates the calcium flow by voltage sensitive L-type calcium channels, which are embedded in the triads and are linked to the other calcium-selective channels (ryanodine receptors) that are located in the SR membrane and thus initiate excitation-contraction (E-C) coupling.

The L-type calcium channel (also called a dihydropyridine receptor because of its dihydropyridine sensitivity) has a high single channel conductance and requires a large depolarization for activation (Bean, 1989). These slow, high threshold channels are products of a single gene (Ma et al., 1996). The protein consists of several subunits from which the \( \alpha_1 \)-subunit forms the pore of the ion channel (Catterall, 1993). The importance of the \( \alpha_1 \)-subunit for E-C coupling is emphasized by studies in mice with muscular dystrophy, a disease occurring with mice homozygote to mdx allele. In T-tubule membranes of dystenic skeletal muscle, the \( \alpha_1 \)-subunit is absent and mice that have this disease are paralysed. Injection of expression plasmids carrying the \( \alpha_1 \)-subunit cDNA into myotubes leads to the appearance of dihydropyridine receptors and restoration of contraction ability of muscle (Tanabe et al., 1988). The other subunits, \( \beta_2 \), \( \gamma \) and \( \delta \), regulate the function of \( \alpha_1 \)-subunit (Gutierrez et al., 1991; Singer et al., 1991).

Electrophysiological studies have shown that high-threshold sustained currents increase postnatally in normal development of mouse muscle fibres (Gonoi, 1993). The conduction velocity in the mouse skeletal muscle also increases significantly along with the postnatal development (Järvillehto and Rissanen, 1987). Although correlation of the development with channels or fibre types was not studied, it may well be assumed that there is an increase in the channel density of voltage
dependent calcium channels since the electrical properties and muscle performance are clearly improved at ageing. In this study we observed in skeletal muscles of developing mouse a clear increase in the density of L-type calcium channels and a difference between different fibre types in the density of these channels.

Materials and methods

Animals and tissue samples

Ten white laboratory mice (Mus musculus, random strain) from different litters were used for skeletal muscle tissue samples. Three of them were 1 day old, three 28 and four 63 days old. The animals were maintained at +22 ± 1°C (12L/12D photoperiod), breast-fed until 21 days of age and then provided with mouse pellets and water ad libitum.

Mice were killed by cervical dislocation, one of the hindlimbs frozen in liquid nitrogen in situ. During freezing the muscle was stretched lightly. The samples were dissected out from the middle part of biceps femoris posterior (newborn mice) or musculus rectus femoris and musculus gastrocnemius (older mice). The whole biceps femoris posterior was used without newborns because of the difficulties to prepare very small muscles individually apart without damaging their structure. In later developmental stages, however, m. rectus femoris was easily localized by microscope.

Labelling of L-type calcium channels

Tissue samples were cut into 10 μm sections, perpendicular to the longitudinal axis of the muscle, on a cryostat microtome (Jung 2800 FrigoCut-E, Leica, Austria). The samples were picked up on glass slides covered by poly-L-lycine, dried for 30 min and treated with cold acetone to prevent autolysis and bacterial activity. The total amount of samples was 30.

The sections were incubated with 15 nM high affinity (–)-enantiomer of dihydropyridine labelled with orange (546 nm) fluorophore (ST-BODIPY®(–)-dihydropyridine, Molecular Probes, Netherlands) for 90 min at dark and at room temperature (+21 ± 1°C). Knaus et al. (1992) have shown, that L-type calcium channels are readily blocked by the binding of dihydropyridines to the pore-forming subunit (α1) of channels. After incubation the samples were rinsed twice in 1% bovine serum albumine and additionally rinsed in phosphate buffer and distilled water. The samples were covered with antifade reagent (FluoroGuard Antifade Reagent, Bio-Rad, USA) and photographed immediately with photomicroscope (Polyvar, Reichert-Jung, Austria, Fuji 400 Super G Plus film) by using 495–580 nm excitation interference green filter. The BODIPY-conjugated dihydropyridine bound to L-type calcium channels was seen as orange marks in the sections. Control sections were incubated in phosphate buffer devoid of dihydropyridine, while otherwise the treatment was like that described above.

The development of L-type calcium channels was assessed from the intensity of orange fluorescence in the sections and registered with symbols in the following way: – negative fluorescence, + weak fluorescence intensity, ++ moderate fluorescence intensity, +++ strong fluorescence intensity.

Histology of fibre types and statistics

Sections for classification of fibre types were processed for succinate dehydrogenase activity (SDH) as described by Nachlas et al. (1957), and for myofibrillar adenosine triphosphatase activity (mATPase) at sodium barbiturate buffer (pH 9.4) as described by Brooke and Kaiser (1970). Fibre type classification was based on (1) the pH sensitivity of mATPase (slow-twitch oxidative fibres, SO, type I, being acid-stable and alkali-labile, and fast-twitch fibres, type II, vice versa), and (2) staining intensity for SDH (fast oxidative glycolytic fibres, FOG, type IIA: high; fast glycolytic fibres, FG, type IIB: low).

The cross-sectional area and fibre diameter of 10 fibres of each identified fibre type were measured with a Micro Scale TM/TC picture analyser (Digithurst Ltd, England). This procedure was also carried out on ten cells labelled with ST-BODIPY. The measurements based on the assumption that in adults of the same species and age, the muscle fibre sizes should be similar among the same fibre type. In order to reveal a possible correlation between histochemically identified fibre types and fibres exhibiting different fluorescence staining intensities, a t-test for Equality of Means was used for statistical comparisons of fibre areas and diameters of different samples.

Results

The development of L-type calcium channels

The muscle tissue of newborn mice was found to be structurally undeveloped. Individual muscle fibres were arranged rather loosely inside the epimysial sheath, and no compact fibre bundles were formed. We found no fluorescence in the samples from 1-day-old mice immunostained for the L type channels (Figure 1A) or in the control sample (Figure 1A, insert).

The muscle tissue of 28-day-old mice was structurally more developed. The fibres were larger and the tissue more dense. The fluorescence intensity in the samples was moderate (Figure 1B), especially at the edges of the fibre. Fluorophore accumulations often seen on the fibre junctions were probably derived from L-type calcium channels of the smooth muscle in the walls of capillaries between the fibres.

The samples from adult (60 days and older) mice m. rectus femoris showed very strong fluorescence