MECHANICAL RESPONSES GENERATED BY ALTERNATING CURRENT OR REPETITIVE SQUARE PULSES IN THE FROG SKELETAL MUSCLE

Hidenobu Mashima and Hisako Tsuchiya

Department of Physiology, School of Medicine, Juntendo University, Hongo, Tokyo

It is well known fact that alternating current (AC) of 50 c/sec is more effective than repetitive square pulses (SP) to generate the contraction of skeletal muscle, suggesting that AC has an action for muscle to contract, besides the generation of action potential. Some experiments have been done on the effectiveness of the internal current for the initiation of contraction (Csapo and Suzuki, 1958; Csapo, 1959). On the contrary, Taylor (1953), Sten-Knudsen (1954, 1960) and Watanabe (1958) has pointed out that the applied current did not act on the contractile substance directly but through depolarization of membrane. And then, Hodgkin and Horowicz (1960) successfully determined the quantitative relation between depolarization and tension of potassium contracture in the single muscle fibre. On the other hand, Katz and Lou (1947), Sten-Knudsen (1954) and Csapo and Wilkie (1956) showed the contraction of non-propagating muscle in excess potassium solution by the application of AC. They suggested that, in excess potassium solution, not the spike potential but the local response would be the cause of contraction. But no information has been submitted whether or not the local responses are also repeated in normal Ringer solution during repetitive stimulation. The answer to this question was clearly given in the previous paper (Mashima and Washio, 1968). Namely, the electrical responses evoked by AC or SP were separated into successive three phases; the phase of spike potentials, the phase of alternating spike and local responses and the phase of summated local responses.

Recently, however, Costantin and Podolsky (1967) have shown the evidence that the skinned fibre could be contracted by electrical current and Lee et al. (1966) also have found that the isolated sarcoplasmic reticulum could release Ca++ by electrical stimulation. These facts imply the possibility that
the muscle could be activated by electrical current irrespective of the changes in membrane potential.

The following experiments were undertaken to examine the mechanical responses generated by AC or SP and to determine the relationship between electrical and mechanical changes during contraction, referring to the results in the previous paper. Some of the results were already described in the preliminary report (MASHIMA and TSUCHIYA, 1967).

METHODS

The semitendinosus muscle of the frog, *Rana nigromaculata*, was mounted horizontally in the Ringer-bath (5 × 5 × 1.5 cm). The whole arrangement is shown in Fig. 1. One end of the muscle was fixed to a glass rod clamped by the movable stand with sliding vernier and the other end was attached to the rectangular isometric lever, by which the muscle tension was conveyed to RCA 5734. A pair of platinum foil electrodes (5 × 1.5 cm) were placed on the opposite walls of the Ringer-bath, and the stimulating current was supplied from AC- or SP-generator through the high power amplifier (30 Watt).

![Diagram of the experimental arrangement](image)

**Fig. 1.** A diagram of the experimental arrangement. A, power amplifier; B, Ringer-bath; C, thermoelectric heat exchanger; E, platinum foil electrode; G, alternating current or square pulse generator; L, isometric lever; M, muscle; R, thermoregulator; V, vernier; W, water circulation; 5734, RCA 5734 tube.

In order to compare the effect of SP with that of AC, the voltage of AC was always described with a half of the peak-to-peak voltage and the pulse duration of SP was adjusted to a half of the pulse interval (see Fig. 12, a and b). The period of stimulation was always 0.5 sec, because in many cases the maximum tension was attained within that time, except when the field intensity was extremely strong. To change the field direction of stimulating current field, the Ringer-bath with stimulating electrodes, together with the underlying thermoelectric heat exchanger was rotated against the base plate. The temperature of Ringer solution was controlled to 20°C by electric current through the thermoelectric heat exchanger.

Ringer solution contained 110 mM NaCl, 12 mM NaHCO₃, 2 mM KCl, 1.8 mM CaCl₂, and pH was 7.0.
RESULTS

1. Mechanical responses generated by longitudinal AC field

   a) Effect of field intensity. When the muscle was stimulated by longitudinal AC field with the frequency of 300 c/sec for 0.5 sec, two peaks were observed in the tension curve at the intensity below 3V/cm (Fig. 2). The peak tension of the early contraction was not increased at more than 1V/cm, but that of the late contraction was gradually increased with the field intensity until it exceeded the first contraction (Fig. 3). Two contractions were fused at more than 3V/cm (Fig. 2).

   ![Fig. 2](image-url)

   **Fig. 2.** Tension curves generated by 300c/sec, 3V/cm, longitudinal AC field for 0.5 sec. Field intensity, A: 0.5V/cm, B: 1V/cm, C: 2V/cm, D: 3V/cm, E: 4V/cm, F: 6V/cm.

   ![Fig. 3](image-url)

   **Fig. 3.** The relation between peak tension and field intensity of AC at 300c/sec. L-A, the early contraction; L-B, the late contraction in the longitudinal field; T, in the transverse field.

   b) Frequency characteristics. For the weak field intensity (2V/cm), two peaks were distinguished in the tension curve between 100-1000 c/sec (Fig. 2, C, or 4, A). Both peaks, especially the second peak, were decreased with the increase in frequency and abolished at 2000 c/sec (Fig. 5, L and 6, L). A twitch response evoked by a sufficiently strong single shock (1msec, 6V/cm) was completely coincided with the early contraction generated by 500 c/sec.
AC (Fig. 5, L, dotted line). This result reveals that only single spike potential was generated at 500 c/sec AC stimulation as shown in the previous paper.

For the strong field intensity (8V/cm), the two peaks were fused below 500 c/sec but they were also separated in the range between 500-2000 c/sec. Both peaks, especially the late contraction, were decreased with increasing frequency, and at 5000 c/sec no contraction was observed (Fig. 7, L and 8, L).

c) Selective inhibition for the early or late contraction. At 200 c/sec and 2V/cm, the two peaks were clearly distinguished in the tension curve (Fig. 4, A). But after the muscle was immersed into excess potassium solution (9 mM K+-Ringer solution), the early contraction selectively disappeared remaining the late one (Fig. 4, B). On the other hand, raising the frequency up to 1000 c/sec, the second contraction was exclusively minimized (Fig. 4, C).

![Fig. 4](image)

In the previous paper it was found that the mean depolarization of membrane potential during AC stimulation was about 30-40 mV in normal Ringer solution as well as in excess potassium solution, in which no spike potential was evoked. Moreover, it was also elucidated that the spike potentials disappeared after 100 msec from the beginning of 200 c/sec AC stimulation and one or few spike potentials were observed at more than 500 c/sec. Therefore, it is concluded that the early contraction is an ordinary tetanus caused by the repetitive spike potentials but the late contraction is a contracture response generated by the steady depolarization of membrane, which consists of the summated local responses.
2. Mechanical responses generated by transverse AC field

a) Effect of field intensity. Generally speaking, the transverse field was more effective than the longitudinal one to generate the tension. Because in the latter the depolarizing effect of the electrode was strong at the end of the muscle but very weak at the centre (CSAPO, 1959), as in the former the half circumference of the membrane was depolarized or hyperpolarized along the whole length of the muscle (SUGI and KOSAKA, 1964). Therefore, it was difficult to distinguish the two peaks in the tension curve developed by the application of transverse AC field in any intensities or frequencies (FIG. 5, T and FIG. 7, T). The peak tension generated by 300 c/sec transverse AC was plotted against field intensity as shown in FIG. 3, curve T. At 3V/cm the peak tension attained almost the maximum tension of the late contraction generated by longitudinal AC, and no more increase in tension was observed in the range beyond 4 V/cm. In the longitudinal AC field the depolarization by weak current would be limited at the both ends of the muscle and expanded gradually to the centre with increasing current intensity, but in the transverse AC field the whole surface could be depolarized rapidly along its whole length. Moreover, the amount of mean depolarization produced by AC was almost independent of the intensity of the current applied, as described in the previous paper. Therefore, the late contraction in the transverse AC stimulation occurred always by the maximum strength and the rise of tension of the late contraction is so quick that two peaks were not separated. Then, it is obvious that in the transverse AC the maximum tension observed is the tension of the late contraction with a nature of contracture.

b) Frequency characteristics. The tension curves obtained by the applica-
tion of transverse AC at various frequencies and the frequency characteristics of the peak tension are shown in Fig. 5, T and Fig. 6, T for the weak field (2V/cm), and in Fig. 7, T and Fig. 8, T for the strong field (8V/cm).

At lower frequency than 100 c/sec, there is not much difference between longitudinal and transverse stimulation, because the main part of contraction was generated by propagating spike potentials at that frequency. At more than 100 c/sec, the late contraction became rather dominant. The decrease in tension with increasing frequency by weak transverse AC (Fig. 6, T) would be interpreted by the rise in the threshold of the local response at higher frequencies. When the current intensity was as strong as 8V/cm, the tension was maintained even at 5000 c/sec (Fig. 8, T). This fact means that the sodium dependent local response could be generated even at higher frequency, provided with a stronger field.

3. Mechanical responses generated by square pulses stimulation.

In order to compare the effect of SP with that of AC, the duration of each pulse was adjusted always to be a half of the pulse interval. The frequency characteristics of the maximum tension generated by longitudinal or transverse SP and AC at 3V/cm are shown in Fig. 9.

In the longitudinal SP stimulation the tension was decreased rapidly at more than 100 c/sec (Fig. 9, SP, L), while in the longitudinal AC stimulation it was decreased gradually at more than 200 c/sec (Fig. 9, AC, L). Of course, in SP stimulation the depolarization occurs only at the cathodal end of the muscle, while in AC stimulation it does at both ends. Moreover, it was shown in the previous paper that the steady depolarization produced by SP was 20-30 mV, while the mean depolarization produced by AC was 30-40 mV. Thus,
it is obvious that the late contraction, which is a contracture caused by the steady depolarization around the cathod, is smaller in SP stimulation than AC.

In the transverse SP stimulation the tension was decreased at more than 500 c/sec (Fig. 9, SP, T), while in the transverse AC it was decreased at more than 2000 c/sec (Fig. 9, AC, T). In the transverse field the one side of the membrane along the whole length will be depolarized by SP but all surface by AC. While the spike potential disappeared soon after the beginning of repetitive stimulation at more than 200 c/sec as shown in the previous paper, the gradual decrease in tension with increasing frequency may represent the decay of steady depolarization or mean depolarization at higher frequencies, due to the rise in the threshold of local response.

4. The effect of external sodium concentration.

When the sodium concentration of the external solution was reduced by replacing with isomolar choline, both the early and late contractions generated by longitudinal AC were decreased (Fig. 10, C). The frequency characteristics of the late contraction in 6V/cm transverse AC field are shown in Fig. 11. In 1/2 or 1/4 Na⁺-Ringer solution, little or no change in tension was observed as compared with the tension developed in normal Ringer solution. But in 1/8 or 1/16 Na⁺-Ringer solution, the tension was greatly decreased especially in the range below 200 c/sec. In sodium-free, choline-Ringer solution, there appeared still some tension in the late contraction below 500 c/sec, but in this
case the tension was increased with decreasing frequency.

The effect of $10^{-8}$-$10^{-7}$ g/ml tetrodotoxin, which has been known to have a specific action in preventing the increase of sodium conductance in nerve or muscle membrane (NARAHASHI et al. 1960, 1964), was almost the same as the sodium reduction (Fig. 10, B). While the local response was evidently abolished by sodium removal as shown in the previous paper, the inhibitory action on the sodium conductance reduced not only the generation of the early contraction or spike potential but also the late contraction on local response. These results also reveal that the sodium-dependent local responses are responsible to the generation of the late contraction. The fact that the optimum frequency for the late contraction or the summation of local responses was in between 500-2000 c/sec would mean that the duration of each local response is longer than 2 msec, and that at least 0.5 msec of pulse duration is necessary to evoke a local response when the intensity is 6V/cm.

The contraction generated in the sodium-free solution attained by about 30% of the maximum tension at 10V/cm, 50 c/sec, transverse AC stimulation. Neither spike potential nor local response is responsible for this contraction. The frequency characteristics of the tension generated in sodium-free solution by transverse 9V/cm AC or SP stimulation are shown in Fig. 12. Generally, the tension was decreased with increasing frequency. At less than 200 c/sec,
AC generated more tension than SP, but at more than 200 c/sec it was reversed. No tension was generated by AC at 1000 c/sec, while about a half of the maximum tension was produced by SP at 10000 c/sec. The tension generated by constant current (DC) with the duration of 0.5 sec is indicated on the ordinate. For the DC with longer duration, more tension was generated.

5. The effect of external potassium concentration.

It has been known that the muscle was rendered non-excitable when it was soaked in Ringer solution containing about 9-12 mM potassium, because no spike potential could be generated at the resting potential less than 57 mV (JENERICK and GERARD, 1953). However, sufficient contraction was observed in this excess potassium solution by the application of AC field (STEN-KNUDSEN, 1954; CSAPO and WILKIE, 1956). The above described results shown in Fig. 4, B, indicates that the tension generated in the potassium solution by AC is a contracture caused by the mean depolarization, in other words by the summated local responses.

The frequency characteristics of the tension in the excess potassium solution is shown in Fig. 13. The tension generated by transverse AC was decreased with the increase in potassium concentration, especially at higher frequencies, but it was increased to more than 90% of the maximum tension by the increase in field intensity. In many cases the maximum tension was
obtained at 500-1000 c/sec, although the rate of rise of tension was slowed with increasing frequency. Of course the transverse field was much more effective than the longitudinal one. These results are roughly the same as those of STEN-KNUDSEN (1954, 1957) and CSAPO and WILKIE (1956).


Many investigations showed the prolongation or the augmentation of potassium contracture by raising the external calcium concentration (FRANK, 1960; MASHIMA and MATSUMURA, 1962; LORKOVIC, 1962; LÜTtgAU, 1963). The contraction generated by AC stimulation in the excess potassium solution was also augmented by raising the external calcium concentration. For example, when calcium concentration was raised to 18 mM, the muscle could exert about 50% of the maximum tension even in 50 mM K⁺ solution by 50 c/sec, 12V/cm, transverse AC stimulation.

In the sodium deficient solution, in which 3/4 of Na⁺ was replaced with choline, the tension generated by AC was decreased. But when the external calcium was raised to 3.6 mM at the same time, the tension was slightly increased between 200-5000 c/sec (FIG. 14). The muscle could develop tension without external calcium, irrespective in the excess potassium or in the sodium deficient solution.
FIG. 14. Effect of calcium on the frequency characteristics of the peak tension generated by 6V/cm transverse AC for 0.5 sec in the sodium deficient solution. A, in normal Ringer; B, in 30 mM Na\(^+\) and 3.6 mM Ca\(^{++}\); C, in 30 mM Na\(^+\) and 1.8 mM Ca\(^{++}\); D, in 30 mM Na\(^+\) and Ca\(^{++}\)-free solution.

FIG. 15. Effect of chloride removal on the tension curves generated by 50 c/sec, transverse AC for 0.5 sec. A and B, in normal Ringer solution; C and D, in the chloride-free solution. Chloride was replaced by propionate. Field intensity, A and C: 2V/cm, B and D: 8V/cm.
7. The effect of external chloride concentration.

The external chloride was replaced by impermeable anion, such as methyl-
sulfate \((\text{CH}_3\text{SO}_4)\) or propionate. In the chloride-free Ringer solution, an increase
in tension was observed by AC stimulation at every frequency between 50-
10000 c/sec, and the relaxation was apparently slowed (Fig. 15). The amount
of augmentation was different from the preparation to the preparation in be-
tween 10-20%. Probably this augmentation results from the prolongation and
slow decline of local contraction due to the potentiation of excitation-contrac-
tion coupling by chloride removal (MASHIMA and MATSUMURA, 1962). For the
longitudinal application of AC, two peaks in the tension curve were easily
separated at less frequency than in normal Ringer solution, probably because
less reproducibility of spike potentials in the chloride deficient solution.

DISCUSSION

The mechanical responses generated by AC or SP shown in the present
paper correspond in many respects to the changes in membrane potential,
which were described in the previous paper (MASHIMA and WASHIO, 1968). Repetitive spike potentials were abolished during AC stimulation within 0.1 sec
at 200 c/sec and only one spike potential appeared at more than 500 c/sec.
Corresponding to the disappearance of spike potential, the early tension gen-
erated by longitudinal AC was reduced at more than 200 c/sec, and only twitch
response was observed at 500 c/sec. The late contraction generated by trans-
verse AC showed an all-or-none property, conforming to the mean depolariza-
tion of local responses produced by AC, which was almost independent of the
current intensity below 500 c/sec. The late contraction in the longitudinal AC
was increased with increasing field intensity, because non-propagating local
depolarization would be limited near the both ends of the muscle and de-
polarized region would be expanded with increasing intensity. Furthermore,
sodium removal or the application of tetrodotoxin reduced both the early and
late contractions. From these results it was concluded that the early contraction
was generated by spike potentials and the cause of the late contraction was local
responses. Thus, it is obvious that the sodium-dependent regenerative de-
polarization, including local response, are responsible for the tension develop-
ment during AC or SP stimulation. The situation was the same in SP stimu-
lation as AC for the mechanical responses as well as electrical changes in
membrane. But the late contraction generated by SP was far smaller than
that by AC, because only a half of the membrane toward the cathod should
be depolarized by SP. Of course, the brief pulse with the duration of 1 msec
is much more difficult to generate local response than the pulse with the dur-
ation of 10 msec. This is the reason why 50 c/sec AC is more effective than
50 c/sec brief pulses for the development of tension.

HODGKIN and HOROWICZ (1960) pointed out that the maximum contracture tension induced by high potassium was about 10% larger than the maximum tetanic tension. In the present results the maximum contracture tension generated by 2000 c/sec, 8V/cm, transverse AC was also about 10% larger than the maximum tetanic tension generated by 20 c/sec, 8V/cm, longitudinal AC (FIG. 8). At 2000 c/sec the spike potential was inhibited almost completely and at 20 c/sec only propagating spike potential could generate tension. GORDON et al. (1966) observed an appreciable amount of creep during the repetitive stimulation at about 20 c/sec at 3-4°C. The creep phenomenon may also result from the steady depolarization maintained between prolonged spike potentials at low temperature.

When the field intensity was sufficiently strong, the muscle exerted some tension even in the sodium-free solution. As the phasic potassium contracture was produced in the sodium-free solution as shown by HODGKIN and HOROWICZ (1960), it is apparent that depolarization can initiate contraction without sodium in the external solution. In the sodium-free solution, \( i_r \) drop of the passing current across membrane will produce depolarization and then contraction, although the regenerative response greatly enhances the depolarization under the presence of sodium. Another explanation for this contraction is a direct action of electrical current to the internal membrane of sarcoplasmic reticulum (SR). According to COSTANTIN and PODOLSKY (1967), skinned muscle fibre could be reversibly activated by electrical stimulation, and the contractions were graded with the strength of the applied current. In our experiments it was difficult to discriminate these two possibilities. Because, while a great decrease in tension with increasing frequency by AC stimulation (FIG. 12, A) would support the idea that \( i_r \) drop may be connected to the release of activator substance, a little decrease in SP stimulation (FIG. 12, B) rather suggested the direct action of the current. LEE et al. (1966) described that monophasic current was more effective than biphasic one for the direct stimulation of SR fragment, but COSTANTIN and PODOLSKY (1967) argued that in the skinned region contractions elicited by AC was somewhat larger than those obtained with SP and claimed that some effect other than a displacement of membrane potential across a single SR fragment would be responsible for LEE's results. In our results AC was more effective than SP below 200 c/sec, but it was reversed beyond 200 c/sec. Then it will be important to determine which is more effective, AC or SP, for the internal membrane.

In the chloride-free solution the maximum tension was potentiated more than 10%. The maximum tetanus tension \( (P_0) \) may represent the intensity of the active state, but this is not a real maximum tension which the muscle can exert. Moreover, the maximum tension could be changed under various conditions, such as in the chloride deficient solution. Then, it is a difficult prob-
lem to determine whether \( P_0 \) is unaffected or not under a certain condition.

**SUMMARY**

1. The mechanical response of frog's semitendinosus muscle generated by AC or SP at various frequencies and intensities were studied, referring to the electrical changes in membrane potential described in the previous paper.
2. The AC stimulation generated the early tetanic contraction due to propagating spike potentials and the late contracture response due to non-propagating local responses. Both the early and late contractions were separated when the longitudinal AC between 200-1000 c/sec was applied. At the frequency higher than 500 c/sec the early contraction became a twitch. In the strong transverse AC field the late contraction was dominant and it was decreased at more than 2000 c/sec.
3. In the excess potassium (9-18 mM) solution the early contraction was abolished.
4. Both contractions were decreased by the sodium removal or the presence of \( 10^{-8} \text{g/ml} \) tetrodotoxin.
5. The effect of SP stimulation was similar to that of AC and the difference in the frequency characteristics was explained by the difference of electrical changes.
6. In the sodium-free solution, strong AC or SP evoked some tension, which was decreased with increasing frequency. Transverse AC was more effective than SP at less than 200 c/sec but the effect was reversed at more than 200 c/sec.
7. Raising the external calcium concentration, the late contraction in the excess potassium or sodium deficient solution was augmented.
8. In the chloride-free solution the late contraction was augmented and the relaxation was slowed.

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