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EVIDENCE FOR THE MAINTENANCE OF MOTONEURONE PROPERTIES BY MUSCLE ACTIVITY

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SUMMARY

1. Electrophysiological properties of soleus motoneurones in adult cats were examined with intracellular electrodes following alterations of activity of the soleus muscle induced by transection of the thoracic spinal cord or by conduction block of the muscle nerve with tetrodotoxin (TTX) cuffs. Attempts were also made to maintain muscle activity by daily stimulation of the peripheral nerve.

2. Within 8 days after transection of the thoracic cord, soleus motoneurones showed a significant decrease in the duration of afterhyperpolarization following action potentials. This change in motoneurone properties induced by cord transection was prevented by daily stimulation of the sciatic nerve.

3. Soleus motoneurones showed a significant decrease in the duration of afterhyperpolarization within 8 days after conduction block of the soleus nerve with TTX. This change in motoneurone properties was prevented by daily stimulation of the nerve peripheral to the TTX cuff but not central to the cuff.

4. The soleus muscle showed a significant decrease in weight relative to body weight within 8 days after transection of the thoracic cord. This decrease in muscle weight following cord transection was prevented by daily stimulation of the sciatic nerve.

5. No fibrillation was detected in the soleus muscle 8 days after conduction block of the soleus nerve with TTX. The maximum twitch tension of the soleus muscle evoked by nerve stimulation showed no significant difference between the two sides treated and untreated with TTX. Fast axoplasmic transport measured with cholinesterase as a marker was not affected by TTX. Thus, there was no sign of functional degeneration of motor nerve fibres following the chronic application of TTX, although morphological abnormalities were found in some nerve fibres.

6. It is concluded that motoneurone properties in an adult depend partly upon some factors associated with activity of the innervated muscle and that such trophic signals are retrogradely carried by the motor axons.

INTRODUCTION

At early developmental stages, a large number of spinal motoneurones die, presumably because they fail to form 'adequate' motor connexions with the muscle

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(Hughes, 1961; Prestige, 1967, 1976; Hamburger, 1975). In fact, if the limb bud (the precursor of limb muscles) is removed before the formation of neuromuscular connexions, all the corresponding motoneurones eventually degenerate (Hamburger, 1958; Hughes, 1962; Prestige, 1967, 1976). Furthermore, the number of motoneurones that die during development is fewer than normal when the peripheral target organ is enlarged by grafting an additional limb bud (Hollyday & Hamburger, 1976). Therefore, it seems reasonable to assume that normal development of spinal motoneurones requires the presence of, or connexion with, skeletal muscle. However, it remains unknown whether normal motoneurone properties in an adult are maintained by an influence from the muscle.

When the soleus muscle is partially denervated in adult cats, the soleus motoneurones whose axons are left intact alter their electrophysiological properties as well as those whose axons had been sectioned (Huizar, Kudo. Kuno & Miyata, 1977). On the other hand, soleus motoneurones show no significant changes in their properties after sensory deprivation by chronic section of the lumbosacral dorsal roots (Kuno, Miyata & Muñoz-Martinez, 1974*a*). It is then possible that adult motoneurone properties depend upon the condition of the innervated muscle, so that normal motoneurone properties are no longer maintained when muscle activity is reduced by partial denervation. The purpose of the present study is to examine the possible influence of muscle activity upon the innervating motoneurones.

For this purpose, activity of motoneurones innervating the hind legs was reduced by transection of the thoracic spinal cord in adult cats, leaving the peripheral neuromuscular connexions intact. In another series of experiments, activity of the soleus muscle was blocked by tetrodotoxin (TTX) cuffs applied to the muscle nerve. Following either method of reducing muscle activity, soleus motoneurones showed changes in their electrophysiological properties, which were similar to those observed in intact motoneurones after partial denervation of the muscle. In addition, the alterations in motoneurone properties induced by these procedures could be prevented when muscle activity was maintained by daily stimulation of the muscle nerve. It is concluded that the adult motoneurone properties are maintained, at least in part, by factors related to activity of the innervated muscle.

METHODS

Cord transection. Adult female cats, 1·8–3·6 kg in weight, were anaesthetized by an I.P. injection of sodium pentobarbitone (35–40 mg/kg). The spinal cord was transected at the twelfth thoracic level with aseptic precautions. The urinary bladder of operated animals was emptied every day either manually or by catheterization until spontaneous micturition was restored. During the first few days after the operation, the hind legs remained flaccid and showed no spontaneous movements. Subsequently, occasional reflex movements appeared, and the hind legs became slightly spastic 4–8 weeks after the operation (see Karpati & Engel, 1968). The body weight decreased by 7–18% within 2 weeks after cord transection but subsequently increased and exceeded the pre-operative weight by 7–20% about 55 days after the operation.

Tetrodotoxin cuffs. Silicone cuffs containing TTX were prepared as described by Lavoie, Collier & Tenenhouse (1976, 1977; also see Brown & Ironton, 1977). One mg TTX buffered with citric acid-sodium citrate (Sankyo Co.) and 65 mg of NaCl were dissolved in 1·2 ml. water, and the solution was lyophilized. The NaCl-TTX powder was then thoroughly mixed with 0·3 ml. of Silastic 382 (Dow Corning Corp.). The mixture was placed in a glass tube with an internal diameter of 4 mm, and the centre of the mixture mould was hollowed with a 15 gauge hypodermic needle (about 2·2 mm in diameter). After polymerization, the silicone cylinder was cut 2–4 mm

in length. Each cuff was estimated to contain approximately 0·1 mg TTX in 20% NaCl. For implantation, the cat was anaesthetized with sodium pentobarbitone, and the nerves to the lateral gastrocnemius and soleus muscles were exposed in the left hind leg under aseptic conditions. The hollow TTX cuff was slit longitudinally, and it was loosely wrapped around the exposed muscle nerves. Contractions of the lateral gastrocnemius-soleus muscles evoked by stimulation of the sciatic nerve were abolished 15–45 min after the application of the TTX cuff. The chronic effect of TTX cuffs was examined twice a day by stimulation of the sciatic nerve with implanted electrodes (see below). All branches of the sciatic nerve were cut in the popliteal fossa, except for the lateral gastrocnemius-soleus (l.g.s.) and flexor digitorum-hallucis longus (f.d.h.l.: equivalent to medial and lateral flexor digitorum longus) nerves (Fig. 1*B*). Thus, contractions of the f.d.h.l. muscles were used as a criterion for the effectiveness of stimulation of the sciatic nerve with implanted electrodes, and the presence or absence of contractions of the l.g.s. muscles was used to test the chronic effect of TTX cuffs (Fig. 1*B*). Contractions of these muscles produced by stimulation of the sciatic nerve could be easily identified after the closure of the wound. Conduction block of the l.g.s. nerves by TTX cuffs lasted for 3–7 days. The period of conduction block appeared to be roughly correlated with the length of the TTX cuff applied (2–4 mm). When nerve conduction was recovered, the cuff was immediately replaced with a new TTX cuff to maintain the block of nerve conduction for the desired period (7 days). Some cats (about 25%) vomited about 30 min after the application of TTX cuffs. From the dose–response relationship for emesis elicited by TTX (Borison, McCarthy, Clark & Radhakrishnan, 1963), it was likely that TTX leaking out of the cuff might have reached the plasma concentration equivalent to an I.V. injection of TTX (1 μg/kg). In no instance did death of the animal result from the application of TTX cuffs. Control cuffs containing 20% NaCl without TTX did not block nerve conduction (also, see Lavoie *et al.* 1976, 1977).

Chronic nerve stimulation. Bipolar silver electrodes were embedded along the internal wall of a hollow silicone (Silastic 382; Dow Corning Corp.) cuff with dimensions: internal diameter, 5 mm; external diameter, 10 mm; length, 15 mm. A portion of the silicone cuff was longitudinally cut out at an angle of about 90 degrees, and the remaining arc-shaped cuff electrodes were encircled around the intact sciatic nerve on the left side at the mid-thigh level (cf. DeVilliers, Nosé, Meier & Kantrowitz, 1964). The cuff electrodes were then capped with the previously removed piece of silicone and tied round with silk thread. Insulating wires attached to silver electrodes were led subcutaneously to a small Winchester plug fixed to the outside of the skin covering the dorsal surface of the sacrum. Implantation of the cuff electrodes was performed under aseptic conditions with sodium pentobarbitone anaesthesia. The threshold for effective nerve stimulation was determined by initiation of contractions of the hind leg muscles (Fig. 1*A*). The threshold did not significantly change for at least 2 weeks after implantation of the cuff electrodes.

Daily stimulation of the sciatic nerve with implanted electrodes was carried out in animals with cord transection. The animal was loosely restrained in a cloth sleeve. The trunk of the cat was supported in a prone position by an aluminium cylinder to which the cloth sleeve was hooked. The hind legs were fixed with metal clamps holding the ankles, and a weight of 600 g was applied to each foot in order to give a constant passive stretch to the ankle extensor (l.g.s.) muscles. Electrical stimuli were applied every 30 sec at a frequency of 10/sec lasting for 10 sec (mean frequency, 200 pulses/min). In some experiments, stimuli were applied every 30 sec at a frequency of 50/sec lasting for 2 sec (mean frequency, 200 pulses/min). The two procedures were identical in the total number of stimuli applied but different in the pattern of stimuli (see Lømo, Westgaard & Dahl, 1974). The pulse duration was 0·1 msec, and the stimulus intensity was adjusted to twice the threshold for initiation of muscle contractions. Chronic nerve stimulation was given for 7–8 hr every day and maintained for 7–13 days (Salmons & Vrbová, 1969). During daily nerve stimulation, the animal usually fell asleep or took food and drink freely since the head and the forelegs were not restrained. Thus, the animal showed no apparent signs of discomfort in response to nerve stimulation.

Stimulation of the intact sciatic nerve causes contractions of the hind leg muscles and antidromic or synaptic activation of their motoneurones in the spinal cord (Fig. 1*A*). The participation of activity of the soleus muscle following stimulation of the sciatic nerve could be eliminated by the application of TTX cuffs to the l.g.s. nerves distal to the site of stimulation (Fig. 1*B*). Attempts were also made to maintain daily activation of the soleus muscle without activation of its motoneurones. For this purpose, a pair of electrodes was implanted distal to the TTX cuff (S_d in Fig. 1*C*). Each of the distal stimulating electrodes was a silver ring (about

2 mm in diameter) tied to the deep surface of the lateral gastrocnemius muscle, so that the soleus nerve emerging through the lateral gastrocnemius muscle was situated between the two electrodes with a distance of about 5 mm to each electrode. Under these conditions, the electrodes were not in direct contact with the nerve, but the spread of stimulating currents caused strong contractions of the soleus muscle without appreciable contraction of the lateral gastrocnemius muscle. The chronic effect of TTX cuffs was examined twice a day by the presence or absence of contractions of the l.g.s. muscles following stimulation with the electrodes implanted in the sciatic nerve (S_p in Fig. 1*C*).

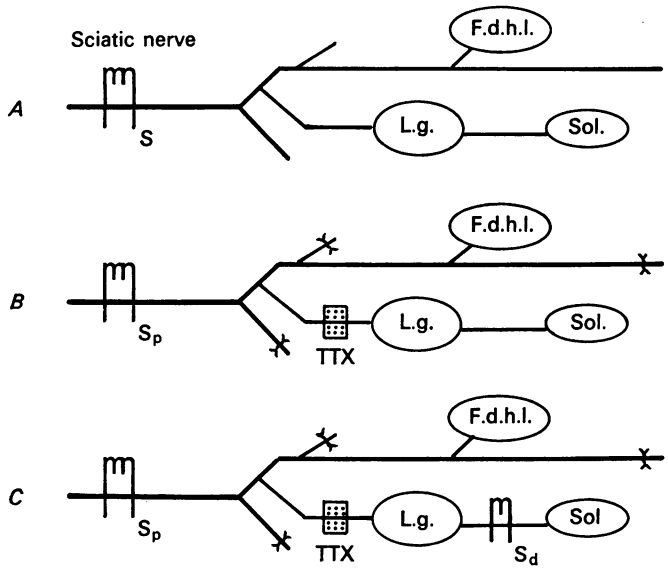

Fig. 1. Schematic diagrams of different experimental conditions in the hind leg. F.d.h.l. flexor digitorum longus and flexor hallucis longus muscles. L.g., lateral gastrocnemius muscle. Sol., soleus muscle. *A*, stimulating electrodes (S) were implanted in the sciatic nerve for daily stimulation in cord-transected cats. *B*, branches of the sciatic nerve were sectioned except for the nerves to the f.d.h.l., l.g. and sol. muscles; activity of the l.g. and sol. muscles was blocked by the application of TTX cuffs; the chronic effect of the TTX cuff was examined by the presence or absence of contractions of the l.g. and sol. muscles in response to stimulation with electrodes implanted in the sciatic nerve (S_p) proximal to the TTX cuff; the same electrodes were used also for daily stimulation in cord-transected animals. *C*, same as in *B*, but daily stimulation was performed with electrodes (S_d) implanted distal to the TTX cuff in cord-transected animals.

Experimental procedure. After varying post-operative periods, the animal was anaesthetized with sodium pentobarbitone (35–40 mg/kg, I.P.) and prepared for intracellular recordings from lumbosacral motoneurones by procedures essentially similar to those described in previous reports (Kuno *et al.* 1974*a*; Huizar *et al.* 1977). In the left hind leg, the nerve to the soleus muscle was isolated from surrounding tissues, leaving its connexions with the muscle intact. Intracellular recordings from soleus motoneurones were identified by antidromic action potentials evoked by stimulation of the soleus nerve. In preparations with TTX cuffs applied to the l.g.s. nerves, the cuff was removed the evening before the experiment. Nerve conduction was completely recovered within 15 hr after removal of the TTX cuff, as evidenced by identical twitch tensions of the soleus muscle in response to stimulation of the nerve central and distal to site of the cuff application. The distal tendon of the soleus muscle was cut and connected to a transducer (Grass FT 10) for measurements of isometric contractions. Muscle contractions evoked by stimulation of the soleus nerve or by intracellular stimulation of each motoneurone were measured at an initial tension of 100 g (Kuno, Miyata & Muñoz-Martinez, 1974*b*; Huizar *et al.* 1977).

The properties of soleus motoneurones examined were resting and action potentials, conduction velocity, and the duration of afterhyperpolarization (a.h.p.) following single action potentials evoked by intracellular stimulation, as detailed previously (Kuno *et al.* 1974*a*). Only those motoneurones with an action potential in excess of 75 mV were studied. Mean values of motoneurone properties under a given condition were calculated from twenty-nine to thirty-two cells. In order to minimize a possible bias of sampling from particular animals, the maximum number of soleus motoneurones observed from each animal was limited to ten. In some experiments, the properties of medial gastrocnemius motoneurones were also examined to compare with those of soleus motoneurones. Statistical analysis of the results was by means of two-tailed t tests with significance limit of $2P < 0{\cdot}05$.

Additional technical details are given in the appropriate sections of Results.

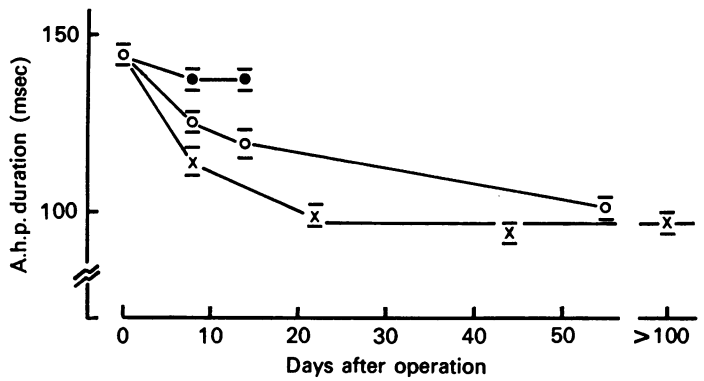

Fig. 2. Changes in the duration of afterhyperpolarization (a.h.p.) of soleus motoneurones after cord transection (open circles) or after section (axotomy) of the soleus nerve (crosses). Filled circles, the results with daily stimulation of the sciatic nerve at 10/sec in cord-transected animals. Each point represents the mean ± S.E. of mean (horizontal bars). The values for axotomized motoneurones (crosses) are reproduced from previous data (Kuno *et al.* 1974*a*). The value for control, unoperated animals (from Huizar *et al.* 1977) is shown on day zero. All durations were measured to the time at which the membrane potential returned to its resting level (Kuno *et al.* 1974*a*).

RESULTS

Experiments with cord transection

Motoneurone properties after cord transection. As illustrated in Fig. 2 (open circles), soleus motoneurones showed a significant decrease in the duration of a.h.p. within 8 days after transection of the spinal cord, and these changes were further enhanced with time after the operation. About 55 days (52–59 days) after cord transection, the mean duration of a.h.p. (101 msec) of soleus motoneurones (open circle in Fig. 2) was not significantly different from that (97 msec) observed over 100 days (110–119 days) after axotomy (crosses in Fig. 2; see Kuno *et al.* 1974*a*). However, axotomized soleus motoneurones are characterized by a significant increase in overshoot of action potentials and a significant decrease in axonal conduction velocity and in resting membrane potential as well as in the duration of a.h.p. (Kuno *et al.* 1974*a*; Huizar *et al.* 1977). In contrast, following cord transection these electrophysiological changes did not occur in soleus motoneurones, except for a decrease in the duration of a.h.p. Therefore, the changes in properties of soleus motoneurones after cord transection were qualitatively similar to those observed in intact soleus motoneurones following partial denervation of the muscle (Huizar *et al.* 1977).

The electrophysiological properties were also examined in fifty medial gastrocnemius motoneurones 52–59 days after cord transection. Their properties were not significantly different from those measured in unoperated cats.

Effects of chronic nerve stimulation. Transection of the thoracic spinal cord caused an apparent paralysis of the hind legs. In order to maintain muscle activity, the sciatic nerve was chronically stimulated at a frequency of 10/sec (see Methods for stimulus paradigm) immediately after cord transection. As shown in Fig. 2, the

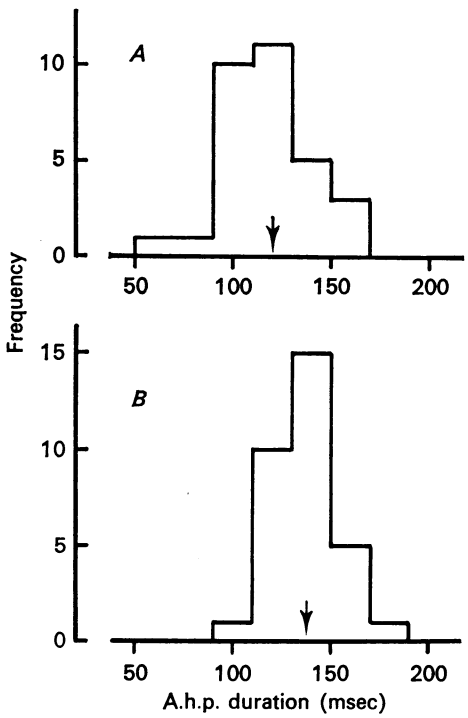

Fig. 3

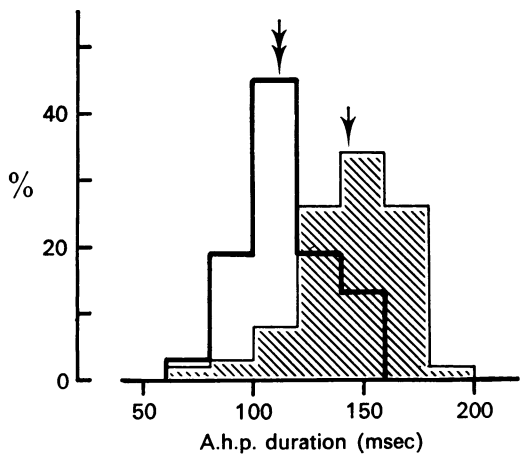

Fig. 4

Fig. 3. Frequency distributions of the duration of a.h.p. of soleus motoneurones 14 days after cord transection with (*B*) and without (*A*) daily stimulation of the sciatic nerve at 10/sec. Their mean values are shown by arrows.

Fig. 4. Frequency distributions of the duration of a.h.p. of soleus motoneurones. Cross-hatched histogram, from control, unoperated cats (reproduced from Huizar *et al.* 1977). Heavy lined histogram, 7 days after conduction block of the soleus nerve with TTX cuffs. Arrows indicate their mean values.

decrease in the duration of a.h.p. of soleus motoneurones following cord transection (open circles) was significantly prevented by daily nerve stimulation (filled circles). The mean duration of a.h.p. (137 msec) of soleus motoneurones observed with daily nerve stimulation 8 or 14 days after cord transection (Fig. 2, filled circles) was not significantly different ($0{\cdot}10 < 2P < 0{\cdot}20$) from the mean value (144 msec) obtained from unoperated, control animals. Fig. 3 shows frequency distributions of the duration of a.h.p. of soleus motoneurones recorded 14 days after cord transection with (*B*) and without (*A*) daily stimulation of the sciatic nerve at a frequency of 10/sec. The

difference between their mean values (137 msec and 119 msec, indicated by arrows in Fig. 3) was highly significant ($2P < 0{\cdot}001$). The difference between the mean values (137 msec and 125 msec; Fig. 2) obtained with (filled circles) and without (open circles) daily nerve stimulation 8 days after cord transection was also highly significant ($0{\cdot}001 < 2P < 0{\cdot}005$). Because of technical difficulties, the experiments with daily nerve stimulation were not extended to periods longer than 2 weeks. However, even from these data alone (Fig. 2, filled circles), it seems clear that changes in motoneurone properties induced by cord transection can be prevented by daily activation of the peripheral nerve.

Effective pattern or amount of stimulation. Under physiological conditions, soleus motoneurones discharge at a frequency of 5–20/sec (Granit, Henatsch & Steg, 1956; Granit, Phillips, Skoglund & Steg, 1957). It is possible that the maintenance of soleus motoneurone properties by daily nerve stimulation may require a particular pattern of stimuli. In eight cats, the sciatic nerve was chronically stimulated at a frequency of 50/sec (see Methods for stimulus paradigm) for 8 days after cord transection. The mean duration of a.h.p. calculated from thirty-two soleus motoneurones was 128 msec. This value was not significantly different from the mean value (125 msec) observed 8 days after cord transection without nerve stimulation (Fig. 2). Thus, daily stimulation of the sciatic nerve at a high frequency apparently failed to prevent the changes in motoneurone properties induced by cord transection.

In this series of experiments (at 50/sec), the total number of stimuli applied per day was kept at the same level as that with daily stimulation at 10/sec (see Methods). Lømo *et al.* (1974) have postulated that the two conditions are identical in the *degree* of muscle activity but different in its *pattern*. However, this may depend upon the definition of 'muscle activity'. In three different soleus muscles, comparison was made for the areas formed by the isometric, tetanic tension curves elicited by 100 stimuli at 10/sec and by 100 stimuli at 50/sec. This area, which indicates the product of tension and time, was found to be consistently greater in the former, by 2·6–3·3 times, presumably because the latter frequency exceeded the tetanic fusion frequency. Thus, 'muscle activity' measured in terms of the maintained tension does not necessarily correspond to the total number of stimuli applied. From the present results, it remains uncertain whether prevention of the changes in motoneurone properties following cord transection requires certain patterns or certain amounts of daily activity.

Experiments with TTX

Effects of nerve block. If the changes in motoneurone properties induced by cord transection are due to a reduction in activity of the innervated muscle, similar changes may be expected to occur in the motoneurones when the muscle nerve is chronically blocked by TTX. TTX cuffs were applied to the l.g.s. muscle nerves (Fig. 1*B*). Activity of the soleus muscle was thus blocked for 7 days, and the experiments were performed on day 8. Fig. 4 shows a histogram (heavy lines) of the duration of a.h.p. for thirty-one soleus motoneurones recorded under such conditions (from six cats). In comparison with the results obtained from control, unoperated animals (Fig. 4, cross-hatched histogram), there was a significant ($2P < 0{\cdot}001$) decrease in the mean value (114 msec *versus* 144 msec; Fig. 4, double and single arrows). The

soleus motoneurones, however, showed no significant changes in axonal conduction velocity, overshoot of action potentials and resting membrane potentials following the chronic application of TTX cuffs. Thus, the changes occurring in soleus motoneurones following cord transection seem to be mimicked by conduction block of the muscle nerve with TTX.

Effects of motoneurone or muscle activity. While the changes in motoneurone properties induced by cord transection can be prevented by daily stimulation of the peripheral nerve at 10/sec, it is uncertain whether this effect is due to daily maintenance of motoneurone activity or muscle activity. To distinguish these possibilities, cord transection was combined with the application of TTX cuffs in the same animal.

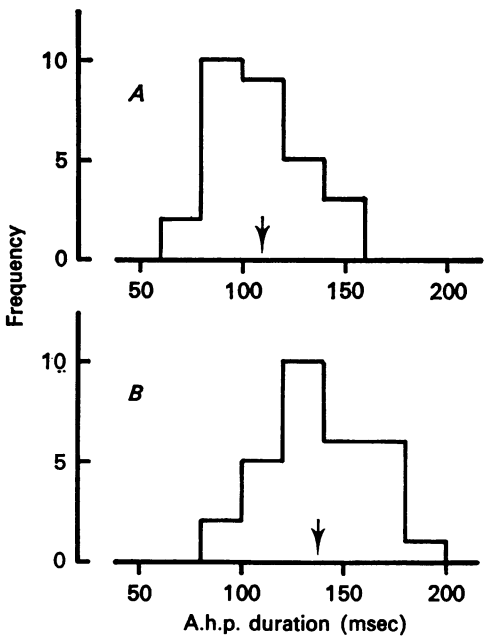

Fig. 5

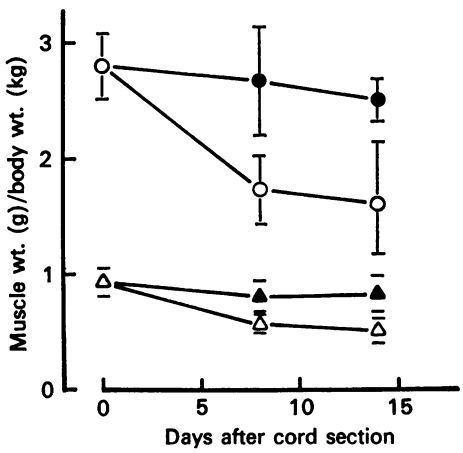

Fig. 6

Fig. 5. Frequency distributions of the duration of a.h.p. of soleus motoneurones 8 days after cord transection with daily stimulation of the peripheral nerve at 10/sec proximal (*A*) and distal (*B*) to the TTX cuff applied to the lateral gastrocnemius and soleus muscle nerves. Their mean values are shown by arrows.

Fig. 6. Changes in the ratio of muscle wt. (g) to the body wt. (kg) with days after cord transection for the medial gastrocnemius (open circles) and soleus (open triangles) muscles. Filled circles and filled triangles, the results with daily stimulation of the sciatic nerve at 10/sec after cord transection. Each point represents the mean ± S.D. (horizontal bars).

In one group of animals (four cats), the sciatic nerve was stimulated with the electrodes implanted proximal to the TTX cuff (Fig. 1*B*, S_p). In another group (six cats), activity of the soleus muscle was maintained by stimulation with the electrodes implanted distal to the TTX cuff (Fig. 1*C*, S_d). Thus, in the former case the participation of activity of the soleus muscle associated with daily stimulation of the sciatic

nerve was eliminated by the TTX cuff, whereas in the latter, muscle activity was maintained without antidromic or synaptic activation of the spinal motoneurones.

Fig. 5 shows frequency distributions of the duration of a.h.p. of soleus motoneurones observed 8 days after cord transection with daily stimulation of the sciatic nerve proximal to the TTX cuff (*A*) and with daily stimulation distal to the TTX cuff (*B*) at a frequency of 10/sec. The mean duration of a.h.p. in the former (110 msec; arrow in Fig. 5*A*) was significantly ($2P < 0{\cdot}001$) shorter than that in the latter (137 msec; arrow in Fig. 5*B*). Also, the mean value obtained with daily stimulation of the nerve proximal to the TTX cuff (Fig. 5*A*) was not significantly ($0{\cdot}40 < 2P < 0{\cdot}50$) different from the value (114 msec; Fig. 4) observed after the application of the TTX cuff alone. Furthermore, the mean value obtained with stimulation of the nerve distal to the TTX cuff (Fig. 5*B*) was not significantly ($0{\cdot}20 < 2P < 0{\cdot}30$) different from the value (144 msec; Fig. 4) observed in control, unoperated animals. From these results, it seems clear that the changes in motoneurone properties induced by a combination of cord transection and nerve block with TTX can be prevented by daily activation of the muscle but not by activation of the motoneurones or their sensory inputs.

Alterations in muscle weight

It is difficult to assess how far muscle activity in the hind leg may be altered by cord transection or by daily stimulation of the peripheral nerve. However, one may assume that changes in muscle activity are reflected, at least to some extent, as changes in muscle weight. Based on this assumption, muscle wet wts. were measured at the end of the experiment, and the muscle wt. (g) was expressed relative to the body wt. (kg) measured on the day of the operation (Eccles, 1941). Fig. 6 shows changes in the ratio of muscle to body wts. for the medial gastrocnemius (open circles) and soleus (open triangles) muscles following cord transection. In both muscles, the value was decreased to about 60 % of that observed in control, unoperated animals within 8 days after cord transection (also, cf. Eccles, 1941). Daily stimulation of the sciatic nerve at a frequency of 10/sec significantly prevented the decrease in muscle weight induced by cord transection (filled circles, filled triangles in Fig. 6). In fact, for the soleus muscle the muscle-to-body wt. ratio observed with daily nerve stimulation 8 or 14 days after cord transection was not significantly ($0{\cdot}10 < 2P < 0{\cdot}20$) different from the control value (filled triangles in Fig. 6).

Table 1 summarizes the ratios of muscle to body wts for the soleus muscle under various experimental conditions. The conditions in which the muscle-to-body wt. ratio showed a significant decrease (Table 1 *B*, *E*, *F*) apparently corresponded to those in which soleus motoneurones showed a significant decrease in the duration of a.h.p. This correlation is consistent with the assumption that alterations in motoneurone properties are associated with changes in some factors related to activity of the innervated muscle. The only condition which was inconsistent with this assumption was daily nerve stimulation at a frequency of 50/sec following cord transection (Table 1 *D*). Under this condition soleus motoneurones displayed a significantly shorter a.h.p. (see above), but the muscle-to-body wt ratio was not significantly ($0{\cdot}05 < 2P < 0{\cdot}10$) different from the control value.

TABLE 1. Ratios of the soleus muscle wt. (g) to the body wt. (kg)

A	*B*	*C*	*D*	*E*	F	*G*
Control	Cord section	Cord section + 10/sec stim.	Cord section + 50/sec stim.	TTX alone	Cord section + TTX + proximal stim.	Cord section + TTX + distal stim.
	8 days	8 days	8 days	7 days	8 days	8 days
$0{\cdot}93 \pm 0{\cdot}13$ ($n = 7$)	$0{\cdot}56 \pm 0{\cdot}11$* ($n = 15$)	$0{\cdot}80 \pm 0{\cdot}14$ ($n = 4$)	$0{\cdot}79 \pm 0{\cdot}13$ ($n = 8$)	$0{\cdot}74 \pm 0{\cdot}10$* ($n = 6$)	$0{\cdot}68 \pm 0{\cdot}10$* ($n = 4$)	$0{\cdot}83 \pm 0{\cdot}19$ ($n = 6$)

* Significant ($2P < 0{\cdot}05$) difference from the control value. All values give the mean $\pm$ S.D.

Validity of nerve block with TTX

In the preparations from which TTX cuffs had been removed the evening before the experiment (see Methods), intracellular stimulation of soleus motoneurones invariably elicited muscle contraction. However, it could be argued that the chronic application of TTX cuffs may have caused degeneration of some soleus motor nerve fibres and that these particular motoneurones may not have been detected by antidromic stimulation of the muscle nerve. If this were the case, the observed changes in soleus motoneurone properties following the application of TTX cuffs might be explained on the basis of partial denervation of the muscle (see Huizar *et al.* 1977). This possibility was, however, unlikely for two reasons. First, in preparations treated with TTX cuffs for 7 days, no fibrillation was detected in the soleus muscle under a dissecting microscope. This was in contrast with the occurrence of fibrillation following a block of the axonal transport in the motor nerve (Fernandez & Ramirez, 1974). Secondly, when the maximum twitch tensions evoked by nerve stimulation were compared between the right (without TTX treatment) and left (with TTX treatment) soleus muscles, the treated side was, on the average, 1·06 times (ranging from 0·95 to 1·18) greater than the control side. This difference in twitch tensions between the two sides was within the normal range (Huizar *et al.* 1977). The mean twitch tension of motor units tested by intracellular stimulation of soleus motoneurones was 1·6 g in preparations treated with TTX cuffs for 7 days. This value was not significantly ($0{\cdot}30 < 2P < 0{\cdot}40$) different from the control value (1·5 g) obtained from unoperated animals. Thus, there was no indication for functional motor connexions by collateral sprouts in preparations treated with TTX.

Fast axoplasmic transport in nerve fibres has been shown to be unaffected by the application of TTX in the concentrations which are sufficient to block impulse conduction (Ochs & Hollingsworth, 1971; Anderson & Edström, 1973; Lavoie *et al.* 1976, 1977; Pestronk, Drachman & Griffin, 1976). In two cats, we measured fast axoplasmic transport with cholinesterase as a marker in collaboration with Dr D. L. McIlwain. The left soleus nerve treated with TTX cuffs for 7 days and the right, control soleus nerve were firmly ligated with a thread (Ethicon 4-0) near the muscle. The soleus nerves on both sides were removed 40 hr after ligation, and two adjoining 5 mm segments central to ligation were obtained from each nerve for assay of cholinesterase by the method of Ellman *et al.* (1961; also, cf. Lavoie *et al.* 1977). Accumulation of cholinesterase so measured was greater by about 100% in the segment closer to the ligation in both control and TTX treated nerves. Thus, our results were consistent with previous observations (see above).

Preliminary morphological observations on the soleus nerve treated with TTX for 7 days were made under the light and electron microscopes by Dr A. Rustioni. To our surprise, about 15% of myelinated fibres showed abnormal pictures which were characterized by darkening of the axoplasm under the light microscope. Electron micrographs showed the presence of Schwann cells and their nuclei within the axoplasm of some myelinated fibres. No abnormality was detected in the soleus nerve treated with the cuff containing 20% NaCl without TTX for 7 days. These results were consistent in two cats prepared for morphological examination. It remains uncertain whether the nerve fibres affected by the chronic application of TTX are motor or sensory fibres; nor is it clear how the presence of morphologically abnormal nerve fibres may be reconciled with the apparent lack of sign of functional degeneration of motor fibres indicated by the physiological experiments (see above). When the soleus muscle is denervated by 7–29%, intact soleus motoneurones have been shown to decrease the mean duration of a.h.p. to 136 msec about 3 weeks after partial denervation (Huizar *et al.* 1977). Compared with this value, the decrease in the duration of a.h.p. (114 msec; Fig. 4) of soleus motoneurones one week after the application of TTX cuffs is significantly greater. Therefore, even if moderate fibre degeneration were present after the chronic application of TTX, this factor alone cannot entirely account for the present results.

DISCUSSION

From the results presented here, it seems clear that the duration of a.h.p. of soleus motoneurones can be modified by factors associated with activity of the innervated muscle. Apparently, the factors responsible for maintenance of the motoneurone properties are not mediated by the sensory fibres since the change in the motoneurones induced by cord transection can be prevented by chronic stimulation

of the peripheral nerve distal to site of conduction block by the TTX cuff but not central to the cuff (Fig. 5). Furthermore, soleus motoneurones show no significant changes in the duration of a.h.p. after chronic section of the lumbosacral dorsal roots (Kuno *et al.* 1974*a*). It is likely that muscle activity is directly or indirectly signalled to the innervating motoneurones through the motor axons. For such retrograde signals, three possible mechanisms may be considered. It should be noted that these three possibilities are not mutually exclusive.

Trophic substances. It is possible that the normal properties of soleus motoneurones are maintained by some trophic substances transported through the motor axons from the muscle. Disuse atrophy of the immobilized muscle has been shown to be associated with a decrease in rates of protein synthesis and an increase in its degradation (Goldspink, 1977*a*, *b*). If the postulated trophic substances show similar changes, depending upon the degree of muscle activity, the changes in motoneurone properties observed in the present study may be accounted for by alterations of the substances in the muscle.

Transmitter release from motor nerve terminals. Changes in muscle activity induced by the present procedures are expected to be accompanied by alterations in the amount of transmitter released at the neuromuscular junctions. It is thus possible that the trophic signal toward the motoneurones may be related to the process of transmitter release from the motor nerve terminals. Pilar & Landmesser (1976) have suggested that the maintenance of neurones may be disturbed by the accumulation of some substances normally utilized at the nerve endings. It is also possible that the uptake of the postulated trophic substances (see above) by motor nerve terminals may occur at the expense of transmitter release (Holtzman, Freeman & Kashner, 1971; Heuser & Reese, 1973). If such were the case, muscle activity *per se* would not be essential for the maintenance of motoneurone properties. These two possible mechanisms cannot account for alterations of intact soleus motoneurones following partial denervation of the muscle, since these motoneurones apparently maintain normal activity (Huizar *et al.* 1977). However, at present it is not absolutely certain whether changes of soleus motoneurones observed in the present study are based on the same mechanisms as those following partial denervation of the muscle.

Collateral sprouting from motor axons. Partial denervation of a muscle results in collateral sprouting from the intact motor nerve fibres (Edds, 1953). It has recently been shown that motor nerve terminals grow collateral sprouts a few days after blocking the motor nerve with TTX (Brown & Ironton, 1977). Similarly, motor nerve sprouting induced by botulinum toxin (Duchen & Strich, 1968) can be prevented by chronic stimulation of the muscle (Brown, Goodwin & Ironton, 1977). Thus, prolonged inactivity of a muscle appears to cause collateral sprouting from the motor nerve terminals. It is possible that the peripheral axonal growth may be responsible for the change in motoneurone properties (see Watson, 1969, 1970, 1973).

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REFERENCES

ANDERSON, K. E. & EDSTRÖM, A. (1973). Effects of nerve blocking agents on fast axonal transport of proteins in frog sciatic nerves in vitro. *Brain Res.* **50**, 125–134.

BORISON, H. L., MCCARTHY, L. E., CLARK, W. G. & RADHAKRISHNAN, N. (1963). Vomiting, hypothermia, and respiratory paralysis due to tetrodotoxin (puffer fish poison) in the cat. *Toxic. appl. Pharmac.* **5**, 350–357.

BROWN, M. C., GOODWIN, G. M. & IRONTON, R. (1977). Prevention of motor nerve sprouting in botulinum toxin poisoned mouse soleus muscles by direct stimulation of the muscle. *J. Physiol.* **267**, 42–43*P*.

BROWN, M. C. & IRONTON, R. (1977). Motor neuron sprouting induced by prolonged tetrodotoxin block of nerve action potentials. *Nature, Lond.* **265**, 459–461.

DEVILLIERS, R., NOSÉ, Y., MEIER, W. & KANTROWITZ, A. (1964). Long-term, continuous electrostimulation of a peripheral nerve. *Trans. Am. Soc. artif. internal Organs* **10**, 357–365.

DUCHEN, L. W. & STRICH, S. J. (1968). The effects of botulinum toxin on the patern of innervation of skeletal muscle in the mouse. *Q. Jl exp. Physiol.* **53**, 84–89.

ECCLES, J. C. (1941). Disuse atrophy of skeletal muscle. *Aust. med. J.* **2**, 160–164.

EDDS, M. V. (1953). Collateral nerve regeneration. *Q. Rev. Biol.* **28**, 260–276.

ELLMAN, G. L., COURTNEY, D., ANDRES, V. & FEATHERSTONE, R. M. (1961). A new and rapid colorimetric determination of acetylcholinesterase activity. *Biochem. Pharmac.* **7**, 88–95.

FERNANDEZ, H. L. & RAMIREZ, B. U. (1974). Muscle fibrillation induced by blockage of axoplasmic transport in motor nerves. *Brain Res.* **79**, 385–395.

GOLDSPINK, D. F. (1977*a*). The influence of immobilization and stretch on protein turnover of rat skeletal muscle. *J. Physiol.* **264**, 267–282.

GOLDSPINK, D. F. (1977*b*). The influence of activity on muscle size and protein turnover. *J. Physiol.* **264**, 283–296.

GRANIT, R., HENATSCH, H. D. & STEG, G. (1956). Tonic and phasic ventral horn cells differentiated by post-tetanic potentiation in cat extensors. *Acta physiol. scand.* **37**, 114–126.

GRANIT, R., PHILLIPS, C. G., SKOGLUND, S. & STEG, G. (1957). Differentiation of tonic from phasic alpha ventral horn cells by stretch, pinna and crossed extensor reflexes. *J. Neurophysiol.* **20**, 470–481.

HAMBURGER, V. (1958). Regression versus peripheral control of differentiation in motor hypoplasia. *Am. J. Anat.* **102**, 365–410.

HAMBURGER, V. (1975). Cell death in the development of the lateral motor column of the chick embryo. *J. comp. Neurol.* **160**, 535–546.

HEUSER, J. E. & REESE, T. S. (1973). Evidence for recycling of synaptic vesicle membrane during transmitter release at the frog neuromuscular junction. *J. cell Biol.* **57**, 315–344.

HOLLYDAY, M. & HAMBURGER, V. (1976). Reduction of the naturally occurring motor neuron loss by enlargement of the periphery. *J. comp. Neurol.* **170**, 311–320.

HOLTZMAN, E., FREEMAN, A. R. & KASHNER, A. (1971). Stimulation-dependent alteration in peroxidase uptake at lobster neuromuscular junction. *Science, N.Y.* **173**, 733–736.

HUGHES, A. (1961). Cell degeneration in the larval ventral horn of Xenopus Laevis (Daudin). *J. Embryol. exp. Morph.* **9**, 269–284.

HUGHES, A. (1962). An experimental study in the relationship between limb and spinal cord in the embryo of *Eleutherodactylus martinicensis*. *J. Embryol. exp. Morph.* **10**, 575–601.

HUIZAR, P., KUDO, N., KUNO, M. & MIYATA, Y. (1977). Reaction of intact spinal motoneurones to partial denervation of the muscle. *J. Physiol.* **265**, 175–191.

KARPATI, G. & ENGEL, W. K. (1968). Correlative histochemical study of skeletal muscle after suprasegmental denervation, peripheral nerve section, and skeletal fixation. *Neurology, Minneap.* **18**, 681–692.

KUNO, M., MIYATA, Y. & MUÑOZ-MARTINEZ, E. J. (1974*a*). Differential reactions of fast and slow alpha-motoneurones to axotomy. *J. Physiol.* **240**, 725–739.

KUNO, M., MIYATA, Y. & MUÑOZ-MARTINEZ, E. J. (1974*b*). Properties of fast and slow alpha motoneurones following motor reinnervation. *J. Physiol.* **242**, 273–288.

LAVOIE, P.-A., COLLIER, B. & TENENHOUSE, A. (1976). Comparison of α-bungarotoxin binding to skeletal muscles after inactivity or denervation. *Nature, Lond.* **260**, 349–350.

LAVOIE, P.-A., COLLIER, B. & TENENHOUSE, A. (1977). The role of skeletal muscle activity in the control of muscle acetylcholine sensitivity. *Expl Neurol.* **54**, 148–171.

LØMO, T., WESTGAARD, R. H. & DAHL, H. A. (1974). Contractile properties of muscle: control by pattern of muscle activity in the rat. *Proc. R. Soc.* B **187**, 99–103.

OCHS, S. & HOLLINGSWORTH, D. (1971). Dependence of fast axoplasmic transport in nerve on oxidative metabolism. *J. Neurochem.* **18**, 107–114.

PESTRONK, A., DRACHMAN, D. B. & GRIFFIN, J. W. (1976). Effect of muscle disuse on acetylcholine receptors. *Nature, Lond.* **260**, 352–353.

PILAR, G. & LANDMESSER, L. (1976). Ultrastructural differences during embryonic cell death in normal and peripherally deprived ciliary ganglia. *J. cell Biol.* **68**, 339–356.

Prestige, M. D. (1967). The control of cell number in the lumbar ventral horn during the development of Xenopus laevis tadpoles. *J. Embryol. exp. Morph.* **18**, 359–387.

PRESTIGE, M. D. (1976). Evidence that at least some of the motor nerve cells that die during development have first made peripheral connections. *J. comp. Neurol.* **170**, 123–134.

SALMONS, S. & VRBOVÁ, G. (1969). The influence of activity on some contractile characteristics of mammalian fast and slow muscle. *J. Physiol.* **201**, 535–549.

WATSON, W. E. (1969). The response of motor neurones to intramuscular injection of botulinum toxin. *J. Physiol.* **202**, 611–630.

WATSON, W. E. (1970). Some metabolic responses of axotomized neurones to contact between their axons and denervated muscle. *J. Physiol.* **210**, 321–343.

WATSON, W. E. (1973). Some responses of neurones of dorsal root ganglia to axotomy. *J. Physiol.* **231**, 41–42*P*.