

ON THE COUPLING RELATION BETWEEN ACTION POTENTIAL AND MECHANICAL RESPONSE DURING REPETITIVE STIMULATION IN FROG SARTORIUS MUSCLE

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Since SANDOW (1952) studied on the excitation-contraction coupling in skeletal muscle response, several works have been reported on the augmentation of twitch tension by specific anions without accompanying the change of action potential contour (HILL & MACPHERSON, 1954; EDWARDS, RITCHIE & WILKIE, 1956; LUBIN, 1957). On the other hand, the separation of tension response from action potential in hypertonic solution (HODGKIN & HOROWICZ, 1957; HOWARTH, 1958) or by stretching (HAKANSON, 1957) was also pointed out. And the coupling mechanism has been analysed, regarding mainly as the activating process for contraction for the steady state of the muscle (CSAPO, 1959; HODGKIN & HOROWICZ, 1960a, b). However, many processes must exist between action potential and mechanical response, at least two groups should be distinguished, namely the activating process for contraction and the mechano-chemical process in contraction.

The present study was carried out with the purpose of learning the properties of coupling relation between action potential and mechanical response during repetitive stimulation.

METHODS

The material used was the sartorius muscle of the frog (*Rana nigromaculata*). Main experimental set-up illustrated in FIG. 1 was a modification from that of NIEDERGERKE (1956) in the cardiac muscle or SCHAECHTELIN (1961) in the rectus muscle of the frog. The plastics bath filled with Ringer solution was separated into two chambers by the horizontal partition plate, through the dip of which the muscle preparation was mounted vertically. The pelvic end of the muscle was clamped to the base of the bath and the tibial tip came out of the solution into air and connected to RCA 5734 tube with fine silver wire, which worked as one of the stimulus electrode. It was necessary to compress the

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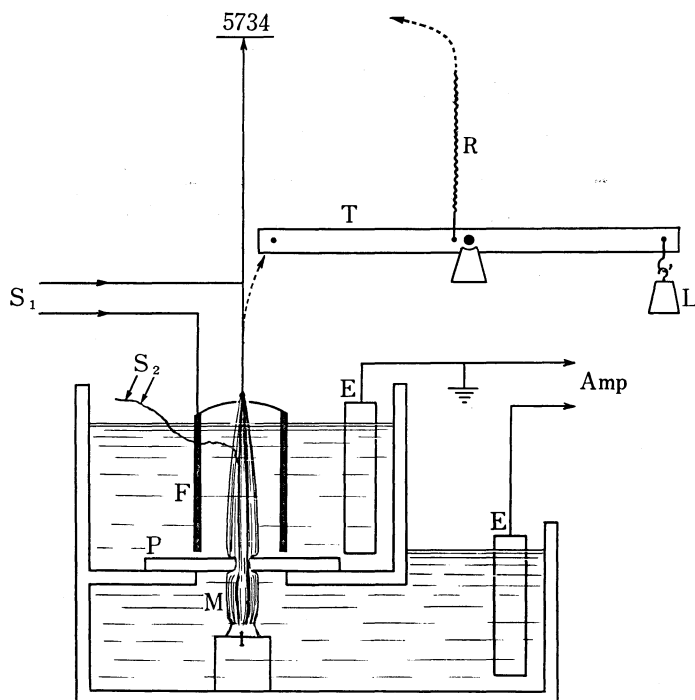


FIG. 1. The arrangement of experimental set-up.

E, Ag-AgCl electrode; F, platinum foil electrode; L, load; M, muscle; P, partition plate; R, rubber thread; S_1 , S_2 , direct and indirect stimulus; T, isotonic lever; Amp, DC-amplifier; 5734, RCA 5734 tube. Dotted line with arrow shows the isotonic recording.

muscle slightly by partition plates with little vaseline from both sides. When the isotonic shortening was to be recorded, the silver wire was led to the one arm of isotonic level (FIG. 1, T) and the nearest point from the axis of the lever was jointed with thin rubber band (FIG. 1, R) to the tube. If the elongation of the rubber band was large enough compared with the force product by the load (FIG. 1, L), an isotonic record was easily obtained (HILL, 1953a). The other stimulus electrode was cylindrical platinum foil placed in the upper bath surrounding the muscle. The lead-off electrodes were non-polarizable Ag-AgCl plates, dipped in upper and lower baths. When it was necessary to stimulate the sartorius nerve, usual silver wire electrodes were used, which hung up the nerve within liquid paraffin superposed on Ringer solution.

In order to obtain monophasic action potential, 0.06% procain was added in the lower bath. Ringer solution contained NaCl 110 mM, KCl 2.0 mM, CaCl_2 1.8 mM, NaHCO_3 10 mM and glucose 0.1%, and pH was 7.2. In most cases 6×10^{-6} D-tubocurarine (Amerizol) was added to Ringer solution to block neuromuscular transmission. Tension curve and action potential were recorded simultaneously with a two-gun oscilloscope through DC-amplifiers, the sweep speed of each beam was adjustable independently from another beam. Stimulus was supramaximal square pulse with duration of 0.1-0.5 msec. The threshold was lower when the cathode was applied to tibial end than the anode was. In supramaximal stimulus, however, anelectrotonic inhibition by large anode in the

bath disturbed the contraction. To avoid this inhibition anode was always fixed to the tibial end.

Checking the action potentials of single muscle fibre, ordinarily intracellular micro-electrode was also used in some experiments. The muscle was fixed horizontally and stretched by 30% of physiological length. Two microelectrodes were inserted into single muscle fibre under binocular more than 3 mm apart from each other to minimize the injury by movement, one of them was stimulus electrode. Resistance of electrode was around 15–20 M Ω .

RESULTS

1. *Preliminary experiment.* The whole muscle preparation was divided longitudinally into two bundles and then it was fixed in the bath. FIG. 2, B was obtained when the one half was stimulated by single shock, and FIG. 2, C was obtained when the other half was stimulated. On the other hand, when two halves were stimulated at the same time, FIG. 2, A was obtained. As for the peak tension of twitch (lower records), the sum of tensions from two halves should be equal to the tension of whole muscle. Actually that was proved. Not only in the peak tension but also in the amplitude of action potential (upper records) the situation was the same. Namely the amplitude in FIG. 2, A was the sum of those in FIG. 2, B and C. From this observation the amplitude of action potential as well as the twitch tension obtained by this partition

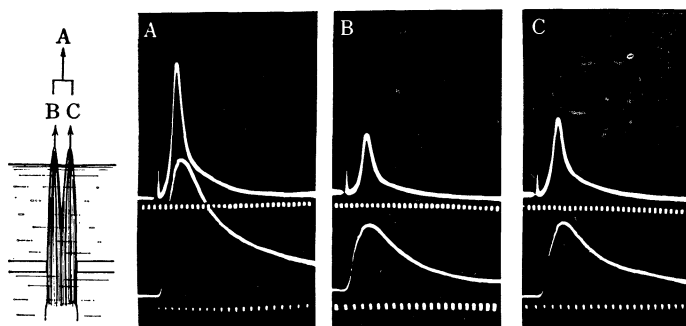


FIG. 2. Action potential and mechanogram obtained from the whole muscle (A) and two halves of it (B and C). The schematic diagram of arrangement is shown on left. 16°C. Time, upper record, 1 msec; lower record, 20 msec. (see text)

method is thought to be the total sum of these from each fibre in whole muscle. Therefore, much advantage accrues to the use of whole muscle instead of single fibre in establishing the height of action potential or tension.

2. *Action potentials and twitch tensions in repetitive stimulation.* As NIEDERGERKE (1952) pointed out, the partition method suffers from some uncertainties, then the experiment was done using only fresh muscles in good condition. In good preparation the shape of action potential did not change for about 30 minutes

after dissection.

When the muscle was stimulated at 2 c/sec, peak tension showed little augmentation, known as the staircase phenomenon, in first several twitches and then it began to decrease (FIG. 3). The relative sizes of action potential and peak tension are shown in TABLE 1. In FIG. 4, these relative sizes were

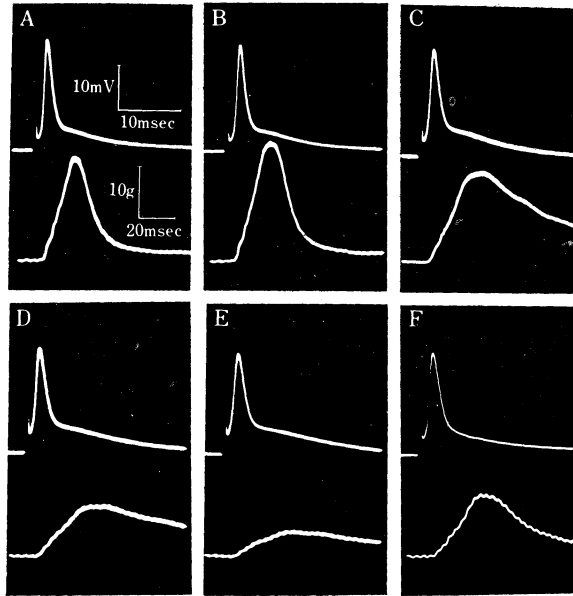


FIG. 3. The effect of repetitive stimulation at 2 c/sec on the action potential and mechanogram. Upper record is action potential, lower record is mechanogram. Both were obtained simultaneously but at the different sweep speed. A, control; B, after 30 stimuli, marked augmentation in peak tension is seen; C, after 150 stimuli; D, after 240 stimuli; E, after 360 stimuli; F, 1 min. rest after E. 25°C. (see text)

TABLE 1.

The effect of the number of stimuli applied at 2 c/sec on the action potential and mechanical response. Average of 6 cases.

number of stimuli	50	100	150	200	300	400	1000
relative size of spike height	99	98	95	93	89	85	63
relative size of peak tension	89	76	65	56	42	28	10
coupling efficiency at 16°C	0.90	0.78	0.68	0.60	0.47	0.33	0.16
coupling efficiency at 24°C	1.12	1.10	1.05	0.93	0.71	0.57	
coupling efficiency $\times 0.85^*$	0.95	0.94	0.89	0.79	0.60	0.48	

* The factor is the ratio of peak tension at 25°C compared with that of room temperature 15°C. Average of 13 cases.

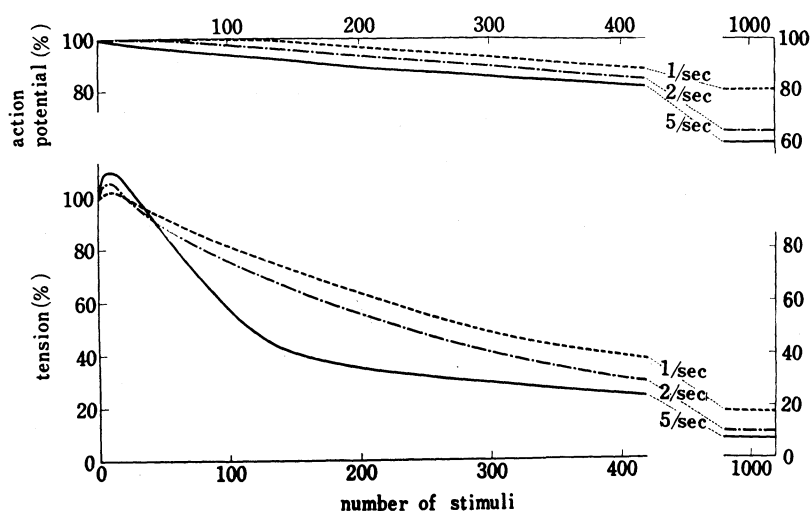


FIG. 4. The course of decrease in the relative size of spike height and peak tension during repetitive stimulation at various stimulating frequencies. 16°C .

plotted against the number of stimuli. The rate of decrease in the relative size of peak tension was clearly faster than that of action potential. Moreover, when the stimulating frequency was raised to 5 c/sec, the peak tension decreased much faster, but action potential did not so. It is convenient to introduce the coupling efficiency (CE) in a given twitch; that is the ratio of relative size of peak tension divided by that of spike potential at the N th twitch in F c/sec stimulation. Then, $\text{CE}_{N(F)}$ was defined as follows:

$$\text{CE}_{N(F)} = \frac{\text{Relative size of peak tension (or shortening)}}{\text{Relative size of spike potential}}$$

Here, the spike duration or the height of negative after-potential was not adequate for the denominator, because they were increased when the peak tension decreased (see FIGS. 3 and 6). The spike height showed the strongest correlation with the peak tension. From TABLE 1, an increase of the number of stimuli clearly resulted in a decrease of CE, that means the fall of coupling efficiency. When the stimulating frequency increased, the coupling efficiency always decreased, except the period of staircase.

In FIG. 3, the spike duration and the height of negative after-potential increased by about 50% after 360 stimuli. After 1 min. rest, the recovery of action potential was so satisfactory and the spike height showed more than 90% recovery. But the peak tension recovered only 60% of the control. Generally, the recovery of peak tension was better in acute fatigue, for example the recovery after 900 stimuli at 30 c/sec is shown in FIG. 5. In this case the peak tension recovered almost completely, but the time course of it was slower

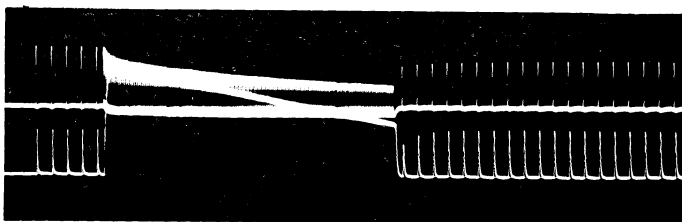


FIG. 5. The recovery of spike height (upper record) and tension (lower record) from acute fatigue. Tetanic stimuli of 30 c/sec were applied for 30 sec. Before and after the tetanization, the muscle was stimulated at 0.6 c/sec. The spike height recovered faster than mechano-gram. 16°C.

than that of spike height.

3. *Diminution and recovery of intracellularly recorded action potential in repetitive stimulation.* The decrease of action potential of whole muscle might be interpreted by two different reasons, one is the diminution in action potential of each muscle fibre, and the other is the firing failure of fatigued fibres. Even if each fibre produces action potential with all-or-none property, action potential of whole muscle could run down when some fibres would escape to fire during few stimuli due to their raising threshold. To avoid the firing failure the stimulus was always supramaximal, and sometimes much stronger stimulus was applied in the middle of experiment, but the course of decrease in the size of action potential was affected little or not at all. Therefore, the possibility of firing escape would be neglected. Moreover, the action potentials of single muscle

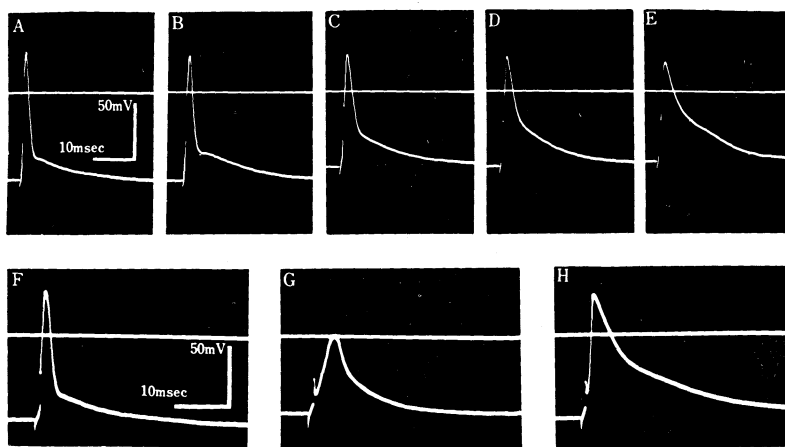


FIG. 6. The effect of repetitive stimulation on the intracellularly recorded action potential. The stimulating frequency in the upper series (A to E) was 1 c/sec. A, control; B, after 60 stimuli; C, after 280 stimuli; D, after 420 stimuli; E, after 1000 stimuli; F, control of another example; G, after 1500 stimuli at 5 c/sec; H, recovery in 2 min. rest after G. 16°C. (see text)

fibre obtained by intracellular microelectrode technique are shown in FIG. 6.

After 280 stimuli at 1 c/sec, the resting potential showed a depolarization of 8 mV, despite no marked decrease in overshoot potential, and after 420 stimuli

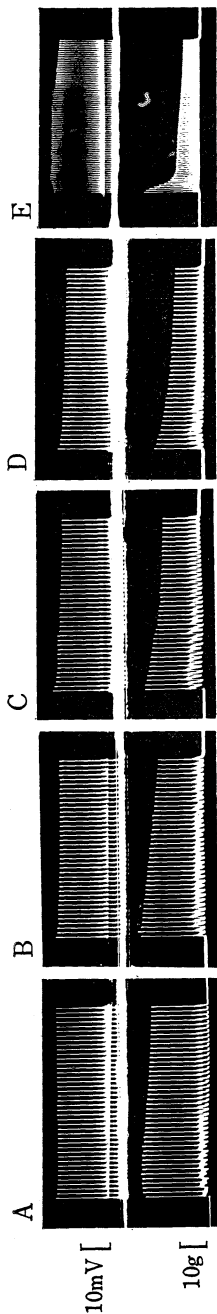


FIG. 7. The effect of fatigue on the spike height (upper record) and tension (lower record). A, the first set of 40 stimuli at 2 c/sec; B, C and D are the second, third and fourth set. One min. rest was inserted between each set. E, another example stimulated at 5 c/sec for 16 sec. 25°C. (see text)

overshoot potential showed little decrease of 5 mV. The relative amplitude of spike potential became 88% at the 280th, 85% at the 420th and 77% at the 1000th action potential (FIG. 6, A to E). These values conformed to the 1 c/sec curve in FIG. 4. Another example is shown in FIG. 6, F to H, in which, after 1500 stimuli at 5 c/sec, overshoot potential disappeared completely, and the recovery of spike height was still satisfactory after 2 min. rest. Then, the injury around the inserted electrodes would not be serious.

As for the spike duration and the height of negative after-potential, the values were 2.5 msec and 18 mV in control, 2.9 msec and 22 mV after 60 stimuli, 3.9 msec and 29 mV after 280 stimuli, 4.3 msec and 34 mV after 420 stimuli and 6.4 msec and 40 mV after 1000 stimuli. These prolongation and augmentation did not recover. But these values showed good agreement with the observations in the whole muscle by the partition method.

4. *The effect of fatigue and temperature on the coupling efficiency.* Four sets of experiment are shown in FIG. 7, A to D. In each of them the whole muscle were stimulated for 20 sec at 2 c/sec, and 1 min. rest was inserted between each set. The coupling efficiency $CE_{40(2)}$ was 0.94 in A, 0.90 in B, 0.69 in C and 0.62 in D. From these figures, it is apparent that the coupling efficiency in given condition decreases progressively by repeating the stimulation. It is natural that in fatigued or unfresh muscle the coupling efficiency fell down. In FIG. 7, E, an extreme example is shown, where $CE_{80(5)}$ fell down to 0.34 by previous stimulation in addition to higher stimulating frequency.

The effect of temperature on the coupling efficiency was not simple. From the figures in TABLE 1, the coupling efficiency was increased

at higher temperature. But at higher temperature, the control size of peak tension decreased, though the spike height did not show much change, in agreement with the observation by MACFARLANE & MEARES (1958). For example, at 25°C the relative peak tension became 85% of the value at 15°C. Then, CE at 24°C multiplied by factor 0.85 were seen in TABLE 1. These are larger than that of 16°C with number of stimuli. This fact suggests that for single stimulus the coupling efficiency is much better at lower temperature, but for repeated stimuli it falls down faster at lower temperature. Actually the staircase phenomenon, during which CE was larger than 1, lasted for much longer time at higher temperature.

5. *Action potential and shortening in repetitive stimulation.* Instead of isometric tension, the isotonic shortening was recorded accompanying action potential. In isotonic condition (FIG. 8), the course of decrease of the shortening depended upon the load which the muscle had to pull up. But it was notable that the

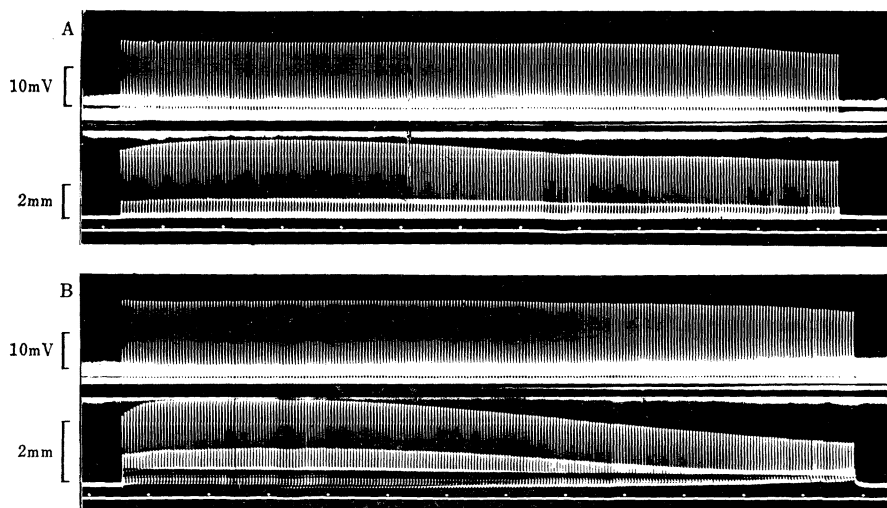


FIG. 8. The effect of the load on the spike height (upper record) and shortening (lower record) during repetitive stimulation at 2 c/sec. The load was 1 g in A, and 5 g in B. Two sartorius muscles from the same frog were used in A and B. In both A and B, the period of augmented twitches lasted for about 32 sec. Time, 5 sec. 29°C. (see text)

period of augmented twitch in staircase phenomenon or the course of decrease of the spike height was hardly affected by the load. Therefore, heavy load or much work could facilitate the dissociation of the shortening from the action potential. These observations suggest that there are two factors within the coupling process; the one can be affected by the external mechanical condition, but the other can not, as if a trigger reaction can not be affected by a modifier of main reaction.

6. *Action potential and tension in indirectly stimulated muscle.* The muscle was stimulated without curarization indirectly through the sartorius nerve. The relative spike heights in muscle during repetitive stimulation are shown in TABLE 2. The relative spike height of indirectly stimulated muscle was smaller

TABLE 2.
The relative size of spike potential during repetitive stimulation. 15°C.

number of stimuli		100	200	300	400
indirect stimulation	2 c/sec	96	92	88	65
	5 c/sec	91	82	68	48
	10 c/sec	79	71	61	53
	20 c/sec	81	70	56	41
direct stimulation	2 c/sec	98	93	89	85
	5 c/sec	94	88	85	82
	10 c/sec*	82	78	76	
	20 c/sec*	82	74	69	

* intracellular recording

than that of directly stimulated muscle in the range beyond 300 stimuli at 2 c/sec, or 200 stimuli at 5 c/sec, of course in these ranges the coupling efficiency became much smaller. In addition, the course of decrease in the spike height was not regular, showing rhythmic fluctuation even in the case of 2 c/sec stimulation.

DISCUSSION

SANDOW (1952) confined the term 'excitation-contraction coupling' in a spike-activation link or the process from membrane depolarization to activating process for contraction. But when we compare the tension with action potential, we can not neglect the contraction process itself. In the present study we adopted the definition proposed by GOODALL (1960) in which the excitation-contraction coupling meant how much mechanical activity followed a membrane depolarization of known amplitude and duration. Following this definition, the excitation-contraction coupling, better say 'electro-mechanical coupling', should be divided into two processes, that is an activating process for contraction and a mechano-chemical process of energy mobilization in contraction; and the former might be a trigger reaction for the latter. For the steady state case or the case in which the mechano-chemical process would not be affected, the relation between action potential and tension could represent the spike-activation link. Thus HODGKIN & HOROWICZ (1960a) assumed the relations from membrane depolarization to the formation of some end-product necessary for contraction. In repetitive stimulation the state in muscle was not steady, it changed from

stimulus to stimulus. However, for the first several twitches it would be stable. Therefore, the augmentation of peak tension in staircase phenomenon could be due to a transient improvement of spike-activation link. Actually the period of augmentation was not affected by external mechanical condition. The fact that the period was greatly prolonged at higher temperature suggests that the activating process is not simply physical reaction, like an ionic exchange through membrane.

On the other hand, the fall of coupling efficiency during repetitive stimulation was attributable to a deficiency of contraction process. MERTON (1956) and EDWARDS & LIPPOLD (1956) described the tension fall with simultaneous augmentation of electromyogram in human muscle. At low frequency stimulation, contraction fatigue would be more prominent than the fatigue in transmission or spike-activation link.

According to LUCO & ROSENBLUETH (1939) and DEL POZO (1942), the transmission fatigue was found when the circulated muscle of the cat was stimulated indirectly at more than 30 c/sec, but at less than 20 c/sec the deficiency in contraction system was rather prominent. But in the frog, the previous stimulation resulted in a depression of action potential of the muscle, even when the interval between two shocks was more than 10 sec (TAKEUCHI, 1958). And the safety factor of transmission is not sufficient in frog. That will be the reason why the transmission fatigue was observed at 2 c/sec stimulation in the range beyond 300 stimuli.

The duration of full active state immediately after action potential has been measured by HILL (1949, 1951, 1953b) and MACPHERSON & WILKIE (1954), and the active state was prolonged by some drugs or ions (RITCHIE, 1954; EDWARDS et al., 1956; LUBIN, 1957; HUTTER & NOBLE, 1960) and by previous stimulation (RITCHIE & WILKIE, 1955). From these findings the staircase and post-tetanic potentiation (BROWN & EULER, 1938) would be attributed to a prolongation of active state, and the efficiency of spike-activation link should have an intimate relation to the duration of active state.

According to SANDOW (1952) and MASHIMA (1959), the safety factor of action potential for contraction was so large that the peak tension did not decrease until the action potential was diminished by 75% deficiency of external sodium. Nevertheless, the coupling efficiency decreased progressively during repetitive stimulation, and the decrease was accelerated by heavy load. These observations indicate that the fatigue or deficiency in mechano-chemical process overcame the improved efficiency in activating process. GRIEVE (1958) reported that during repetitive stimulation the height of action potential decreased in parallel with the contraction height. But it seems unreasonable to measure the height of diphasic action potential which could be changed depending upon the conduction velocity.

In the intracellular recording, the resting potential showed some depolari-

zation at first, and then overshoot potential decreased, too. This observation conformed to the report by DRAPER & KARZEL (1961). The fact that the course of decrease of spike height by intracellular recording coincided with that by partition method, indicates that the partition method is an equivalent to the method using single fibre, as far as the relative size or shape of action potential is concerned. This is a great advantage.

The negative after-potential was increased in fatigued muscle or at higher temperature (MACFARLANE & MEARES, 1954), but in both conditions the peak tension decreased. Therefore, the correlation between negative after-potential and mechanical activity seems to be poor.

SUMMARY

1. The coupling relation between action potential and mechanical response during repetitive stimulation was investigated using the sartorius muscle of the frog.
2. The course of decrease in spike height obtained by partition method coincided with that by intracellular recording.
3. The coupling efficiency between spike height and peak tension during repetitive stimulation was improved at first during staircase phenomenon and then it decreased progressively.
4. Fatigue, higher stimulating frequency, and heavy load resulted in a deficiency of coupling process, especially the mechano-chemical process.
5. At higher temperature, the coupling efficiency for single stimulus was decreased, but for repetitive stimulation the period of staircase phenomenon was greatly prolonged and the rate of decrease in spike height became much slower.
6. The height of negative after-potential or the spike duration showed a poor correlation with peak tension during repetitive stimulation.
7. When the muscle was stimulated at 2 c/sec through nerve, transmission fatigue was observed in the range beyond 300 stimuli.
8. The electro-mechanical coupling, as the complex of the activation process for contraction and the energy mobilizing process in contraction, was discussed.

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