DISUSE ENHANCES SYNAPTIC EFFICACY IN SPINAL MONONEURONES

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SUMMARY

1. Monosynaptic excitatory post-synaptic potentials (e.p.s.p.s) were recorded from triceps surae motoneurones in the cat following section or chronic conduction block of the medial gastrocnemius (m.g.) nerve with tetrodotoxin (TTX) or after daily stimulation of the sciatic nerve.

2. The mean maximum amplitudes of homonymous and heteronymous monosynaptic e.p.s.p.s evoked by stimulation of the m.g. nerve were reduced significantly between 1 and 2 weeks after section of the muscle nerve. The mean amplitudes of monosynaptic e.p.s.p.s produced in the same motoneurones by afferent volleys from the intact synergists showed no significant alterations.

3. Reduction of the amplitude of monosynaptic e.p.s.p.s evoked by the sectioned m.g. afferent volleys was not prevented by daily stimulation of the sciatic nerve. The chronic stimulation of the sciatic nerve did not increase the amplitude of monosynaptic e.p.s.p.s evoked by stimulation of the intact, lateral gastrocnemius (l.g.) or soleus nerve.

4. Chronic conduction block of the intact m.g. nerve with TTX cuffs for 2 weeks resulted in a significant increase in the homonymous e.p.s.p. amplitude. The amplitude of the heteronymous e.p.s.p.s evoked in the same m.g. motoneurones by stimulation of the intact l.g. or soleus nerve showed no significant changes.

5. It is concluded that decreased central synaptic transmission following section of the peripheral nerve is not due to elimination of impulse activity (disuse) of the sensory input and that prolonged disuse of the sensory fibres causes an increase, rather than a decrease, in central synaptic efficacy.

INTRODUCTION

There have been several attempts to assess the effects of prolonged disuse on monosynaptic transmission in the mammalian spinal cord. Eccles & McIntyre (1953) have shown that spinal monosynaptic reflexes are strikingly depressed after 3 weeks of total disuse induced by section of the dorsal roots just distal to the ganglia. Similarly, following section of a muscle nerve in the hind leg, monosynaptic excitatory post-synaptic potentials (e.p.s.p.s) recorded from the homonymous and heteronymous motoneurones are significantly reduced in amplitude within 2 weeks (Eccles, Krnjević & Miledi, 1959). These results are consistent with the idea that disuse of central synapses decreases their efficiency. However, decreased synaptic trans-

mission could be due to alterations of the sensory neurones associated with axonal injuries (axon reaction) rather than to the deprivation of impulse activity (disuse).

Monosynaptic spinal reflexes produced by stimulation of a muscle nerve are enhanced a few weeks after severance of the tendon (tenotomy) of the muscle (Beránek & Hnik, 1959; Beránek, Hnik, Vyklický & Zelená, 1961; Kozak & Westerman, 1961; Robbins & Nelson, 1970; Goldfarb & Muller, 1971). Based on the assumption that tenotomy results in disuse of the sensory fibres arising from the muscle, it has been suggested that disuse may cause an increase rather than a decrease in central synaptic efficacy (Beránek & Hnik, 1959; Beránek *et al.* 1961; Hnik, Beránek, Vyklický & Zelená, 1963; April & Spencer, 1969). However, disuse of the afferent pathway from tenotomized muscles has not been proven experimentally. Thus, the effect of disuse on central synapses remains uncertain.

The present study was undertaken to examine the changes in the mean amplitude of monosynaptic e.p.s.p.s recorded from spinal motoneurones in association with use or disuse of their sensory inputs. The principal question to be posed is twofold. (1) Is the decrease in central synaptic efficacy observed following section of a peripheral nerve prevented by daily stimulation of the cut sensory fibres? (2) Does chronic conduction block of the peripheral nerve with tetrodotoxin (TTX) induce alterations in central synaptic efficacy comparable to those produced by nerve section?

METHODS

Preparation. Adult cats, 2·6–4·0 kg in weight, were anaesthetized by an I.P. injection of sodium pentobarbitone (35 mg/kg). With aseptic precautions, the nerve to the medial gastrocnemius (m.g.) muscle on the left side was exposed and sectioned near the muscle. The central end of the cut nerve was ligated and deflected centrally to prevent reconnexions with the muscle. After a post-operative period varying from 1 to 3 weeks, monosynaptic e.p.s.p.s were recorded from lumbosacral motoneurones (see below). In five cats, bipolar silver electrodes embedded in a hollow silicone cuff were implanted around the sciatic nerve using procedures described in a previous report (Czéh, Gallego, Kudo & Kuno, 1978). A miniature stimulator (Smith, 1978) was mounted on a pet-harness saddled to the animal. Insulated wires attached to the silver electrodes were led subcutaneously to the stimulator output. Following section of the m.g. nerve, electrical stimuli were applied to the sciatic nerve at a frequency of 100/sec for 5 sec out of every 30 sec over a 2-week period. The pulse duration was 0·1 msec, and the stimulus intensity was adjusted every day to 1·2–1·5 times the threshold for initiation of muscle contractions. At this intensity, no flexion reflexes were noted. Also, the animal showed no behavioural response to the stimuli except movement of the hind leg. In five other cats, silicone (Silastic 382, Dow Corning Corp.) cuffs containing TTX (Sankyo Co.) were applied to the intact m.g. nerve. Detailed procedures for preparation and implantation of TTX cuffs have been described previously (Czéh *et al.* 1978). The effectiveness of the block was examined by looking for the presence or absence of contractions of the m.g. muscle following stimulation with the electrodes implanted in the sciatic nerve central to the TTX cuff (Czéh *et al.* 1978). Conduction block by a TTX cuff lasted for 3–9 days. When nerve conduction was recovered, the cuff was immediately replaced with a new one. In this manner, nerve conduction was blocked for 2 weeks. The TTX cuff was removed the evening before the experiment. At the time of the acute experiment, the size of the afferent volley evoked in the lumbosacral dorsal roots by stimulation of the nerve distal to site of the cuff application was 86–97 % of that produced by stimulation of the nerve proximal to the site. It is uncertain whether this difference was due to incomplete recovery of nerve conduction or to partial degeneration of the sensory fibres. Monosynaptic e.p.s.p.s from the m.g. nerve were evoked by stimulation of the muscle nerve central to site of the cuff application. No fibrillation was detected in the m.g. muscle after a 2-week period of nerve block.

Experimental procedure. One to three weeks after the operation, the cats were anaesthetized with sodium pentobarbitone (35 mg/kg, I.P.). The brain was ischaemically impaired by bilateral occlusion of the common carotid and vertebral arteries. After cessation of respiration and dilatation of the pupils, the spinal cord was transected at the first cervical level, and the brain rostral to the transection was pithed. The animal was then maintained on artificial respiration and immobilized by I.V. injections of gallamine triethiodide throughout the experiment. The lumbosacral spinal cord was exposed by laminectomy. Intracellular recordings from spinal motoneurones were made with glass micro-electrodes filled with 2 M-potassium acetate. The resistance of the electrodes was between 5 and 15 MΩ. The input time constant of the high-impedance preamplifier (Fein, 1966) connected to the electrode was kept at 30–80 μsec by capacitative feed-back. In the left hind leg, the nerves to the m.g., lateral gastrocnemius (l.g.) and soleus muscles were dissected and cut distally. For intracellular recordings, these moto-neurones were identified by antidromic action potentials evoked by stimulation of the central ends of the cut muscle nerves. All exposed tissues were covered with pools of paraffin oil, and external heat aided in keeping the rectal temperature between 36 and 38 °C. Artificial ventilation was adjusted to maintain end-tidal CO_2 levels at 2·5–3·5 %.

Fig. 1. Monosynaptic e.p.s.p.s recorded from a m.g. motoneurone. *A*, the antidromic action potential was blocked by post-synaptic hyperpolarization (17·5 nA), leaving only the M-spike (downward arrow) in order to disclose the homonymous e.p.s.p. (up-ward arrow). The falling phase of the M-spike was estimated (dots) by extrapolation, the decay being assumed to be exponential. *B*, heteronymous e.p.s.p. evoked by stimulation of the l.g. muscle nerve with the same amount of hyperpolarizing current (17·5 nA). *C*, same as *B* but without post-synaptic hyperpolarization. Each record is the average of sixteen responses. Calibration, 1 mV and 1 msec.

Monosynaptic e.p.s.p.s were recorded from the ankle extensor (m.g., l.g., soleus) motoneurones in response to stimulation of each of these muscle nerves at a frequency of 0·5/sec. Monosynaptic e.p.s.p.s were evoked at the intensity of stimulation which yielded the maximum amplitude. With the aid of a computer (Fabri-Tek 1072), the mean maximum amplitude was obtained by averaging sixteen e.p.s.p.s elicited by afferent volleys from each of the muscle nerves. Moto-neurones with an action potential of less than 75 mV were excluded from the analysis. The mean values of the results obtained under different experimental conditions were examined by two-tailed *t* tests with significance limit of $P < 0.05$.

Sources of possible errors. The observations on monosynaptic e.p.s.p.s were made 5–17 hr after pentobarbitone anaesthesia. The mean maximum amplitude of e.p.s.p.s recorded 12–17 hr after anaesthesia was greater by 12 % than that observed during a post-anaesthetic period of 5–10 hr; however, this difference was statistically insignificant ($0.30 < P < 0.40$).

Since both the dorsal and ventral roots were left intact, the antidromic action potential was occasionally (about 12 % of motoneurones examined) produced at a stimulus intensity weaker than that required to evoke the maximum homonymous e.p.s.p. In such cases, stimuli were applied at the supramaximal intensity for the e.p.s.p.s. At the same time, DC currents were applied through the intracellular electrode, and the motoneurone was hyperpolarized to the level at which the antidromic motor impulse failed to invade the initial segment, leaving only the M-spike (Coombs, Eccles & Fatt, 1955*a*; Eccles, 1957). The peak of the monosynaptic e.p.s.p. could be revealed by this procedure (upward arrow in Fig. 1*A*; also, see Eccles, Eccles & Lund-berg, 1957; Burke, 1968; Burke, Rymer & Walsh, 1976). The falling phase of the M-spike (downward arrow in Fig. 1*A*) was then estimated by extrapolation (dots in Fig. 1*A*), assuming exponential decay. The value of the extrapolated falling phase at the e.p.s.p. peak was then subtracted from the e.p.s.p. amplitude measured from the resting level (Burke, 1968; Burke *et*

al. 1976). In a few motoneurones, the rising phase of monosynaptic e.p.s.p.s intersected the M-spike before or immediately after its peak, so that the falling phase of the M-spike could not be estimated. The results from these motoneurones (about 2 % of total motoneurones studied) were discarded.

The mean amount of hyperpolarizing currents used to block antidromic invasion of impulses was 25 nA. It was possible that the e.p.s.p. amplitude may have been increased by the hyperpolarizing current (Coombs, Eccles & Fatt, 1955b). This possibility was tested by comparing the maximum amplitudes of the heteronymous e.p.s.p.s in the presence (Fig. 1B) and absence (Fig. 1C) of the same amount of post-synaptic hyperpolarization as that used to block invasion of the motor impulse (Fig. 1A). In seventeen motoneurones so tested, the heteronymous e.p.s.p. amplitude showed no difference (two cells) or a slight increase (six cells) or a slight decrease (nine cells); on the average, post-synaptic hyperpolarization caused a decrease in the heteronymous e.p.s.p. amplitude by about 1 %. It should be noted that the spatial distribution of synapses on a given motoneurone is not different between the homonymous and heteronymous afferent fibres (Kuno & Miyahara, 1969; Jack, Miller, Porter & Redman, 1970). Since the effect of hyperpolarizing currents on the e.p.s.p. amplitude was thus practically negligible (also, see Coombs *et al.* 1955b; Nelson & Frank, 1967; Shapovalov & Kurchavyi, 1974; Klee, 1975; Edwards, Redman & Walmsley, 1976; Werman & Carlen, 1976), no correction was made. Post-synaptic hyperpolarizing currents (2–6 nA) were also applied to block synaptically evoked action potentials when monosynaptic e.p.s.p.s exceeded the threshold for excitation.

In many motoneurones, the shape of monosynaptic e.p.s.p.s was apparently distorted by co-existent inhibitory post-synaptic potentials (i.p.s.p.s) which were presumably evoked through Group Ib afferent fibres or through recurrent motor-axon collaterals or both. In twenty motoneurones, the i.p.s.p.s were reversed by post-synaptic hyperpolarization in order to measure their latencies. The i.p.s.p.s were invariably initiated after monosynaptic e.p.s.p.s reached the peak amplitude. Therefore, it was assumed that the e.p.s.p. amplitudes were not significantly influenced by the i.p.s.p.s.

The onset of monosynaptic e.p.s.p.s was often masked by field potentials due to antidromic invasion of neighbouring motoneurones (Eccles *et al.* 1959). In many motoneurones, the size of the field potential was deliberately altered by changing the stimulus intensity and hence, changing the number of antidromically recruited motoneurones. This procedure did not affect the maximum e.p.s.p. amplitude measured from the level of resting membrane potential.

RESULTS

Effects of nerve section. Fig. 2A shows the mean maximum amplitudes of monosynaptic e.p.s.p.s recorded from m.g. motoneurones in response to stimulation of the m.g. (homonymous, open circles) or l.g. (heteronymous, filled circles) nerves. The results obtained from control, unoperated cats are shown at day 0. In three other groups of animals, the monosynaptic e.p.s.p.s were recorded 1, 2 and 3 weeks after section of the m.g. nerve, respectively. Each group comprised four cats, and each point was obtained by averaging the maximum amplitudes of e.p.s.p.s recorded from twenty-one to thirty-eight motoneurones. In agreement with previous observations (Eccles *et al.* 1959), the homonymous e.p.s.p.s recorded from m.g. motoneurones (open circles in Fig. 2A) showed no changes in amplitude 1 week after section of the m.g. nerve but were significantly ($P < 0.001$) decreased after post-operative periods of 2 and 3 weeks. The heteronymous e.p.s.p.s evoked by stimulation of the intact l.g. (filled circles in Fig. 2A) or soleus (not illustrated) nerve were not affected at least up to 3 weeks after section of the m.g. nerve.

Fig. 2B shows the mean maximum amplitudes of monosynaptic e.p.s.p.s recorded from l.g. motoneurones as a function of days after section of the m.g. nerve. The monosynaptic e.p.s.p.s produced by stimulation of the sectioned m.g. nerve again

showed a significant ($P < 0.001$) decrease in amplitude within 2 weeks after the operation (open circles in Fig. 2B). On the other hand, the e.p.s.p.s evoked by afferent volleys from the intact l.g. (filled circles in Fig. 2B) or soleus (not illustrated) nerve were not significantly altered 3 weeks after section of the m.g. nerve. The number of soleus motoneurones recorded from each group of animals was relatively small (twelve to fourteen). However, monosynaptic e.p.s.p.s evoked in these motoneurones by stimulation of the m.g. nerve were also significantly ($P < 0.001$) decreased 2 and 3 weeks after section of the m.g. nerve, whereas those produced by stimulation of the l.g. or soleus nerve showed no significant changes.
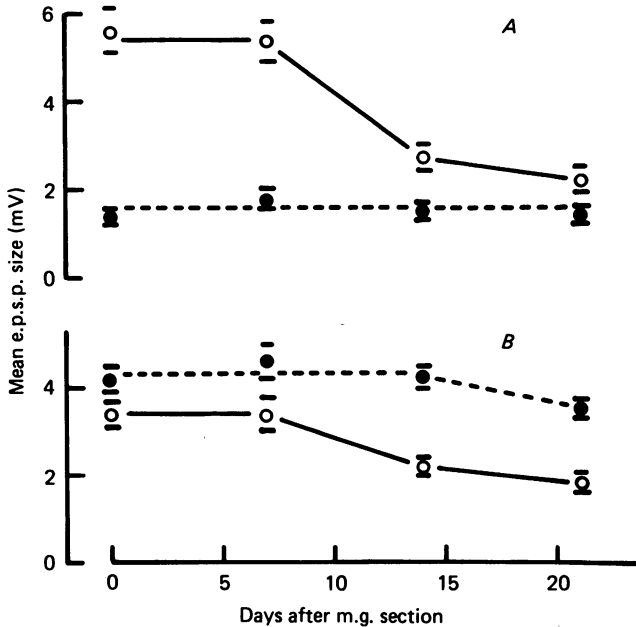

Fig. 2. *A*, change in the mean maximum amplitudes of monosynaptic e.p.s.p.s recorded from m.g. motoneurones after section of the m.g. muscle nerve. *B*, same as in *A* but e.p.s.p.s recorded from l.g. motoneurones. Open circles, e.p.s.p.s evoked by stimulation of the m.g. nerve. Filled circles, e.p.s.p.s evoked by stimulation of the l.g. nerve. Each point represents the mean ± S.E. of mean (horizontal bars) obtained from twenty-one to thirty-eight motoneurones.

From these results it seems clear that no significant alterations in central synaptic transmission occur for at least 1 week after section of a muscle nerve and that the subsequent reduction in the amplitude of monosynaptic e.p.s.p.s is confined only to those synapses formed by sensory fibres which were sectioned.

One week after section of the muscle nerve, m.g. motoneurones showed a significant ($P < 0.001$) increase in overshoot of action potentials (also, see Kuno, Miyata & Muñoz-Martinez, 1974). At this stage, the mean axonal conduction velocity of m.g. motoneurones (about 95 % of normal) showed no significant ($0.05 < P < 0.10$) change; its significant ($P < 0.001$) decrease (about 91 % of normal) was observed 2 weeks after axotomy (also, see Eccles *et al.* 1959; Kiraly & Krnjevic, 1959; Cragg & Thomas, 1961; Kuno *et al.* 1974; Mendell, Munson & Scott, 1976). The amplitude of monosynaptic e.p.s.p.s is known to be decreased within 2 weeks in motoneurones axotomized by section of the ventral root (Eccles, Libet & Young, 1958; McIntyre, Bradley &

Brock, 1959; Shapovalov & Grantyn, 1968; Kuno & Llinás, 1970). As shown in Fig. 2A, however, e.p.s.p.s from the intact heteronymous (l.g.) nerve were not depressed even when recorded from axotomized m.g. motoneurones. Therefore, decreased monosynaptic e.p.s.p.s in response to stimulation of the sectioned m.g. nerve (Fig. 2A, open circles) cannot be attributed to the effects of axotomy of the motoneurones. In fact, e.p.s.p.s from the sectioned m.g. nerve were also depressed when recordings were made from non-axotomized l.g. motoneurones (Fig. 2B). As suggested by Mendell *et al.* (1976), reaction of motoneurones to axotomy appears to occur more slowly following severance of the muscle nerve than after ventral root section.

Effects of chronic nerve stimulation. After section of the m.g. nerve, impulse activity would no longer be present in the sensory fibres arising from the muscle. It is possible that the decrease in the e.p.s.p. amplitude following section of the muscle nerve is due to prolonged disuse of the sensory fibres (Eccles & McIntyre, 1933; Eccles *et al.*
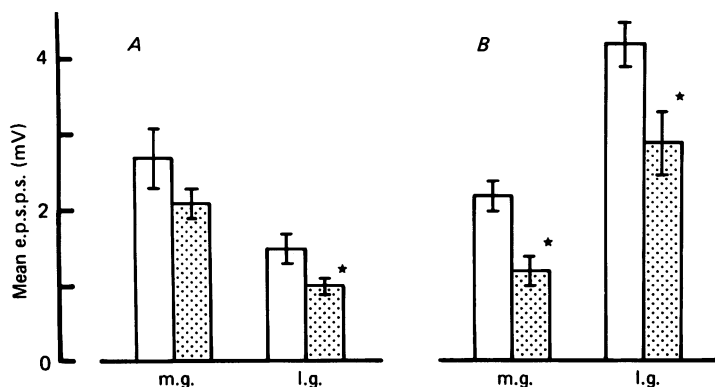

Fig. 3. *A*, the mean maximum amplitudes of monosynaptic e.p.s.p.s recorded from m.g. motoneurones 2 weeks after section of the m.g. nerve with (stippled columns) and without (open columns) daily stimulation of the sciatic nerve; m.g., e.p.s.p.s evoked by stimulation of the m.g. nerve; l.g., e.p.s.p.s evoked by stimulation of the l.g. nerve. Vertical bars, s.e. of mean. *B*, same as in *A* but monosynaptic e.p.s.p.s recorded from l.g. motoneurones. Asterisks, significant ($P < 0.05$) decrease in the mean e.p.s.p. amplitude by daily nerve stimulation.

1959). If this were the case, the reduction in central synaptic transmission might be prevented by daily stimulation of the sectioned peripheral nerve. To test this possibility, the sciatic nerve was chronically stimulated (see Methods for stimulus paradigm) for 2 weeks after section of the m.g. nerve.

Fig. 3A shows the mean maximum amplitudes of monosynaptic e.p.s.p.s recorded from m.g. motoneurones 2 weeks after section of the m.g. nerve with (stippled columns) and without (open columns) daily stimulation of the sciatic nerve. The homonymous (m.g. in Fig. 3A) e.p.s.p. amplitudes in the presence (stippled column) and absence (open column) of chronic nerve stimulation were not significantly ($0.10 < P < 0.20$) different. However, daily stimulation of the sciatic nerve caused a significant decrease in amplitude of the heteronymous e.p.s.p.s from the intact l.g. nerve (l.g. in Fig. 3A). Fig. 3B illustrates the results obtained from l.g. motoneurones. As indicated with asterisks (Fig. 3B), monosynaptic e.p.s.p.s produced by afferent volleys from both the sectioned m.g. and intact l.g. nerves were significantly reduced by chronic nerve stimulation. In response to stimulation of the intact, soleus nerve,

the e.p.s.p.s evoked in m.g. motoneurones were not significantly decreased by daily stimulation, but those evoked in l.g. motoneurones were.

The stimulus frequency (100/sec) used in the present study was comparable to the maximum frequency of spindle sensory discharges (50–130/sec) observed in the freely moving cat (Prochazka, Westerman & Ziccone, 1976). However, the pattern of daily stimulation was highly artificial and certainly did not exactly mimic the normal sensory discharges. Also, it is not clear how chronic nerve stimulation caused a significant depression in some synapses but not in others. In spite of these uncertainties, there was at least no evidence that decreased synaptic transmission following section of the peripheral nerve can be prevented by added sensory stimuli.

Implantation of the stimulating electrodes resulted in proliferation of connective tissues around the nerve. It is possible that such a proliferation of connective tissues may influence the sensory neurones by some as yet unknown mechanisms, thereby changing their central synaptic efficacy. This possibility was tested in two cats in which a pair of stimulating electrodes was implanted around the sciatic nerve for 2 weeks but no stimuli were added. In these animals, the e.p.s.p. amplitude showed no significant alterations.

Effects of nerve block with TTX. Section of a peripheral nerve, while certainly producing disuse of sensory fibres, also causes chromatolytic changes in sensory neurones (see Discussion). The latter changes might be related to the observed synaptic depression. In order to produce disuse conditions without complications of nerve injury, impulse conduction of the m.g. nerve was blocked for 2 weeks, using TTX cuffs (see Methods). Fig. 4A shows the mean amplitudes of monosynaptic e.p.s.p.s recorded from m.g. motoneurones 2 weeks after conduction block of the m.g. nerve (stippled columns). Open columns represent the results obtained from control, unoperated cats. The mean amplitude of the homonymous (m.g. in Fig. 4A) e.p.s.p.s showed a significant ($0.005 < P < 0.01$) increase after the block of the muscle nerve. This effect cannot be attributed to the possible alterations of m.g.
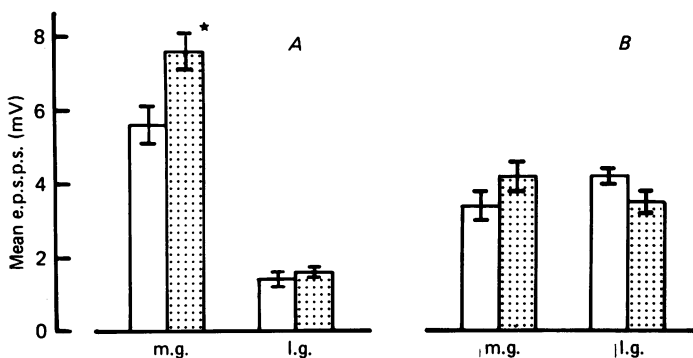

Fig. 4. A, the mean maximum amplitudes of monosynaptic e.p.s.p.s recorded from m.g. motoneurones in control, unoperated cats (open columns) and in cats in which the m.g. nerve was blocked for 2 weeks with TTX (stippled columns); m.g., e.p.s.p.s evoked by stimulation of the m.g. nerve; l.g., e.p.s.p.s evoked by stimulation of the l.g. nerve. Vertical bars, s.e. of mean. Asterisk indicates a significant ($P < 0.01$) increase in the mean e.p.s.p. amplitude after the application of TTX cuffs. B, same as in A but monosynaptic e.p.s.p.s recorded from l.g. motoneurones.

motoneurone properties since the heteronymous (l.g. in Fig. 4A) e.p.s.p.s were not significantly (0·20 < P < 0·30) affected. Also, the increase in the homonymous e.p.s.p. amplitude after conduction block of the m.g. nerve was not associated with any significant change in the mean time-to-peak of e.p.s.p.s. Fig. 4B shows the results obtained from l.g. motoneurones in these animals. There was an increase in amplitude of e.p.s.p.s evoked by stimulation of the disused m.g. nerve (Fig. 4B, m.g.), but this was statistically insignificant (0·10 < P < 0·20). The apparent decrease in the mean amplitude of e.p.s.p.s from the l.g. nerve (Fig. 4B, l.g.) was also statistically insignificant. Similarly, monosynaptic e.p.s.p.s evoked in m.g. or l.g. motoneurones by stimulation of the soleus nerve were not significantly altered by chronic conduction block of the m.g. nerve.
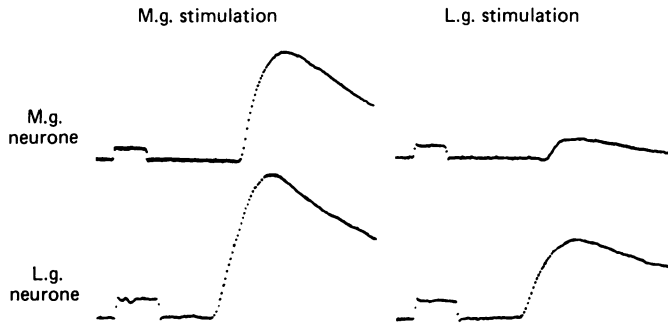

Fig. 5. Monosynaptic e.p.s.p.s recorded from m.g. (upper records) and l.g. (lower records) motoneurones following stimulation of the m.g. (left records) or l.g. (right records) muscle nerve. Conduction of the m.g. nerve had been blocked with TTX cuffs for 2 weeks. Each record is the average of sixteen responses. Calibration, 1 mV and 1 msec.

It was somewhat puzzling that chronic conduction block of the m.g. nerve caused a significant increase in the homonymous e.p.s.p. amplitude (Fig. 4A, m.g., stippled column), whereas the e.p.s.p.s recorded from the hetronymous motoneurones (Fig. 4B, m.g., stippled column) were not significantly enhanced. The maximum amplitudes of monosynaptic e.p.s.p.s from the homonymous and heteronymous nerves may vary widely from neurone to neurone, depending upon the motoneurone (motor unit) type (Burke et al. 1976). Thus, the possible increase in the mean e.p.s.p. amplitude following conduction block of the m.g. nerve might have been masked by the relatively large variation in amplitudes of e.p.s.p.s recorded from different motoneurones. As a more sensitive test, the amplitudes of homonymous and heteronymous e.p.s.p.s in each motoneurone may be compared since the ratio of these e.p.s.p. amplitudes is independent of the type of motoneurone (Burke et al. 1976). Fig. 5 shows monosynaptic e.p.s.p.s recorded from m.g. (upper records) and l.g. (lower records) motoneurones in response to stimulation of the m.g. (left records) and l.g. (right records) nerves 2 weeks after conduction block of the m.g. nerve with TTX. In both neurones, the e.p.s.p.s evoked by stimulation of the m.g. nerve (Fig. 5, left records) were appreciably greater than those produced by the l.g. afferent volleys (Fig. 5, right records). After conduction block of the m.g. nerve, the heteronymous e.p.s.p. evoked by stimulation of the m.g. nerve was greater than the homonymous

e.p.s.p. in twenty out of thirty-two l.g. motoneurones (63%) recorded. This is in contrast with control, unoperated cats in which the heteronymous e.p.s.p. produced by the m.g. afferent volleys was larger than the homonymous e.p.s.p. only in three out of twenty-five (12%) l.g. motoneurones. These results suggest that following prolonged disuse of the m.g. nerve there is a relative increase in efficacy of synapses formed by the m.g. sensory fibres even in l.g. motoneurones. As a quantitative measure, the amplitudes of e.p.s.p. were expressed as the ratio of those evoked by stimulation of the m.g. to those by the l.g. nerve and this was examined in each motoneurone. Fig. 6 shows frequency distributions of this ratio for both m.g. (A, B) and l.g. (C, D) motoneurones in control, unoperated cats (A, C) and in cats in which
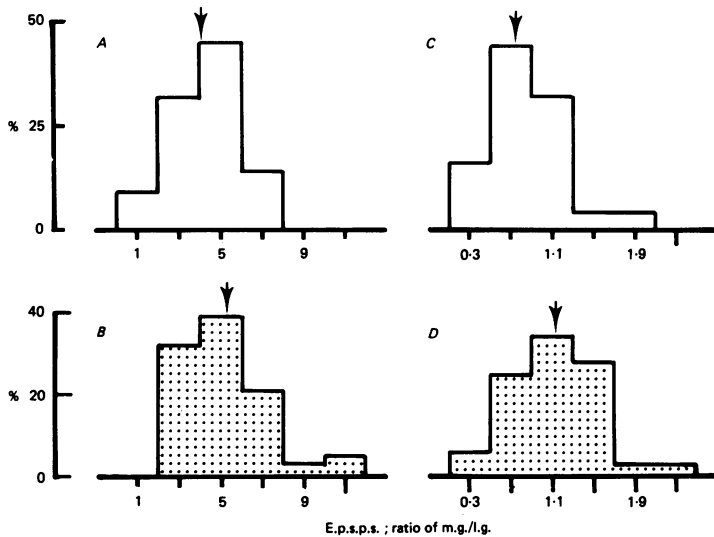

Fig. 6. Histograms of the ratio of the maximum amplitude of e.p.s.p.s evoked by m.g. nerve stimulation to that by l.g. nerve stimulation for m.g. (A, B) and l.g. (C, D) motoneurones. A and C, results obtained from control, unoperated cats. B and D, 2 weeks after conduction block of the m.g. nerve with TTX cuffs. Arrows indicate the mean ratios.

the m.g. nerve was blocked $(B, D;$ stippled histograms). Following prolonged disuse of the m.g. nerve, the mean ratio was significantly $(0.01 < P < 0.02)$ increased from 4·2 (Fig. 6A, arrow) to 5·3 (Fig. 6B, arrow) in m.g. motoneurones. Similarly, a significant $(P < 0.001)$ increase in the ratio from 0·82 (Fig. 6C, arrow) to 1·2 (Fig. 6D, arrow) was observed in l.g. motoneurones. Therefore, it is concluded that the chronic conduction block of the m.g. nerve significantly enhances the efficacy of synapses formed by the m.g. sensory fibres on both the homonymous (m.g.) and heteronymous (l.g.) motoneurones.

The above conclusion is based on the assumption that the efficacy of synapses formed by the l.g. sensory fibres remains unchanged following prolonged conduction block of the m.g. nerve. Of course, the relative increase in the amplitude of e.p.s.p.s from the m.g. nerve (Fig. 6) may arise from a decrease in the efficiency of synapses formed by the l.g. sensory fibres. One may conceive that conduction block of the m.g. nerve may result in excessive use of the sensory

fibres arising from the l.g. muscle and that this may tend to decrease the efficacy of synapses formed by the l.g. sensory fibres. This possibility is unlikely since chronic section of the m.g. nerve does not produce any significant changes in monosynaptic e.p.s.p.s from the intact l.g. nerve (Fig. 2). It should be noted that the chronic application of TTX cuffs might have caused degeneration of 3–14 % of the sensory fibres (see Methods). If this possibility is taken into consideration, the true increase in the e.p.s.p. amplitude due to prolonged disuse may be even slightly greater than the present evaluation.

Post-tetanic potentiation. Post-tetanic potentiation of monosynaptic e.p.s.p.s in response to stimulation of sectioned afferent fibres is known to be significantly increased in both amount and duration (Eccles *et al.* 1959). This behaviour has been considered to be indicative of partial restoration of the efficiency of disused central synapses by intense activation (Eccles *et al.* 1959; also, see Eccles & McIntyre, 1953).
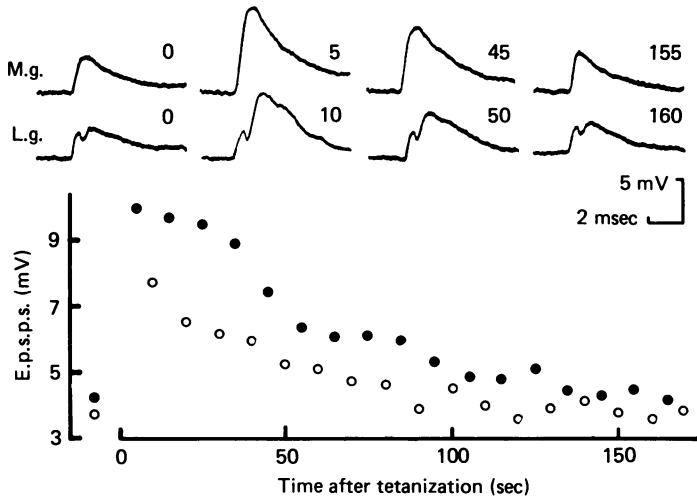

Fig. 7. Potentiation of monosynaptic e.p.s.p.s recorded from a l.g. motoneurone in response to stimulation of the m.g. and l.g. nerves following tetanic stimulation of the two muscle nerves in the cat in which conduction of the m.g. nerve had been blocked with TTX cuffs for 2 weeks. Upper records, monosynaptic e.p.s.p.s recorded before (indicated by 0) and at the indicated intervals in sec after the tetanus. Note, invasion of antidromic motor impulse from the homonymous input (l.g.) was blocked by hyperpolarization. Lower graph, time course of post-tetanic potentiation of the e.p.s.p.s evoked by the m.g. (filled circles) and l.g. (open circles) volleys.

In the present study, attempts were made to examine whether a similar augmentation of post-tetanic potentiation may occur at synapses disused for 2 weeks because of the application of TTX. Post-tetanic potentiation of monosynaptic e.p.s.p.s was studied in eight motoneurones (six l.g., one m.g. and one soleus) 2 weeks after conduction block of the m.g. nerve. In the experiment illustrated in Fig. 7, monosynaptic e.p.s.p.s were elicited in a l.g. motoneurone by stimulating the m.g. and l.g. nerves alternately every 5 sec before (Fig. 7, sample records indicated by 0) and after tetanic stimulation of the two muscle nerves at 500/sec for 10 sec (Fig. 7, sample records at the indicated intervals in sec after the tetanus). The motoneurone was hyperpolarized by applying DC currents through the intracellular electrode in order to prevent initiation of action potentials by the e.p.s.p.s as well as invasion of

the antidromic motor impulse from the homonymous input (note, M-spikes preceding the e.p.s.p.s from a l.g. motoneurone in Fig. 7; also, see Methods). The time courses of post-tetanic potentiation of the e.p.s.p.s produced by stimulation of the m.g. (filled circles) and l.g. (open circles) nerves are shown in the lower graph (Fig. 7). Potentiation of both homonymous and heteronymous e.p.s.p.s lasted for 2–3 min. Similar results were obtained in seven other motoneurones. No difference in the duration of post-tetanic potentiation was observed between normal and disused synapses. The amount of potentiation was taken as the ratio of the largest e.p.s.p. obtained after tetanization to the mean e.p.s.p. amplitude before the tetanus (Eccles *et al.* 1959). This ratio was $1\cdot78 \pm 0\cdot58$ (s.d.), on the average, for the e.p.s.p.s evoked from the chronically blocked m.g. nerve. This was essentially the same as the value ($1\cdot79 \pm 0\cdot56$) for the e.p.s.p.s elicited from the intact l.g. nerve. Thus, there was no indication that either the amount or duration of post-tetanic potentiation is significantly altered by prolonged disuse of the sensory fibres.

DISCUSSION

The principal finding in the present study is that prolonged conduction block of an intact muscle nerve with TTX significantly enhances synaptic efficacy in the homonymous and heteronymous motoneurones. Our interpretation of this result is that elimination of impulse activity (disuse) of sensory nerve fibres increases the efficiency of their central synaptic transmission. It has been suggested that enhancement of monosynaptic spinal reflexes evoked by afferent volleys from chronically tenotomized muscles results from prolonged disuse of the sensory pathway (Beránek & Hník, 1959; also see Introduction). This suggestion is consistent with the present results, although it is uncertain how much sensory activity from the muscle may be reduced by tenotomy. It should be noted that chronic immobilization of knee and ankle joints of the cat causes a significant decrease in monosynaptic e.p.s.p.s recorded from the ankle extensor motoneurones (Burke, Mayer, Kanda, Walmsley & Hodgson, 1978). It is not clear how this observation may be reconciled with the present results or with those seen after tenotomy.

The factors responsible for an increase in the homonymous e.p.s.p. amplitude following the conduction block of the m.g. nerve remain unclear. This increase was not accompanied by significant alterations in the amplitude of e.p.s.p.s evoked by stimulation of the synergic (l.g. or soleus) afferent fibres or in the time-to-peak of the homonymous e.p.s.p.s. Therefore, the present results cannot be accounted for by an increase in the motoneurone input resistance or by redistribution of the disused afferent terminals on the motoneurone (cf. Robbins & Nelson, 1970). Three possible mechanisms may be considered. First, afferent terminals may show sprouting following prolonged disuse, thereby increasing functional synaptic connexions with the motoneurones. Secondly, the amount of transmitter available for release may increase after prolonged disuse of the presynaptic fibres. Thirdly, sensitivity of the postsynaptic receptors for the transmitter may increase following prolonged disuse of the synapses. At present it is difficult to distinguish among these possibilities.

In agreement with previous observations (Eccles *et al.* 1959), section of a muscle nerve causes a reduction of monosynaptic e.p.s.p.s evoked by stimulation of that

nerve. Daily stimulation of the cut peripheral nerve failed to prevent reduced synaptic transmission in motoneurones. The maximum frequency (100/sec) and the over-all mean frequency (about 17/sec) of our stimulus paradigm would probably have been at the upper limits of normal sensory activity (see Prochazka et al. 1976). With this paradigm, the chronically applied stimuli were either ineffective or tended to decrease central synaptic transmission whether the sensory nerve stimulated was normally active (intact) or quiescent (sectioned). Thus, there was no evidence that central synaptic efficacy following section of peripheral nerve depends upon the presence or absence of impulse activity in the sensory fibres or that central synaptic efficacy may increase after excessive usage of the intact, afferent fibres. Admittedly, electrical stimuli chronically applied to the peripheral nerve by no means had a resemblance to the normal pattern of sensory discharges. It cannot completely be excluded that different patterns of stimulation may have produced qualitatively different effects on central synaptic transmission.

Monosynaptic spinal reflexes evoked by afferent volleys from a muscle have been reported to increase following denervation of its synergists (Eccles, Kozak & Westerman, 1962). This effect was attributed to an increase in sensory activity resulting from 'compensatory over-use' (Eccles et al. 1962). However, the homonymous monosynaptic e.p.s.p.s evoked by afferent volleys from the m.g. muscle show no significant increase in amplitude after removal of its synergists (Walsh, Burke, Rymer & Tsairis, 1978).

From the present study it seems clear that the effect of severance of a peripheral nerve on central synaptic transmission is not simply the result of disuse of the sensory fibres. It is well known that the cell body of a sensory neurone shows a chromatolytic reaction to section of its peripheral process (Anderson, 1902; Ranson, 1914; Hare & Hinsey, 1940; Carmel & Stein, 1969). This reaction is associated with a decrease in axonal conduction velocity of both the peripheral (Eccles & McIntyre, 1953; Eccles et al. 1959; Kiraly & Krnjević, 1959; Czéh, Kudo & Kuno, 1977) and central (dorsal root) processes (Czéh et al. 1977). Thus, reaction of sensory neurones to peripheral axotomy apparently spreads centrally beyond the cell bodies. In fact, Knyihár & Csillik (1976) have shown that section of the sciatic nerve in the rat causes degenerative atrophy of primary afferent terminals in the dorsal horn. If similar changes occur in terminals of Group Ia afferent fibres of the cat following peripheral axotomy, this might account for the decrease in monosynaptic e.p.s.p.s observed after section of a muscle nerve. Degenerative alterations in afferent terminals also follow the application of colchicine or vinblastine to the peripheral nerve (Csillik, Knyihár & Elshiekh, 1977). Therefore, it is possible that decreased central synaptic transmission after axotomy may be the result of interrupted axonal transport in the sensory fibres. It should be noted that the application of TTX cuffs does not interfere with the fast axoplasmic transport (Ochs & Hollingsworth, 1971; Anderson & Edström, 1973; Lavoie, Collier & Tenenhouse, 1976; Pestronk, Drachman & Griffin, 1976; Czéh et al. 1978).

Another factor which must be considered in relation to decreased synaptic transmission is the possibility of neurone death in the dorsal root ganglia following peripheral nerve section (Ranson, 1914; Cavanaugh, 1951). At present, few data are available on the magnitude and time course of cell death in the dorsal root ganglia after peripheral axotomy. Hence, it is uncertain whether cell death may contribute

to the observed decrease of the composite e.p.s.p.s. However, it should be pointed out that synaptic function appears to recover in association with peripheral regeneration of the sectioned nerve (Eccles *et al.* 1959).

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