Effects of reinnervation on denervated skeletal muscle by axons of motor, sensory, and sympathetic neurons

ANDREW A. ZALEWSKI

Laboratory of Neuropathology and Neuroanatomical Sciences, National Institute of Neurological Diseases and Stroke, National Institutes of Health, Bethesda, Maryland 20014

ZALEWSKI, ANDREW A. Effects of reinnervation on denervated skeletal muscle by axons of motor, sensory, and sympathetic neurons. Am. J. Physiol. 219(6): 1675-1679. 1970.—Although the effects of denervation of skeletal muscle are reversed after reinnervation by axons of any somatic motor neurons, reinnervation by dendrites of sensory neurons has always proved unsuccessful. In order to determine whether the trophic function of nerve on muscle was specific to motor neuron axons, the sternomastoid muscle of adult rats was studied from 1 to 5 months after denervation and after reinnervation by the following axons: its original motor nerve, the motor hypoglossal nerve, the central fibers of the sensory neurons of the vagal nodose ganglion, and the preganglionic cervical sympathetic nerve fibers. Functional reinnervation was accomplished only by the axons of the motor sternomastoid (original nerve) and hypoglossal nerves. Muscles reinnervated by axons of sensory or sympathetic neurons all resembled chronically denervated muscle. The results demonstrate that axons of only motor neurons can restore denervated muscle. It is concluded therefore that the trophic influence of nerve on muscle is a property of axons of motor neurons and not of axons of sensory or sympathetic neurons.

DENERVATION CAUSES a number of changes in skeletal muscle, including paralysis, change of color, loss of weight, reduction of muscle fiber diameter, and alterations of muscle enzyme activities (5, 9). Most of these effects can be reversed by reinnervating the muscle with any motor nerve (original or foreign) (5, 9), whereas none is altered after sensory nerve reinnervation (8, 15). It is important to note that in the case of motor nerve reinnervation the muscle was reinnervated by the axons of the motor neurons, while after sensory nerve regeneration the reinnervation was by the dendrites (peripheral fibers) of the sensory neurons. Before the trophic influence of nerve on muscle can be ascribed specifically to the axons of motor neurons, denervated muscle should be studied after reinnervation by axons of other types of neurons (e.g., sensory, autonomic). The present experiment therefore was performed to determine whether axons of sensory (central fibers) and sympathetic neurons could functionally reinnervate muscle and exert a trophic influence similar to that of a motor nerve.

MATERIALS AND METHODS

Osborne-Mendel male rats (250-275 g) were anesthetized with chloral hydrate (40 mg/100 g ip) and the left sternomastoid muscle and nerve, vagus nerve and nodose ganglion, hypoglossal nerve, and cervical sympathetic nerve trunk were exposed. In some animals a sham denervation operation was performed on the sternomastoid muscle, while in others the muscle was subjected, after denervation, to nerve-anastomosis reinnervation by joining one of the following nerves to the cut distal sternomastoid nerve: a) its own nerve, b) axons (central fibers) of the sensory neuron of the vagal nodose ganglion, c) dendrites (peripheral fibers) of the sensory neurons of the vagal nodose ganglion, and d) reinnervation by the peripheral vagal nerve stump after excision of the nodose ganglion (performed to control for retrograde passage of motor fibers up the vagus nerve). Nerve anastomosis was accomplished by apposing the appropriate cut nerve ends in an arterial graft and "gluing" them in place by the application to topical fibrinogen. Nerve ends not used in the reinnervation were buried deeply into another nearby muscle.

In a second type of reinnervation procedure the axons of the motor neurons of the hypoglossal nerve, the axons (central fibers) of the sensory neurons of the vagal nodose ganglion, the preganglionic cervical sympathetic axons, or the peripheral vagal nerve stump minus the nodose ganglion were implanted directly into the caudal end of the muscle at least 9.0 mm away from the point where the sternomastoid nerve entered the muscle. Nerve implantation was made into normally innervated (hypoglossal nerve only) and denervated muscles (all nerves). The specific aim of the nerve implantation procedure was to see which nerve fibers could induce the formation of new motor end plates. The sternomastoid nerve could not be used for the purpose because it is too short to reach beyond the normal end-plate region. Since the hypoglossal nerve has been shown to induce new end-plate formation at the end of the muscle (4), this nerve served as a control motor nerve.

The general features of four normal, four sham denervation-operated, four chronically denervated, and four nerve-anastomosis reinnervated muscles from each group were studied after 3 and 5 months. Similarly, four muscles from each nerve implant group and four normal and four chroni-
cally denervated muscle were examined for end plates after 1 and 3 months. At the appropriate time, the animals were again anesthetized and the appearance and contraction (if present) of the muscle were compared to those of the normal contralateral sternomastoid muscle. The muscle was then excised, weighed, and frozen in liquid nitrogen. Frozen sections, 8 or 16 μ in thickness, were cut and stained with periodic acid-Schiff (PAS)-hematoxylin, an actomyosin adenosinetriphosphatase stain (6), or cholinesterase-hematoxylin (11). Only representative sections from the ends and middle of the muscles reinnervated by nerve anastomosis were studied, whereas in muscles subjected to nerve implantation, serial sections of the implant region were investigated for end plates. Sections of normal sternomastoid muscle were always stained with the experimental muscles, and control enzyme sections were incubated without added substrate. The arterial graft containing the nerve-anastomosis site was also studied with the PAS and cholinesterase stains.

**RESULTS**

**Normal sternomastoid muscles.** The normal sternomastoid muscle is a mixed muscle; its lateral portion is white and its medial portion is red. The sternomastoid nerve enters the muscle on the lateral side, and when the nerve is pinched with jeweler's forceps the muscle contracts rapidly. The stain for adenosinetriphosphatase revealed the presence of three types of muscle fibers (Fig. 1A). Under alkaline staining conditions the α fiber stains intensely, the αβ fibers moderately, and the β fiber is unreactive (7). Only α and αβ fibers were found in the white portion of the muscle, while the red portion was composed of all three fiber types. The β fibers were randomly found throughout the red portion of the muscle generally in groups consisting of one to four fibers (Fig. 1A). Nerve fibers were stained by the PAS (Fig. 3A) and cholinesterase-hematoxylin reactions (Fig. 2A) and found only in the middle of the muscle. The motor end-plate region of the muscle fibers was identified by the cholinesterase stain (Fig. 2A) and extended about 3–4 mm on either side of the point where the sternomastoid nerve entered the middle portion of the muscle. Beyond this distance no end plates were found, and only muscle spindles and their associated nerves in the end regions of the muscle gave a cholinesterase reaction. The characteristic cholinesterase staining of muscle fiber end plates was readily distinguished from that of muscle spindles and nerves. Control enzyme sections that were incubated without any added substrate failed to reveal any adenosinetriphosphatase or cholinesterase staining.

**Denervated sternomastoid muscles.** None of the operative procedures (denervation or various reinnervations) had an ill effect on the general growth of the animals. The body weights of control and experimental animals were similar at each time interval that animals were killed. A number of changes were found 3 months after denervating the muscles. The muscles were smaller than normal, and they had a uniformly pale pink color. No tension could be felt in the denervated muscles as they were cut free of their attachments. In the normal muscle strong tension, contraction, and relaxation followed in sequence when the muscle was removed. Muscle weights were 40-45% of that found in the contralateral muscle. The stain for adenosinetriphosphatase revealed the presence of atrophied muscle fibers (Fig. 1D). There did not appear to be any change in the distribution or groupings of any fiber type, although β fibers seemed to atrophy less than the other two fiber types. Various abnormal-looking muscle fibers were also found 3 months after denervation. These fibers were large and round and often their diameter exceeded that of normal muscle fibers (Fig. 1D). The enlarged fibers were scattered mainly in the red portion of the muscle and usually one to two were found in any particular muscle fascicle. These fibers exhibited moderate adenosinetriphosphatase activity, but the fiber type to which this abnormal fiber belongs was not determined. The PAS and cholinesterase stains failed to reveal the presence of any nerve fibers. End plates still gave an intense cholinesterase reaction and were again localized to the middle region of the muscle. No end plates were found at the ends of the denervated muscle fibers. The findings in muscles studied 5 months after denervation were similar to those described at 3 months. However, at this time muscle weights were only 20–25% of normal. The abnormally large fibers seen at 3 months were still present and the end plates stained for cholinesterase activity.

**Effects of nerve-anastomosis reinnervation on denervated muscles.** Within 3 months after reinnervation by the original sternomastoid nerve, the muscle appeared normal. The size and appearance of the muscles were normal, and they con-

![FIG. 1. Adenosinetriphosphatase stain (× 85). All micrographs are cross sections from red portion of sternomastoid muscle. A: normal muscle. Three fiber types are present: α fibers stain intensely, αβ fibers (arrow) moderately, and β fibers (arrow) are unreactive. Note that β fibers occur only in groups of 1–4 fibers. B: C: sternomastoid (B) and hypoglossal (C) nerve-anastomosis reinnervated muscle. Within 3 months after sternomastoid or hypoglossal reinnervation, muscle fiber size appears normal and large groups of β fibers (arrow) are now present. D: central sensory (axon) reinnervated muscle. Muscle fibers are highly atrophied and large, abnormal-looking fibers (arrow) are present 3 months after central sensory reinnervation. Similar findings were present in peripheral sensory, sympathetic and peripheral vagal stump reinnervated, and chronically denervated muscles.](image-url)
tracted after pinching the sternomastoid nerve. Muscle weights were 80–90% of normal. Fiber diameters of all three muscle fiber types appeared normal (Fig. 1B). Large groups of β fibers were found in the red portion of the muscle (Fig. 1B), and none of the abnormally large fibers that had been observed in the denervated muscles were present. Many nerve fibers were identified within the muscle by the PAS or cholinesterase stain, and end plates were confined to their midbelly region. The only added change observed in muscles 5 months after reinnervation was an increase in their weights, which now were 95–98% of normal.

Reinnervation of the denervated muscles by the axons (central fibers) or dendrites (peripheral fibers) of the sensory nodose ganglion neurons or by the peripheral vagal nerve stump failed to restore the muscle toward normal. These muscles appeared atrophied and no contraction was observed when the nerve was pinched. The histological findings were similar to those observed and described in chronically denervated muscle. The notable difference between the sensory reinnervated and the chronically denervated muscle was that nerve fibers could be identified within the sensory reinnervated but atrophied muscles.

Effects of nerve implantation on motor end-plate formation. As previously described, end plates in normal muscle are confined to the midbelly of the muscle, and none are found at the ends of normal or chronically denervated muscle. No contractions of the muscle or new end plates were observed after implantation of the hypoglossal nerve into the end of normally innervated muscle (Fig. 2B). The implanted hypoglossal fibers grew out only a short distance into the muscle and apparently terminated. New end plates were found, however, when the hypoglossal nerve was implanted into denervated muscle (Fig. 2, C–E). The morphology of the cholinesterase-stained newly formed end plates clearly resembled that of end plates found in the middle of the muscle (compare Fig. 2C with 2A). Although few in number (i.e., 15–25) when first seen 1 month after implantation (Fig. 2, C–E), their number greatly increased by 3 months, and as a result of functional reinnervation the diameter of most muscle fibers closely resembled normal or sternomastoid nerve reinnervated muscle (Fig. 1C). Large groups of β fibers were also present (Fig. 1C).

No new end plates were found in the end of the muscle after reinnervation by axons of sensory (Fig. 3A) or sympathetic (Fig. 3B) neurons or by the peripheral vagal nerve stump. These muscles all resembled chronically denervated muscle (Fig. 1D).

Observations on nodose ganglion and nerve-anastomosis reinnervation sites. In order to demonstrate that neurons survived the surgical procedures and regenerating nerve fibers crossed the anastomosis site, the nodose ganglion and all
nerve-anastomosis sites were 1 with the PAS and cholinesterase stains. In all cases peripheral or central sensory fibers, sympathetic fibers, and sternomastoid and hypoglossal fibers were seen crossing the anastomosis site. Furthermore, the sensory vagal nodose neurons were normal histologically (Fig. 3C) and in cholinesterase activity (Fig. 3d).

DISCUSSION

The results of the present study demonstrate that only axons of motor neurons can functionally reinnervate denervated muscle. The restoration of weight and contractility, the presence of larger groups of a given muscle fiber type (12, 16), and the formation of new motor end plates (1) clearly show that the sternomastoid and hypoglossal nerves reinnervated the muscle. On the other hand, reinnervation by axons or dendrites of sensory neurons or by axons of sympathetic neurons failed to restore the muscle, and all these muscles resembled chronically denervated muscle. This failure of axons of sensory neurons to functionally reinnervate muscle differs with the results of a previous study in the cat (14). In that study the axons (central fibers) of the sensory neurons in the nodose ganglion were implanted into the longus capitis muscle, and several months later a contraction of the muscle was observed after stimulation of the peripheral vagus nerve in 1 of 2 animals tested. However, the longus capitis muscle was not denervated at the time of nerve implantation; consequently the basis of the recorded contraction is questionable because a normally innervated muscle cannot be reinnervated (hyper-neurotized) by a second motor nerve (1, 3, 4, 10). This fact was again demonstrated in the present study by the failure of the normally innervated sternomastoid muscle to contract or to develop new motor end plates after implantation of the motor hypoglossal nerve (Fig. 2B). Furthermore, even if the observed contraction in the cat study was real (14), the results could be explained by the functional reinnervation of muscle fibers (injured during nerve implantation) by motor nerve fibers which travel up the vagus nerve. In order to demonstrate that the muscle contraction was not due to retrograde passage and innervation by motor fibers in the vagus nerve, the longus capitis muscle should have been studied after implantation of the vagus from which the nodose ganglion was excised. Since this control procedure was not employed, the muscle contraction observed (14) cannot safely be ascribed to innervation by axons of sensory neurons.

REFERENCES

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