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EFFECTS OF DRUGS ON PARTIALLY AND COMPLETELY DENERVATED SKELETAL MUSCLE OF THE FROG

BY

G. B. FRANK

From the Department of Pharmacology and Therapeutics, University of Manitoba Faculty of Medicine, Winnipeg, Canada

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The sensitivity of completely denervated and the innervated end-plate regions of partially denervated frog's sartorius muscles to stimulation by drugs has been investigated. The drugs were tested on muscles isolated 12 to 39 days following denervation. On the basis of the results obtained most of the drugs could be put into one of two main groups. The first group included choline, tetramethylammonium, carbachol and decamethonium ; these drugs readily stimulated non-denervated muscles, and chronic denervation, either partial or complete, greatly increased the sensitivity of the muscles. The second group included pilocarpine and carbamate of (2-hydroxypropyl) trimethyl ammonium chloride (Bethanechol, U.S.P.). The muscles were relatively insensitive to stimulation by these two drugs. They could stimulate the muscles if applied in high enough concentrations, but the responses thus obtained could not be distinguished from responses produced by similar concentrations of sucrose and chronic denervation did not increase the sensitivity of the muscles to stimulation by these two drugs. Methacholine, like the drugs in the second group, was unable to stimulate the muscles in low concentrations, but chronic denervation rendered the muscles sensitive to stimulation by this drug. Tetraethylammonium presented a completely different pattern of activity which was consistent with a hypothesis that in low doses this drug stimulates skeletal muscle by causing a release of acetylcholine from nerve endings in the muscle.

It has been known for a long time that a denervated structure develops an increased sensitivity to many drugs in addition to its normal transmitter (Cannon & Rosenblueth, 1949). Recently it was reported (Frank, 1959) that the normally innervated end-plate regions of frog sartorius muscle fibres develop an increased sensitivity to their normal chemical mediator following denervation of another end-plate region on the same cells. In a subsequent report, Miledi (1960a) reached the opposite conclusion, although he did not adequately test for an increased sensitivity of innervated end-plates in partially denervated muscles. Because this point is relevant to any theory which attempts to explain the mechanism of denervation sensitization, it was decided that further work was warranted. In the present study on the sensitivity of partially and completely denervated frog sartorius muscles, several nicotinic and muscarinic drugs were tested.

METHODS

The methods used were described previously in detail (Frank, 1959). In the frog sartorius muscle the individual fibres extend the full length. There are two end-plate regions, one

proximal and one distal, with a distinct nerve supply to each, and most fibres have at least one neuromuscular junction in each end-plate region (Katz & Kuffler, 1941).

One sartorius muscle of each frog was denervated several days prior to testing; the sartorius muscle of the other leg served as a control. For complete denervation the whole nerve was severed at its site of entry into the muscle. For partial denervation, all the nerve branches in the muscle running towards the distal end were cut. Nerve section was done under a dissecting microscope. Only results obtained with muscles denervated 12 to 39 days prior to testing were used to determine the effects of denervation.

For testing, both sartorii were removed from the frog, leaving proximal ends attached to the pelvic girdle. The muscle pair thus formed was mounted vertically in a bath with the distal end of each muscle attached by a nylon thread to a critically balanced isotonic lever above the bath. The bath was drained or filled from below to permit the test solution to cover the proximal end-plate region without touching the distal end-plate zone. For testing, all drugs were dissolved in a solution containing the following salts (in mM): sodium chloride, 111.3; potassium chloride, 2.5; calcium chloride, 1.08; sodium bicarbonate, 2.4; sodium dihydrogen phosphate, 0.087; glucose, 11.1. The drug concentrations in the test solutions were increased in steps of approximately threefold, and, for ease in comparing sensitivity to various drugs, concentrations are reported in mM. The proximal end-plate regions of both muscles were exposed simultaneously to a test solution for at least 30 sec, and between tests the muscles were kept in a drug-free solution for at least 15 min. No more than two drugs were tested on any one muscle pair. No attention was paid to the order of drug application, with the exception of decamethonium, which was always the last drug tested on any muscle pair.

The tests were designed to find the minimum drug concentration which would produce a discernible response in the muscle. This was considered the threshold concentration, provided that the effect was reproducible and that the muscle responded to the next higher drug concentration.

In each test the thresholds for the two sartorius muscles from a single frog were determined with the muscles mounted in a single bath. Since only one muscle from each frog was chronically denervated, the non-denervated sartorius muscle from the other leg served as a control. Using the results thus obtained, a separate threshold ratio was determined for each muscle pair, and these are listed in the Tables under the columns headed "Ratio (C/D)."

After every experiment with a partially denervated muscle it was examined under a dissecting microscope to confirm that the nerve section had been properly performed, to see if the nerve supply to the distal end-plate region had regenerated, and to ascertain that the nerve supply to the proximal end-plate region was viable. The results obtained with muscles showing signs of nerve regeneration are reported separately.

RESULTS

The sensitivity of sartorius muscles from different frogs to the same drug varies greatly. Thus it was previously reported (Frank, 1959) that the acetylcholine thresholds of sartorius muscles from different frogs may differ by a factor of 1,000 times. This factor was smaller for each drug reported here, although differences were still present and made it necessary to establish some criterion for denervation sensitization. It is generally assumed that the homologous muscles from the two sides of an animal are equally sensitive to applied drugs, and thus if only one is chronically denervated the other can be used as a control. The thresholds of acetylcholine for 24 pairs of sartorius muscles in which the innervation remained intact were previously determined (Frank, 1959), and the threshold sensitivities of pairs of innervated muscles reported here also were tested for all but one of the drugs (see below). In all tests the homologous muscles from the same frog had the same threshold.

Another indication that homologous muscles were equally sensitive to applied drugs was the effect of regeneration on the sensitivity of partially denervated muscle. When the nerve supply to the distal end-plate region had regenerated, the difference in the drug sensitivity of the proximal end-plate regions in the denervated and control muscles tended to disappear. This always occurred when testing thresholds of acetylcholine (Frank, 1959) and usually occurred when testing the thresholds of the other drugs included in the present study.

All soluble chemicals tested could produce a muscle response, provided that a large enough concentration was used. Since the drugs were added to the test solution for testing and since often extremely large drug concentrations were necessary, it was thought that the responses might have been produced by the tonicity of the

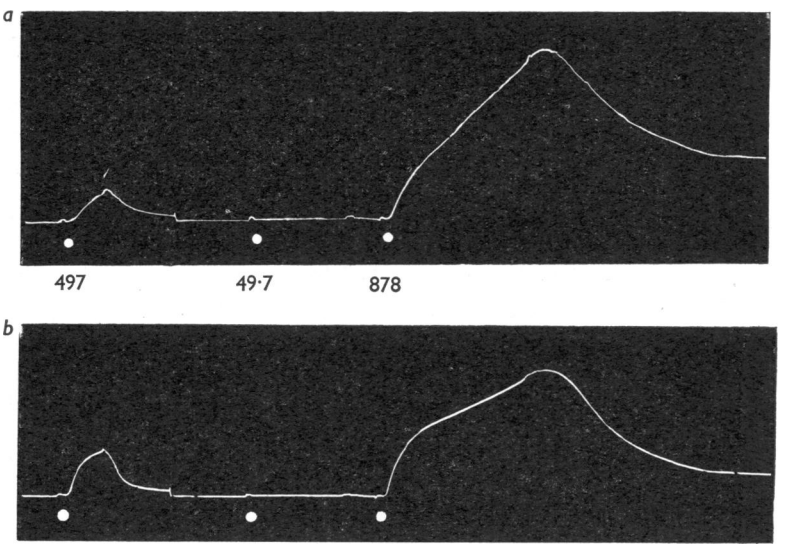

Fig. 1. Responses of frog sartorius muscles produced by the application of hyperosmotic sucrose-containing solutions to the lower halves of the muscles. (*a*) Partially denervated muscle; (*b*) control muscle from other leg of frog. Dots below records indicate point at which test solutions were added to the bath. Numbers between the records indicate the concentrations of sucrose (in mM). Small rectangular artifacts on the records were caused by emptying the bath.

test solutions rather than by the action of the drug. To test this possibility the response of the muscles to high concentrations of sucrose was tested. Responses produced by sucrose are shown in Fig. 1. Three pairs of muscles were thus tested, and, in all three, sucrose concentrations of 497 mM or larger produced a response. In two of these pairs a sucrose concentration of 49.7 mM failed to produce a muscle response.

In the experiments using drugs, any muscle which did not respond when tested with a concentration of 50 mM or more was considered to be relatively insensitive to the drug. All muscles responded to any drug tested in a concentration of 410 mM or greater, but for these the response would have to be attributed to the tonicity

of the test solution. Because of the responses to high osmotic concentrations, it was impossible to determine if a muscle was completely insensitive to the effect of a drug.

Choline chloride. It has long been known that choline can stimulate skeletal muscle, differing from acetylcholine only in being less potent. Eleven pairs of muscles were tested with choline (Table 1). These included 12 innervated control muscles. The most sensitive of these control muscles had a choline chloride

TABLE 1

CHOLINE CHLORIDE CONCENTRATIONS REQUIRED TO PRODUCE THRESHOLD CONTRACTIONS OF FROG SARTORIUS MUSCLES

Ranges given except where not applicable. n, number of muscle pairs; C/D, ratio of threshold doses for control and denervated muscle pairs except in the pairs without a chronically denervated muscle, when the ratio represents right sartorius (R)/left sartorius (L); >=greater than, indicates that the muscle did not contract, but higher drug concentrations were not tested

Preparation	n	Control (C) (mM)	Denervated (D) (mM)	Ratio (C/D)
No denervation	1	R, 7·16; L, 7·16		1
Complete denervation	5	7·16–>71·6	0·716–7·16	3–>100
Partial denervation	3	7·16–>71·6	2·15–7·16	3–>10
Partial denervation with regeneration	2	7·16	7·16	1

threshold of 7.16 mM. Using acetylcholine (Frank, 1959), the most sensitive muscles responded to a solution containing 5.37×10^{-4} mM, or a difference of about 10,000 times.

As would be expected, chronic complete denervation increased the sensitivity of frog sartorius muscles to stimulation by choline. Similarly the innervated end-plate regions of the partially denervated muscles also developed an increased sensitivity to choline stimulation. In the two examples in which the partially denervated muscles were observed to have a viable nerve supply to their distal end-plate regions following drug testing (Table 1), the partially denervated muscles and their controls did not differ in their sensitivity to choline.

Pilocarpine hydrochloride. This alkaloid has marked parasympathomimetic effects, but apparently is completely devoid of nicotinic properties. Five pairs of muscles were tested with solutions containing pilocarpine. Three of the muscles had been completely denervated 9, 13 and 34 days prior to testing. Two of these pairs were tested with a maximum pilocarpine concentration of 41 mM and did not contract. The other three responded when tested with a solution containing 410 mM; one of the pairs tested with 123 mM did not respond. There was no observed effect of complete denervation on the threshold or response to pilocarpine. In these tests pilocarpine had an effect and potency comparable to that of sucrose.

Tetramethylammonium chloride. In agreement with the known nicotinic properties of tetramethylammonium, Dale & Gasser (1926) demonstrated that denervated mammalian skeletal muscle developed an increased sensitivity to stimulation by this drug. Similar results were obtained in the present experiments. It was observed that both completely denervated frog sartorius muscles and the

TABLE 2

TETRAMETHYLAMMONIUM CHLORIDE CONCENTRATIONS REQUIRED TO PRODUCE THRESHOLD CONTRACTIONS OF FROG SARTORIUS MUSCLES

Ranges given except where not applicable. n, number of muscle pairs; C/D, ratio of threshold doses for control and denervated muscle pairs except in the pairs without a chronically denervated muscle, when the ratio represents right sartorius (R)/left sartorius (L); >=greater than, indicates that the muscle did not contract, but higher drug concentrations were not tested

Preparation	n	Control (C) (mM)	Denervated (D) (mM)	Ratio (C/D)
No denervation	1	R, 0·274; L, 0·274		1
Complete denervation	6	0·0913–0·913	0·0274–0·0913	3·3–10
Partial denervation	3	0·274–0·913	0·0913	3–10
Partial denervation with regeneration	2	0·274	0·274	1
	1	0·274	0·0913	3

innervated end-plate regions of partially denervated muscles had a lower threshold to stimulation by tetramethylammonium chloride than did control muscles (Table 2).

An interesting observation was that the proximal end-plate region of one of the partially denervated muscles had an increased sensitivity to tetramethylammonium stimulation, despite the fact that the nerve supply to the distal end-plate region had regenerated. This persistent increase in sensitivity in the presence of regeneration was never found in experiments with acetylcholine (Frank, 1959), but was observed with some of the other drugs used in the present study (see below).

Tetraethylammonium bromide. Although solutions containing tetraethylammonium readily stimulate frog sartorius muscles, both the type of contraction obtained and the effects of denervation on the response to this drug indicate that, at least in lower concentrations, it produces its stimulation by a mechanism of action different from that of the other drugs included in this study.

In the innervated muscle the typical threshold response produced by all the other active drugs studied was a slow, smooth shortening. In contrast, tetraethylammonium either produced no response or a violent twitching of the muscle (Fig. 2*b*).

Seven pairs of muscles, including a completely denervated muscle, were tested with tetraethylammonium (Table 3). In all but one of these, the control muscle was more sensitive than the denervated muscle to stimulation by this drug. Another result of the complete denervation was elimination of the violent twitching, the

TABLE 3

TETRAETHYLAMMONIUM BROMIDE CONCENTRATIONS REQUIRED TO PRODUCE THRESHOLD CONTRACTIONS OF FROG SARTORIUS MUSCLES

Ranges given except where not applicable. n, number of muscle pairs; C/D, ratio of threshold doses for control and denervated muscle pairs except in the pairs without a chronically denervated muscle, when the ratio represents right sartorius (R)/left sartorius (L); >=greater than, indicates that the muscle did not contract, but higher drug concentrations were not tested

Preparation	n	Control (C) (mM)	Denervated (D) (mM)	Ratio (C/D)
No denervation	1	R, 4·76; L, 4·76		1
Complete denervation	6	4·76–47·6	47·6–>47·6	<0·1–<1
	1	14·3	4·76	3
Partial denervation	3	14·3	0·476–4·76	3–30
	2	4·76, 47·6	4·76, 47·6	1
Partial denervation with regeneration	1	4·76	4·76	1

response of the denervated muscle to this drug being similar in appearance to the responses to all other stimulant drugs studied (Fig. 2*a*). The records obtained in the one case in which the completely denervated muscle had a lower threshold than its control are shown in Fig. 2.

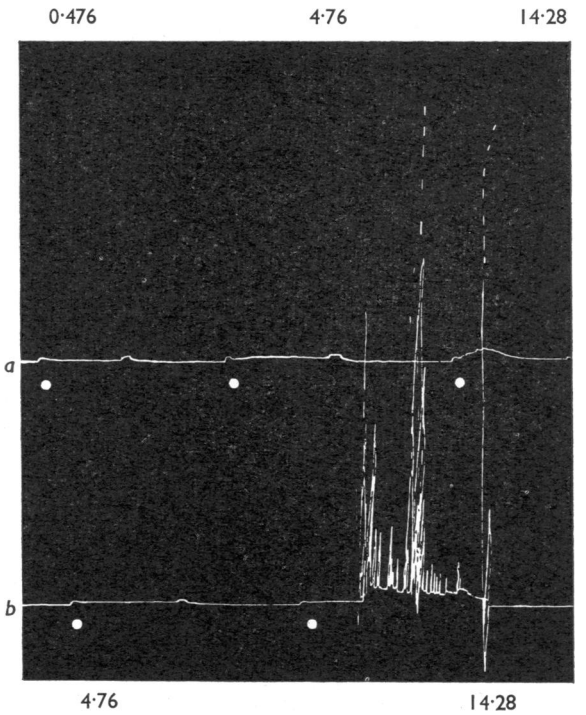

Fig. 2. Responses of frog sartorius muscles to the application of tetraethylammonium-bromide-containing solutions. (*a*) Completely denervated muscle; (*b*) control muscle from other leg of frog. 18 days post-denervation. Levers not aligned. Dots below records indicate point at which test solutions were added to the bath. Numbers above and below record indicate the tetraethylammonium concentrations (in mM). Small rectangular artifacts on the records were caused by emptying the bath.

The type of response produced by the application of tetraethylammonium to the innervated end-plate region of the partially denervated sartorius muscle was the same as produced in the control muscles. In contrast to the changes produced by complete denervation, 3 of the 5 partially denervated muscles had a lower threshold to stimulation by this drug than did their controls.

These results can best be explained if it is assumed that the stimulation produced by tetraethylammonium is brought about by the release of acetylcholine from the nerve endings in the muscle and only in the completely denervated muscle is a direct stimulation of the muscle fibres by this drug uncovered.

Bethanechol chloride, methacholine chloride, and carbachol chloride. These three closely related drugs presented an interesting spectrum of activities, with carbachol having marked nicotinic properties, bethanechol devoid of nicotinic activity and methacholine intermediate (Table 4).

TABLE 4

BETHANECHOL, METHACHOLINE, AND CARBACHOL CONCENTRATIONS REQUIRED TO PRODUCE THRESHOLD CONTRACTIONS OF FROG SARTORIUS MUSCLES

Ranges given except where not applicable. n, number of muscle pairs; C/D, ratio of threshold doses for control and denervated muscle pairs except in the pairs without a chronically denervated muscle, when the ratio represents right sartorius (R)/left sartorius (L); >=greater than, indicates that the muscle did not contract, but higher drug concentrations were not tested

Drug and preparation	n	Control (C) (mM)	Denervated (D) (mM)	Ratio (C/D)
Bethanechol				
Complete denervation	5	>5·09–>50·9	>5·09–>50·9	—
Partial denervation	1	>50·9	>50·9	—
Partial denervation with regeneration	1	>50·9	>50·9	—
Methacholine				
No denervation	2	R>51·2, 512 L>51·2, 512		—, 1
Complete denervation	6	>51·2	0·512–5·12	>10–>100
Partial denervation	3	>51·2	5·12–51·2	>1–>10
Partial denervation with regeneration	1	>51·2	>51·2	—
	2	>51·2	5·12	>10
Carbachol				
No denervation	3	R, 0·0548–0·164 L, 0·0548–0·164		1
Complete denervation	5	0·0548–0·548	0·00164–0·0164	10–100
Partial denervation	4	0·0164–0·548	0·00164–0·00548	10–100
Partial denervation with regeneration	1	0·0548	0·0548	1
	1	0·0548	0·0164	3·3

In none of the muscles of the seven pairs tested with bethanechol was a contraction obtained. Although 50.9 mM was the largest concentration tested, the effects of higher concentrations could have been attributed to osmotic effects.

In contrast, carbachol had marked nicotinic stimulant properties. It readily stimulated both control and denervated muscles, and in this it was the most potent drug included in the present study. Both the completely denervated muscles and the innervated end-plate regions of the partially denervated muscles had an increased sensitivity to stimulation by this drug.

An intermediate response pattern was obtained in tests with methacholine. All the control muscles were relatively insensitive to stimulation, but the chronically denervated muscles, both complete and partial, were sensitive to stimulation by methacholine. Even 2 of the partially denervated muscles in which the nerve supply to the distal end-plate region had regenerated had a threshold of 5.12 mM.

Decamethonium bromide. Zaimis (1951) showed that the response of denervated mammalian skeletal muscle to decamethonium was identical to its response to acetylcholine. In the present study only the stimulation produced by this neuromuscular blocking agent in low doses has been investigated. As can be seen from the results presented in Table 5, both completely denervated muscles and the innervated end-plate regions of the partially denervated muscles were sensitized to the stimulant action of this drug. Although at the start of these tests the possibility was considered that the muscles might become blocked by the decamethonium without showing any measurable signs of stimulation, this did not prove to be a

TABLE 5

DECAMETHONIUM BROMIDE CONCENTRATIONS REQUIRED TO PRODUCE THRESHOLD CONTRACTIONS OF FROG SARTORIUS MUSCLES

Ranges given except where not applicable. n, number of muscle pairs; C/D, ratio of threshold doses for control and denervated muscle pairs except in the pairs without a chronically denervated muscle, when the ratio represents right sartorius (R)/left sartorius (L); >=greater than, indicates that the muscle did not contract, but higher drug concentrations were not tested

Preparation	n	Control (C) (mM)	Denervated (D) (mM)	Ratio (C/D)
No denervation	1	R, 1·20; L, 1·20		1
Complete denervation	3	0·0718–0·239	0·00718	10–33
Partial denervation	3	0·239	0·00239–0·0239	10–100
	1	>23·9	0·239	>100
Partial denervation with regeneration	2	0·239	0·239	1

problem. However, in one pair of muscles the threshold for the control muscle was greater than 23.9 mM (Table 5), and, since this is considerably above all the other thresholds recorded, it is possible that in this one instance the muscle was blocked without a response being recorded.

DISCUSSION

Dale & Gasser (1926) divided drugs which act like acetylcholine into two groups, those which stimulated and those which failed to stimulate denervated mammalian muscles. They pointed out that the active drugs were characterized by also having nicotine-like effects on ganglion cells, and that chronic denervation had made the mammalian skeletal muscle comparable to muscles from amphibia and birds which are normally sensitive to stimulation by nicotinic drugs.

With two notable exceptions, the drugs tested in the present study fit the pattern that they described. Thus the chronically denervated muscles developed an increased sensitivity to stimulation by choline, tetramethylammonium, carbachol and decamethonium, all of which were relatively potent in stimulating the control muscles. On the other hand, pilocarpine and bethanechol were unable to stimulate either control or denervated muscles in reasonably low doses, and any stimulation obtained could be attributed to an osmotic effect comparable to that produced by sucrose solutions.

Methacholine presented an interesting exception to this pattern in that the control muscles were relatively insensitive to stimulation by this drug, whereas the denervated muscles had developed a marked sensitivity (Table 4).

The results obtained with tetraethylammonium differed from those obtained using the other drugs in almost every respect. Tetraethylammonium markedly antagonizes the blocking action of curare on neuromuscular transmission (Riker, 1953). Stovner (1958) concluded that tetraethylammonium produced its anticurare effect by increasing the amount of acetylcholine released by each nerve impulse. The present results also support the concept that tetraethylammonium can act by facilitating the release of acetylcholine from nerve endings in muscle. Thus the threshold response to tetraethylammonium is a violent twitching of the muscle (Fig. 2), as would be expected if there was a sudden release of acetylcholine from the nerve endings once the concentration of tetraethylammonium in the extracellular fluid reached a critical

level. In contrast, drugs such as acetylcholine or decamethonium which depolarize the end-plates by a direct action produce a smooth muscle shortening at threshold concentrations. This would be expected from the conditions of the present experiment which result in a gradual rise in the drug concentration in the extracellular spaces of the muscle. It is of interest that the stimulation by tetraethylammonium of the completely denervated muscle, in which the nerve endings have degenerated, results in a response similar in shape to that produced by drugs known to depolarize directly the muscle end-plates.

The hypothesis that tetraethylammonium can release acetylcholine from the nerve endings in muscle, and that this release can be brought about by lower doses than those needed to produce a measurable direct response, also explains the observed increase in threshold in most of the completely denervated muscles. However, in one instance the threshold for the completely denervated muscle was lower than that of its control (Fig. 2). This indicates that tetraethylammonium can directly stimulate the muscle and that the chronically denervated muscle can develop an increased sensitivity to this direct effect. The above hypothesis also would explain the observed increase in sensitivity of the innervated end-plate regions of some of the partially denervated muscles to tetraethylammonium, since it has previously been shown that these regions develop an increased sensitivity to direct depolarization by acetylcholine (Frank, 1959). Since the response of these innervated regions of partially denervated muscles would not depend only on their acetylcholine thresholds but also on the amounts of acetylcholine released by the tetraethylammonium from the nerve endings, it is not surprising that two of the five partially denervated muscles did not show a lower tetraethylammonium threshold than their controls.

In a recent study, Miledi (1960a) concluded that the innervated end-plate regions of partially denervated frog sartorius muscle fibres were not more sensitive to acetylcholine than the end-plate regions of innervated muscle fibres. However, he did not make this comparison experimentally, but based his conclusion on the observation that the responses recorded in the region of innervated end-plates of partially denervated and innervated muscles were not very different following acetylcholine administration. Without comparing the sensitivity of matched pairs of sartorius muscles to drug stimulation it would often be difficult and at times impossible to detect an increased sensitivity following either partial or complete denervation.

More difficult to explain is the marked difference in sensitivity to acetylcholine stimulation of the innervated and denervated end-plate regions of partially denervated muscle fibres observed by Miledi (1960a). Frank (1959) did not find this difference in tests carried out in the presence of neostigmine. Since at times the complete nerve supply to the denervated end-plate region was not sectioned and since the muscles were not examined for evidence of regeneration, it is possible that in the experiments of Miledi (1960a) regeneration of the nerve supply to the denervated end-plate region had occurred. If this was so, the innervated end-plate region would no longer be supersensitive to acetylcholine stimulation (Frank, 1959), but the denervated end-plates would maintain their increased sensitivity for a considerable period of time (Miledi, 1960b). Thus it is possible that under the conditions of the experiments of Miledi (1960a) the innervated end-plate regions of the partially denervated muscles did not have an increased sensitivity to acetylcholine, but this

does not mean that the innervated end-plates cannot develop this supersensitivity under the appropriate conditions.

In 1943 Kuffler reported that the non-innervated areas of frog sartorius muscle fibres as well as their end-plate regions develop an increased sensitivity to acetylcholine following complete denervation of the muscle. However, he emphasized the fact that the end-plate regions were still considerably more sensitive. Recently this increase in the area of membrane sensitive to acetylcholine following denervation has been more clearly demonstrated by the iontophoretic application of acetylcholine to minute areas of membrane in denervated mammalian skeletal muscle (Axelsson & Thesleff, 1959) and in denervated frog skeletal muscle (Miledi, 1960a). These investigators have put forward the hypothesis that this increase in the sensitive area of membrane is the change responsible for the increased sensitivity to applied drugs which follows denervation.

Two main lines of experimental evidence are used to support this hypothesis (Miledi, 1960a ; Axelsson & Thesleff, 1959 ; Thesleff, 1960). First is the demonstration itself of an enlarged area of increased sensitivity. Second, and most important, neither group observed an increased response to iontophoretically applied acetylcholine of the denervated end-plate region itself but only an increased response of the surrounding areas of membrane. In fact, in denervated mammalian skeletal muscle, the entire membrane appeared to have become uniformly sensitive to acetylcholine. However, the above workers neither compared dose-response relationships nor acetylcholine thresholds in innervated and denervated end-plates.

There is considerable evidence that the cholinesterase activity in the end-plate area decreases following denervation (Thesleff, 1960 ; Strömblad, 1960), which results in an increased response to applied acetylcholine (Thesleff, 1960 ; Axelsson & Thesleff, 1959 ; Frank, 1959). Since the responses to iontophoretically applied acetylcholine, when tested in the absence of an anticholinesterase, were no greater in the region of a denervated end-plate than in the region of an innervated end-plate (Axelsson & Thesleff, 1959 ; Miledi, 1960a), this would indicate that the acetylcholine doses were producing maximal responses. It is known that the maximal response of a skeletal muscle does not increase following denervation (Dale & Gasser, 1926 ; Cannon & Rosenblueth, 1949). Thus the responses to threshold or submaximal drug doses must be tested before rejecting the possibility that denervation results in an increased sensitivity of the end-plate areas to applied acetylcholine. Furthermore, it would be difficult to explain many of the results obtained in the present study as well as the results obtained by Dale & Gasser (1926) if it is assumed that there is no true increased sensitivity to applied drugs following denervation, but only an increased area of membrane sensitive to stimulation.

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