Trophic Influence of Sensory Axons in Denervated Skeletal Muscles

Kwong WH¹, Ochi M², Kimori K³, Chow SP⁴
¹Department of Anatomy, Chinese University of Hong Kong; ²Department of Orthopaedics, Shimane Medical School; ³Department of Orthopaedic Surgery, Hiroshima University School of Medicine; ⁴Department of Orthopaedic Surgery, University of Hong Kong.

Scientific background
Are there myotrophic factors?

The lower motor neurons, sensory ganglionic neurons, and preganglionic and postganglionic autonomic neurons constitute the nervous supply of our body tissues. These peripheral neurons regulate the activity of their target tissues. Conversely, their well-being depends on the integrity of their connections with the targets. They are known to be regulated by trophic factors produced by the peripheral tissues (7,9,16,24,25). The nerve growth factor, for example, is produced by peripheral targets of sensory ganglionic neurons, sympathetic neurons, fibroblasts and Schwann cells; and it promotes the survival of sensory and sympathetic neurons (17,42).

Much less is known about the trophic effects in the reverse direction. The theory that nerves exert a trophic effect on skeletal muscles is an old, widely accepted one (2,7). According to this theory, axotomy not only interrupts the passage of electric impulses (which elicit contractile activity), but also the flow of certain substances to the neuromuscular junctions. Many studies had pointed to the existence of neurogenic "myotrophic" substances but so far none has been identified (reviewed in 11,13). A skeletal muscle undergoes less severe atrophy when only the neuromuscular transmission, but not the physical length of its axons, was interrupted. This suggests that some beneficial factors flow in the axons towards muscle fibres. Extract of the peripheral nerves, specifically the sciatic nerve in most studies, was shown to ameliorate atrophy of denervated skeletal muscle fibres, reduce shrinkage of their organelles, and increase acetylcholinesterase activity of cultured muscles (5,15,27). The exact chemical nature of the trophic substance(s) in the extract is not known. A once hopeful candidate, named "sciatin", was shown to be just a special form of the iron-binding protein transferrin (35,36). Although transferrin is known to promote myoblast proliferation and growth of muscle cells in culture, its neuronal origin and in vivo effect on muscle fibres remain uncertain.
Most of the studies on the neural regulation of muscle properties focused on the motor innervation of the skeletal muscle fibres (6,10,20,21,43). This is naturally so as only motor axons can form synapses (neuromuscular junctions) with muscle fibres, and their firing frequencies have profound effects on properties of muscle fibres. Motor axons secrete acetylcholine as the neurotransmitter, whereas this substance does not appear to occur in significant quantity in sensory axons (37,40). Since the work of Langley and Anderson (22), it is generally accepted that only cholinergic axons can reinnervate a denervate muscle (4). Motor reinnervation prevents atrophy of the muscle. On the other hand, sensory axons could only ramify among the denervated muscle fibres (12,44,45). They fail to form neuromuscular contacts and could not prevent muscle atrophy. These properties of sensory axons led workers of the early studies to conclude that sensory axons did not have any "trophic effect" on muscle fibres (44). It should be pointed out that the "trophic effect" of these studies merely referred to any neural influence that could prevent muscle atrophy, including the influence of nerve impulses. It did not specify that they came from diffusible substances secreted at the nerve terminals. Later studies on the trophic regulation of muscles became focused on diffusible substances from motor axons. It had then been taken for granted that sensory axons did not produce any myotrophic substance. So far only a few studies had touched upon the topic (see Why this study? below). These in vivo studies did not specifically deal with the trophic influence of sensory axons on normal skeletal muscle fibres. The lack of publication on this topic testifies its unpopularity.

**Sensory innervation of skeletal muscles**

The apparent non-involvement of sensory axons in the trophic regulation of muscles contradicts sharply with their preponderance in the muscle. Skeletal muscles, although obviously an effector organ, are richly supplied with sensory axons (1). In fact, sensory axons of a muscle outnumber the motor axons. They form nerve endings in the intramuscular connective tissue, blood vessels, muscle spindles and tendon organs. Of these endings, only those in muscle spindles come in direct contact with muscle fibres. These specialised muscle fibres (intrafusal fibres) constitute only a small portion of the total fibre population, and they are much smaller than extrafusal fibres (1). They contribute only a negligible proportion to the mass or contractile force of the whole muscle (1,28). Therefore any change in the neurotrophic
influence of sensory axons on the intrafusal muscle fibres, should it exist, would barely be reflected in the mass or tension of the whole muscle.

Clinical background

In some cases of brachial plexus injuries the spinal roots and even their ganglia may be avulsed. To prevent atrophy of the upper limb muscles, a nearby intact nerve may be used as a "donor" nerve for the reinnervation of the muscle (3,8,19,29,30). The spinal accessory nerve or intercostal nerves were often used as they are "motor" in nature and contain a relatively rich supply of motor axons. The avulsed ganglia were not sutured to the original site, since regeneration of axons from the ganglia to the spinal cord invariably failed because of gliosis at the entry to the cord (39). In other nerve injuries involving major branches of the sciatic nerve, a "motor" nerve may not be available in the denervated region and a cutaneous (sensory) nerve, such as the sural nerve or saphenous nerve, may be used instead. A severed nerve and a denervated muscle will undergo fibrosis if they are not reinnervated after a long time (14). The purpose of this sensory nerve grafting is to keep the axonal pathway of the muscle occupied with axons (26,41). With this method, an intact axonal pathway will still be available to the regenerating axons even after a long delay to their return. Some of us, who had been doing nerve repair for patients, had been wondering whether some use could be made of the avulsed ganglia, instead of simply discarding them. What about, for example, grafting the ganglia to the muscles? Would they serve the same function as the sensory nerve? What about the contractile ability of the ganglion-reinnervated muscle? But first of all, would a transplanted ganglion survive in the new environment after disconnection from the spinal cord? For these, the answers would await results from animal experiments.

Why this study?

As reviewed above, sensory reinnervation would not bring back the contractile activity of a denervated muscle, and the muscle will still undergo atrophy. At first sight, the scientific background would not support any hope for fruitful results from the ganglion transplant experiment. However, we visualised something positive. First, there had been no evidence to prove or disprove the existence of a myotrophic factor from sensory axons. A careful measurement of the contraction force and fibre size of a ganglion-reinnervated muscle, the motor supply of which had been surgically removed, may provide a clue as to its
existence. Previous culture studies showed that dorsal root ganglion promoted the formation of budings along chick muscle fibres (38) and maintains the cholinesterase activity of whole muscles from the newt (23). These results suggest that sensory ganglia may secrete some factors that affect muscle properties. However, they do not show that the influence could prolong the survival of a denervated muscle. In another in vitro study, muscle fibres became less vacuolated when co-cultured with dorsal root ganglia than when cultured alone (18). (However, contradictory to their observations, the authors summarised that the ganglia had no effects on muscle fibres.) Again, whether the results may be translated as indicating a beneficial effect to denervated fibres in vivo is uncertain.

Second, we need to answer the questions we raised in Clinical background above. Only when the answers are known that we can decide if ganglion grafting can be put up for clinical use.

The following account summarises our findings which had been reported in Ref. 33 and 34.

**Ganglion grafting experiments**

**Methods** The ganglion of cervical dorsal root C5 or C6 was removed from one group of adult Wistar-Kyoto rats (the donors) and transplanted to another group of the same strain (the recipients) as follows. In the recipient rats a long segment of the sciatic nerve was removed and the proximal stump, together with its branches, was reflected. The distal stump remained connected to the leg muscles. Removal of a long sciatic segment and reflection of the proximal stump ensured that there would be no regeneration of the sciatic axons. A ganglion from a donor rat was then sutured to the distal stump of to the recipient rat (henceforth called experimental rat). For control rats, the same surgical denervation was performed but no ganglion was transplanted. The rats were then kept for 72-286 days. At the end of the survival period the condition of two leg muscles, tibialis anterior (TA) and extensor digitorum longus (EDL) was assessed by isometric tension recording and histological study. TA muscles were stained with the routine H & E method and EDL muscles were stained with a silver-cholinesterase method for axons and motor endplates (31; modified according to 32). Ganglia were stained with Nissl method.
Results  Stimulation of distal stump of the sciatic nerve or the common peroneal nerve (which innervates the TA and EDL muscles) did not cause detectable contraction of the TA or EDL muscles. This suggests that motor axons are absent in the distal stump, but it does not rule out the presence of other kinds of axons. However, stimulation of the muscles directly did elicit some tension. The twitch and tetanic tensions decreased rapidly with survival time during the first 6 months of the postoperation period. During that period, the experimental muscles (which received the ganglion graft) developed more tension than the control muscles (Figure 1). Subsequently, the difference between the two groups became much smaller.

The mass of the anterior crural muscles (TA and EDL muscles together) was compared between the experimental and control groups. The decrease in muscle mass followed the same pattern of decline as the tension, and the two groups showed significant difference (Figure 2). The decrease was due to death of muscle fibres and atrophy of the surviving fibres (Figure 3). Extensive inflammatory reaction, as indicated by the presence of numerous neutrophils and macrophages, was also observed in both groups. Sections of the TA muscle taken at 11, 27 and 31 postoperation weeks indicated that muscles reinnervated by grafted ganglion had larger muscle fibres than the control muscles (Figures 3 and 4).

The extent of reinnervation was estimated by counting terminal branches of the axons which ramified among muscle fibres. Terminal branches within the whole EDL muscle were counted. Fine axonal branches were observed in both groups, but no axon actually terminated at the endplates. This agreed with the lack of contraction on stimulation of the nerve supply to the TA or EDL muscle. There were much more terminal branches in the experimental group than in the control group (Figures 5 and 6).

In all of the experimental rats, the ganglion could be readily located at the end of the survival period. Slight adhesion between the ganglion and the surrounding tissue was noted. In all cases neurons were found along the periphery of the ganglion (Figure 7). Those in the deeper part appeared to have died and been replaced by scar tissue. The nucleus of some neurons was located at the centre of the neurons, and the neurons exhibited a substantial to normal amount of Nissl substance. Other neurons had their nucleus displaced towards the periphery. Thus the ganglionic neurons had recovered to different extents from chromatolysis.
Little difference in recovery was observed between the ganglia examined at the early and late stages of the survival period.

**Conclusion**

The severance of the sciatic nerve removed the motor and sensory innervation from the muscles. With the grafting of the dorsal root ganglion to the distal stump, the sensory innervation was restituted, while the motor innervation remained permanently absent. This grafting experiment allowed us to study the effect of sensory axons in the absence of neuromuscular transmission or trophic influence from the motor axons. It should be superior to the model in which the dorsal and ventral roots are severed from the spinal cord, as motor axons in the ventral roots may find their way back along any nerve which they encounter and which supplies to the leg. The proximity of the severed dorsal and ventral roots would ensure such contamination.

The present results show that deterioration of denervated muscles proceeds more slowly if they receive innervation from a sensory ganglion. The beneficial effect is to delay rather than prevent muscle atrophy. Since the sensory axons could not revive neuromuscular transmission and contractile activity, the only possible explanation for the beneficial effect is that sensory axons exert certain trophic effect on the denervated muscles. The effect is modest and declines with time. It is probably because of this, and because of the preoccupation with the belief that sensory axons are not involved in the trophic regulation of the muscle, that the effect had been overlooked for so long.

**References**


Legends

Figure 1. Twitch tension (upper figure) and tetanic tension (lower figure) of experimental and control muscles. The tension of each rat is expressed as percentage of the tension of the contralateral normal muscle. The tensions declined exponentially with postoperation survival time. Regression analysis (see ref. 34) showed that the experimental muscles developed more tension at the beginning of the decline than the control muscles (p<0.002).

Figure 2. Mass of the anterior crural muscles (TA and EDL together) of the experimental and control rats. The mass of experimental muscles is expressed as percentage of the mass of the contralateral normal muscles. Statistical analysis, showing heavier mass of the experimental muscles (p=0.002), is the same as that for tensions.

Figure 3. Transverse sections of an experimental (upper figure) and a control (lower figure) TA muscles at 30 week postoperation. H & E staining. Bar: 100 mm

Figure 4. Area of muscle fibres of the experimental (shaded bars) and control TA muscles at 11 week (upper figure) and 31 week (lower figure) postoperation. Mean fibre areas (in 100 x mm²) of the experimental and control muscles were 8.5±9.4 and 1.9±1.2 respectively at 11 week, and 4.3±5.6 and 0.7±0.7 respectively at 31 week. The difference at both stages are significant (p<0.05 on Wilcoxon's test).
Figure 5. Innervation of a normal unoperated (upper figure) and a ganglion-innervated (lower figure) EDL muscles. Motor endplates in the ganglion-innervated muscle (arrowheads) appeared shrunken and remained devoid of nerve supply. Bar: 100 mm.

Figure 6. Total number of terminal profiles of axons in the experimental and control EDL muscles. The number did not correlate with survival time, but there were consistently more profiles in the experimental group than the control group (p<0.01 on t-test).

Figure 7. Transverse section of a dorsal root ganglion at 30 week postoperation. Nissl staining. Bar: 50 mm. (From Ochi et al. 1992. Exp. Neurol. 118, p291-301.)
Figure 1
Figure 3
Figure 4

Area of muscle fibre (100 x μm²)

% of fibres counted

11 weeks
- Exp
- Con

17 19

31 weeks
- Exp
- Con

Area of muscle fibre (100 x μm²)

Figure 4
Figure 6