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Muscle Twitch Tension—Influence of Electrical Stimulating Conditions at Different Temperatures

III. Analysis and Correlation

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§ 1. Introduction

In the previous two papers of this series (Kelly, Fry and Fry 1964, Kelly and Fry 1964), which will be referred to as I and II, we have reported values of the electrical stimulation parameters for eliciting the maximum amplitude of twitch tension of frog sartorius muscle held at various temperatures in the range from 0° to 24°c and in various ionic environments—chloride and nitrate Ringer's. The importance of achieving maximum response is apparent since the observed variations of twitch tension with temperature and ionic environment are ordinarily interpreted to support the currently accepted active state theory of muscle contraction and, therefore, play a major rôle in the description of muscle contraction mechanisms. Our results indicate that the maximum response to single stimuli (at temperatures above 18°c) is considerably greater than values reported by other investigators, and that the large increases in twitch tension reported by previous investigators when a muscle is stimulated in nitrate Ringer's compared with the responses obtained in chloride Ringer's are not observed when the muscle is stimulated to produce maximum twitch tension under *all* conditions. It is shown in I and II that two parameters describing the electrical stimulus, amplitude and *duration* (in our experiments this was between 0·2 and 10·0 msec), must be varied in order to achieve a maximum response, that is, increasing the stimulus amplitude alone for short pulse durations does not result in the maximum response capability of the muscle to single stimuli at all temperatures.

This paper includes a discussion of the results given in I and II, their relation to observations reported by previous investigators, and an interpretation of the new results in terms of basic muscle mechanisms.

§ 2. Analysis

The essential finding of the investigations reported in I is that the maximum amplitude of tension of uncurarized frog sartorius muscle at temperatures above 18°c, in response to a single stimulus delivered by a mass electrode, is considerably greater than indicated by previous

investigators. This finding is critical, since the large number of experiments at such temperatures, previously reported by a number of investigators dealing with a variety of problems, rely for the authenticity of their conclusions on the premise that all fibres within the muscle are responding simultaneously to the stimulus with maximum tension. Optimization of the electrical stimulating conditions accounts for only a part of the additional observed tension. The influence of the dynamic characteristics of the attachments employed to fasten the muscle to the transducer and the frequency response characteristic of the recording system will be discussed in a later paper.

Since stimulation of the whole muscle at high temperatures, with single pulses of longer duration than normally applied, results in high values of the twitch tension amplitude, a question of fundamental importance is whether uncurarized, isolated *single muscle fibres* at high temperature respond with increasing amplitude of twitch tension to increasing stimulus pulse durations. The question is, of course, closely associated with the intensively investigated problem of gradation of tension amplitude in muscle fibres (Lucas 1905, Pratt and Eisenberger 1919, Pratt 1930, Gelfan 1930, 1931 and 1934, Ramsey and Street 1938, 1941 a and 1941 b; Buchthal and Lindhard 1939, Sichel and Prosser 1935, Brown and Sichel 1936). Of this group of investigators the work most directly related to the present study is that of Brown and Sichel (1936) who studied the twitch contraction of single, uncurarized fibres of frog skeletal muscle (at temperatures above 20°C), stimulated by electrodes extending almost the entire length of the fibre. In addition to their finding that the contractions of such fibres are graded, Brown and Sichel indicated that for a constant voltage the total tension developed varies as a function of the *duration* of the applied electrical stimulus.

In view of these results it is of interest to consider the work of Sichel and Prosser (1940) and of Ramsey and Street (1941 a) on single fibre preparations. Both groups of investigators were concerned with the relation between the refractory period and the summation of twitch tension following a second single stimulus. Sichel and Prosser (1940), working in the temperature range 15° to 25°C, used isolated cut lengths (approximately 20 mm) of single fibres of skeletal muscle, mounted between two silver strip electrodes so that the fibres were stimulated all along their lengths. These authors point out that although the twitch may be graded and non-propagated in such preparations, it resembles in its form and time relations the isometric twitch of whole muscles and, therefore, it has been assumed that the release of energy involves the same mechanism in both cases. The experimental procedure consisted in determining whether a second, single stimulus applied within a limited time period after the first stimulus (usually 0 to 30 msec) would result in an increase in the isometric *peak* twitch tension compared with the peak tension elicited with a single stimulus. The results indicated that the second stimulus causes an increase in peak twitch tension and that the amount

of this increase is most pronounced when the time interval between the initial and second stimulus is *brief* (only a minor increase occurs for spacing intervals greater than 10 msec). The greatest increase in peak tension was recorded when the time between the two stimuli was zero, i.e. when the shocks were ' synchronous '. In fact, under these circumstances, the tension developed was always more than double that due to either stimulus alone. Since the single fibre section always responded to the second stimulus with a greater peak twitch tension, and since there was no indication of the existence of a time interval following the first stimulus during which the muscle was unresponsive, Sichel and Prosser concluded that for such isolated lengths of single fibres they obtained no evidence in support of the existence of a refractory period. However, there is another possible interpretation of the data which these authors apparently did not consider. Namely, that the greatly increased peak twitch tension observed for ' synchronous ' stimuli is due to the application of a stimulus of double the pulse duration of the ' single ' stimuli (the experimental method indicates the time constant for single stimuli was 1·1 msec, while for the ' synchronous ' stimuli the time constant was 2·2 msec).

Ramsey and Street (1941 a), in similar experiments on summation in response to two separated stimuli, used isolated, uninjured single fibres of skeletal muscle stimulated by means of a pore electrode. The stimulus pulse duration was 0·3 msec, and the temperature was in the range 8·5° to 14°C. Their results indicate that both the propagated and the local excitatory process exhibit absolute refractory periods with durations which vary inversely with the temperature. The observed increase in peak twitch tension due to the second stimulus was greater the *longer* the interval between stimuli (which must not exceed the time limit for summation), a result opposite to that found by Sichel and Prosser. In addition, Ramsey and Street found that at low temperature the twitch tension amplitude approached the magnitude of tetanus tension. These investigators apparently feel that a linear relation exists between the twitch tetanus ratio and the duration of the refractory period. In a recent discussion of this early work, Ramsey (1962) shows a twitch tetanus ratio of approximately 0·8 for single fibres at low temperatures and 0·1 to 0·2 for such fibres at 20°C. It appears reasonable to conjecture that the apparently opposing results of the two groups of investigators may well be the result not only of the different techniques used for stimulation but also the result of the different temperatures and stimulus pulse durations employed.

Consider now Ramsey and Street's finding of a twitch tetanus ratio of 0·1 to 0·2 for single fibres at 20°C. In our investigations on whole muscle (I), twitch tetanus ratios of 0·7 to 0·8 were obtained at temperatures of approximately 20°C, if stimuli of appropriate durations were used. These high values were obtained despite the fact that the tetanus tension magnitudes on a per unit area basis were, on the average, in the upper part of the range (some are higher) recently quoted by Ramsey (1962) as

the maximum tension capability for single skeletal muscle fibres ($3–4 \times 10^6$ dyne/cm^2). It should be considered, therefore, that the twitch tension amplitudes obtained by Ramsey and Street at high temperatures for single muscle fibres stimulated with short duration stimuli by means of a pore electrode may be considerably below maximum twitch capability.

It is of interest to compare the results obtained on isolated single fibre preparations (Sichel and Prosser 1940, Ramsey and Street 1941 a) with the results obtained on whole muscle, in regard to the effect of a second single stimulus on the amplitude of tension responses. Hill (1949) indicates that the first single maximal stimulus applied to a muscle elicits the full active state process of the muscle, and that a second single stimulus can " do no more than bring the active state back to its full intensity again ". In considering what interval of time between the two stimuli will result in the greatest amplitude of total tension, Hill reasons that if the second stimulus is applied when the series elastic component has already been stretched, i.e. when the force developed by the first stimulus is maximum, then the greatest total tension will be developed for that time interval. As indicated by Hill, this hypothesis is apparently substantiated by an experiment of Hartree and Hill (1921) on frog sartorius muscle at 10°c stimulated directly with two single pulses with varying time intervals between shocks, but is not in agreement with results obtained by Cooper and Eccles (1930) on the effect of indirect second single stimuli on amplitude of tension of a cat soleus muscle at temperatures in the range 34° to 37°c. The studies of Eccles and O'Connor (1939) and Brown and Matthews (1960) are also of interest, since both found that a second single stimulus applied to the nerve of a cat muscle, *a brief interval* after the first stimulus (of the order of 2 msec), resulted in a tension response with an amplitude which was at least twice that elicited by the single maximal stimulus. Since the generally accepted view is that a single maximal stimulus elicits the full twitch response, the increased amplitude of tension recorded after a double stimulus is regarded by the above authors as a double response, irrespective of the time interval between stimuli. However, it is of interest to consider the possibility that single maximal stimuli of the type generally applied at the present time in both direct and indirect stimulation experiments do not elicit complete twitch responses of the muscle at all temperatures. Walker (1960) found for indirectly stimulated rat muscle that the maximum tension response is obtained when the interval between shocks is very short (of the order of 2·8 msec) and he also observed an increase in velocity of tension development compared with that elicited by a single shock. It is also of considerable interest that in recent studies on locust flight muscle at 35°c (fig. 5 of Neville and Weis-Fogh 1963) the authors found: (*a*) a response resembling a *single* twitch of increased tension is elicited when a second single stimulus is applied 2 msec (which is considerably less than the spike duration) following the first, (*b*) a small increase is observed in the shortening velocity of the response when a second stimulus

is applied after a brief interval, and (*c*) a response showing either a change of slope or a second peak is observed when the interval between stimuli is prolonged.

Walker's (1948, 1949 and 1960) finding for rat muscle that the effect of a second indirect single stimulus, *applied after a brief interval,* on the amplitude of twitch tension is temperature dependent, i.e. as the temperature is decreased the large potentiation due to the second stimulus is abolished or greatly reduced, correlates with the temperature effect found by us in frog muscle (I), namely, that at high temperatures large increases of peak twitch tension occur when the pulse duration of the single stimulus is extended within the range 0·2 to 10·0 msec, but only minor increases occur for such extensions of pulse durations if the muscle is at a low temperature. It is of interest that in an evaluation of his earlier studies on repetitive stimulation and quick stretch, Walker (1960) concludes that the active state is *not* fully developed in a normal twitch at high temperature and suggests that " isometric twitch tension is smaller than that in tetanus because the muscle does not contract completely in the twitch responses ".

It can be postulated that the potentiation of tension observed when a second single stimulus is applied directly after an interval such that a second peak or change in slope of the rise time of the myogram can be detected, is probably associated with a ' summation mechanism ' of individual responses which is related to the summation process operating in a tetanus. However, the mechanism involved in the twitch potentiation observed by Walker (1960), when the time interval between the indirectly applied stimuli is short and the resultant response appears to resemble a single twitch with increased velocity of tension development, may be related to the mechanism underlying the effects observed in the present series of investigations for uncurarized muscle when the pulse duration of the stimulus is increased.

After considering all of the factors previously discussed it would appear of some importance that the peak response of uncurarized skeletal muscle at relatively high temperatures can be increased by a number of methods (double stimuli, post-tetanic stimuli, increased duration of stimulus, treppe and substitution of a certain class of anions for the chloride of Ringer's), but the effect of all these on the amplitude of tension is considerably decreased when the temperature of the muscle is reduced. Another method not already mentioned for influencing the amplitude of peak twitch tension of skeletal muscle, namely, application of high pressure to the muscle, is also of interest to the present study, particularly with regard to the pressure-induced twitch potentiation observed at high temperature and its absence at low temperature (Cattell and Edwards 1928 and 1932, Brown 1934 and 1935, Cattell 1935).

In attempting to identify possible common features of basic mechanisms underlying the operation of all these potentiating methods, and in order to correlate the effects of temperature variation and curarization on the

operation of these mechanisms, the studies of Bigland, Goetzee, Maclagan and Zaimis (1958) and Cannard and Zaimis (1959) on the effect of lowered temperatures on the action of neuromuscular blocking drugs on mammalian skeletal muscle are also of interest. The essential finding of these investigators is that reduction of temperature results in different actions for depolarizing and non-depolarizing drugs respectively. For the two depolarizing drugs tested (decamethonium and suxamethonium) it was found that lowering of the temperature increases the magnitude of the effect of the drugs on the myoneural junction and prolongs the duration of their action. For the non-depolarizing drug, tubocurarine, it was found that lowering of the temperature *reduces* the magnitude of its neuromuscular effect. Since tubocurarine presumably acts as a neuromuscular blocking agent by competing with acetycholine, thus preventing its depolarizing action by excluding it from the endplate receptors, the above authors postulated that the decreased effectiveness of tubocurarine at low temperatures could be explained by an *increased* effectiveness of the acetylcholine at low temperatures (similar to that exhibited by decamethonium and suxamethonium).

One obvious conjecture regarding the action involved in all the methods listed for potentiating the twitch tension amplitude of the *uncurarized* muscle at high temperatures is that such action is intimately associated with the acetylcholine mechanism operative at the myoneural junction,† since the effectiveness of all the techniques is greatly reduced at low temperatures. Thus, the low amplitudes of twitch tension at *high temperatures* observed by previous investigators (employing stimuli durations which resulted in large twitch amplitudes at low temperatures) may be associated with the fact that the acetylcholine mechanism was not adequately stimulated by the short duration stimuli, whereas the large amplitudes of tension obtained for uncurarized muscles in response to a single stimulus at high temperatures in our investigations (I) could be attributed to adequate stimulation of the acetylcholine system by the longer duration stimuli. The effect of certain anions, such as nitrate, on the twitch response of skeletal muscle may be associated with an action to increase the effectiveness of the acetylcholine mechanism at the myoneural junction similar to the effect of decreased temperatures. The latter postulate would explain why agents such as nitrate Ringer's do not produce any substantial effect on twitch tension at the lower temperatures because the acetylcholine system, more effective at lower temperature, causes essentially adequate stimulation of the muscle in the chloride Ringer's. However, the application of such agents to inadequately

† The results of end plate potential studies should also be considered in formulating basic mechanisms underlying muscle stimulation, but this aspect will not be discussed here. However, the studies of N. Takeuchi (1958), Eccles, Katz and Kuffler (1941), Fatt and Katz (1951), and Kostiuk (1957) are of particular interest in regard to some of the points discussed in this paper.

stimulated muscles results in increased magnitudes of twitch tension because the electrical stimuli, which are inadequate in chloride Ringer's, are in this environment more capable of stimulating the acetylcholine mechanism. This is consistent with the fact that when ' adequate ' stimulation of the muscle in chloride Ringer's is provided (for example, by applying a long duration stimulus) then only a small increment in the amplitude of twitch tension is observed in nitrate Ringer's (II). It is also consistent with the finding that in nitrate Ringer's, increasing the duration of the stimulus in the range 0·2 to 10·0 msec results in only minor increases of tension (II). The above outlined conjecture would obviously require the further assumption that despite the use of mass electrodes in our experiments, the myoneural junction would be involved in the stimulating mechanism because of the uncurarized state of the muscle (Buchthal and Lindhard 1939).

However, consideration of the data on the curarized muscle at high temperatures presented in (I) requires an extension of the acetylcholine mechanism hypothesis. In this regard it should be recalled that we have found that in the high temperature range curarization did not affect the amplitude of tetanus tension, but the peak twitch tension was considerably smaller than the tension observed for an uncurarized muscle in response to a single stimulus of optimum duration. Furthermore, the peak twitch tension of the curarized muscle could not be increased by prolonging the duration of the stimulus beyond 0·2 msec. If the applied electrical stimulus is assumed to depolarize the curarized muscle directly (i.e. without intervening steps in the mechanism), then it is difficult to explain why the twitch tension amplitude observed for a curarized muscle is less than the twitch tension amplitude of the uncurarized muscle (both stimulated by single ' supermaximal ' pulses) while the tension amplitude in response to a tetanus stimulus is essentially the same for both the curarized and uncurarized preparations. In considering this dilemma, it should be recalled that the long duration single stimuli applied to uncurarized preparations do not elicit the twitch response of the muscle by some repetitive discharge mechanism not operating when the muscle is stimulated by the short duration single pulses (I).

The results obtained by us for both the uncurarized and curarized muscles may be explained by postulating that the excitation-contraction mechanism involving acetylcholine and the action of its antagonist, curare, is not restricted to the neuromuscular junction. In the case of the uncurarized muscle, at both low and high temperatures, this postulate does not require any change in the previously outlined arguments regarding the acetylcholine mechanism except that it must be assumed that the acetylcholine is acting all along the muscle membrane rather than being restricted to the endplate region. For the curarized muscle the low twitch tension output at high temperatures, compared with the high tetanus tension output at the same temperatures, can be explained if it is postulated that electrical stimulation at a high repetition rate is an

effective means of eliciting along the length of the fibre a sufficient concentration of acetylcholine so that, despite the presence of curare, maximum tetanus tension is developed. However, a single stimulus, even one of long duration, is not capable at these high temperatures of causing sufficient concentration of acetylcholine to overcome the action of curare (which is most effective at high temperatures) with the result that the twitch response is not maximal. At low temperatures, the single stimulus elicits a large amplitude of twitch tension for the curarized muscle. Again this may be explained by the more effective action of acetylcholine at low temperatures and the less effective action of curare.

In this respect it is of interest to consider the results of Ochs and Mukherjee (1959) who suggested that acetylcholine acts as a depolarizing agent all along the muscle membrane and that its antagonist, curare, also has a generalized action along the membrane. Further, Ochs, Annis and Mukherjee (1960) found that succinylcholine (a drug which belongs, like acetylcholine, to the general class of so-called depolarizing neuromuscular blocking agents) decreased the amplitude of the isotonic twitch tension of a frog sartorius muscle stimulated by a *direct* supermaximal stimulus. Pre-treatment of the muscle with curare prevented the action of succinylcholine on the tension amplitude. As indicated by Ochs *et al.* these results suggest that theories which relate the effects of depolarizing blocking agents exclusively to muscle endplate mechanisms do not appear justified, since the muscles in the above-mentioned experiments were *directly* stimulated and, therefore, a disturbance restricted to the endplate should not have affected the amplitude of twitch tension. Katz and Miledi (1961) in an attempt to duplicate the experiment by Ochs *et al.* found that addition of succinylcholine to a directly stimulated muscle did *not* result in any diminution of the twitch tension amplitude. In view of the results discussed here, it is of interest that Katz and Miledi applied single stimulus pulses, ranging in duration from 0·2 to 0·5 msec, while Ochs *et al.* used rather long duration stimulus pulses, namely 5 msec.

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Summary

Previous investigators have explained many of the effects induced by changes in both physical and chemical parameters on the amplitude of peak twitch tension as a direct consequence of changes in the duration of the active state. It is pointed out that recent experimental results indicate that the application of the active state concept to correlate earlier data on the variation of twitch tension with changes in temperature and chemical environment may now be open to question.

A basis for correlating the data of a number of previous investigators on the amplitude of the single response of skeletal muscle under various conditions of stimulation with that of the present authors is presented. Arguments are formulated for the viewpoint that the acetylcholine coupling mechanism is not restricted to the endplate region.

Résumé

Tension de secousse musculaire—l'influence de conditions stimulantes électriques à différentes températures. III. Analyse et corrélation

Les investigateurs antérieurs avaient expliqué beaucoup d'effets causés par les changements de paramètres physiques et chimiques sur l'amplitude de la tension de crête de la secousse comme la conséquence directe des changements de la durée de l'état actif. On montre que d'après les résultats expérimentaux récents l'emploi de l'idée de l'état actif pour mettre les résultats antérieurs, relatifs à la variation de la tension de secousse, en corrélation avec les changements de la température et dans l'ambiance chimique, peut être mis en question à présent.

On présente une base pour mettre les résultats d'un nombre d'investigateurs antérieurs, relatifs à l'amplitude de la réponse unique du muscle strié dans les différents conditions de stimulation, en corrélation avec les résultats des auteurs. On formule les thèses pour le point de vue, que le mécanisme d'accouplage à l'acétylcholine n'est pas limité à la région de la plaque motrice terminale.

Zusammenfassung

Muskelzuckungsspannung—der Einfluss elektrischer Reizbedingungen bei verschiedenen Temperaturen. III. Analyse und Korrelation

Die früheren Forscher haben zahlreiche Effekte, die von den Veränderungen der physikalischen und chemischen Parametern in der Amplitude der Spitzen-Zuckungsspannung hervorgerufen wurden, als unmittelbare Folge der Veränderungen in der Dauer des aktiven Zustands erläutert. Es wird gezeigt, dass, den jüngsten Experimentaldaten gemäss, die Anwendung des Begriffs des aktiven Zustands für die Korrelation früherer Ergebnisse der Zuckungsspannungänderung mit der Veränderung der Temperatur sowie der chemischen Umgebung nunmehr als fraglich betrachtet werden kann.

Man legt eine Basis dar für die Korrelation der von einer Anzahl früherer Forscher erhaltenen Ergebnisse betr. die Amplitude des Einzelansprechens eines Skelettmuskels bei verschiedenen Reizbedingungen mit den von den Verfassern erhaltenen. Es werden Beweise für den Gesichtspunkt formuliert, dass der Azetylcholinkopplungsmechanismus nicht auf die Endplatte eingeschränkt wird.

Резюме

Напряжение мышечной судороги—влияние электрических стимулирующих условий при разных температурах. III. Анализ и корреляция.

До сих пор исследователи объясняли многие эффекты, вызываемые изменениями физических и химических параметров, в амплитуде пикового напряжения судороги, как непосредственное следствие изменений продолжительности активного состояния. Указывается, что, согласно последним экспериментальным данным, применение понятия активного состояния для коррелирования прежних данных по изменению напряжения судороги с изменениями температуры и химической среды может требовать дальнейших доказательств.

Предлагается исходный пункт для коррелирования данных, найденных многими исследователями, по амплитуде единичной реакции скелетной мышцы при разных условиях, стимуляции, с данными авторов. Формулируются доводы для точки зрения, согласно которой механизм ацетилхолиновой связи применим не только к концевой пластинке.

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