Further Study of the Electrical and Mechanical Responses of Slow Fibers in Cat Extraocular Muscles

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ABSTRACT Electrical and mechanical responses have been obtained in situ and in vitro from the superior oblique muscle stimulated by single and repetitive electrical pulses, applied to the trochlear nerve. Two different types of muscle fibers are described, the twitch and the slow. The slow type is characterized electrically by the presence of junctional potentials, which have reversal potentials between $-10$ and $-20$ mv, and do not show propagated responses or spikes, during nerve stimulation. When the slow muscle fibers are repetitively stimulated in situ, a prolonged contraction is maintained during stimulation. At the time, the recorded electrical activity is produced locally, at the level of the neuromuscular junctions of the slow fibers. These results indicate that the contractile mechanism of the slow muscle fibers is activated locally and segmentally.

INTRODUCTION

In mammalian extraocular muscles, there are two types of muscle fibers characterized by morphological and physiological differences. In one, the "twitch" type, the fiber has a single motor end plate and upon stimulation exhibits propagated action potentials. The other type of fiber, the "slow," has multiple nerve terminals of a more delicate nature and is, usually, incapable of generating an action potential during activation (Hess and Pilar, 1963). Further morphological differences in internal structure and nerve terminals of both kinds of muscle fibers have been described (Pilar and Hess, 1966).

The physiological characteristics of these two types of fibers in cat extraocular muscles have been disputed recently by Bach y Rita and Ito (1966). They have questioned, specifically, the previous assertion that slow fibers produce only junctional potentials upon stimulation (Hess and Pilar, 1963) and have contended that such fibers are capable of producing propagated
responses. However, Bach y Rita and Ito (1966) did not provide adequate evidence to prove that the action potentials recorded by them arose from the same muscle fibers that Hess and Pilar (1963) called slow.

The present study, designed specifically to answer their criticism, presents data showing that the junctional potentials recorded from slow muscle fibers are not movement or electrical artifacts, as suggested by Bach y Rita and Ito (1966), and that during normal activation in situ, the slow fibers do not necessarily exhibit spike activity.

METHODS

The superior oblique muscle of the cat was used. Experiments were performed in vitro and in vivo. For the in vitro experiments, the experimental techniques have been described (Hess and Pilar, 1963). In vivo or in situ experiments were performed in cats previously anesthetized with 35 mg/kg of sodium pentobarbital given intraperitoneally. The head of the animal was fixed in a cat frame. The eyeball was collapsed and removed. The distal tendon of the superior oblique muscle was separated from its insertion on the eyeball and the muscle exposed. Special care was taken to maintain an adequate blood supply. The trochlear nerve was exposed and cut at its point of emergence from the orbit. The nerve-muscle preparation was bathed in an oil pool at 35°C.

The nerve was stimulated with rectangular electrical pulses of 0.1 msec duration by means of platinum electrodes. The electrical activity of the muscle fibers was recorded with two steel “spring action” electrodes or calomel half-cells. The electrodes were connected to an ac low level preamplifier, and the electrical responses were displayed on an oscilloscope. Intracellular records were obtained with conventional microelectrodes filled with 3 M KCl and connected to the oscilloscope by a cathode-follower probe. Current was passed through the intracellular micropipette using a bridge circuit previously described by Martin and Pilar (1963). For mechanical recording, the muscle fibers were at optimal length, at which the peak twitch tension was maximal for the twitch fibers (Close, 1964). The distal tendon of the muscle was attached firmly to a transducer while the isometric contractions were recorded with a strain gauge (Statham G1-8, Statham Instruments, Inc., Los Angeles, Calif.), the output of which was displayed on an oscilloscope and photographed.

RESULTS

Intracellular Recordings from Slow Fibers

Fig. 1 is an intracellular record obtained from a slow fiber which responded to increasing strengths of nerve stimulation with three slow depolarizations of different amplitudes, 0.2, 5, and 4 mv; latencies, 8, 15, and 5 msec; maximal rate of rise, 0.3, 1.5, and 5.2 mv/msec; and half-decay time 48, 60, and 11 msec, respectively (Fig. 1 A, B, C). In Fig. 1 A, at threshold stimulus strength, a junctional potential appears arising at some distance from the recording electrode. In B, with increased stimulus strength a second potential is ob-
observed, superimposed on the junctional potential with the lowest threshold. Fig. 1 C shows a composite depolarization elicited now with maximal stimulation where a new junction potential precedes the previous ones. The latter potential was generated probably from a locus near the electrode because it had the fastest rise and decay times. The half-decay has been extrapolated, assuming that it follows a time course similar to that observed at the beginning of repolarization. These sequences in potential appearance did not always follow this order. In some cases, the larger potentials were observed at threshold stimulation concealing smaller potentials produced, presumably, by activation of different nerve fibers.

The observed junctional potentials were probably generated by different current sources scattered along the muscle membrane.

![Figure 1](image)

**Figure 1.** Intracellular recording in vitro of junctional potentials of a slow muscle fiber elicited by increasing strength of stimulation applied to the nerve from A to C. Dots indicate the stimulus artifact. Dashed line in C represents the assumed decay of the last evoked potential. Note different amplitudes, latencies, and time course of the potentials.

Similar complex potentials have been recorded in all the muscle fibers called slow (Hess and Pilar, 1963). The presence of numerous components is an indication of multiple innervation, and the variation in threshold and latency showed that the multiple innervation is polyneuronal. Furthermore, since these potentials could be graded by varying the strength of nerve stimulation and since they had different time courses, they could not be due to small "spike activity," as suggested by Bach y Rita and Ito (1966).

These junctional potentials are similar to those described in slow muscle fibers of other animals (Kuffler and Vaughan Williams, 1953 a; Ginsborg, 1960).

During impalement of a fiber in which junctional potentials were evoked by nerve stimulation, the membrane polarization was changed by passing current through the recording pipette with the bridge circuit (Fig. 2). The amplitude of the junctional potentials increased with an increase in membrane potential, and it was possible to extrapolate the reversal level to a value lower than the zero membrane potential, between −10 and −20 mv (Burke and Ginsborg,
1956 a). This value for the reversal potential is quite different from the value expected at the peak of an action potential. In addition, since these junctional potentials have an equilibrium potential, they cannot be movement artifacts, as suggested by Bach y Rita and Ito (1966). It should be pointed out that the method used for determination of the junctional potentials equilibrium level offers only an approximation because of the distribution of the endings along the muscle membrane. The transmitter release drives the whole membrane toward the equilibrium potential, and the current injected through the recording microelectrode is applied only to a small area of the membrane (Burke and Ginsborg, 1956 a).

![Figure 2](https://example.com/fig2.png)

**Figure 2.** Intracellular recording in vitro of a junctional potential elicited by maximal nerve stimulation. In each record, upper beam, current pulses; lower beam, membrane potential changes. The potential consists of three distinct junctional potentials elicited at different stimulation strengths. The membrane polarization of the same cell was changed by passing current through the recording electrode with a bridge circuit. The amplitude of the evoked junctional potential increases with membrane polarization. Membrane resting potential, 50 mv. Further membrane shifts imposed by current in both directions were complicated by the presence of electrode polarization and/or membrane rectification.

**Ability of Slow Muscle Fibers to Generate a Propagated Response**

Under the present experimental conditions, the slow fibers were unable to generate an action potential either by a depolarization produced by the junctional potentials or by passing depolarizing or hyperpolarizing current through the membrane (Figs. 1 and 2). However, the possibility existed that these fibers did not generate spikes because their firing threshold was higher than that of ordinary twitch fibers. This possibility was tested by applying repetitive stimulation to the nerve while the junctional potential was recorded intracellularly (Fig. 3).

The resting potential of the fiber was 65 mv and nerve stimulation induced a membrane depolarization with a peak amplitude of 30 mv. At this depolarization level action potentials were not elicited. Still the possibility remained
that in situ, during normal operation, these fibers might be activated by action potentials. Tetanic stimulation of the nerve was used in order to mimic the natural condition of contracting muscle fibers. Twitch muscle undergoes a decrease of tension during a prolonged tetanus at frequencies higher than 60 per sec (Del Pozo, 1942). Cat extraocular muscles in situ also undergo this "fatigue." In the experiment illustrated in Fig. 4, the superior oblique muscle was stimulated indirectly at 72/sec and intracellular potentials were recorded from its proximal portion. After several seconds of activation, neuromuscular transmission failed as seen in the record, which consists of three consecutive superimposed sweeps. Fully developed spikes appear in the first two sweeps, and end plate potentials alone are present in the third sweep. After the activity of twitch fibers is reduced by tetanic stimulation, it should be possible to correlate the remaining mechanical and electrical events. This remaining activity, after fatigue of the twitch fibers, should be produced by slow muscle fibers only.
The fusion frequency of the twitch fibers of the superior oblique muscle occurs at nerve-stimulating frequencies of from 250 to 300 impulses/sec (Pilar and Close, unpublished observation). The muscle was, therefore, stimulated in situ through its nerve at a frequency of 250 impulses per sec (Fig. 5). During prolonged tetanic contraction evoked for 12 sec, the initially recorded tension decreased after several seconds to approximately one-third of the initial tension and stayed at that level for the remainder of the stimulation period. This residual tension was probably maintained by slow muscle fiber contraction during repetitive nerve stimulation. The degree of tension probably sustained by the slow muscle fibers in this experiment is very similar to the proportion of total tension produced by slow muscle fibers during in vitro superfusion with acetylcholine (10 μg/ml) as shown elsewhere (Hess and Pilar, 1963).

Further evidence that slow fiber activity was contributing to the residual tension during long tetanic nerve stimulation was obtained as follows: the electrical activity of the muscle was recorded simultaneously with tension as shown in Fig. 6. Two electrodes were placed on the distal portion of the muscle where only slow fiber-nerve junctions occur (Hess and Pilar, 1963). Recordings were displayed on a double beam oscilloscope sweeping at 2 msec per cm. Three sweeps were superimposed on each photographic frame which advanced at 0.5 sec intervals.

At the onset of the tetanus, produced by nerve impulses at 180/sec, fast diphasic spike-like responses appeared (Fig. 6 A). A small spike activity also occurred later, which could have been obtained from muscle fibers innervated by more slowly conducting nerve fibers (Pilar and Hess, 1966). After a latent period, the tension rose rapidly to a maximum in 2 sec (Fig. 6 B and C). Each stimulus produced a tension increment since the fusion frequency of mechanical responses occurs at frequencies higher than 250/sec (Pilar and Close, unpublished observation). Each increment was accompanied by a propagated response. After a few seconds, the tension began to diminish and at the same time action potentials appeared with longer latency and smaller amplitude (Fig. 6 D). Seconds later, the tension leveled off to about one-third of the peak tension and became smooth. This "contracture" was maintained during the
rest of the stimulation while the electrical record was a monophasic response with a slower time course (Fig. 6 E). Stimulation was stopped for 5 sec (Fig. 6 F). Upon restimulation (Fig. 6 G) the tension rose considerably, increments appeared, and spike activity was also present (Fig. 6 H). However, the tension promptly fell again to a lower level and only monophasic electrical activity occurred (Fig. 6 I).

This behavior is different from that observed in a twitch muscle fiber. The frog sartorius, composed mainly of twitch fibers (Kuffler and Vaughan Williams, 1953 b), has been studied under similar experimental conditions. At
stimulating frequencies of from 30 to 50 shocks/sec, the tension gradually subsided to nil in spite of prolonged stimulation. Even after tension subsided, spike activity was recorded, although of smaller amplitude than that initially obtained. At higher stimulating frequencies (75 to 125/sec) the tension and the electrical activity decreased progressively to nil during prolonged stimulation (Pilar, unpublished observations). These findings are in agreement with the observations of Sandow and Eberstein (1963) when muscles were stimulated at lower frequencies. They suggested that fatigue recorded at low frequencies of nerve stimulation may be attributed to some changes in the excitation coupling mechanism. However, when muscles are stimulated indirectly at higher frequencies, fatigue occurs at the neuromuscular junction (Del Pozo, 1942; Krnjević and Miledi, 1958).

The electrical activity recorded during tetanic fusion had the characteristics of a locally produced nonpropagated response. It probably arose from the delicate, multiple nerve terminals of the slow muscle fibers, since it was recorded from the distal end of the muscle where essentially only these nerve endings are present (Hess and Pilar, 1963). It is unlikely that this activity was recorded electrotonically from the end plates of the twitch muscle fibers, since such nerve terminals are restricted to the proximal portions of the muscle (Hess and Pilar, 1963). When one electrode was placed on the muscle-tendon junction of the distal end of the muscle, a monophasic spike was recorded upon nerve stimulation as expected from the ends of aligned muscle fibers (Katz and Miledi, 1965). Tetanic nerve stimulation was applied as in the previous experiment, and the monophasic spike appeared in conjunction with nonfused tetanic contraction (Fig. 7 A and B). During steady contraction, no potential occurred (Fig. 7 C). Since in this case, the nearest neuromuscular junction was only 2–3 mm away from the recording electrode and still no potential was recorded, it is unlikely that in the previous experiments (e.g., Fig. 6) a potential would have spread electrotonically from the “end plate” endings (on the proximal end of the muscle) which were about 10 mm away from the recording electrodes.

The experiments already described show that in situ, as well as in vitro, the superior oblique muscle has two contractile systems with different fusion frequencies (Hess and Pilar, 1963). At the onset of prolonged stimulation, both groups of muscle fibers are activated, and the tension recorded shows increments with each stimulus. Each increment of tension is accompanied by spike activity. The system responsible for such a response, with a very high fusion frequency, has twitch-type characteristics. When the propagated action potentials are blocked because of neuromuscular transmission failure, smooth tension is achieved, and the electrical activity is produced locally. This contracture is maintained by the other muscle fiber group, with a much slower fusion frequency and is not activated by propagated action potentials. Instead,
locally produced depolarization sustains the muscle tension. These are the characteristics of the slow type of muscle fibers described by Hess and Pilar (1963) in the extraocular muscles in vitro.

**Figure 7.** Simultaneous recording of electrical activity and tension as in Fig. 6. Upper traces, electrical records; middle traces, tension records; lower traces (dashed lines), base line for tension recordings. Nerve was stimulated at 100/sec and electrical activity was recorded with one electrode placed on the junctional region between muscle fibers and tendon. A, 0.5 sec after initiation of tetanic stimulation, B, 5 sec; C, 9 sec later; D, no stimulation. (See text for explanations.)

**DISCUSSION**

The present study presents evidence for the existence of two different types of muscle fibers in the superior oblique muscle of the cat: a twitch type and a slow type. Since some confusion exists in the literature about this nomenclature, the differences between the muscle fibers should be pointed out.

The dissimilarity is inherent in the mode of activation of the contractile apparatus which is essentially similar for all striated muscle fibers. In the case of twitch type fibers, the muscle response is triggered at once by a propagated action potential. In the slow type, the contraction is achieved by local depolarization. In the first case, the response is all-or-nothing, because the action potential is all-or-nothing, while in the latter, the tension is graded because
the depolarization also can be graded. Anatomical foundations have been described for such different modes of activation. In the twitch type, the muscle fibers have focal innervation and well-developed sarcoplasmic reticulum and transverse tubular systems. The slow fibers have multiple motor nerve terminals, and a rather poorly organized sarcoplasmic reticulum essentially devoid of the transverse tubular element (Pilar and Hess, 1966). It is not feasible to deny the possibility that some slow fibers may give rise to an action potential. It may very well be that under the experimental conditions used the slow fibers studied were already depolarized and the spike mechanism for that reason was inactivated. Indeed, an action potential was elicited from one multiply innervated muscle fiber in a previous study (Hess and Pilar, 1963). Likewise, the small spikes recorded after the large spikes in Fig. 5 of the present study may have arisen from muscle fibers innervated by smaller nerve fibers. However, the present results indicate that the contractile mechanism of the slow muscle fibers is activated locally and segmentally. These conclusions contrast with the view put forward by Bach y Rita and Ito (1966) that nerve stimulation produces action potentials in the slow fibers and that the slow fibers are consequently similar to fast fibers.

The following comments suggest a possible explanation for the differences between their observations and those reported here and elsewhere (Hess and Pilar, 1963). To study isolated responses of slow fibers, Bach y Rita and Ito (1966) used anodal nerve block (referred to by them as “anode break”) of rapidly conducting nerve fibers (Burke and Ginsborg, 1956b). However, when this procedure is used, it is necessary to monitor nerve responses to determine whether only small nerve fibers are being activated selectively (Kuffler and Vaughan Williams, 1953a). Such a control was not reported. Furthermore, it has been pointed out by Burke and Ginsborg (1956) that the anodal block technique for selective stimulation of axons innervating frog slow fibers “cannot be used” at stimulating frequencies greater than 10/sec. Above these frequencies axons to twitch fibers are also stimulated. Bach y Rita and Ito (1966) used the technique of Burke and Ginsborg to selectively stimulate axons of slow muscle fibers at frequencies of 15 and 25/sec. However, adequate evidence was not provided that only axons to slow muscle fibers were stimulated under these conditions.

As another criterion to determine which type of fiber was impaled by the electrode, Bach y Rita and Ito (1966) used resting potential values and assumed that slow fibers had resting potentials lower than twitch fibers. However, the values for resting potentials showed considerable overlap; fibers responding to anodal block stimulation had membrane potential values of 20–65 mv, while fibers which did not respond to anodal block showed values of from 20–110 mv. This overlap in resting potentials and a similar overlap reported by Hess and Pilar (1963) emphasize that this criterion does not
discriminate between slow and twitch fibers. It was suggested furthermore, that the low resting potential of slow fibers was caused by mechanical damage produced by micropipette insertion, to which slow fibers were presumably more prone because of their small diameter. However, slow fibers are not necessarily smaller than twitch fibers as found in the guinea pig extraocular muscles (Hess, 1961) and in the cat superior oblique muscle (Hess and Pilar, unpublished observations).

Bach y Rita and Ito (1966) used the asymmetry in the amplitude distribution of miniature potentials (found during spontaneous activity of the muscle fiber) as an indication of multiple innervation. Since a skewed amplitude distribution of miniature and end plate potentials occurs in individually innervated twitch muscle fibers (e.g., Liley, 1956), this criterion by itself does not indicate the presence of slow muscle fibers. The time course of miniature end plate potentials must be considered. The three miniature junctional potentials shown by Bach y Rita and Ito (1966) all have about the same slope of rise time and decay. Since a histogram of the distribution of the temporal course of miniature junction potentials was not presented, it could not be ascertained that the miniature junction potentials arose from slow fibers.

In summary, the contentions put forward by Bach y Rita and Ito (1966) about the similarity between the electrical activity of slow and twitch fibers suffer from the possibility that their intracellular recordings arose from only twitch muscle fibers. This possibility exists since they did not show that they were recording from multiply innervated fibers. Multiple innervation has been established as a distinct criterion for the identification of slow fibers in the cat extraocular muscles (Hess and Pilar, 1963). The present results add further evidence for the existence in the cat extraocular muscles of two distinct systems of extrafusal muscle fibers.

Part of this work was done during the tenure of a visiting fellowship in the Department of Physiology, Australian National University, Canberra, Australia.

The author wishes to thank Professor J. C. Eccles for his hospitality and Drs. C. Eyzaguirre, A. Hess, and R. Close for their criticism during the preparation of this manuscript. He also acknowledges the collaboration of Dr. R. Close in some of the experiments.

This investigation was partially supported by Grant NB 05244 from the United States Public Health Service.

Received for publication 6 April 1967.

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