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EFFECT OF IMPLANTATION OF AN EXTRA NERVE ON THE RECOVERY OF NEUROMUSCULAR TRANSMISSION FROM BOTULINUM TOXIN

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SUMMARY

1. The common peroneal nerve was implanted into soleus in the mouse and 2 weeks later a sublethal dose of botulinum toxin injected causing a block of neuromuscular transmission at the terminals of the soleus nerve. Most muscle fibres became innervated by the common peroneal nerve.

2. Recovery of neuromuscular transmission at the soleus nerve terminals was delayed in the common peroneal nerve implanted muscles.

3. Stimulation of the soleus nerve after botulinum-evoked subthreshold end-plate potentials (e.p.p.s) in virtually every fibre tested in unoperated muscles. In common peroneal nerve-implanted muscles stimulation of the soleus nerve failed to evoke e.p.p.s in about 40 % of fibres tested and where e.p.p.s were recorded their amplitudes were generally smaller.

4. When the common peroneal nerve was cut 2 months after botulinum, neuromuscular transmission at soleus nerve terminals occurred after 4 weeks. When the common peroneal nerve was cut 6 months after botulinum, transmission was found at soleus nerve terminals within 1 week.

5. Recovery of transmission at soleus nerve terminals from the effects of botulinum toxin is delayed if the muscle fibres become innervated by the common peroneal nerve and a proportion of soleus nerve terminals cease to release acetylcholine (ACh) until after the peroneal nerve has been cut.

INTRODUCTION

The injection of a sublethal dose of botulinum toxin into skeletal muscle causes prolonged local paralysis (Guyton & MacDonald, 1947) due to the inhibition of the release of acetylcholine (ACh) from motor nerve terminals (Burgen, Dickens & Zatman, 1949). In skeletal muscle of the mouse, axonal

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sprouting occurs from the affected motor nerve terminals and new end-plates are formed (Duchen & Strich, 1968; Duchen, 1970, 1971). It seems likely that this may promote the recovery of neuromuscular transmission.

It is known that skeletal muscle paralysed by botulinum toxin can become innervated by a foreign nerve although the original nerve terminals are still present (Fex, Sonesson, Thesleff & Zelena, 1966). In the present investigations the common peroneal nerve was implanted into soleus in the mouse and 2 weeks later botulinum injected. Most fibres in soleus became innervated by common peroneal nerve within a few weeks. Histological observations indicated that the axonal sprouting at soleus nerve terminals, usually seen after botulinum, did not occur (Duchen, Rogers, Stolkin & Tonge, 1975) and preliminary results (Tonge, 1974*a*) indicated that re covery of neuromuscular transmission was inhibited if the muscle fibres became innervated by the common peroneal nerve.

METHODS

Surgery. Approximately 200 adult albino mice of both sexes were used in the experiments. The mice were anaesthetized with an I.P. injection (0·5 g/kg) of tribromoethyl alcohol (Bayer). The common peroneal nerve was cut and the proximal stump inserted into the end-plate-free region of soleus close to its proximal tendon. During the same operation the femoral nerve was crushed with a pair of forceps to immobilize temporarily the right leg in order to prevent the implanted nerve from being pulled out of the muscle. Two weeks later, a sublethal dose of botulinum toxin (type A) was injected into the calf muscles of the right leg. The toxin was dissolved in 0·1 % gelatin phosphate buffer (pH 6·8) and the dose (0·05–0·1 ml.) was sufficient to kill about 10 % of the mice during the first week. In some mice the common peroneal nerve was cut in the thigh of the right leg 2 or 6 months after botulinum. In control mice this nerve was implanted without further treatment or botulinum injected into unoperated animals.

Electrophysiological methods. After varying periods of time the mice were anaesthetized and soleus together with short lengths of the two nerves were removed for *in vitro* studies. The proportions of muscle fibres effectively innervated by the soleus and the common peroneal nerves were determined by the relative tensions developed in response to direct and indirect stimulation and by recording end-plate potentials (e.p.p.s) or action potentials with glass micro-electrodes filled with 3 M-KCl. The tensions developed by soleus were measured isometrically using a Grass force-displacement transducer (Type FTO3C).

The nerve was stimulated at 100/sec for 1–2 sec and the tension developed was compared with that developed during direct stimulation of the muscle by placing platinum wire electrodes on either side of the muscle, and passing current through the muscle and the contents of the bath. The voltages used for both direct and indirect stimulation were supramaximal. The measurements were made within 5 min of removing soleus from the mouse when the tension developed in normal muscles in response to indirect stimulation is at least 90 % of that developed during direct stimulation.

The sensitivity of muscle fibres to ACh was determined by iontophoresis. A glass micropipette filled with 1 M-ACh chloride was positioned using a double headed

micromanipulator (Narashigi) so that its point was within a few microns of that of the recording electrode. The relative positions of the points of the electrodes were such that the muscle fibre could be impaled with the recording electrode, leaving the ACh pipette close to (but not penetrating) the muscle fibre. ACh was ejected from the pipette by positive pulses of 0·05–0·5 sec duration and regulated at values between 1 nA and 1 μA by a constant current supply. A negative backing voltage prevented leakage of ACh from the pipette. This voltage was adjusted so that a muscle fibre gave a maximal response to a given pulse applied to the ACh pipette but without any depolarization occurring prior to the test pulse. ACh sensitivity was expressed in units of membrane potential change in mV per nC of current applied through the pipette (Miledi, 1960).

The amplitude of the quantal components (quantum size) of e.p.p.s was calculated by dividing the mean amplitude of a series of e.p.p.s by their mean quantal content as estimated by the coefficient of variation method (del Castillo & Katz, 1954). The nerve was stimulated at 50/sec for 1–2 sec. The first ten e.p.p.s were disregarded (because their amplitudes were generally greater than the rest) and the quantal content and quantum size estimated from the next twenty-five e.p.p.s. The magnesium content of the bathing solution was raised to up to 15 mM where necessary to block neuromuscular transmission. In thirty-one muscle fibres, the quantal content of e.p.p.s was determined using both the coefficient of variation method and the method of failures (del Castillo & Katz, 1954). The values obtained by both methods were very similar.

The input resistance of muscle fibres was determined by impaling the fibre with two micro-electrodes whose tips were 100–200 μm apart. A rectangular depolarizing pulse of 100 msec duration was passed between one micro-electrode and another electrode in the bathing solution. The voltage of this pulse was adjusted to cause a membrane depolarization (recorded by the second micro-electrode) of about 5 mV. The current passing between the stimulating electrode and the bath was measured using a micro-ammeter.

The mammalian Ringer solution had the following composition (in m-mole/l.): NaCl 115; KCl 3·5; $CaCl_2$ 2; $MgSO_4$ 1; $NaHCO_3$ 25; KH_2PO_4 1; glucose 10. The solution was continuously gassed with 95% O_2/5% CO_2. The experiments were carried out at room temperature (24–27° C).

RESULTS

Formation of neuromuscular junctions by the implanted nerve. After implantation the common peroneal nerve grew further into the muscle. Stimulation of the nerve after intervals of 2–10 weeks in seven control muscles (without further treatment) caused the contraction of a few fibres adjacent to the site of implantation of the nerve. The tension developed during stimulation of the common peroneal nerve in these muscles was generally too small to measure (Fig. 1). Within 1 week of the injection of botulinum, stimulation of common peroneal nerve caused a strong contraction of soleus indicating the formation of neuromuscular junctions. After 6 weeks, the tension developed during stimulation of this nerve was more than 70% of the response to direct stimulation indicating that it had innervated most muscle fibres (Fig. 1).

Stimulation of common peroneal nerve 2 weeks after implantation

evoked only subthreshold e.p.p.s in a few fibres close to where the nerve had been implanted. It is likely that in these fibres, end-plates had been formed as a result of local damage to the muscle (Miledi, 1963). Within 1 or 2 days of the injection of botulinum, stimulation of this nerve evoked

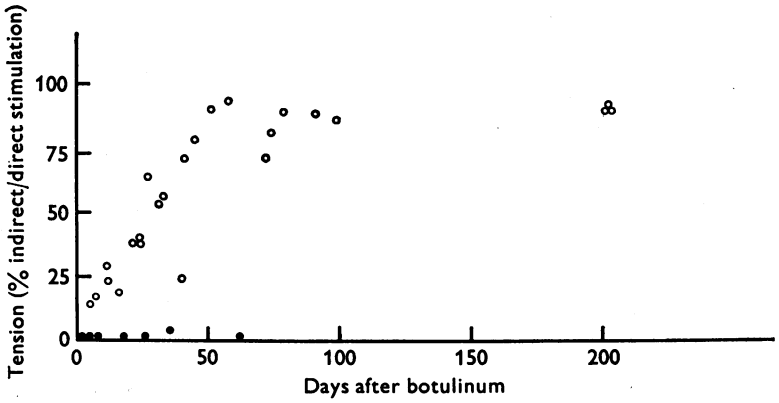

Fig. 1. The tension developed by soleus during repetitive stimulation (100/sec) of the common peroneal nerve as % response to direct stimulation in control- (●) and botulinum-injected mice (○). In both groups of mice the nerve was implanted 2 weeks before day 0.

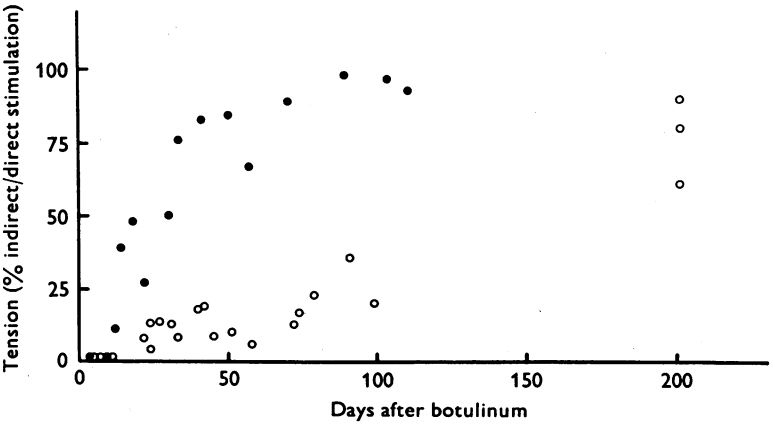

Fig. 2. The tension developed by soleus during repetitive stimulation (100/sec) of the soleus nerve as % response to direct stimulation after botulinum in unoperated (●) and common peroneal nerve-implanted muscles (○).

action potentials or subthreshold e.p.p.s in many muscle fibres. At first the e.p.p.s were recorded in fibres close to where this nerve had been implanted but after 1–2 weeks the area in which e.p.p.s could be recorded had spread across the muscle and also towards the original end-plate zone.

In most muscles the common peroneal nerve end-plates and those of soleus nerve remained separated by 1–2 mm, although occasional e.p.p.s evoked by stimulation of peroneal were recorded in the end-plate zone of the soleus nerve.

TABLE 1. The tensions developed by soleus during repetitive stimulation (100/sec) of the soleus (SN) and the common peroneal nerves (CPN) separately and simultaneously. The tensions are expressed as % responses to direct stimulation

Days after botulinum	Simultaneous stimulation of SN and CPN	Sum of responses to stimulation of SN and CPN	Days after botulinum	Simultaneous stimulation of SN and CPN	Sum of responses to stimulation of SN and CPN
5	14	14	45	80	87
7	17	17	51	92	100
11	29	29	58	93	99
12	23	33	72	79	84
16	39	39	74	84	98
21	38	46	79	95	112
24	39	43	91	90	124
27	64	78	99	86	106
31	60	66	203	97	174
33	58	64	203	91	166
40	27	42	203	97	170
41	75	90			

Responses to stimulation of the soleus nerve. Implantation of common peroneal nerve by itself had no effect on the response of soleus to stimulation of its own nerve in any of the seven control muscles. After botulinum both peroneal-implanted and unoperated muscles were paralysed for about 10 days, after which neuromuscular transmission was restored in some fibres. In unoperated muscles recovery of transmission was quite rapid so that 12 weeks after botulinum the tension developed by soleus during stimulation of soleus nerve was similar to that developed during direct stimulation (Fig. 2). In contrast, the rate of recovery in common peroneal nerve-implanted muscles was much slower and had not fully occurred at 28 weeks. Stimulation of the soleus nerve caused contraction of fibres in the part of the muscle on the opposite side to where the common peroneal nerve had been implanted, whereas those adjacent to the implanted nerve did not respond unless the latter were stimulated. In many muscles the sum of the tensions developed during stimulation of each nerve independently was greater than that developed during stimulation of both nerves simultaneously (Table 1), indicating that some muscle fibres were innervated by both nerves.

The release of ACh from the soleus nerve. During the first week after botulinum, stimulation of the soleus nerve evoked occasional e.p.p.s of less than

1 mV at every end-plate tested in both common peroneal nerve-implanted and unoperated muscles (Table 2). The e.p.p.s recorded in both groups of muscles were first similar in size (Table 3) but in the unoperated muscles they began to increase so that by 4–6 weeks they were large enough to

TABLE 2. Numbers of muscle fibres in which e.p.p.s or action potentials could be evoked during stimulation of soleus nerve (50/sec for 2–3 sec) in unoperated and common peroneal nerve (CPN)-implanted muscles

Unoperated muscles			CPN-implanted muscles		
Days after botulinum	No. of fibres giving response	No. of fibres tested	Days after botulinum	No. of fibres giving response	No. of fibres tested
5	10	10	5	10	10
6	15	15	5	24	24
11	12	12	7	15	15
12	11	11	11	12	12
14	11	11	11	0	25
15	12	12	12	15	20
30	17	18	21	5	22
34	14	14	33	13	18
41	12	12	40	16	16
50	17	17	41	14	21
56	15	15	45	8	27
70	14	14	51	7	16
			58	11	25
			64	12	20
			79	19	28
			91	7	18
			99	13	19

evoke action potentials in most muscle fibres. The increase in the size of the e.p.p.s was partly due to an increase in the quantal content of e.p.p.s (Table 3) which occurs during recovery from botulinum (Tonge, 1974*b*) and partly due to an increase in the quantum size of e.p.p.s (Fig. 3). In common peroneal nerve-implanted muscles, the e.p.p.s evoked by stimulation of the soleus nerve were generally smaller than those recorded in unoperated muscles (Table 3) and in many muscle fibres remained less than 1 mV for up to 3 months after botulinum. The smaller size of the e.p.p.s was due to the fact that they contained fewer quanta (Table 3) and that the mean amplitude of the individual quanta was smaller than in the unoperated muscles (Figs. 3 and 4).

About 2 weeks after botulinum, stimulation of the soleus into the common peroneal nerve-implanted muscles failed to evoke e.p.p.s in many muscle fibres (Table 2). It seemed that the nerve terminals were failing to release

ACh since in four muscles examined between 33 and 51 days after botulinum, raising the calcium content of the Ringer solution to 10 mM enabled e.p.p.s to be recorded at end-plates where there had been no response to

TABLE 3. The amplitudes and quantal contents (mean ± S.D.) of e.p.p.s in common peroneal nerve (CPN)-implanted and unoperated muscles after botulinum. The e.p.p.s were recorded from ten fibres in each muscle during repetitive stimulation (50/sec) of the soleus nerve (SN)

Days after botulinum	E.p.p. amplitude in mV		Mean quantal content	
	Unoperated muscles	CPN-implanted muscles	Unoperated muscles	CPN-implanted muscles
1	0·05 ± 0·01	0·05 ± 0·02	0·15 ± 0·09	0·09 ± 0·06
5	0·27 ± 0·17	0·07 ± 0·04	0·98 ± 0·57	0·18 ± 0·09
11	0·31 ± 0·12	0·12 ± 0·08	0·93 ± 0·31	0·32 ± 0·23
15	0·98 ± 0·14	0·08 ± 0·05	1·89 ± 1·23	0·21 ± 0·10
20	0·43 ± 0·35	0·08 ± 0·06	0·66 ± 0·57	0·24 ± 0·14
25	1·57 ± 1·00	0·05 ± 0·04	2·09 ± 1·34	0·24 ± 0·23

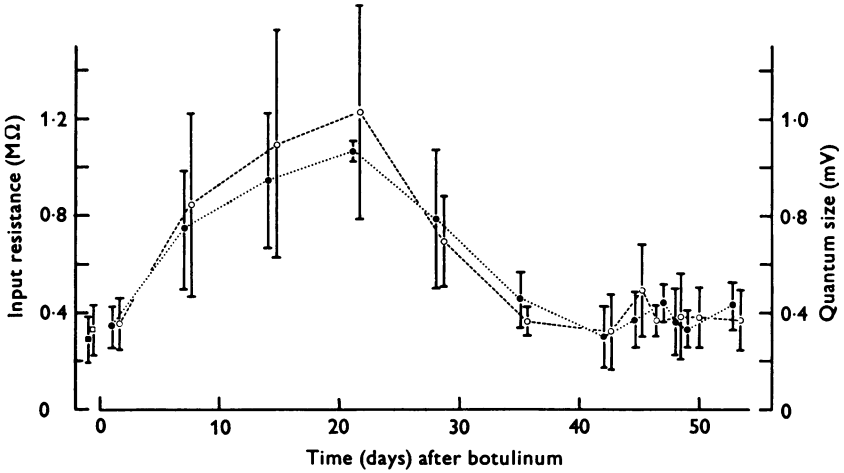

Fig. 3. Input resistance (MΩ) and quantum size (mV) of e.p.p.s evoked by stimulation of soleus nerve in unoperated muscles. Input resistance of normal muscle fibres (■). Input resistance after botulinum (●). Quantum size of e.p.p.s in normal muscle (□). Quantum size of e.p.p.s after botulinum (○). Each symbol represents the mean of ten fibres per muscle. S.D. indicated by vertical line. Note the increase in both input resistance and quantum size during 3 weeks after botulinum.

stimulation of the soleus nerve in normal Ringer. This effect of calcium was not observed in any of the four muscles examined between 58 and 91 days after botulinum. In these muscles it was not known whether soleus nerve

terminals were still in contact with muscle fibres, although there was no known reason why they should not have been.

Quantum size of e.p.p.s and input resistance of muscle fibres. The quantum size of e.p.p.s evoked by stimulation of the soleus nerve in normal soleus was 0·3–0·4 mV and this was virtually unchanged by the implantation of common peroneal nerve (Fig. 3). After botulinum the quantum size of e.p.p.s in the unoperated muscles increased fourfold within three weeks and then declined to normal after about 6 weeks (Fig. 3). In contrast, in

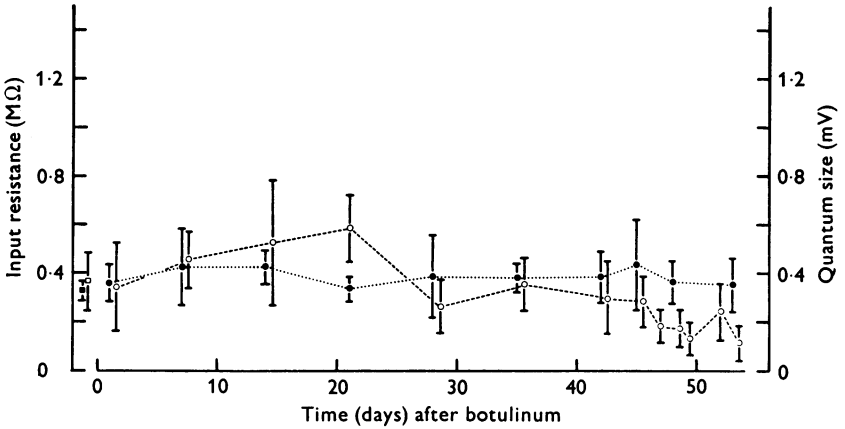

Fig. 4. Input resistance (MΩ) and quantum size (mV) of e.p.p.s evoked by stimulation of the original nerve in common peroneal nerve-implanted muscles. Input resistance before botulinum (■). Input resistance after botulinum (●). Quantum size of e.p.p.s before botulinum (□). Quantum size of e.p.p.s after botulinum (○). Each symbol represents the mean of ten fibres per muscle. S.D. indicated by vertical line. Note that the input resistance of muscle fibres shows little change. The quantum size of e.p.p.s increases only slightly during weeks 1–3, returns to normal and then declines after 6 weeks.

muscles implanted with common peroneal nerve, there was only a slight increase in quantum size during the first 3 weeks after botulinum (Fig. 4).

In order to investigate why the quantum size of e.p.p.s evoked by stimulation of the soleus nerve differed in the two groups of muscles the input resistance of muscle fibres was measured, since this is one factor known to influence the amplitude of spontaneous m.e.p.p.s (Katz & Thesleff, 1957) which normally are equivalent to the quantal components (quantum size) of evoked e.p.p.s (del Castillo & Katz, 1954; Boyd & Martin, 1956). Normally the input resistance of muscle fibres in soleus is 0·2–0·4 MΩ and is not significantly changed by implantation of the common peroneal nerve (Figs. 3 and 4). The input resistance in the non-operated muscle increased about fourfold 3 weeks after botulinum and then declined, whereas there

was little change in the implanted group. The change in input resistance in the unoperated muscles was probably due to atrophy since their weight fell by two thirds during the first 4 weeks after botulinum and then slowly recovered to normal. The weight of common peroneal nerve-implanted muscles decreased only slightly and then recovered to normal within 1 month. The changes in quantum size of e.p.p.s after botulinum generally correlated well with the input resistance of fibres in both groups of muscles (Figs. 3 and 4).

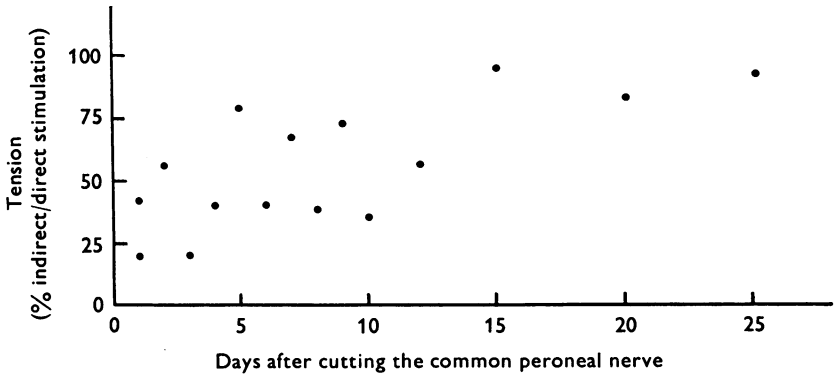

Fig. 5. The tension developed by soleus during repetitive stimulation (100/sec) of the soleus nerve as % response to direct stimulation. The common peroneal nerve was cut 2 months after the injection of botulinum.

TABLE 4. The numbers of fibres in common peroneal nerve (CPN)-implanted muscles in which e.p.p.s or action potentials could be evoked during stimulation of soleus nerve (SN) (50/sec for 2–3 sec). The CPN had been cut 2 months after the injection of botulinum toxin

Days after cutting CPN	No. of fibres giving e.p.p. or action potential	No. of fibres tested
1	9	24
2	14	22
3	17	20
5	11	15
7	15	18
9	23	23
15	17	18

Sensitivity to ACh. Both unoperated and common peroneal nerve-implanted muscles became supersensitive to ACh after botulinum. In five unoperated muscles examined between 5 and 25 days after botulinum, extrajunctional sensitivity of more than 50 mV/nC was recorded 1–2 mm from the original end-plate zone in all fibres tested (twenty per muscle). In the nerve-implanted group, similar extrajunctional sensitivity was found

in two muscles examined 5 and 11 days after botulinum. In three muscles studied 15–25 days after botulinum, extrajunctional sensitivity had fallen to less than 1 mV/nC in the fibres tested.

The effects of cutting the common peroneal nerve. In two groups of mice, injected with botulinum 2 or 6 months previously, the nerve was cut in the thigh. In the fifteen mice in which the common peroneal nerve was cut 2 months after botulinum there was a slow increase in the tension developed during stimulation of soleus nerve so that after 2–3 weeks the response was similar to that produced by direct stimulation (Fig. 5). The proportion of fibres in which e.p.p.s could be evoked by stimulation of the soleus nerve increased during the first week after cutting common peroneal nerve (Table 4) but the e.p.p.s were generally too small to evoke action potentials until about 2–3 weeks.

In the second group of twenty-one mice in which common peroneal nerve was cut 6 months after botulinum the tension developed during stimulation of soleus nerve was usually equal to that developed during direct stimulation after about 1 week (Fig. 6).

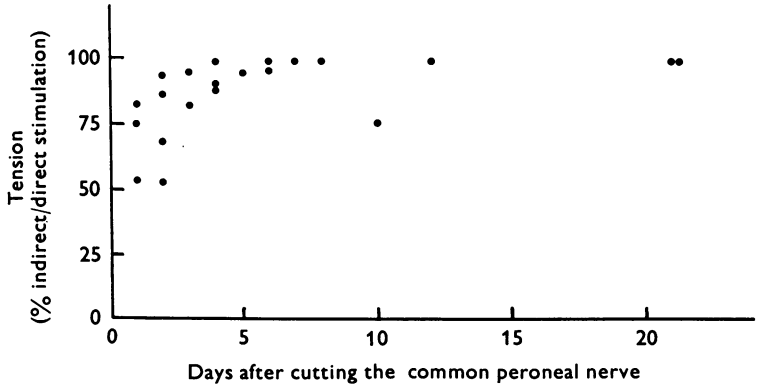

Fig. 6. The tension developed by soleus during repetitive stimulation (100/sec) of the soleus nerve as % response to direct stimulation. The common peroneal nerve was cut 6 months after the injection of botulinum.

DISCUSSION

The results of the present investigations indicate that the implantation of a foreign nerve into muscle paralysed by botulinum toxin inhibits the recovery of transmission at the neuromuscular junctions of its original nerve. The inhibitory effect of the implanted nerve may be due to a number of factors which are probably mediated through the muscle fibre.

The injection of botulinum toxin caused a great reduction in the evoked release of ACh at the end-plates of the soleus nerve in both common peroneal nerve-implanted and unoperated muscles. Occasional subthreshold e.p.p.s

could, however, be evoked by stimulation of the soleus nerve at every end-plate tested in both groups during the first week after botulinum. The differences between the two groups in recovery from botulinum became apparent during the next few weeks as the implanted common peroneal nerve innervated the soleus muscle.

In unoperated muscles, recovery of neuromuscular transmission at end-plates of the soleus nerve was associated with a progressive increase in the amplitude of evoked e.p.p.s during the first few weeks after botulinum. The increase was due to the release of more quanta per e.p.p. and also an increase in the amplitude of each quantum. The increase in the quantal content of e.p.p.s occurring during recovery from botulinum could be due to two factors. It is possible that the effects of the toxin somehow decrease with time so that more ACh can be released from the affected terminals. It also seems likely that the axonal sprouts which develop from motor nerve terminals after injection of botulinum (Duchen & Strich, 1968; Duchen, 1970, 1971) release ACh and that part of the observed increase in the quantal content of e.p.p.s was due to the maturation of these new neuromuscular junctions.

In muscles in which the common peroneal nerve had been implanted the amplitude of e.p.p.s evoked by stimulation of the soleus nerve after botulinum did not increase as quickly as in unoperated muscles and at a varying proportion of end-plates, e.p.p.s could not be evoked after the end of the second week. The quantal content of e.p.p.s in peroneal nerve-implanted muscles was lower than in unoperated muscles. This may in part have been due to the absence of axonal sprouting after botulinum in the implanted muscles (Duchen *et al.* 1975). It also seems likely that the probability of release of ACh from soleus nerve terminals became reduced as the neuromuscular junctions formed by common peroneal nerve matured. In some fibres, e.p.p.s were only recorded during stimulation of soleus nerve after the calcium content of the Ringer solution had been increased.

The quantum size of e.p.p.s after botulinum were smaller in common peroneal nerve-implanted than in unoperated muscles. It seems likely that this was due to the fact that innervation of soleus by the peroneal nerve largely prevented the atrophy and the associated increase in the input resistance of muscle fibres after botulinum.

When the common peroneal nerve was cut 2 months after the injection of botulinum there was an increase during the first week in the proportion of muscle fibres at which e.p.p.s could be evoked by stimulation of the soleus nerve. Restoration of neuromuscular transmission took place slowly over the next few weeks. It seemed that the terminals of the soleus nerve behaved as if still affected by botulinum at the time of cutting the common peroneal nerve although in unoperated muscles recovery of transmission

had occurred by this time. The delay in recovery from the effects of botulinum could have been due to the lack of axonal sprouting in the implanted muscles.

When the common peroneal nerve was cut 6 months after botulinum there was an increase in the tension developed by soleus during stimulation of its nerve so that after 1 week the responses to direct and indirect stimulation were similar. It was not certain whether the recovery was due to the formation of new neuromuscular junctions or the reactivation of previously existing but electrically silent junctions.

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REFERENCES

BOYD, I. A. & MARTIN, A. R. (1956). The end-plate potential in mammalian muscle. *J. Physiol.* **132**, 74–91.

BURGEN, A. S. V., DICKENS, F. & ZATMAN, L. J. (1949). The action of botulinum toxin on the neuromuscular junction. *J. Physiol.* **109**, 10–24.

DEL CASTILLO, J. & KATZ, B. (1954). Quantal components of the end-plate potential. *J. Physiol.* **124**, 560–573.

DUCHEN, L. W. (1970). Changes in the motor innervation and cholinesterase localization induced by botulinum toxin in skeletal muscle of the mouse: differences between fast and slow muscles. *J. Neurol. Neurosurg. Psychiat.* **33**, 40–54.

DUCHEN, L. W. (1971). An electron microscopic study of the changes induced by botulinum toxin in the motor end-plates of slow and fast skeletal muscle fibres of the mouse. *J. neurol. Scis* **14**, 47–60.

DUCHEN, L. W., ROGERS, M., STOLKIN, C. & TONGE, D. A. (1975). Suppression of botulinum toxin induced axonal sprouting in skeletal muscle by implantation of an extra nerve. *J. Physiol.* **248**, 1–2*P*.

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

FEX, S., SONESSON, B., THESLEFF, S. & ZELENA, J. (1966). Nerve implants in botulinum poisoned mammalian muscle. *J. Physiol.* **184**, 872–882.

GUYTON, A. C. & MACDONALD, M. A. (1947). Physiology of botulinus toxin. *Archs Neurol. Psychiat., Chicago* **57**, 578–592.

KATZ, B. & THESLEFF, S. (1957). On the factors which determine the amplitude of the miniature end-plate potential. *J. Physiol.* **137**, 267–278.

MILEDI, R. (1960). The acetylcholine sensitivity of frog muscle fibres after complete or partial denervation. *J. Physiol.* **151**, 1–23.

MILEDI, R. (1963). Formation of extra nerve-muscle junctions in innervated muscle. *Nature, Lond.* **199**, 1191–1192.

TONGE, D. A. (1974*a*). Synaptic function in experimental dually innervated muscle in the mouse. *J. Physiol.* **239**, 96–97*P*.

TONGE, D. A. (1974*b*). Chronic effects of botulinum on neuromuscular transmission and sensitivity to acetylcholine in slow and fast skeletal muscle of the mouse. *J. Physiol.* **241**, 127–139.