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THE IMPORTANCE OF PERIPHERAL CHANGES IN DETERMINING THE SENSITIVITY OF STRIATED MUSCLE TO DEPOLARIZING DRUGS

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SUMMARY

1. The sensitivity of the flexor digitorum longus and soleus muscles to depolarizing drugs was tested after cross-union of their respective motor nerves.

2. The alien innervation did not affect the sensitivity of either the flexor digitorum longus (FDL) or soleus muscles, which retained their normal characteristic responses to decamethonium and suxamethonium. The time course of muscle contractions was, however, altered by the cross-union operation.

3. A considerable increase in sensitivity to depolarizing drugs was shown after de-afferentation and after tenotomy of the soleus muscles. Both these conditions are associated with muscle atrophy.

4. It is suggested that hypersensitivity to depolarizing drugs can be expected in any situation where the muscle is undergoing atrophy.

INTRODUCTION

It is not yet fully understood how the sensitivity of a muscle to its chemical transmitter is determined. Experiments with denervated muscle seem to suggest that it is the influence of the motoneurone which normally controls the sensitivity of the membrane to acetylcholine. As is well known, section of the motor nerve produces hypersensitivity to acetylcholine and a spread of the receptor area along the membrane (Axelson & Thesleff, 1959; Miledi, 1960*a*). However, denervation immediately interferes with the normal pattern of activity of the muscle and is followed by considerable chemical and morphological changes which make it

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difficult to single out any one factor as the cause of denervation hypersensitivity.

There is already a reasonable accumulation of evidence that changes in sensitivity to depolarizing drugs can be produced in muscles with intact motor nerves. For example, Johns & Thesleff (1961) found an increased sensitivity to acetylcholine, applied both iontophoretically and close arterially, in muscles inactivated by spinal cord and dorsal root section. A similar increase in sensitivity was shown by Solandt, Partridge & Hunter (1943) for immobilized, non-denervated muscles. Consistent with this is the observation that leg muscles become less sensitive to tubocurarine when the whole limb is immobilized in a plaster cast (Thomsen & Luco, 1944). In addition, it is known that when a muscle is tenotomized it becomes more sensitive to depolarizing drugs and less sensitive to tubocurarine (Jewell & Zaimis, 1954*b*).

In all these situations, although the motor nerves are intact, motoneurone activity is changed. It has been suggested that the change in sensitivity to acetylcholine is due to lack of transmitter release (Johns & Thesleff, 1961) or to lack of some other neural factor (Miledi, 1960*b*). However, in these cases, as in the denervated muscle, it is possible that the metabolic changes which have occurred in the muscle are primarily responsible for the increase in acetylcholine sensitivity.

In the present experiments, an attempt was made to distinguish between these possibilities. Advantage was taken of the different sensitivity of the slow and fast muscles of the cat's hind limb to depolarizing blocking drugs. The slow soleus muscle is known to be approximately 3 times less sensitive to decamethonium and suxamethonium than a fast muscle, such as the tibialis anterior (Paton & Zaimis, 1951). If this difference in sensitivity of slow and fast muscles is determined by the motoneurone, then it could be expected that when the motor nerves to these muscles are crossed, their drug responses should also be changed.

METHODS

Adult cats were used in all experiments. The surgical procedures of cross-union of motor nerves, section of muscle tendons, spinal cord section or de-afferentation of one hind limb were performed with full aseptic precautions under sodium pentobarbitone anaesthesia (Nembutal, Abbott Laboratories Ltd. 45 mg/kg I.P.). For the acute experiments, a mixture of Choloralose (70 mg/kg Roche Products Ltd.) and pentobarbitone sodium (2 mg/kg) was administered into the radial vein of the forelimb.

Cross-union experiments were performed using the nerves supplying the flexor digitorum longus (FDL) and soleus muscles of one hind limb as described by Buller, Eccles & Eccles (1960). These nerves were approached through an incision in the lateral aspect of the lower hind limb, ligated and cut. The central stump of the FDL nerve was sutured to the peripheral stump of the soleus nerve using human hair. Then the central stump of the soleus nerve was sutured to the peripheral stump of the FDL nerve. Care was taken to see that the sutured

regions of the nerves did not lie over one another in order to ensure cross-regeneration of nerve fibres uncomplicated by self-re-innervation.

The muscles which had been supplied with alien innervation in this way were studied 18–24 weeks later, when re-innervation was expected to be complete. They were compared with the normal muscles of the unoperated contralateral hind limb.

In several experiments a slightly different procedure was used. The nerve supplying the soleus muscle was cut and the central stump sewn into the skin. The FDL nerve was also cut and the central stump split longitudinally into two approximately equal parts. One part was sutured to the peripheral stump of the soleus nerve; the other part was rejoined to the peripheral end of the FDL nerve. In this way the FDL nerve was made to re-innervate both soleus and FDL muscles.

In the second series of experiments, disuse atrophy of the muscles of the hind limb was produced in several different ways:

(i) In several experiments the Achilles tendon was cut in one leg in an aseptic operation and the tenotomized soleus muscle was studied 6–21 days later.

(ii) Atrophy was produced by de-afferentation of one hind limb, either alone or in combination with tenotomy. The dorsal roots from L 5 to S 2 were transected extradurally in an aseptic operation and the acute experiments were performed 7–10 days later.

In all acute experiments, indirectly elicited isometric muscle contractions were recorded and the sensitivity of the muscles to I.V. administration of depolarizing drugs was studied using the methods described in the previous paper (Maclagan & Vrbová, 1966). Muscle temperature was adjusted to 36–37° C and kept constant through each experiment.

In the case of the cross-innervated muscles, individual motor nerves were stimulated after careful dissection.

At the end of each experiment both operated and control muscles were dissected out, weighed and preserved for histological examination.

The drugs used were decamethonium di-iodide (Allen and Hanburys Ltd.), suxamethonium chloride (Allen and Hanburys Ltd.) and tubocurarine chloride (Burroughs Wellcome Ltd.), and they were administered intravenously into a jugular vein.

RESULTS

The sensitivity of cross-innervated muscles to depolarizing drugs

It is known that fast and slow muscles show sharp contrasts in their responses to neuromuscular blocking drugs. In particular, it has been shown that fast muscles, such as the tibialis anterior muscle, are much more sensitive to depolarizing neuromuscular blocking drugs than the slow soleus muscle (Paton & Zaimis, 1951; Jewell & Zaimis, 1954*a*). In the present experiments attempts were made to alter the relative sensitivities of the fast FDL muscle and the slow soleus muscles by cross-union of their motor nerves.

Figure 1 shows the response normally obtained from the FDL and soleus muscles in response to I.V. administration of a small dose of decamethonium (20 μg/kg). The contractions of the soleus and FDL muscles were recorded simultaneously from one hind limb.

This shows that a dose of decamethonium which produces complete paralysis of the normal fast muscle for 8 min has no effect on the slow muscle.

In the first series of experiments, the nerves to FDL and soleus were crossed over and 4–6 months allowed for regeneration to occur. In the acute experiments, the sensitivity to depolarizing drugs of the cross-innervated FDL and soleus muscle was compared with the sensitivity of the control muscles of the unoperated contralateral hind limb.

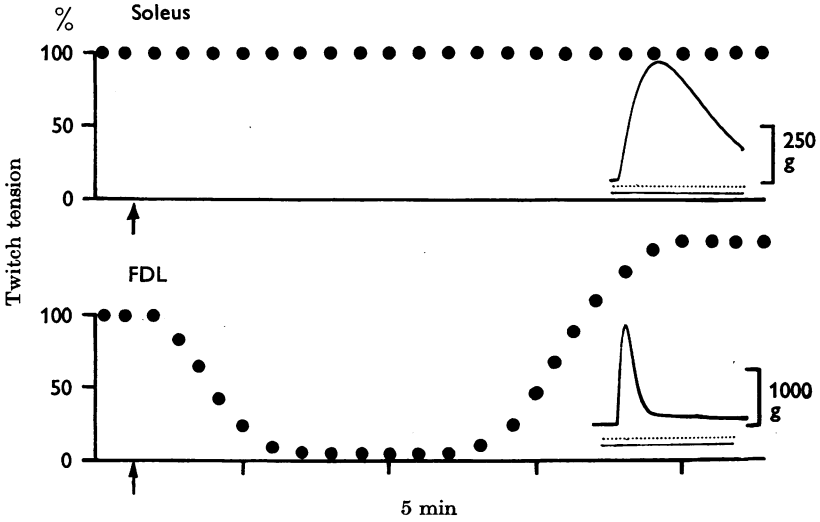

Fig. 1. The graphs show indirectly elicited isometric maximal twitch tension, expressed as a percentage of the value just before drug administration, plotted against time. The inserts under the graphs show the time course of a single contraction and the actual tension developed in a twitch at the start of the experiment. Time calibration 10 msec interval between dots. Records were taken simultaneously from the normal soleus muscle (upper section) and normal FDL muscle (lower section). At arrow, 20 μg/kg of decamethonium was administered intravenously.

Figure 2 shows the results of such an experiment on a FDL muscle which had been re-innervated with the soleus nerve (top section). The response of the control FDL muscle is shown in the lower section. The time course of the twitches are shown as inserts under the graphs.

It is clear that cross-innervation of the FDL muscle with the soleus nerve has markedly slowed the time course of the twitch as previously reported by Buller *et al.* (1960). However, the effect of intravenous administration of 20 μg/kg of decamethonium is identical in the cross-innervated and control muscles. Thus innervation of the FDL muscle with the soleus nerve has not produced any change in sensitivity to depolarizing drugs.

It was found in these experiments that it was never possible to achieve complete re-innervation of the FDL muscle fibres with the soleus nerve.

This is probably due to the fact that the soleus nerve contains fewer fibres than the larger FDL nerve. The presence of denervated fibres within the cross-innervated muscle could always be demonstrated, as the denervated fibres were very sensitive to depolarizing drugs and always responded with a contracture. This contracture is shown in Fig. 2 in the cross-innervated FDL muscle as a short-lasting upward deflexion of the base line.

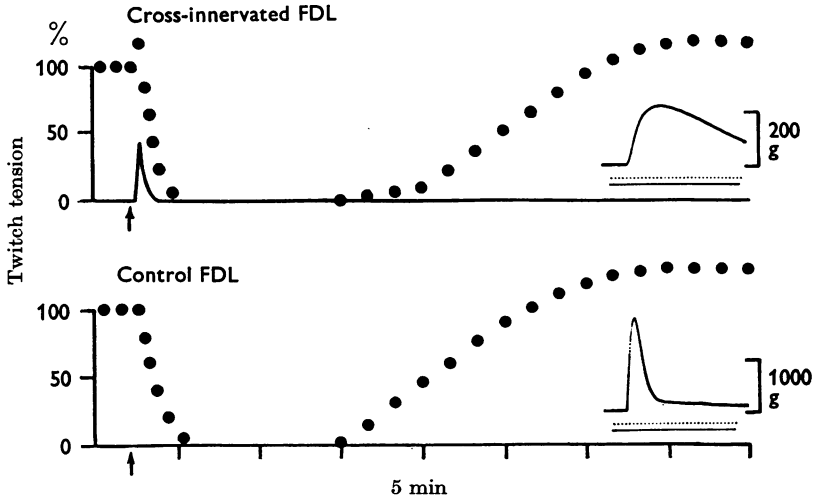

Fig. 2. Graphs and inserts as in Fig. 1. Upper section: cross-innervated FDL muscle. Lower section: control FDL muscle. At arrow 20 μg/kg of decamethonium was given I.V.

The fact that complete re-innervation of the muscle with the alien nerve was never established was shown also by the much smaller tensions exerted by the cross-innervated muscles (see Figs. 2–4). The cross-innervated muscles also weighed less than the control muscles.

When soleus muscles were cross-innervated with FDL nerves no effect of the alien innervation on the sensitivity to depolarizing drugs could be detected. Results obtained from such a cross-innervated soleus muscle are shown in Fig. 3. The response of the control muscle of the other hind limb is shown in the lower section. In this experiment, the time course of the twitch of the cross-innervated muscle had been speeded up by the alien innervation. However, the depth of the block and the occurrence of repetitive firing and twitch potentiation was similar in both muscles.

In all these experiments, the sensitivity to tubocurarine was also tested, and was found to be unaffected by the alien innervation.

These results suggested that the motoneurone was not responsible for the pharmacological characteristics of the end-plate region of fast and

slow muscles. This was confirmed in another series of experiments in which a slightly different experimental approach was used.

In these experiments the FDL nerve was cut and the central stump split and sutured so that it was made to re-innervate both soleus and FDL muscles. In the acute experiment, stimulation of the FDL nerve above the longitudinal division produced simultaneous contraction of soleus and FDL muscles. Results obtained in such an experiment are

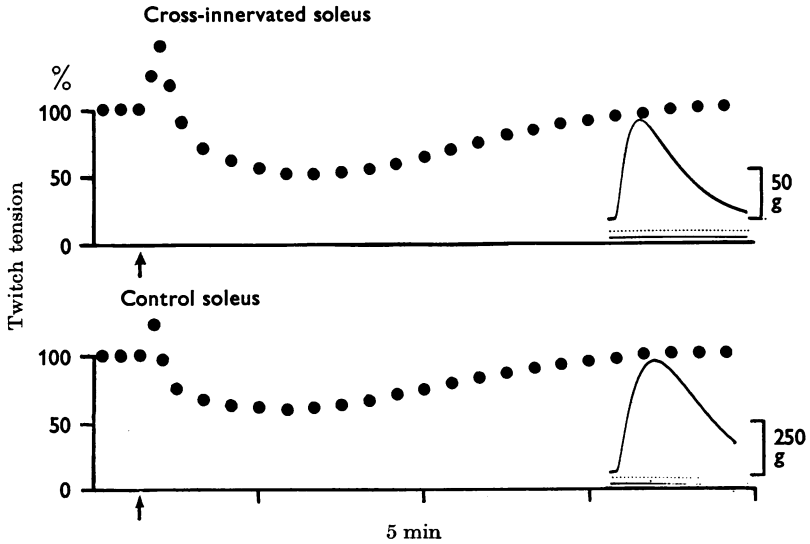

Fig. 3. Graphs and inserts as in Fig. 1. Upper section: cross-innervated soleus muscle. Lower section: control soleus muscle. At arrow 50 μg/kg of decamethonium was administered intravenously.

shown in Fig. 4. The twitch tensions exerted by the muscles were of course small, as a large proportion of the muscle fibres had not been re-innervated. This figure shows that although both muscles are now supplied by the same motor nerve, they do not possess the same sensitivity to decamethonium. The insensitivity of the cross-innervated soleus muscle relative to the FDL muscle which is shown in Fig. 4 should be compared with the exactly similar effect shown in Fig. 1 for normal soleus and FDL muscles.

Influence of atrophic changes on sensitivity to depolarizing drugs

It has been shown by Jewell & Zaimis (1954*b*) that if the tendon of the soleus muscle is cut, the muscle membrane becomes more sensitive to depolarizing drugs and less sensitive to tubocurarine. In addition it is known that tenotomy abolishes the continuous electromyogram activity normally displayed by the soleus muscle (Vrbová, 1963) in which it also

causes marked degenerative changes (McMinn & Vrbová, 1964). On the basis of this evidence, the increased sensitivity of tenotomized muscle to depolarizing drugs could be due to changes in motoneurone activity, or it could be secondary to the atrophic changes which occur in the muscle.

The experiments described in the previous section did not show any marked influence of the motoneurone on the pharmacological characteristics of the muscle membrane. Consequently, the possibility that the hypersensitivity of the tenotomized solei was related to the degenerative changes was investigated.

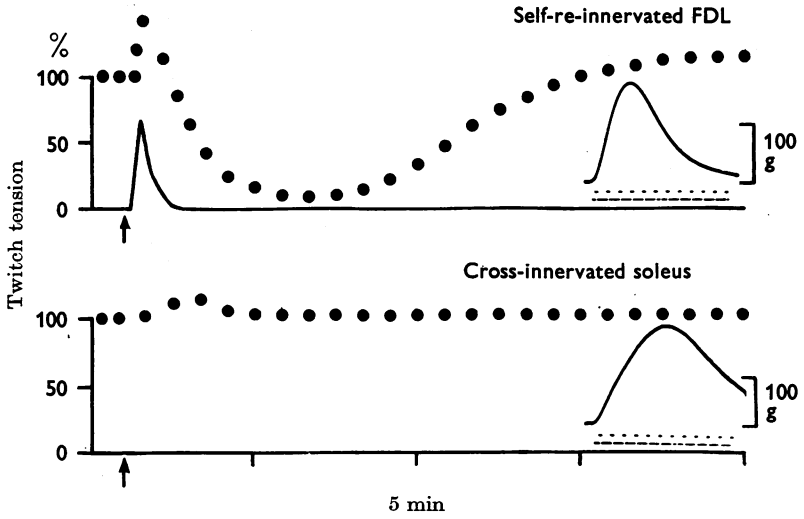

Fig. 4. Graphs and inserts as in Fig. 1. In this experiment the FDL nerve of one leg had been cut and the central stump split and made to re-innervate both soleus and FDL muscles. The figure shows records of twitch tension obtained simultaneously from the self-re-innervated FDL (upper section) and the cross-innervated soleus muscle (lower section) in response to stimulation of the FDL nerve above the division. At arrow, I.V. administration of 20 μg/kg of decamethonium di-iodide.

Figure 5 shows the results obtained from a soleus muscle which had been tenotomized for 20 days. The responses of the tenotomized muscle (upper section) and the control muscle from the unoperated contralateral hind limb (lower section) to I.V. administration of decamethonium are compared. The atrophic tenotomized muscle was clearly much more sensitive to decamethonium than the control muscle. This increase in sensitivity was observed in some experiments as early as 6 days after tenotomy. These results confirm the observations reported by Jewell & Zaimis (1954*a*).

De-afferentiation also produces atrophic changes in the muscle, and in three cats the effect of this operation on the sensitivity of the soleus muscle to depolarizing drugs was studied.

Seven to ten days after section of dorsal roots between L 5 and S 2 on one side, the weight of the soleus muscle and its isometric tension were less than those of the soleus of the unoperated control limb. Figure 6 shows that the de-afferented muscle is considerably more sensitive to decamethonium (30 μg/kg) than the control muscle.

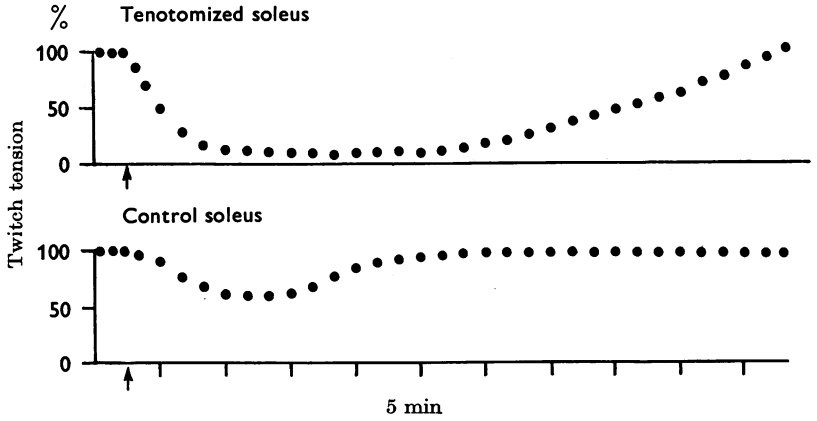

Fig. 5. Graphs as in Fig. 1. Upper section: tenotomized soleus muscle, 20 days after operation. Lower section: control soleus muscle. At arrow 30 μg/kg of decamethonium was administered intravenously.

Conversely, the de-afferented soleus muscle was less sensitive to tubocurarine.

It was also apparent from these experiments that tenotomy produced a greater hypersensitivity than could be produced by de-afferentation. The degree of atrophy and decrease in muscle tension were also much greater in the tenotomized than in the de-afferented muscle. In the next experiments an attempt was made to reduce the degree of atrophy produced by tenotomy in order to see whether the change in sensitivity was also reduced.

It has recently been shown that in rats, tenotomy of the soleus muscle combined with dorsal root section produces a smaller degree of atrophy in the muscle than tenotomy alone (Hník, 1964). Therefore, in two cats the degree of atrophy and the sensitivity to depolarizing drugs of the tenotomized soleus muscle of one hind limb was compared with that of the contralateral hind limb which had been subjected to a combination of tenotomy and de-afferentation.

Figure 7 shows results recorded simultaneously from the tenotomized

and de-afferented soleus muscle (upper section) and from the contralateral tenotomized muscle (lower section). The tenotomized muscle had undergone greater atrophy, as both muscle weight and twitch tension were smaller in this muscle than in the tenotomized and de-afferented muscle. When a dose of decamethonium (45 μg/kg) was injected intravenously the effect was greater in the tenotomized muscle. Thus it appears that the more atrophic of these two muscles also exhibits the greater sensitivity to depolarizing drugs.

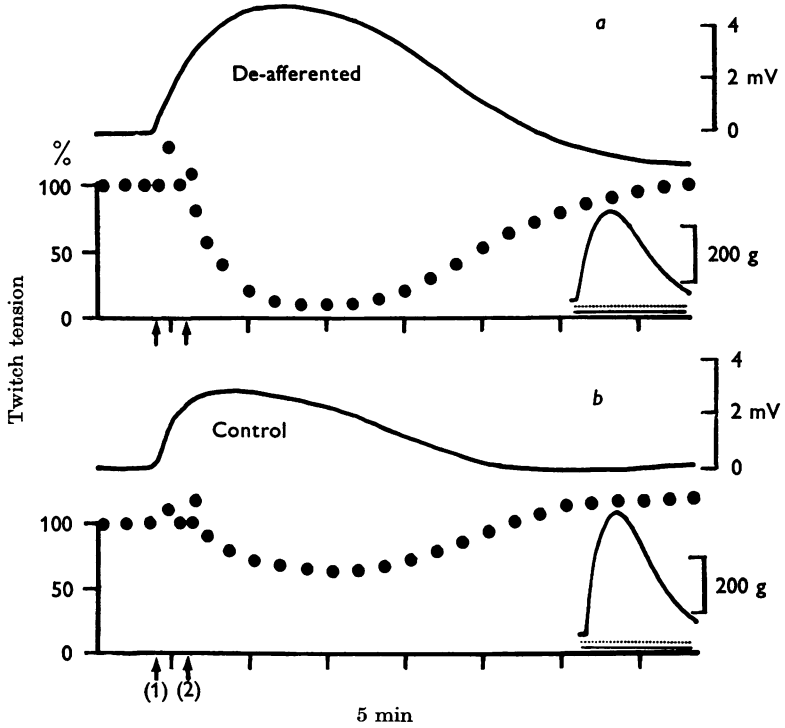

Fig. 6. Simultaneous records of isometric twitch tension of de-afferented (*a*) and control (*b*) soleus muscles plotted as in Fig. 1. The upper continuous record in each section shows changes in potential at the end-plate region of each muscle, recorded with reference to a paired, second electrode fixed on the bone. At arrow (1) 30 μg/kg and at arrow (2) 10 μg/kg decamethonium was administered intravenously.

The results which have been obtained with de-afferented muscles, tenotomized muscles and with muscles subjected to a combination of tenotomy and de-afferentation suggest that when a muscle is undergoing atrophy its sensitivity to depolarizing drugs increases.

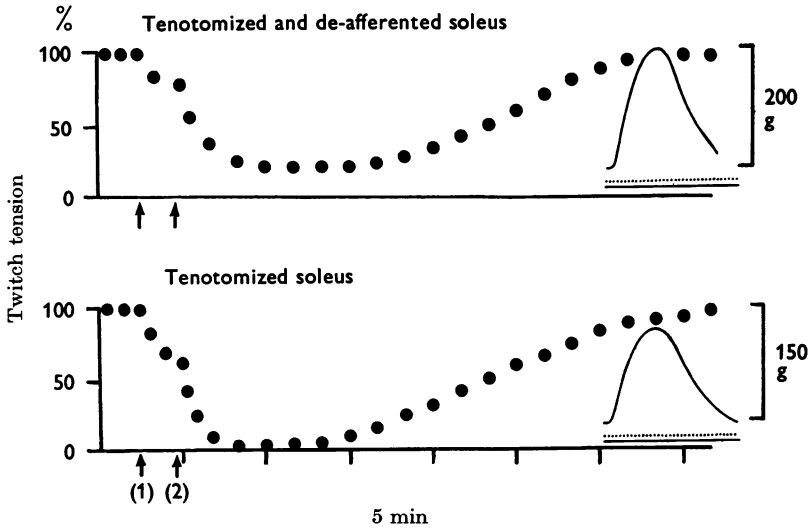

Fig. 7. Graphs and inserts as in Fig. 1. At arrow (1) 10 μg/kg and at arrow (2) 20 μg/kg decamethonium was administered intravenously. Upper section: tenotomized and de-afferented soleus muscle 8 days after the operation. Lower section: tenotomized soleus muscle 8 days after the operation.

DISCUSSION

The results described in this paper show that muscles innervated with an alien motor nerve retain their characteristic responses to depolarizing drugs. In these experiments sufficient time elapsed between the cross-union operation and the acute experiments to be certain that the changes associated with the period of early re-innervation were not complicating the results. The influence of the cross-innervation could be observed on the mechanical response of the muscle, as already reported by Buller *et al.* (1960) and Eccles, Eccles & Kozák (1962). However, in no case did the drug response change; even in experiments in which both soleus and FDL muscle were supplied by the same motor nerve their characteristics remained entirely different. Thus it appears that the pharmacological properties of the muscle membrane are not determined by the motoneurone. Other factors must be more important in determining the sensitivity to depolarizing neuromuscular blocking drugs, and, by analogy, the sensitivity to acetylcholine.

There seems to be a relation between the drug sensitivity and the condition of the muscle fibres. Although it is very difficult to produce direct evidence that the atrophic changes are responsible for the increased sensitivity to depolarizing drugs there is indirect evidence that this may be so.

The results of the present experiments show that atrophic changes are accompanied by hypersensitivity of the muscle membrane. De-afferentation, which caused atrophy of the muscle, was accompanied by an increased sensitivity to depolarizing drugs, and the increase in sensitivity could be altered by changing the degree of atrophy of the muscle. Tenotomy alone produced a more severe atrophy and greater hypersensitivity than tenotomy combined with dorsal root section.

The hypothesis that the increased sensitivity to depolarizing drugs is secondary to atrophic changes in the muscle fibres can explain results obtained in a number of other investigations.

Recent results of Katz & Miledi (1964) have shown that after local injury, due to transection of frog muscle fibres, the nerve-free segment became very sensitive to acetylcholine applied iontophorically. This increased sensitivity could be attributed to atrophic changes although there is no information concerning the condition of the fibres in these experiments.

It was shown by Jewell & Zaimis (1954*b*) that following tenotomy of the cat soleus and tibialis muscles the weight loss and decrease of muscle twitch tension was associated with an increase in sensitivity to decamethonium and suxamethonium.

In the experiments of Solandt & Magladery (1942) muscle atrophy was produced by spinal cord section. Again, a correlation between the sensitivity to acetylcholine and weight loss was found. The sensitivity to acetylcholine increased a few days after spinal cord section while the muscles were undergoing atrophy, but in the later stages after the operation, the muscle weight and fibre diameter returned to normal, and so did the acetylcholine sensitivity. Johns & Thesleff (1961) have shown that if the innervated muscles of the cat's hind limb are inactivated by section of the spinal cord at L 4 together with section of all the dorsal roots below this level, then the size of the acetylcholine-sensitive area starts to increase 1 week after the operation. After 3 weeks of inactivity the muscles show a considerable increase in the receptor area and a tenfold increase in sensitivity to intra-arterial injections of acetylcholine. These authors did not record muscle weights or tensions, but it has previously been shown by Eccles (1941) that 3 weeks after such an operation, the cat's hind limb muscles have atrophied to 60% of normal and both twitch and tetanic tensions are reduced to low values. This is therefore another experimental situation in which hypersentivity to acetylcholine can be correlated with atrophic changes.

This evidence, together with the fact that cross-union of the motor nerves failed to produce a change in sensitivity to depolarizing drugs, strongly suggests that hypersensitivity to these drugs can be expected in any situation in which atrophic changes are present in the muscle fibres.

The question which remains is, whether other factors are involved in the hypersensitivity of *denervated* muscles. In seeking an answer, experiments in which the metabolic and atrophic changes following denervation could be prevented by electrical stimulation would be of great importance.

Ginetzinsky (1956) reported that if the muscle atrophy, which normally follows motor nerve section, was prevented by electrical stimulation of the muscle, the membrane still remained hypersensitive to acetycholine. In these experiments the method of close arterial injection described by Brown, Dale & Feldberg (1936) was allegedly used to test the sensitivity to acetylcholine. However, Brown *et al.* (1936) obtained maximal contractions from normal muscles by injecting 5–20 μg of acetylcholine, and from denervated muscles by 0·005 μg, whereas Ginetzinsky had to use 1000 μg of acetylcholine to obtain a response from normal muscles, and 5 μg to obtain a response from the denervated and stimulated muscle. In these circumstances, the latter results cannot be taken as conclusive, and further experiments are needed to investigate the mechanism of denervation hypersensitivity.

Although this important piece of evidence is lacking the literature does contain some indirect findings which are relevant to this point. Denervation produces a large number of chemical and morphological changes in skeletal muscle (Gutmann, 1962). Many of these changes appear a few hours after section of the motor nerve. The ability of the tibialis anterior muscle to synthesize glycogen is reduced as early as 6 hr following denervation (Gutmann, Vodička & Vrbová, 1954) and a disturbance of protein metabolism was reported to occur after 24 hr (Žák & Gutmann, 1960). However, the increase in sensitivity to acetylcholine cannot be demonstrated until much later—48 hr following denervation in rat muscle and only after 4 days in the case of cat muscle (Thesleff, 1963; Axelson & Thesleff, 1959)—long after changes in the metabolic activity of the muscle have taken place. It is possible, therefore, that denervation hypersensitivity is due in part at least to the metabolic changes which have occurred during the earliest stages of denervation.

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