REACTION OF INTACT SPINAL MOTONEURONES TO PARTIAL DENERVATION OF THE MUSCLE

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SUMMARY

1. The properties of soleus motoneurones of the cat were examined with intracellular electrodes about 3 weeks after partial denervation of the soleus muscle. Soleus motoneurones whose axons had been left intact were distinguished from those whose axons had been sectioned by the presence or absence of muscle contraction in response to intracellular stimulation of each motoneurone.

2. The average twitch tension of motor units evoked by intracellular stimulation of intact soleus motoneurones after partial denervation of the muscle was not significantly different from that observed in control, unoperated cats. Therefore, it was assumed that the majority of intact motoneurones had not been subject to injuries in their axons upon partial denervation.

3. Soleus motoneurones whose axons had been sectioned showed a significant increase in overshoot of action potentials and a significant decrease in resting membrane potential, in axonal conduction velocity and in the duration of after-hyperpolarization.

4. Soleus motoneurones whose axons had been left intact also showed a significantly shorter after-hyperpolarization than that seen in control, unoperated cats. Other electrophysiological properties of the intact soleus motoneurones were indistinguishable from those observed in unoperated animals.

5. The decrease of the duration of after-hyperpolarization in intact soleus motoneurones was greater in highly denervated preparations than in moderately denervated preparations.

6. The decrease of the duration of after-hyperpolarization in intact soleus motoneurones was associated with a decrease in contraction times of the innervated muscle fibres, the former preceding the latter by one to two weeks.

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7. It is concluded that motoneurone properties can be modified without injury to their axons and that alterations in the properties of intact motoneurones depend upon the degree of partial denervation of the muscle. The possible signal for alterations of motoneurone properties is discussed.

8. It is also concluded that the contractile properties of muscle fibres can be modified without cross-union of the nerves. It is suggested that the contractile properties of muscle fibres may be linked to the duration of after-hyperpolarization or to some mechanism related to this factor in the innervating motoneurones.

INTRODUCTION

Spinal motoneurones undergo chromatolytic changes about a week after section of their axons (see review by Liberman, 1971). These changes are associated with alterations in several electrophysiological properties of the motoneurones (Eccles, Libet & Young, 1958; McIntyre, Bradley & Brock, 1959; Shapovalov & Grantyn, 1968; Kuno & Llinás, 1970a, b; Kuno, Miyata & Muñoz-Martinez, 1974a, b; Mendell, Munson & Scott, 1976). If the cut motor axons are allowed to regenerate into a muscle, the motoneurones recover, at least in part, their original properties (Kuno *et al.* 1974b). However, at early stages of muscle re-innervation, recovery of the original properties was not seen even in those motoneurones which had already re-established functional motor connexions with the muscle (Kuno *et al.* 1974b). At late stages of re-innervation, on the other hand, the original properties were restored even in those motoneurones which failed to form functional motor connexions with the muscle (Kuno *et al.* 1974b). It is then possible that restoration of the original motoneurone properties may be triggered by some factors associated with an increase in the degree of re-innervation of the muscle, regardless of the presence or absence of functional motor connexions of each motoneurone. If this were the case, motoneurone properties would be subject to an influence from the muscle, such that even normal motoneurones may change their properties according to the degree of motor innervation of the muscle.

The degree of motor innervation of a muscle can be modified by partial denervation of the muscle. Under such conditions, the axons of some motoneurones are sectioned, whereas the axons of other motoneurones are left intact. The present results will show that intact motoneurones change their properties in response to partial denervation of the muscle and that these changes depend upon the degree of partial denervation of the muscle. Some preliminary results have been included in a recent review (Kuno, 1976).

METHODS

Preparations. Adult cats, 2·2–3·9 kg in weight, were anaesthetized by an I.P. injection of pentobarbitone sodium (35–40 mg/kg). The nerve to the soleus muscle on the left side was exposed and a fraction of the soleus nerve was sectioned (partial denervation) about 2–8 mm from the muscle with aseptic precautions. The soleus nerve was found to diverge into two to four branches at the entry to the muscle. In some cases, one or two of these nerve branches were cut. In other cases, an arbitrary fraction of the nerve trunk was sectioned. The fraction of the soleus nerve to be sectioned was deliberately varied from animal to animal.

After a post-operative period varying from 8 to 22 days, the cats were anaesthetized by pentobarbitone sodium (I.P.). The brain was anaemically impaired by bilateral occlusion of the common carotid and vertebral arteries. Anaemic destruction of the brain was evidenced by cessation of respiratory movements and by dilatation of the pupils. The spinal cord was then transected at the first cervical level, and the brain rostral to the section was pithed. The animal was maintained on artificial respiration. After lumbosacral laminectomy, the dorsal roots from the sixth lumbar through the first sacral segments were cut on the left side. In the left leg, branches of the sciatic nerve were cut in the popliteal fossa, except for the soleus nerve whose connexions with the muscle were left intact. The distal tendon of the soleus muscle was severed, and the distal portion of the muscle was freed of surrounding tissues, care being taken to preserve the blood supply. The cut tendon was attached to a transducer (Grass FT 10) operated into a carrier amplifier (Tektronix 3C66) to measure isometric contractions of the muscle. The initial tension applied to the muscle was 100 g in all experiments. The exposed tissues were covered with pools of paraffin oil and external heat aided to maintain the rectal temperature between 36° and 38° C. The pool temperature around the soleus muscle ranged from 30° to 33° C. Artificial respiration was adjusted to keep end-tidal CO_2 levels at 2·5–3·5 %. Hyperventilation seemed to be helpful for maintaining muscle contractions throughout the experiment.

Experimental procedure. Intracellular recording from spinal motoneurones was performed with glass micro-electrodes filled with 2 M potassium acetate. The resistance of the electrodes ranged from 5 to 15 MΩ. Recordings from soleus motoneurones were identified by antidromic action potentials evoked by electrical stimuli applied to the soleus nerve central to the site of partial denervation (Fig. 1 A, S_n). Once the motoneurone was identified, contraction of the soleus muscle was examined in response to stimulation of the motoneurone by depolarizing pulses applied through the intracellular electrode (Fig. 1 A). The presence of muscle contraction following intracellular stimulation of motoneurones was used as a criterion for identification of soleus motoneurones whose axons had been left intact upon partial denervation (see below; also cf. Mendell *et al.* 1976). The properties of soleus motoneurones were measured in terms of axonal conduction velocity, duration of after-hyperpolarization and resting and action potentials by the methods described in a previous report (Kuno *et al.* 1974a). Motoneurones with an action potential of less than 75 mV were excluded from the analysis. The analyses were made on, in total, 465 soleus motoneurones recorded from sixty-three cats.

Identification of 'intact' and sectioned motoneurones. When the soleus nerve is sectioned and immediately reunited to the muscle, no functional motor re-innervation of the muscle can be seen for at least 30 days after the operation (Eccles, Eccles & Kozak, 1962; Kuno *et al.* 1974b). Therefore, it is unlikely that muscle contraction could be elicited by intracellular stimulation of the motoneurones whose axons had been sectioned 8–22 days previously by partial denervation of the muscle. It might

be argued that the absence of muscle contraction to intracellular stimulation of motoneurones may have been due to deterioration of neuromuscular transmission or to conduction block of the nerve during the experiment. However, sixty-four soleus motoneurones examined for motor connexions in ten control, unoperated cats invariably evoked muscle contraction when they were intracellularly stimulated.

Estimate of the degree of partial denervation. At the beginning of every experiment, the ratio of the maximum twitch tension of the soleus muscle evoked by nerve stimulation (Fig. 1*A*, S_n) to that produced by direct stimulation of the muscle (Fig. 1*A*, S_m) was measured. This ratio may provide a rough estimate for the degree of partial denervation (Eccles *et al.* 1962; Kuno *et al.* 1974*b*). Direct stimuli were applied through silver plate (about 2·5 cm²) or multiforked electrodes placed on the
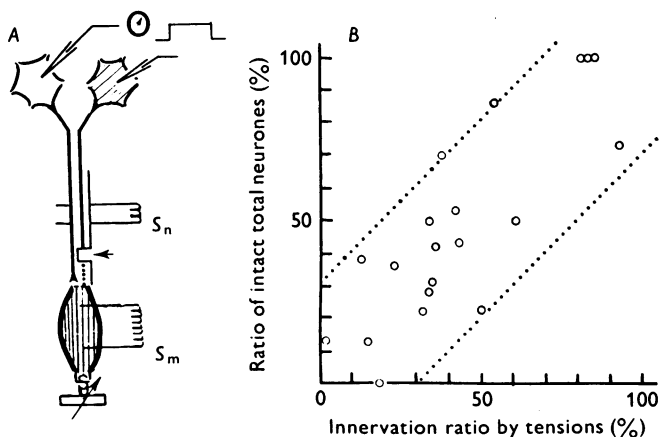

Fig. 1. *A*, schematic diagram of the experiment on partial denervation (horizontal arrow) of the soleus muscle. Hatched neurone represents a soleus motoneurone whose axon had been sectioned; the open neurone represents a motoneurone whose axon had been left intact. S_n, nerve stimulation. S_m, direct stimulation of the soleus muscle. Micro-electrodes inserted into soleus motoneurones serve for intracellular recording as well as for intracellular stimulation. Twitch tensions of the soleus muscle are recorded with a transducer attached to the tendon. *B*, relation between the percentage of intact soleus motoneurones over the total soleus motoneurones examined in each animal (ordinate) and the innervation ratio estimated by the ratio of twitch tensions of the soleus muscle (abscissa). Only those experiments in which eight or more soleus motoneuronse were recorded are included in this Figure.

dorsal and ventral surfaces of the soleus muscle. To prevent repetitive excitation of the muscle, the pulse duration was limited to 1 msec which is shorter than the refractory period of the soleus muscle (1·6 msec; Buller & Lewis, 1965*a*; Lewis, 1972). When the stimulating electrodes were placed close to the nerve entry to the muscle, direct stimulation occasionally resulted in multiple nerve discharges, thereby leading to tetanic activation of the muscle. This disturbance could be avoided by i.v. injection of gallamine triethiodide (0·8–2·0 mg/kg). The maximum twitch tension evoked by nerve stimulation 2 hr after the application of gallamine triethiodide was at least 90% of that examined before the application. Thus, identification of intact or

sectioned motoneurones was not significantly impaired by this procedure at the beginning of the experiment. Twitch tensions to direct stimulation of the muscle usually reached the maximum at an intensity of about 15 V (at the output of a stimulus isolation unit; output impedance, about 1 kΩ) but in relatively large soleus muscles, stimulus intensities over 100 V were still insufficient to obtain the maximum twitch (Rosenblueth, Alanis & Rubio, 1958). In such preparations, the degree of partial denervation was estimated by the ratio of the maximum twitch tension evoked by nerve stimulation to that similarly obtained from the contralateral (unoperated side) soleus muscle. The difference in twitch tensions evoked by nerve stimulation between the left and right soleus muscles in five, unoperated animals ranged from 0·2 to 35 % with a mean of about 14 %. This difference would have caused an error in estimating the degree of partial denervation. In addition, there might have been some atrophy in denervation muscle fibres and some functional re-innervation by collateral sprouting from intact motor axons following partial denervation (Edds, 1953; but cf. Results). The magnitude of the errors arising from these sources was difficult to determine.

Theoretically, the innervation ratio of a given soleus muscle after partial denervation should be proportional to the probability of recording intact soleus motoneurones over the total soleus motoneurones examined in that animal. The number of soleus motoneurones recorded from one animal varied from two to twenty-six. Eccles & Sherrington (1930) have found an average of about 200 myelinated motor fibres in the soleus nerve of the cat. Since about 30 % of these are gamma motor fibres, approximately 140 would be a fair estimate for the total number of soleus alpha motoneurones (also, see Bagust, 1974). The innervation ratio of the soleus muscle after partial denervation was estimated by the percentage of intact soleus motoneurones over the total soleus motoneurones examined in each animal in which eight or more soleus motoneurones were recorded (sampling size > 5 % of the population). The innervation ratio so measured (ordinate) is plotted in Fig. 1 *B* against the innervation ratio estimated from the ratio of twitch tensions of the soleus muscle (abscissa). The values calculated from the two methods agreed only with allowance for an error of about 30 % (Fig. 1 *B*, dotted lines). The accuracy of measurements of the degree of partial denervation was thus considerably limited. Therefore, effects of the degree of partial denervation were studied only in two groups in which the soleus muscle was highly (66–98 %) or moderately (7–29 %) denervated, judging from the ratio of twitch tensions of the muscle (see Results).

RESULTS

Definition of intact motoneurones

When intracellular stimulation of soleus motoneurones produced detectable (> 100 mg) increment in the muscle tension, they were considered to be those motoneurones whose axons had been left intact upon partial denervation (see Methods). Eccles & Sherrington (1930) have shown that some motor nerve fibres divide into 'daughter fibres' (branching axons) about 50 mm before reaching the muscle. It is then possible that some of the 'daughter fibres' might have been sectioned upon partial denervation of the muscle. If so, twitch tension of motor units innervated by such intact motoneurones would become considerably smaller than that of control, normal soleus motor units. Contractions of individual motor units

were evoked by single and tetanic (at 50–75/sec) stimulation of each soleus motoneurone with depolarizing pulses applied through the intracellular electrode. Fig. 2 shows the relation between the maximum tetanic tension and the twitch tension for control, normal soleus motor units (open circles in the dotted line boundary) and for those innervated by intact soleus motoneurones 20–22 days after partial denervation of the muscle (filled circles in the continuous line boundary). In partially denervated preparations, about 8 % of the soleus motoneurones evoked twitch or tetanic
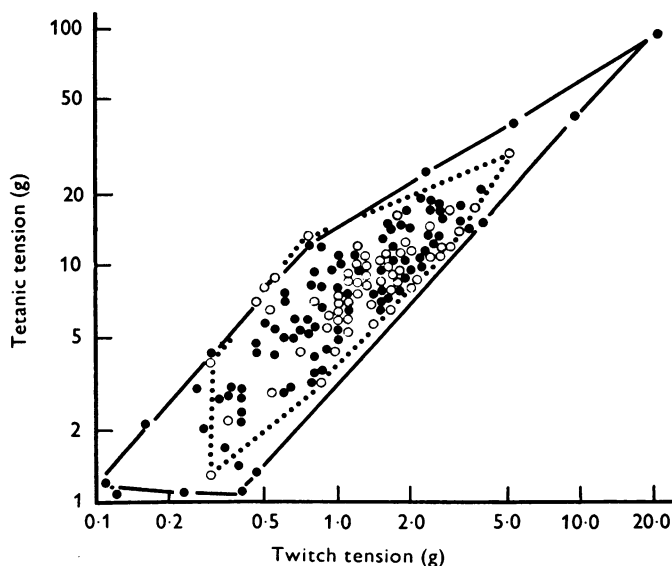

Fig. 2. Relation between the maximum tetanic tension and the twitch tension of soleus motor units observed in control, unoperated cats (open circles in the dotted line boundary) and 20–22 days after partial denervation of the soleus muscle (filled circles in the continuous line boundary).

tensions smaller than the normal range. However, mean twitch and tetanic tensions of motor units showed no significant differences between the unoperated and partially denervated preparations. Therefore, the majority of intact motoneurones examined in the present study cannot be considered to be those whose 'daughter fibres' were partly sectioned.

One may note that about 4 % of the soleus motoneurones in partially denervated preparations produced greater twitch (up to 21 g) or tetanic (up to 95 g) tensions than the normal range (Fig. 2). This could be due to functional motor connexions of newly sprouted branches from intact motor axons as a result of partial denervation (Edds, 1953). It might be argued that the effects of section of 'daughter fibres' may have been masked

by collateral sprouting from the parent motor axon. This possibility was tested on partially denervated preparations in a post-operative period of 8 days which was presumably too short for collateral sprouts to form functional motor connexions with denervated muscle fibres (van Harreveld, 1945; Edds, 1953). Mean twitch tensions of motor units innervated by intact soleus motoneurones 8 days (1·5 g; thirty-eight units) and 20–22 days (1·7 g; 109 units) after partial denervation showed no significant difference. It is suggested that functional motor connexions by collateral sprouts in the cat may be formed only over 3 weeks after partial denervation of the muscle (cf. van Harreveld, 1945; Edds, 1953).

From these results, it was assumed that most, if not all, intact motoneurones had not been subject to injuries in their axons upon partial denervation (see Discussion).

Differencesbetween intact and sectioned motoneurones. Based on the presence or absence of muscle contraction to intracellular stimulation of motoneurones, in total, 120 intact and eighty-five sectioned soleus motoneurones were recorded from twenty-three cats 20–22 days after partial denervation of the soleus muscle. As control, ninety-four soleus motoneurones were examined in sixteen normal, unoperated cats. The mean values of the results obtained from these three groups were statistically examined by two-tailed t tests with significance limit of $2P < 0.05$.

It has previously been reported that soleus motoneurones show no significant changes in the resting membrane potential after chronic section of their axons (axotomy) and that a significant increase in the amplitude of overshoot of action potentials can be seen only over 50 days after axotomy (Kuno et al. 1974a). In the present study, however, sectioned soleus motoneurones 20–22 days after partial denervation showed a slightly but significantly lower resting potential (mean, 66 mV) and a significantly greater mean overshoot potential (29 mV) than control soleus motoneurones (mean values, 70 and 24 mV) in unoperated animals. The mean resting potential (69 mV) and the mean amplitude of overshoot potentials (23 mV) of intact soleus motoneurones after partial denervation were not significantly different from those in control soleus motoneurones.

Fig. 3 illustrates frequency distributions of axonal conduction velocities of control soleus motoneurones (Fig. 3A) and intact (Fig. 3B) and sectioned (Fig. 3C) soleus motoneurones 20–22 days after partial denervation of the muscle. In agreement with previous observations (Kiraly & Krnjević, 1959; Cragg & Thomas, 1961; Kuno et al. 1974a; Mendell et al. 1976), the mean conduction velocity of sectioned soleus motoneurones (57 m/sec) was significantly slower than that of control soleus motoneurones (69 m/sec). Soleus motoneurones whose axons had been left

intact upon partial denervation showed no significant difference in conduction velocity (67 m/sec) from control soleus motoneurones (also see Mendell *et al.* 1976).

The duration of after-hyperpolarization following each action potential is shown in Fig. 4 for the three groups of soleus motoneurones (Fig. 4*A–C*). Sectioned soleus motoneurones examined 20–22 days after partial denervation were characterized by a drastic decrease in the duration of after-hyperpolarization (mean, 101 msec; Fig. 4*C*), compared with control
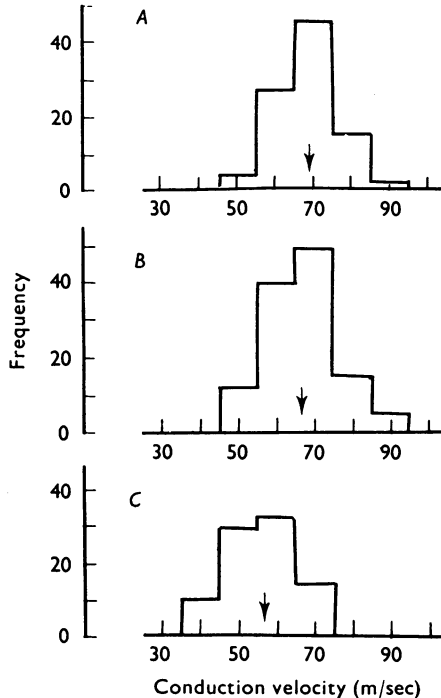

Fig. 3. *A*, histograms of axonal conduction velocities of soleus motoneurones observed in control, unoperated cats and, *B*, for intact and, *C*, sectioned soleus motoneurones 20–22 days after partial denervation of the soleus muscle. Ordinates, the number of observed motoneurones. Mean conduction velocities indicated by arrows.

soleus motoneurones (mean, 144 msec; Fig. 4*A*). The duration of after-hyperpolarization of intact soleus motoneurones (mean, 123 msec; Fig. 4*B*) was significantly shorter than control soleus motoneurones (Fig. 4*A*) but was significantly longer than that of sectioned motoneurones (Fig. 4*C*). It seems clear that the properties of spinal motoneurones can be modified, at least in part, without section of their axons and that the properties of motoneurones so modified are different from those sectioned motoneurones.

Effects of the degree of partial denervation

If the properties of intact soleus motoneurones are modified by a decrease in the innervation ratio of the muscle following partial denervation, one may expect that the changes in the properties of intact motoneurones become more prominent with an increase in the degree of partial denervation. The duration of after-hyperpolarization of intact soleus
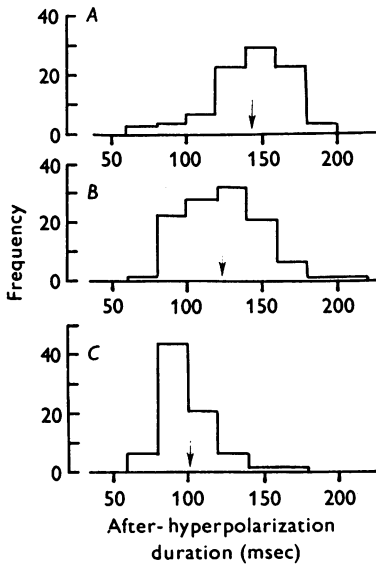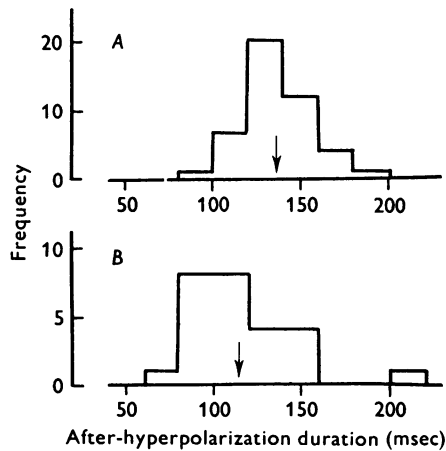

Fig. 4   Fig. 5

Fig. 4. *A*, histograms of the duration of after-hyperpolarization of soleus motoneurones observed in control, unoperated cats and, *B*, for intact and, *C*, sectioned soleus motoneurones 20–22 days after partial denervation of the soleus muscle. Ordinates, the number of observed motoneurones. Mean values of the duration of after-hyperpolarization indicated by arrows.

Fig. 5. Histograms of the duration of after-hyperpolarization of intact soleus motoneurones 20–22 days after partial denervation of the soleus muscle. *A*, moderately (7–29%) denervated preparations (average innervation ratio, 90%). *B*, severely (66–98%) denervated preparations (average innervation ratio, 27%). Ordinates, the number of observed motoenurones. Mean values of the duration of after-hyperpolarization indicated by arrows.

motoneurones was compared in two extreme groups of preparations examined 20–22 days after partial denervation: one group comprised the preparations in which 66–98% of the soleus muscle was denervated; and the other comprised those in which only 7–29% of the muscle was denervated.

In the former group, a total of ninety-six soleus motoneurones was recorded from eight cats but only twenty-six of them were found to be intact motoneurones (average innervation ratio, 27 %). In contrast, forty-five intact motoneurones were obtained out of a total of fifty soleus moto-neurones recorded from seven moderately denervated preparations (average innervation ratio, 90 %).

The frequency distributions of the duration of after-hyperpolarization of intact soleus motoneurones are shown in Fig. 5*A* for moderately and severely (Fig. 5*B*) denervated preparations. The mean duration of after-hyperpolarization (136 msec) shown in Fig. 5*A* was still significantly shorter than that of control soleus motoneurones (144 msec; Fig. 4*A*), and the mean values of the results illustrated in Fig. 5*B* (114 msec) was significantly longer than that of sectioned motoneurones (101 msec; Fig. 4*C*). However, the mean duration of after-hyperpolarization of intact soleus motoneurones in moderately denervated preparations (Fig. 5*A*) was significantly longer than that in severely denervated preparations (Fig. 5*B*). Thus, alterations in the properties of intact soleus motoneurones appear to depend upon the degree of partial denervation of the muscle.

Effects on contraction time of the muscle

The axonal conduction velocity and the duration of after-hyperpolariza-tion in motoneurones are both closely related to contraction times of the innervated muscle fibres (Devanandan, Eccles & Westerman, 1965; Burke, 1967; Bagust, 1974; Bagust, Lewis & Westerman, 1974; Hammarberg & Kellerth, 1975). As shown in Fig. 6, similar relations can be seen for contractions of individual soleus motor units evoked by intracellular stimulation of the motoneurones in control, unoperated cats. The inverse relation between the axonal conduction velocity and the contraction time (Fig. 6*A*) as well as the positive correlation between the duration of after-hyperpolarization and the contraction time (Fig. 6*B*) was statistically significant.

The pool temperature of paraffin oil around the exposed soleus muscle ranged from 30° to 33° C in the present study. Relatively long contraction times shown in Fig. 6 as compared with those reported by Burke (1967) may be accounted for by low temperatures (Gordon & Phillips, 1953). Soleus motor units are generally considered to be a homogeneous group (McPhedran, Wuerker & Henneman, 1965; Henneman & Olson, 1965; Burke, 1967; Burke, Levine, Salcman & Tsairis, 1974). However, two soleus motor units (about 3 %) indicated by arrows in Fig. 6 were similar in both motoneurone and muscle properties to fast (e.g. gastrocnemius) motor units (also see Bagust, 1974).

Since soleus motoneurones left intact after partial denervation of the muscle show a significant decrease in the duration of after-hyperpolariza-tion (Fig. 4*B*) without alteration in their mean conduction velocity

(Fig. 3 *B*), the measure of contraction times of these motor units may provide a favourable test as to which parameter of motoneurones is more directly related to the contractile properties of the muscle. Fig. 7 illustrates the results obtained from intact soleus motor units 20–22 days after partial denervation of the muscle. The conduction velocity of motoneurones was no longer correlated with the contraction time of the
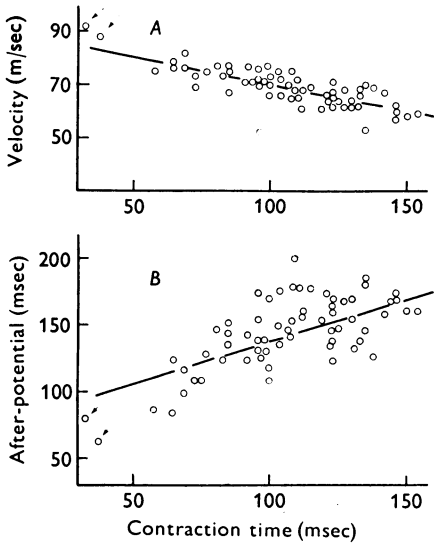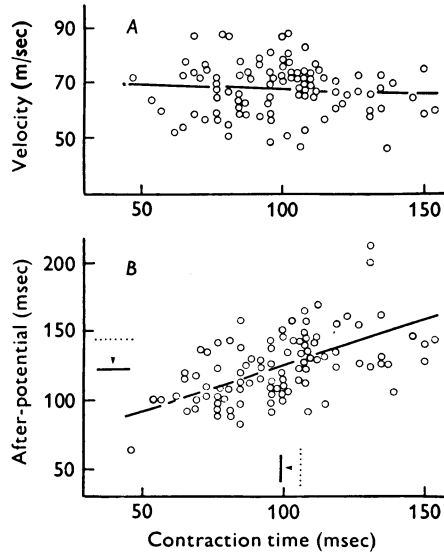

Fig. 6. *A*, relations between the axonal conduction velocity of soleus motoneurones and the contraction time of the motor units and, *B*, between the duration of after-hyperpolarization of soleus motoneurones and the contraction time of the motor units observed in control, unoperated cats. The correlation coefficients of the regression lines (continuous lines) are 0·79 (*A*) and 0·72 (*B*). Arrows indicate fast type soleus units.

Fig. 7. Results obtained from intact soleus motor units 20–22 days after patrial denervation of the muscle. *A*, relation between the conduction velocity of motoneurones and the contraction time of the motor units. *B*, relation between the duration of after-hyperpolarization and the contraction time of the motor units. The correlation coefficients of the regression lines (continuous lines) are 0·10 (*A*) and 0·56 (*B*). Arrows in *B* indicate a shift of mean values from the control values.

muscle fibres (Fig. 7 *A*), whereas the relation between the duration of after-hyperpolarization and the contraction time was still statistically significant (Fig. 7 *B*). Evidently, it is the duration of after-hyperpolarization of motoneurones that is more closely related to the contractile properties of muscle fibres.

A decrease in the mean duration of after-hyperpolarization following partial denervation (vertical arrow in Fig. 7*B*) was associated with a decrease in the mean contraction time (from 107 to 98 msec; horizontal arrow in Fig. 7*B*). Thus, it seems clear that contraction times of muscle fibres innervated by intact soleus motoneurones are also modified after partial denervation of the muscle. Furthermore, the maintenance of correlation between the duration of after-hyperpolarization and the contraction time (Fig. 7*B*) suggests that the changes in contraction time of
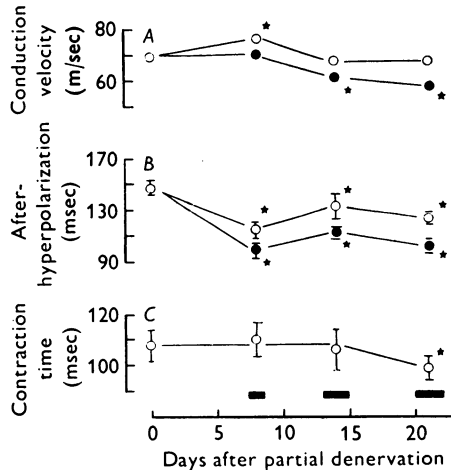

Fig. 8. *A*, time courses of the changes of the mean values for conduction velocity, *B*, the duration of after-hyperpolarization and, *C*, contraction time of soleus motor units after partial denervation of the soleus muscle. The mean values obtained from control, unoperated animals are shown at day 0. Open circles, intact soleus motor units. Filled circles, sectioned soleus motoneurones. Asterisks indicate significant differences from the control mean values. Each point represents the mean ± 2 s.e. of mean. Horizontal bars below indicate post-operative periods examined. The average innervation ratios estimated by the ratio of 'intact' motoneurones to the total soleus motoneurones recorded are 45 % (44/97 from fifteen cats) for day 8, 48 % (33/69 from nine cats) for day 13–15 and 59 % (120/205 from twenty-three cats) for days 20–22.

muscle fibres are coupled to the changes in the duration of after-hyperpolarization of the innervating intact motoneurones by some mechanism.

A question may then arise as to whether changes in the duration of after-hyperpolarization of intact motoneurones *precede* or *follow* the changes in contraction times of the innervated muscle fibres after partial denervation. Fig. 8 shows time courses of the changes of the mean values for the axonal conduction velocity (Fig. 8*A*), the duration of after-hyperpolarization (Fig. 8*B*) and the contraction time of motor units (Fig. 8*C*)

after partial denervation of the soleus muscle. These points may be compared with the mean values obtained from control, unoperated cats (points at day 0 in Fig. 8). A significant (marked with asterisks in Fig. 8) decrease in the duration of after-hyperpolarization was observed within 8 days after partial denervation in both intact (open circles) and sectioned (filled circles) soleus motoneurones (Fig. 8), whereas a significant decrease in the mean contraction time of motor units innervated by intact soleus motoneurones occurred only about 3 weeks after partial denervation (asterisk in bottom of Fig. 8). Thus, it is clear that the changes in contraction time of the innervated muscle fibres have a time lag of 1–2 weeks behind the changes in the duration of after-hyperpolarization of the motoneurones (see Discussion).

In agreement with previous observations (Kuno *et al.* 1974*a*; Mendell *et al.* 1976), sectioned motoneurones (filled circles in Fig. 8) showed a significant (marked with asterisks) decrease in conduction velocities in a post-operative period between 1–2 weeks (Fig. 8, top). However, a puzzling point was that intact soleus motoneurones (open circles) showed a transient increase in conduction velocity 8 days after partial denervation (Fig. 8*A*). The conduction velocities of intact soleus motoneurones examined about 2 and 3 weeks after partial denervation were not significantly different from the mean conduction velocity of control soleus motoneurones (Fig. 8*A*). The explanation for these results remains unclear.

<center>DISCUSSION</center>

From the present study, there seems little doubt that intact spinal motoneurones can modify, at least in part, their properties in response to partial denervation of the muscle and that these changes in motoneurone properties are associated with the changes in the contractile properties of the innervated muscle fibres. The possible mechanisms underlying the two phenomena are discussed below.

How do 'intact' motoneurones react to partial denervation of the muscle?

Sensory deprivation. Upon partial denervation, sensory fibres arising from the muscle as well as the motor nerve are sectioned. However, it has been demonstrated that soleus motoneurones show no significant changes in conduction velocity and duration of after-hyperpolarization 29–46 days after section of the lumbosacral dorsal roots (Kuno *et al.* 1974*a*). Therefore, alterations in the properties of 'intact' soleus motoneurones would not be due to sensory deprivation associated with partial denervation of the muscle.

Section of 'daughter' motor fibres. Some 'daughter' branches arising from

a motoneurone might have been sectioned upon partial denervation of the muscle. Thus, stimulation of such motoneurones may still produce muscle contraction but alterations of the motoneurone properties could be a reaction to partial injury of the axon. The number of motor nerve fibres a few millimetres from a muscle has been reported to be greater by 10–25 % than the number of motor fibres a few centimetres from the muscle (Eccles & Sherrington, 1930). If some motoneurones bifurcate more than once before reaching the muscle, motoneurones that show dichotomy before entering the muscle would comprise even less than 10–25 % of the total population. Alpha motor fibres may branch to a greater extent than gamma fibres (Eccles & Sherrington, 1930). Even if this factor is taken into consideration, the probability of cutting one of the 'daughter' fibres while leaving the other intact would not exceed 15–35 % of the motoneurones examined in the present study. Furthermore, the mean tension of contractions of motor units was not significantly different between the control, unoperated cats and the partially denervated preparations (Fig. 2). Thus, reaction of intact motoneurones to partial denervation of the muscle cannot entirely be accounted for by partial injuries in their axons.

Motoneurone interaction. One might conceive that axotomized motoneurones may affect neighbouring intact motoneurones by some as yet unknown mechanisms, thereby resulting in alterations of the intact motoneurone properties. If such a motoneurone interaction exists, the changes in the properties of intact motoneurones would appear to be correlated with the degree of partial denervation of the muscle. This possibility may be remote but it cannot completely be excluded at present.

Collateral sprouting of motor axons. Partial denervation of a muscle is known to induce collateral sprouting from the intact motor nerve fibres (Edds, 1953). It is possible that the axonal growth may be responsible for initiation of the cell reaction of intact motoneurones (Watson, 1969, 1970, 1973). The degree of collateral sprouting of intact motor axons varies in proportion to the degree of partial denervation of the muscle (Edds, 1953). Thus, this possibility is consistent with the present results. It has previously been shown that restoration of the original motoneurone properties following peripheral motor re-innervation depends on the degree of re-innervation of the muscle rather than on the presence or absence of motor connexions of the motoneurone under study (Kuno *et al.* 1974*b*; also see Introduction). This observation may also be adequately explained by assuming that the original motoneurone properties are restored by suppression of peripheral sprouting of the motor axon associated with an increase in the degree of re-innervation of the muscle. However, it remains uncertain how the signal for collateral sprouting of motor axons is initiated by partial denervation of the muscle.

Retrograde trophic action. It is possible that normal motoneurone properties may be maintained by some trophic signal from the muscle. If such retrograde trophic signals are dependent upon the degree of innervation of the muscle or upon muscle activity, the present observations may be accounted for on this basis.

How do innervated muscle fibres react to partial denervation?

The present study has shown that the changes in contraction times of muscle fibres following partial denervation occur after alterations in the duration of after-hyperpolarization in the innervating motoneurones (Fig. 8). However, the correlation between the contraction time of muscle fibres and the duration of after-hyperpolarization in the innervating motoneurones was still maintained under such conditions (Fig. 7 *B*). These results suggest that the contractile properties of muscle fibres are determined, at least in part, by some factor related to the duration of after-hyperpolarization in the innervating motoneurones.

It is known that the speed of contraction of the fast and slow twitch muscles can be partly transformed after cross-union of their nerves (Buller, Eccles & Eccles, 1960; Eccles *et al.* 1962; Buller & Lewis, 1965*b*; Close, 1969). A possibility was suggested that a specific trophic substance emanating from a particular *type* of motoneurone may determine the contractile properties of the innervated muscle (Buller *et al.* 1960). In contrast to the cross-union experiments, however, the present study showed that the same innervating motoneurones can modify the contractile properties of the muscle. This may imply that the signal responsible for the contractile properties of muscles is not directly related to the type of motoneurone.

A possibility exists that the nature of trophic substances emanating from motoneurones may change following partial denervation of the muscle, thereby altering the contractile properties of the muscle. If this were the case, such changes would be coupled to the changes in the duration of after-hyperpolarization of the motoneurones (Fig. 7 *B*). It is generally agreed that the duration of after-hyperpolarization is one of the factors responsible for the frequency of motoneurone discharge (Eccles, Eccles & Lundberg, 1958; Kuno, 1959; Kernell, 1965). Therefore, the discharge pattern of motoneurones may be a primary determinant of contraction times of the innervated muscle (Eccles *et al.* 1962; Salmons & Vrbová, 1969; Lømo, Westgaard & Dahl, 1974). Whatever the underlying mechanism, the duration of after-hyperpolarization seems to be the only electrophysiological parameter of motoneurones which cannot be divorced from contraction times of the innervated muscle.

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