

## Development of Postural Muscles and Their Innervation

J. IJkema-Paassen and A. Gramsbergen

*Medical Physiology, University Medical Centre Groningen, the Netherlands*

### ABSTRACT

**Control of posture is a prerequisite for efficient motor performance. Posture depends on muscles capable of enduring contractions, whereas movements often require quick, forceful muscle actions. To serve these different goals, muscles contain fibers that meet these different tasks. Muscles with strong postural functions mainly consist of slow muscle fibers with a great resistance against fatigue. Flexor muscles in the leg and arm muscles are mainly composed of fast muscle fibers producing relatively large forces that are rapidly fatigable. Development of the neuromuscular system continues after birth. We discuss in the human baby and in animal experiments changes in muscle fiber properties, regression from polyneural into mononeural innervation, and developmental changes in the motoneurons of postural muscles during that period. The regression of poly-neural innervation in postural muscles and the development of dendrite bundles of their motoneurons seem to be linked to the transition from the immature into the adult-like patterns of moving and postural control.**

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Reprint requests to: J. IJkema-Paassen, Medical Physiology, A. Deusinglaan 1, bldg 3215, 9713 AV Groningen, the Netherlands; e-mail: j.ijkema-paassen@med.rug.nl

### INTRODUCTION

Movement of the extremities on the one hand and the maintenance of posture on the other require different properties of muscles. During standing and sitting, the postural muscles provide joint stiffness in order to resist gravity and to maintain balance. The muscles involved in these tasks require not only a long lasting resistance to fatigue because postures might have to be maintained for large parts of the day but also the possibility to contract quickly when sudden perturbations occur.

During walking, the abdominal and back muscles keep the trunk erect, and the antigravity muscles in the one leg provide stiffness, allowing the other leg to flex and swing. During the swing phase of the leg, the flexor muscles produce short and strong bursts, whereas the extensor muscles in this leg are flaccid. During standing, however, both the flexor and extensor muscles are active. To serve these different goals, namely the resistance to fatigue on the one hand and high contraction speed and large force production on the other, the trunk and leg muscles contain a specific mixture of slow, fatigue-resistant fibers, fast-fatigable fibers, and an intermediate-fiber type, which is fast but relatively fatigue resistant.

In 1678, Stefano Lorenzini described white and red muscles in the same animal and since then, the differences in color have been related to the metabolic and physiological properties of muscles. For an historical review on the identification of the different muscle-fiber types, see Dubowitz (1973). The properties of muscle fibers can be studied by recording the contraction speed of muscle fibers,

by identifying the enzyme systems related to the metabolism in the muscle fiber, or by their type of myosin, the contractile protein.

When the nerve to a muscle is electrically stimulated (e.g. McPhedran et al., 1965; Wuerker et al., 1965) or a single motoneuron through intracellular micro-electrodes (e.g. Burke et al., 1973), a contraction of the muscle fiber occurs that can be measured by means of a force transducer. The time between the onset of the contraction and the peak value of tension is defined as the twitch contraction time. Such measurements indicate considerable differences in twitch contraction times among the muscle fibers. In the cat, contraction times vary between 20 ms for the fastest muscle fibers in the medial gastrocnemius and 130 ms for the slowest fibers in the soleus muscle (Burke et al., 1974). Fibers that reach the peak value relatively fast are called fast twitch or fast fibers, those that slowly build up the force are called slow twitch or slow fibers. In the human, twitch contraction times of the fibers of the extensor hallucis brevis muscle, for example, vary between 20 ms to 140 ms, indicating that this foot muscle contains both slow and fast fibers (Sicca & McComas, 1971).

Differences in muscle fibers can also be identified by their metabolism and morphology. Muscle fibers consist of thick myofilaments called myosin, surrounded by a hexagonal array of thin actin filaments. The myosin filament is an aggregate of around 100 myosin molecules, their 'tails' forming the filament. The 'heads' of the molecules, situated at an angle with their tails, form the cross bridges with the actin. Upon contraction, the cross bridges alter their angle with the myosin filament, giving a power stroke to the actin filament. For this process, ATP is used as the energy source. 'White' muscle fibers are dependent upon anaerobic metabolism and very little ATP is stored in them. Short-term replenishment of ATP is conducted via creatine phosphate, which transfers

its phosphate group to ADP. When additional ATP is required, it will be derived from the anaerobic metabolism of glucose reserves in muscle fibers or of glucose from the blood. These fibers have a very high speed of contraction and a high myosin ATPase activity. ATP is used faster than it can be produced, thus these fibers fatigue rapidly.

'Red' fibers contain many mitochondria and acquire their energy from the aerobic metabolism of glucose and from free fatty acids. The glucose metabolism forms pyruvate, which enters the citric acid cycle in the mitochondria, as do the fatty acids. Red fibers have a low contraction speed and a low myosin ATPase activity. This property, in combination with a high production of ATP, explains why these fibers are fatigue resistant.

Enzyme-histochemical techniques make use of the differences in metabolism. Anaerobic and aerobic muscle fibers are respectively classified as type II or type I fibers. A further classification into type IIa and IIb can be made. Type IIa fibers are the intermediate fibers containing high contents of glycogen and mitochondrial enzymes and thus working under both anaerobic and aerobic conditions. The myosin ATPase technique has been used extensively to study fiber type distributions (Engel, 1962; Brooke & Kaiser, 1970; Lind & Kernell, 1991; Ijkema-Paassen et al., 2001). Anaerobic and aerobic fibers differ in their sensitivity to the pH of the staining solutions, resulting in darker or lighter staining of the fibers.

Myosin ATPase resides in the heavy chain of myosin (Weiss et al., 1999). It has been established that the myosin has several isoforms (Pette & Staron, 1990). Based on the differences in isoforms, antibodies against slow or fast myosin have been developed that can be visualized in histological sections. (Pette & Staron, 1990, 2000; Canepari et al., 2000). Therefore, slow or type I fibers and fast or type II fibers can also be studied with the techniques mentioned above (Staron & Pette, 1986; Bottinelli et al., 1991; 1994; Galler et al., 1994).

Almost all muscles contain both slow and fast fibers. Percentages of slow and fast muscle fibers have been calculated in cross sections of muscles in the rat (Gramsbergen et al., 1996; Ijkema-Paassen et al., 2001) and in the human (Johnson et al., 1973; Gollnick et al., 1974; Kumagai et al., 1984; Bellemare et al., 1986; Bottinelli & Reggiani, 2000; Polla et al., 2004). From these studies, it became obvious that trunk muscles and extensor muscles in the legs in humans contain relatively large percentages of slow muscle fibers, whereas most other muscles are predominantly composed of fast muscle fibers. The percentages of type I muscle fibers are 57% in the m. erector spinae, 46% in the m. rectus abdominus, 54% in the m. trapezius (an extensor), and 35% in the m. sternomastoid (a flexor). The m. soleus in the leg contains on average 88% type I fibers (Johnson et al., 1973).

#### DEVELOPMENT OF MUSCLES AND DIFFERENTIATION OF MUSCLE FIBERS

The skeletal muscles of vertebrates are derived from the paraxial mesoderm. This mesoderm develops into bilaterally paired somites at the end of the 5<sup>th</sup> postmenstrual (PMA) week in the human embryo (Cossu et al., 1996; Larson, 2001a). The somites give rise to the muscles of the neck, trunk and limbs, and the axial skeleton. At the end of the 7<sup>th</sup> week, the myotomes have developed, and then the muscles form by a process of fusion, splitting, or migration (for review see Mastaglia, 1981). Limb buds are formed from the lateral plate mesoderm, the upper limbs around the 38<sup>th</sup> day PMA, and the lower limbs around the 43<sup>rd</sup> day (Larson, 2001b). The limb buds consist of an ectodermal cap and the inner mesodermal core, giving rise to the skeleton, ligaments, and vasculature of the limbs. The mesenchymal cells located dorsally (the dorsal plate) later give rise to the

flexors, whereas the cells in the ventral plate later on develop into the extensor muscles (Francis-West et al., 2003)

The mesenchymal cells develop into myoblasts, and these mononucleated cells fuse with each other to form multinucleated myotubes. These so-called primary myotubes appear at about the 7<sup>th</sup> week PMA and thereafter rapidly increase in number (Fenichel, 1966; Barbet et al., 1991). The innervation of the primary myotubes is observed from the 9<sup>th</sup> week onward, the youngest age investigated (Fidzianska, 1980). This observation is in line with the data of Juntunen and Teräväinen (1972), who described the development of the myoneural junctions starting at age 8.6 weeks PMA in the intercostal muscles and at 10 weeks in the tibial muscle. The first general movements (movements in which all parts of the body are involved) can be observed between 8 and 10 weeks PMA, implying that the primitive myoneural junctions are functional by that time (de Vries et al., 1982). Secondary and subsequent generations of myotubes develop from undifferentiated mononucleated cells lying in close proximity to the primary myotubes (Ontell, 1977).

Much research on muscle development has been performed in rats, and most of the following results have been obtained in these animals. The primary myotubes, formed by the fusion of myoblasts, are already observed at embryonic day 9 (E9), and at E12 the nerves reach the myotubes (rats are born after a gestation period of 22 to 23 days); (Jansen & Fladby, 1990; Wigmore & Dunglison, 1998). The secondary myotubes develop beneath the basal lamina of the primary myotube, and as soon as the myotube has reached its tendons, an independent basal lamina is formed for the secondary myotube (Kelly & Zacks, 1969; Ontell & Kozeka, 1984; Duxson et al., 1986). Research in rodents and chick embryos has demonstrated that primary myotubes develop and differentiate even in the absence of innervation (Butler et al., 1982; Condon et al., 1988;

1989; 1990). The appearance and development of secondary myotubes, on the other hand, seems to be dependent on innervation (Betz et al., 1980; Harris, 1981; McLennan, 1983; Ross et al., 1987a; 1987b). Shortly after their generation, the primary myotubes in fetal rats display immunoreactivity against slow myosin, whereas the secondary myotubes, emerging from E18 are immunoreactive to fast myosin (Rubinstein & Kelly, 1981; Dhoot et al., 1985; Narusawa et al., 1987; Condon et al., 1989). These properties can change during development because from E15 onward, the superficially located primary myotubes of most muscles lose their reactivity to slow myosin and become reactive to fast myosin (Condon et al., 1989). In the human embryo, differences in the primary and secondary myotubes can be observed as early as 12 to 13 weeks PMA, using the myofibrillar ATPase method (Fenichel, 1966). Dreager and coworkers (1987) found that the primary myotubes in the quadriceps muscle at 10 to 12 weeks react with antibodies against slow myosin. At 14 to 15 weeks, secondary myotubes emerge and react with an antibody to fast myosin; this reaction similarly holds for a third wave of tertiary myotubes emerging at 16 to 17 weeks. During further development, the myotubes enlarge but by 33 to 36 weeks, around 50% of the secondary myotubes change their fast myosin into slow myosin (Dreager et al., 1987). Obviously, both in rats and in humans, rearrangements occur in the properties of part of the myotubes, but via different developmental trajectories.

At the end of the intra-uterine period, the number of myofilaments increases and the nucleus is pushed toward the cell membrane. From this moment, the fibers are called muscle fibers. In the human fetus, the change from myotubes into muscle fibers takes place around 22 weeks PMA (Mastaglia, 1981) Research in rats has indicated that during postnatal development, further changes occur in the properties of muscle fibers, mainly by

changes in patterns of activation. The percentage of slow fibers in the rat's soleus muscle increases because the animal is beginning to use the muscle for support. Unloading the hindleg demonstrated that this development does not occur then (Lowrie et al., 1989; Sakuma et al., 1995). Reloading the soleus muscle for at least 2 weeks will undo the changes in properties of the muscle (Lee et al., 2004). In line with this evidence, the properties of the muscle fibers of the human baby are also subject to changes during the first years of life. The percentage of type I fibers in the diaphragm of preterm babies (<37 weeks) is around 10%, increasing to 25% at term age and to 55% at 2 years of age. Similar changes were observed in the intercostal muscles. These muscles contain around 20% type I fibers in preterms, changing to 46% in full terms and to 65% in young children. (Keens et al., 1978; DeLuca et al., 1986; Bottinelli & Reggiani, 2000; Baldwin & Haddad, 2002; Polla et al., 2004).

#### DEVELOPMENT OF NEUROMUSCULAR INNERVATION

The neuroepithelium in the ventricular zone of the neural tube in rats starts proliferating from embryonic day 12 (Nornes & Das, 1974). The future motoneurons are the first neuroblasts to migrate toward ventrolateral positions in the developing spinal cord. Initially, these neuroblasts form a continuous column along the rostrocaudal extent of the spinal cord, and later on, after differentiation, motoneuronal pools develop. Axons of the motoneurons are the first to emerge from the spinal cord. Their initial guidance as far as axons to the trunk muscles are concerned depends on the sclerotomes of the somite (Keynes & Stern, 1984; Hughes & Salinas, 1999). The axons of the motoneurons heading for the muscles in the extremities form the spinal nerves. The

dorsal ramus of these nerves head for the dorsal plate in the limb bud and the ventral ramus towards the ventral plate. From the dorsal plate, the flexor muscles develop and from the ventral plate the extensor muscles. The axons of a particular motoneuronal pool reach their muscles even after experimental perturbations at early developmental stages such as partial deletion and reversal of a spinal cord segment (Lance-Jones & Landmesser, 1980a; 1980b). Additionally, the experimental repositioning of muscle targets does not prevent correct connections being formed, indicating that both along the trajectory and by the target muscle, trophic factors are secreted that guide the outgrowing axons (Ebbens et al., 1996). In the human, an example of such specificity is observed in the Martin-Gruber anastomosis. The thumb is normally innervated by the median nerve but in the Martin-Gruber anastomosis, a crossover of some axons occurs from the median to the ulnar nerve. These nerves, however, still are able to locate their proper target muscle in the hand (McComas, 1996; Lee et al., 2005).

Upon contacting the muscle, synaptic transmission is established within 1 to 2 hours (Chow & Cohen, 1983; Xie & Poo, 1986). The nerve secretes agrin, a molecule that, after incorporation into the basal lamina of the muscle fibers, induces the aggregation of acetylcholine receptors (AChR) (Rupp et al., 1991; Bove & Fallon, 1995). Muscle-specific kinase (MuSK), a tyrosine kinase receptor, is also activated by agrin and this, in turn, induces the nerve to stop growing, and then the growth cone changes into a presynaptic nerve terminal (Valenzuela et al., 1995; DeChiara et al., 1996; Gautam et al., 1996). In the human embryo, the first primitive motor endplates are observed at around 9 weeks PMA in the quadriceps femoris (Fidzianska, 1980), intercostals, and tibial muscles (Jutunen & Teräväinen, 1973).

The motor endplates are initially innervated by axonal branches from different motoneurons. This

so-called polyneural innervation has been studied in rats by intracellular recordings of endplate potentials in response to a graded stimulation of the muscle nerve (Redfern, 1970). With increasing stimulus intensity, more and more axons are recruited, leading to a stepwise increase of the endplate potentials. The number of these steps in the endplate potential indicates the number of axons innervating the muscle fiber. In rats, the numbers of axons impinging on one muscle fiber can vary from two axons in lumbrical muscles (Betz et al., 1979) to six axons in the soleus muscle (Bennet & Pettigrew, 1974). The maximum in polyneural innervation is reached one day before birth in the diaphragm and the intercostal muscles (Bennet & Pettigrew, 1974) and a few days after birth in the soleus and the extensor digitorum muscle (Balice-Gordon & Thompson, 1988; Brown et al., 1976).

Additionally, the period of time in which polyneural innervation is replaced by mononeural innervation differs among the various muscles. The rat's extensor digitorum muscle is already mononeurally innervated from the third day of life (Balice-Gordon & Thompson, 1988), whereas in the soleus and psoas muscle polyneural innervation lasts until the third week (Brown et al., 1976; IJkema-Paassen & Gramsbergen, 1998).

The regression of polyneural innervation probably is related to muscle activity. Electrical or pharmacological stimulation of the soleus muscle accelerates the process of regression (O'Brien et al., 1978; Thompson, 1983; Vrbova et al., 1988; Zu & Vrbova, 1992; Vyskocil & Vrbova, 1993), whereas a decrease in activity by tendinotomy of the Achilles' tendon, nerve conduction block, or blocking synaptic transmission results in a retarded regression of polyneural innervation (Benoit & Changeux, 1975; Thompson & Jansen, 1977; Riley, 1978; Brown et al., 1982; Duxson, 1982; Caldwell & Ridge, 1983; Callaway & Van Essen, 1989; Greensmith & Vrbova, 1991).

Probably the activity in the muscle fibers

releases  $K^+$  that accumulates in the synaptic cleft and induces an increase of  $Ca^{2+}$  in the nerve terminal. When surpassing a certain level,  $Ca^{2+}$  activates the neural protease calpain (CANP), which in turn leads to the breakdown of cytoskeletal proteins and the subsequent degradation of the supernumerary nerve endings (Vrbova et al., 1995). Small nerve terminals take the lead in this withdrawal because the accumulation of  $Ca^{2+}$  reaches its critical concentration more easily. It has been suggested that slow muscle fibers are more heavily innervated than fast fibers (Jansen & Fladby, 1990). Indeed, primary myotubes, the precursors of slow muscle fibers, are innervated by many more nerve endings than are secondary myotubes (Sheard et al., 1991). These findings indicate that polyneuronal innervation is also a prominent feature in postural muscles in their initial stages of development, as they contain a large percentage of type I fibers (for review see Ribchester, 2001).

Polyneuronal innervation for certain muscles persists in the postnatal period, whereas in other muscles, regression is completed at the day of birth. The regression of polyneuronal innervation in the diaphragm and intercostal muscles is complete at the day of birth, at the onset of continuous respiration (Bennet & Pettigrew, 1974), whereas the end of polyneuronal innervation in the soleus muscle of the rat coincides with the emergence of the adult type of walking (Brown et al., 1976; Westerga & Gramsbergen 1990; Geisler et al., 1993). This seems to indicate a relation with the function of the muscles.

Only limited knowledge is available on polyneuronal innervation and its regression in the human. Fidzianska (1980) studied in fetuses until 20 weeks PMA the ultrastructural pattern of the early stages of end-plate formation in the quadriceps femoris muscle. She observed polyneuronal innervation until 20 weeks PMA. Unfortunately, this stage did not extend until older ages. The regression of poly-

neuronal innervation was studied in the psoas muscle in human fetuses from 15.5 weeks PMA and in babies until 80 weeks PMA (Gramsbergen et al., 1997). The psoas muscle is important for stabilizing the vertebral column and leg adduction and thus is involved in postural control. The end stage of polyneuronal innervation in this muscle was reached at the age of 12 weeks post term, a few months ahead of the age at which sitting is developing.

#### DEVELOPMENTAL CHANGES IN MOTONEURONS INNERVATING POSTURAL MUSCLES

The motoneurons innervating postural and anti-gravity muscles, at adult age, are coupled by dendrite bundles. With the Golgi technique, a silver impregnation technique for staining neurons, these dendrite bundles have been observed in the spinal cord of rats and cats (Cajal, 1911; Scheibel & Scheibel, 1970; Anderson et al., 1976; Roney et al., 1979; Schroder, 1980; Nicouloupoulos-Stournaras & Iles, 1983; Furicchia & Goshgarian, 1987; Bellinger & Anderson, 1987a; 1987b; Anderson et al., 1988). Remarkably, in rats at early postnatal ages, the motoneurons innervating the soleus muscle had their dendrites still running in all directions. From postnatal day 14 (P14) until P16, the dendrites reorganized into bundles (Westerga & Gramsbergen, 1992). The age at which this reorganization occurred in the soleus muscle coincided with the point at which rats start to walk fluently in an adult like fashion (Westerga & Gramsbergen, 1990).

No such reorganizations have been found in the tibialis anterior muscle, a hind limb flexor. In later research, we studied the occurrence of dendrite bundles in a large variety of muscles in the trunk and in extremities. We demonstrated that dendrite bundles are observed only in the dendrites

of motoneurons innervating antigravity muscles and trunk muscles (Gramsbergen et al., 1996). Further results indicated that such reorganization is not dependent upon contacts with muscles or segmental afferent input but rather upon descending influences. Spinal cord transection prevented this reorganization from occurring (Gramsbergen et al., 1995).

Dendrite bundles have also been observed in the human spinal cord (Schoenen, 1982). Schoenen found dendrite bundles to be the tightest in the ventromedial motoneuronal column, in which motoneurons are located innervating the axial muscles, and in the central column with motoneurons innervating extensor muscles of leg and thigh.

Later research showed that the dendrites in dendrite bundles are interconnected by means of gap junctions (van der Want et al., 1998). The junctions enable the transmission of ionic currents and play a role in electrotonic coupling of the motoneurons. These dendrodendritic connections therefore might play a role in synchronizing the activity of the motoneurons to antigravity and trunk muscles. The coincidence of their development and the onset of fluent walking, as well as their dependency on the integrity of descending fiber projections, suggest a causal relation with postural control mechanisms in the central nervous system (Farmer, 1998).

### CONCLUSION

Research in the human and in the rat indicates that the development of the muscles and their innervation continues after birth. Changes in the myosin composition of muscle fibers result from changes in the activity of the muscles. Activity is also of great importance for the regression of polyneuronal innervation. The formation of dendrite bundles, on the other hand, is not related to activity

but rather to connections with supraspinal systems. When activity is reduced or completely halted at an early stage, we know from animal experiments that this leads to abnormalities in myosin composition in the muscles and a delayed regression of polyneuronal innervation. When extrapolating these results to the human situation, we hypothesize that abnormal motor and postural control in babies resulting from complications in the perinatal period can induce abnormal fiber type compositions in the affected muscles and deviances in the development of mononeuronal innervation patterns.

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