Action Potential in the Transverse Tubules and Its Role in the Activation of Skeletal Muscle

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ABSTRACT The double sucrose-gap method was applied to single muscle fibers of *Xenopus*. From the "artificial node" of the fiber, action potentials were recorded under current-clamping condition together with twitches of the node. The action potentials were stored on magnetic tape. The node was then made inexcitable by tetrodotoxin or by a sodium-free solution, and the wave form of the action potential stored on magnetic tape was imposed on the node under voltage-clamp condition (simulated AP). The twitch height caused by the simulated AP's was always smaller than the twitch height produced by the real action potentials, the ratio being about 0.3 at room temperature. The results strongly suggest that the transverse tubular system is excitable and is necessary for the full activation of twitch, and that the action potential of the tubules contributes to about 70% of the total mechanical output of the normal isotonic twitch at 20°C. Similar results were obtained in the case of tetanic contraction. At a temperature near 10°C, twitches produced by the simulated AP were not very different (85% of control amplitude) from the twitches caused by real action potentials. This indicates that the excitability of the tubules becomes less necessary for the full activation of twitch as the temperature becomes lower.

INTRODUCTION

The transverse tubular system (T system) of muscle plays an important role in transmitting the electrical signal of the surface membrane deep into the central part of the cell, bringing about a rapid and synchronous initiation of contraction of myofibrils that are located at varying depths from the surface. The importance of the T system was first demonstrated by the experiment of Huxley and Taylor (1958), in which they showed that local stimulation of the surface membrane was effective in eliciting contraction only when the location of the stimulus coincided with the place where the T system would exist.

As for the mode of transmission of electrical signals through the T system, the graded contraction observed in Huxley and Taylor's experiment sug-

gested a nonregenerative, decremental conduction rather than a mechanism involving a regenerative action potential. Nevertheless, several investigators have obtained suggestive evidence favoring the presence of some kinds of regenerative activities, occurring either in the sarcoplasmic reticulum or in the tubular system (Natori, 1954; Strickholm, 1966; González-Serratos, 1966, 1971; Costantin and Podolsky, 1967; Sugi and Ochi, 1967; for critical review of this subject see Huxley, 1971). A definite answer to this question has recently come from the experiments by Costantin (1970) and Costantin and Taylor (1971). They showed that square pulse depolarizations of the surface membrane of muscle bathed in 50% $[Na]_o$ produced a pattern of radial spread of contraction which indicated the presence of regenerative activity, and that this pattern was changed by adding tetrodotoxin.

Still, there is one important question that remains unanswered: Is the action potential of the T system necessary for normal activation of muscle? More specifically, to what extent does the action potential of the T system contribute to the elicitation of normal contraction? The question seems pertinent since Adrian, Costantin, and Peachy (1969) have shown that electrotonic conduction along the T system was just about enough, with a small safety factor, to elicit movements of the myofibrils at the central part of the fiber. They interpreted this smallness of safety factor of the electrotonic conduction as suggestive for the presence of regenerative activity in the T system. But the result also suggests, at least to us, a possibility of nonessentiality, or nonimportance, of the regenerative activity, even if it exists, for the full activation.

The experiments described in this paper were designed to answer this question. For this purpose the double sucrose-gap technique described in the previous paper (Nakajima and Bastian, 1974) was used. It will be shown that there is an Na-dependent action potential in the T system, and that this process contributes to roughly 70% of the magnitude of mechanical output in normal isotonic twitch at room temperature. A preliminary paper was read at a meeting of the American Physiological Society (Bastian and Nakajima, 1972).

METHODS

Single isolated fibers from toe muscles (m. flexor brevis digiti V) of *Xenopus laevis* were used. Experiments were performed from May, 1971 to March, 1972, and all the data obtained at room temperature in this paper have been derived from the group of fibers listed in Table III of the previous paper (Nakajima and Bastian, 1974): the sampling procedure was explained in the legend of the table.

The methods of recording electrical and mechanical events of the "artificial node" of the single fiber and several problems concerning this technique have already been described in the previous paper. Tris Ringer was made by substituting 125 mM Tris for 120 mM Na of the normal Ringer, the phosphate buffer being omitted. This

solution had the same osmolarity (within 2% error) as that of the normal Ringer solution.

RESULTS

Twitch Produced by Real and Simulated Action Potentials

A single muscle fiber was stretched to 130% of the slack length, and set in the sucrose-gap apparatus. Flows of the solutions were started, and the current clamping was applied to the artificial node. Action potentials and twitches were continuously recorded at an interval of once every 16.4 s. After the start of the flows, 20–30 min were allowed for stabilization. The sucrose-gap hyperpolarization was usually bucked up by unbalancing the current-clamping circuit, and the membrane potential was adjusted to a level which required a critical depolarization of about 40 mV for the initiation of an action potential (see Fig. 4 of Nakajima and Bastian, 1974). Examples of action potentials and twitch force recorded at this stage are illustrated in Fig. 1, A1 (slow sweep) and A2 (fast sweep). Several of these control action potentials were also fed to an FM tape recorder.

After this control period, the solution in the center pool was switched to a Ringer solution containing 2.5×10^{-8} g/ml tetrodotoxin (TTX). This blocked the excitability of the node completely. The current-clamp circuit was loosened, switched to the voltage-clamping mode, and the clamping was tightened. Care was taken not to change the resting potential of the fiber during these maneuvers. The tape recorder was started and the action potentials stored on the tape were applied to the command input of the clamping circuit. This resulted in nearly the same potential changes in the node as the real action potential. This potential change of the node will be referred to as "simulated action potential" (simulated AP). This technique is similar to that used by Adrian, Costantin, and Peachy (1969) under different conditions.

Fig. 1 B1 and B2 shows a simulated action potential and the twitch force of the node resulting from it. The height of the twitch force produced by the simulated AP was considerably smaller than that produced by the real action potential. The time-course of the twitch was also somewhat different. While in the control situation it was characterized by the presence of a rapid phase of relaxation, the relaxation of the twitch caused by simulated action potential lacked this rapid phase, and the rising and falling phases became more symmetrical in shape. As was discussed in the previous paper, the presence of the rapid relaxation is not an artifact from the recording system. The lack of the rapid relaxation under the simulated AP is not a result of the smaller twitch, since the rapid relaxation persisted with the real AP, even when the node was made very short, rendering the recordable force very small (cf. Fig. 9, A1, A2 and B1, B2).

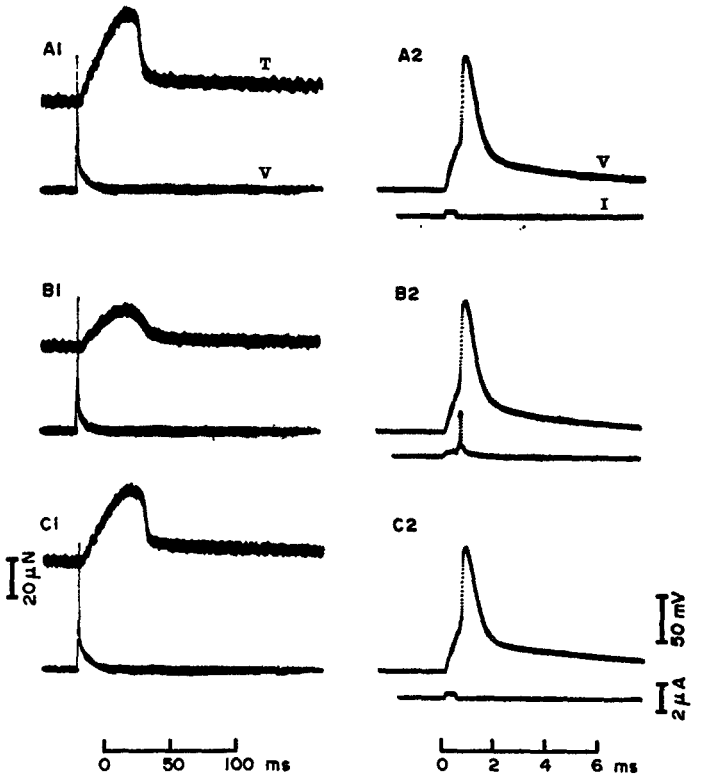

FIGURE 1. Action potential and twitch of a single muscle fiber of *Xenopus* recorded with the sucrose-gap technique. A1, B1, C1: Records on slow time base. A2, B2, C2: Records on fast time base. The records of the slow and the fast time bases were photographed simultaneously on a four-beam scope and were split photographically. Cross-over points of the beams were retouched. A1, A2: Real action potential (V), current (I), and twitch force (T) recorded in normal Ringer solution. Length of the artificial node was 261 μm. B1, B2: Simulated AP and the resultant current and twitch in a Ringer solution containing TTX (2.5×10^{-8} g/ml). Nodal length was 261 μm. C1, C2: Normal action potential, current, and twitch 17 min after starting the replacement of TTX with the normal Ringer. Nodal length was 246 μm. Fiber diameter 96 μm. Bath temperature 23.5°C.

After several simulated AP's were recorded, the voltage clamping was released, the solution in the center pool was switched back to normal Ringer, and the current clamping was applied. As can be seen from Fig. 2, which illustrates the entire time-course of the experiment, the effect of TTX was reversible. Action potentials reappeared 3 min after the switching of the solution to normal Ringer. This time lag represents, at least in part, the dead space of the flow system. The twitch recovered more slowly, and the half-time of the recovery was 7.5 min. (This half-time included the time lag of about 3 min due to the dead space between the node and the stopcock. The average

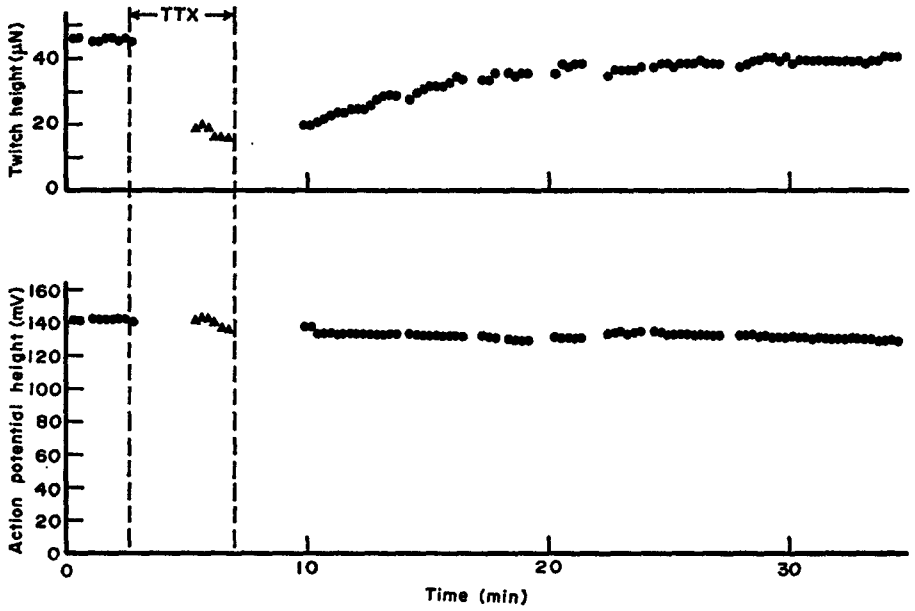

FIGURE 2. Time-courses of changes in action potentials and heights of twitch during the experiment shown in Fig. 1. First, real action potentials and twitches were recorded in the normal Ringer (circles). Nodal length was 261 μm. Then, TTX (2.5×10^{-8} g/ml) was applied to abolish the action potential, and simulated action potentials and twitches were recorded under voltage clamping (triangles). The slight scatter of the height of successive simulated action potentials was brought about intentionally by changing the output voltage of the tape recorder. Nodal length was 261 μm. Finally, TTX was washed out by the normal Ringer, and the time-courses of the recovery of the action potential and twitch were followed (circles). Nodal length was 246 μm. Diameter 96 μm. Bath temperature 23.5°C.

half-time of the 13 fibers in Table I A was 6 min ranging from 2.5 to 13.4 min.) This slow recovery of the twitch is somewhat puzzling. It might represent, at least in part, a slow diffusion of TTX in the T system. Fig. 1, C1 and C2 are records of the action potential and twitch force 17 min after the start of TTX washout, showing that not only the magnitude returned toward the control, but also the characteristic shape of the normal twitch, with its rapid relaxation phase, reappeared.

Similar experiments were performed, in which Na-free Ringer (Tris-substituted) was used, instead of TTX, to make the node inexcitable. As shown in Fig. 3, the results were essentially the same as in the TTX experiment, except that the twitches recovered more quickly, and the time-course of the recovery of the action potential and twitch was not as markedly different as in the TTX experiments.

Table I A summarizes the results of the experiments. As shown in the columns VI and VII, the heights of twitch caused by the simulated AP were

TABLE I

TWITCH AND TETANUS BY REAL AND SIMULATED ACTION POTENTIALS

	I Fiber	II Bath temp	III Diameter	IV Nodal length	V Height of AP	VI Height of twitch and tetanus during: control period	VII simulated AP	VIII recovery attained	IX Ratio of VII and VI	X Ratio of VIII and VI
		(°C)	μm	μm	(mV)	(μN)	(μN)	(μN)	(%)	(%)
A. Twitch at room temperature										
	X 50		115	368	155	73.0	14.2	73.0	19.5	100
	X 53	22.5	110	353	188	76.5	36.9	58.5	48.2	76.5
	X 56	24.5	83	292	131	48.6	11.9	32.6	24.5	67.1
	X 59	23.0	108	338	137	43.3	20.0	42.9	46.2	99.1
	X 93	23.5	158	200	145	46.4	10.2	16.2	22.0	34.9
	X 95	23.5	96	261	142	45.6	19.2	38.5	42.1	84.4
TTX	X 96	24.0	97	350	138	49.4	11.3	32.0	22.9	64.8
	X 99	22.5	169	246	136	171	74.4	138	43.5	80.7
	X105	23.5	112	353	141	31.4	10.6	27.9	33.8	88.9
	X107	24.0	130	368	138	141	26.5	32.8	18.8	23.3
	X109	23.5	120	368	136	97.6	30.0	82.2	30.7	84.2
	X113	23.5	108	313	138	46.0	12.5	22.9	27.2	84.1
	X114	23.5	105	378	135	55.8	25.9	46.9	46.4	49.8
Tris	X 64	24.0	104	338	173	63.8	24.6	58.4	38.6	91.5
	X 65	23.5	118	347	167	90.6	34.0	84.8	37.5	93.6
Mean ± SEM		23.5	116	325		72.0	24.1	52.5	33.5	74.9
									±2.7	±5.9
B. Twitch at low temperatures										
X 55		11.5	116	276	139	37.3	32.7	35.3	87.7	94.6
X 57		12.5	125	338	140	116	108	109	93.1	94.0
X103		10.0	113	307	144	91.7	74.4	93.9	81.1	102
X185		13.0	126	399	180	127	102	105	80.3	82.7
Mean ± SEM		11.8	120	330		93.0	79.3	85.8	85.6	93.3
									±3.0	±4.0
C. Tetanus at room temperature										
X105		23.5	112	353	143	136	38.1	64.0	28.0	47.1
X107		24.0	130	368	137	488	112	139	23.0	28.5
X113		23.5	108	313	141	201	44.6	50.5	22.2	25.1
X114		23.5	105	378	134	194	48.8	97.7	25.2	50.4
Mean ± SEM		23.6	114	353		255	60.9	87.8	24.6	37.8
									±1.3	±6.4

Of all the TTX experiments at room temperature, about 10 fibers were not listed in the table, since in these fibers the recovery of twitch height after the TTX treatment was less than 10% of the control twitch. "Diameter" is the value at relaxed length. "Nodal length" is the value during the control period. There were small drifts in the flow rate in some of the experiments; therefore the average nodal lengths during the period of simulated AP were 99.6% of the nodal length during the control period in A, 98% in B, and 97% in C. The average nodal lengths at the end of recovery period were 97% of the nodal length during the control period in A, 98% in B, and 94% in C. The "action potential height" is the value during the control period. The average height of the simulated AP was the same as the control. The height of action potentials when the recovery was attained were 97% of the control in A, 102% in B, and 92% in C. The height of action potential was the value after bucking up the sucrose-gap hyperpolarization. No buck up voltage was applied in X50, X53, X64, X65, and all the fibers in B. Fibers were stretched to 130% of the slack length, except X105, X109, X113, and X114, in which the stretch was between 135–145%. For seven fibers (from A) in which the hyperpolarization was bucked up and the stretch was 130%, the magnitude of twitch shortening was 10.5% of the slack nodal length, and the shortening speed was 3.46 L/s (L = slack nodal length; not corrected for the end effect). These values were based on the Young's modulus (see the previous paper), and are subject to large errors.

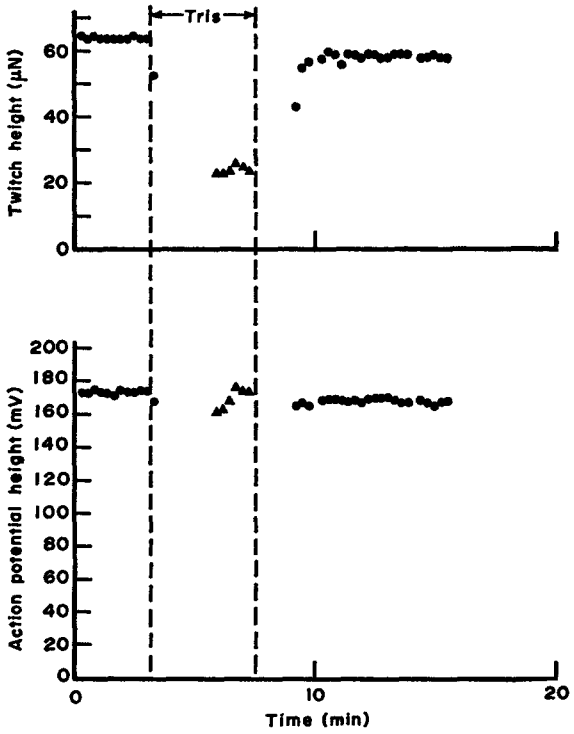

FIGURE 3. Twitches produced by simulated action potentials in Na-free solution. During the control period action potentials and twitches were recorded in normal Ringer (circles). Nodal length was 338 μm. Then, normal Ringer was replaced by a Na-free solution (Tris Ringer), and simulated action potentials and twitches were recorded under voltage clamping (triangles). Nodal length was 347 μm. Finally normal Ringer was reintroduced resulting in an almost full recovery of normal action potentials and twitches. The sucrose-gap hyperpolarization was not bucked up; therefore the action potentials are larger. Nodal length was 338 μm. Diameter 104 μm. Bath temperature 24.0°C.

always less than those of the control twitch and the ratio of these two quantities was on the average 33.5% (column IX). The twitch height attained after the recovery averaged 74.9% of the control in this sample of fibers (column X). The recovery of the action potential was better than the recovery of twitch and averaged 97% of the control.

As shown in the previous paper, the "twitches" illustrated in Fig. 1 approximately represent isotonic shortenings of the node. In two fibers, time-courses of isotonic shortening of the node elicited by a real action potential and that elicited by a simulated AP were recovered by Fourier analysis. It was revealed that the ratio of the height of the twitch force caused by the real AP to that caused by the simulated AP was not measurably different

from the ratio of the magnitude of the shortening by the real AP to that by simulated AP.

The above results of TTX and Tris experiments seem to be good evidence for the presence of an Na-dependent action potential in the T system. As will be shown below, TTX itself has no direct effect on excitation-contraction coupling other than abolishing the Na spike. Thus, if the T-system membranes were not excitable under normal conditions, the real action potential and the simulated AP should have resulted in the same mechanical output of the node. For in both cases the potential changes of the surface would have conducted decrementally along the T system, and the potential and current distribution along the T system should have been the same. The fact that the normal action potential produced larger twitches indicates that under normal conditions the T system, with its regenerative activity, is depolarized more than it would be by electrotonic conduction of the surface action potential. Since the ratio of the heights of the twitch forces resulting from the simulated and the real action potentials was 33.5%, we can conclude that without the regenerative activity in the T system about 70% of the magnitude of the mechanical output of the normal twitch (under isotonic condition) would be lost. It is noteworthy that the relaxation phase of the normal twitch was quicker than that of the twitch by simulated AP. This might be a manifestation of a more synchronous activation of all myofibrils due to the regenerative activity.

Experiments at Low Temperatures

Fig. 4 shows an experiment at 12.5°C. A1 and A2 are real action potentials (V), current (I), and twitch (T), at a slow and a fast time base. B1 and B2 are simulated action potentials, twitch and current after the node was made inexcitable by TTX (2.5×10^{-8} g/ml). C1 and C2 are the records obtained after the TTX was washed out and the excitability recovered. As seen from these records, the twitch force developed by the simulated action potential at the low temperature was not markedly different from the twitch caused by the real action potential. Table I B, summarizing the experiments at low temperatures, indicates that the twitch height produced by the simulated AP was on the average 86% of the control.

The time-course of action potential in the cold is slow and of long duration. Slower potential changes on the surface membrane are expected to be transmitted to the interior more effectively by passive electrotonic spread than rapid changes are. And this seems to explain satisfactorily the result that the simulated AP produced nearly full-size activation of the contractile mechanisms. Although the tubular system would probably still be excitable, the tubular action potential becomes less important and less necessary in producing full-sized contraction at low temperatures.

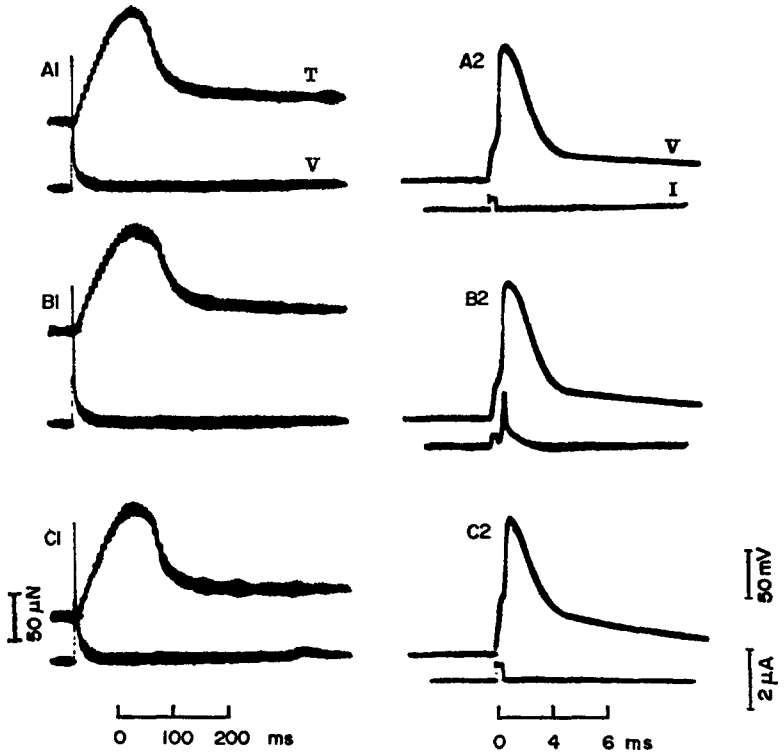

FIGURE 4. Action potentials and twitch at 12.5°C. A1, B1, C1: Records on a slow time base. A2, B2, C2: Records on a fast time base. A1, A2: Real action potential (V), current (I), and twitch (T) recorded in normal Ringer solution. Length of the artificial node was 338 μm. B1, B2: Simulated action potential and the resultant current and twitch in TTX (2.5×10^{-8} g/ml). Nodal length was 338 μm. C1, C2: Normal action potential, current and twitch 4 min after switching to the normal Ringer solution again. The sucrose-gap hyperpolarization was not bucked up. Nodal length was 347 μm. Fiber diameter 125 μm.

Tetanic Stimulation

Fig. 5 shows an experiment using tetanic stimulation. The tetanus was elicited by stimulating the node with 10 pulses 10 ms apart. In A1 a train of real action potentials and the resulting tetanic contraction are displayed, and in A2 are shown the first of the series of action potentials and currents on a fast time base. B1 and B2 are the records of a train of simulated AP's, contraction, and current, after the node was treated with 2.5×10^{-8} g/ml TTX. As in the case of twitch, the mechanical output resulting from simulated AP's was substantially smaller than that produced by the real action potential. The results of the tetanic stimulation are summarized in Table I C. It shows that the height of tetanus in response to simulated AP's averaged 24.6% of the control.

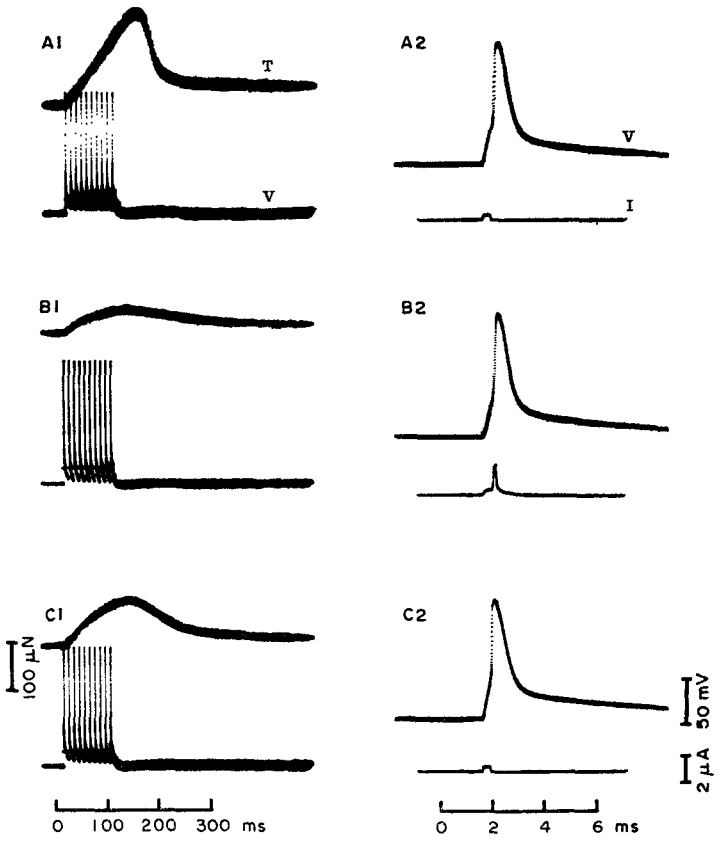

FIGURE 5. Tetanic stimulation. Stimulus frequency 100 per s. A1, B1, C1: Records on a slow time base. A2, B2, C2: Records on a fast time base, showing only the first action potential of the train of impulses. A1, A2: Real action potential (V), current (I), and tetanic contraction (T) recorded in normal Ringer solution. Nodal length was 378 μm. B1, B2: Simulated action potential and the resulting current and tetanic contraction in the Ringer solution containing TTX (2.5×10^{-8} g/ml). Nodal length was 338 μm. C1, C2: Normal action potential, current and tetanic contraction 14 min after switching back to normal Ringer solution. Only partial recovery of contraction is seen. Nodal length was 307 μm. Fiber diameter 105 μm. Bath temperature 23.5°C.

The recovery from TTX was usually less satisfactory than in the case of twitch experiments (Fig. 5 C).

In the case of the twitch experiments, the action potential ends almost completely before the start of the mechanical event. In the tetanic experiments, on the other hand, the second and the subsequent action potentials of the train occurs in the node that is contracting, and more and more sarcomeres would be pulled into the node from the sucrose part of the gap. Therefore, the average nodal area that is activated during tetanic stimulation would be larger in the case of real action potential than in the case of simulated AP.

From the standard Young's modulus of the resting fiber (Nakajima and Bastian, 1974), it was possible to estimate the extent of the shortening, and thus to estimate the average nodal length in each experiment. If this factor were taken into account, the height of tetanus caused by the simulated AP would have been 27% of the control instead of 24.6% in the experiments listed in Table I C. In conclusion, it is likely that roughly the same proportion of the mechanical output of the twitch and tetanus is due to the excitability of the T system.

Effects of TTX on Activation

The above conclusion about the presence of an action potential in the T system is heavily dependent on an assumption, which has hitherto never been proven, that TTX has only one effect, the abolition of Na-permeability increase in the action potential, without affecting other activation mechanisms. This assumption was tested by experiments in which effects of TTX were studied on the nodes that had already been rendered inexcitable by the Na-free solution. In Fig. 6, solid circles are real action potentials and twitches of the node in the normal Ringer solution. The solution was switched to Tris Ringer, and the simulated AP's and resulting twitches were recorded. These are represented by solid triangles. The solution was then switched to a Tris Ringer containing 5×10^{-8} g/ml TTX. The simulated AP's and twitches under this condition are plotted by open triangles. Finally the solution was switched to normal Ringer again (solid circles) and the recoveries of the action potential and twitch were followed. As seen from the record, the addition of TTX at this concentration had no effect on the activation of contraction.

Other Sources of Errors

IMPERFECTNESS OF VOLTAGE-CLAMPING It was difficult to produce a simulated action potential of exactly the same shape as the real action potential. Particularly, the rate of rise of the simulated AP was usually slower than the real AP. This was mainly caused by the difficulty in applying a tight clamp to a node that was not short enough and thus had a fairly large phase lag. Application of a tight negative feed-back to such an element inherently produces instability. In the experiment of Fig. 1, for example, the maximum rate of rise of simulated AP (B2) was 1,120 V/s compared with 1,430 V/s in the real action potential (A2). In a few experiments, by choosing a large fiber and/or a small nodal length, we could apply a very tight clamping. Fig. 7 shows two examples. In the case of A the maximum rate of rise of the simulated AP (A2) was 1,450 V/sec, compared with 1,330 V/s in the control (A1). The twitch caused by simulated AP is smaller than that resulting from

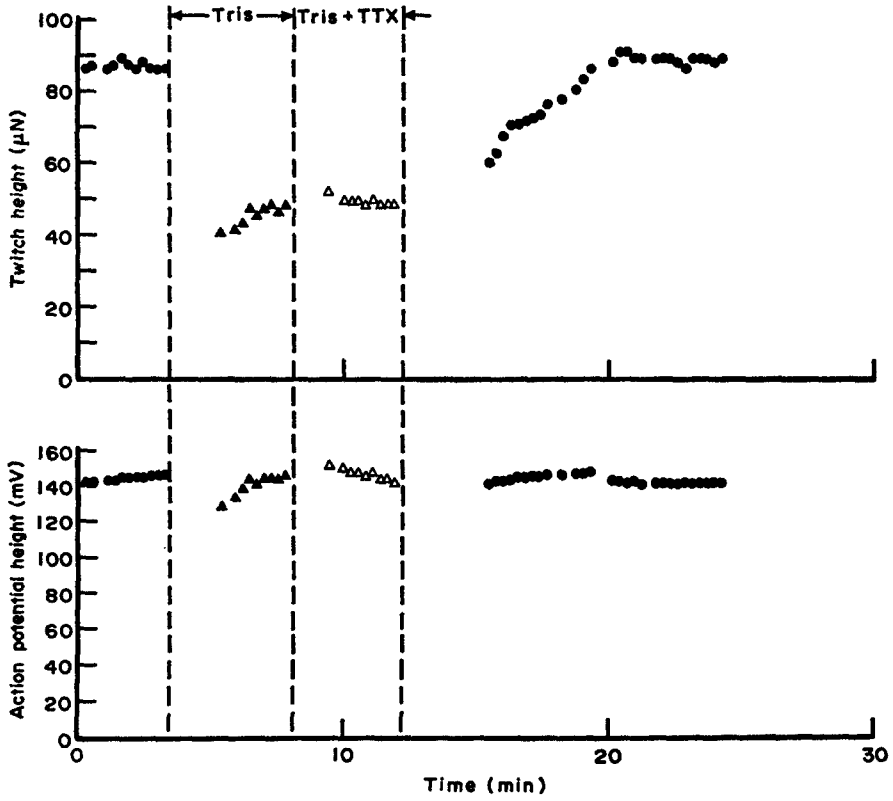

FIGURE 6. Noneffectiveness of TTX on activation of contraction. In the control period, real action potentials and twitches were recorded in normal Ringer (solid circles). The normal Ringer was replaced by Na-free solution (Tris Ringer), and simulated action potentials and twitches were recorded under voltage clamping (solid triangles). TTX (5×10^{-8} g/ml) was added to the Na-free solution (open triangles). Finally, normal Ringer was reintroduced and resulted in a full recovery of normal action potentials and twitches (solid circles). Nodal length was 338 μm throughout. Fiber diameter 118 μm. Bath temperature 23.5°C.

real AP. The rate of rise of the simulated AP was then slowed down to about 1,100 V/s by inserting an RC low pass filter with a time constant of 30 μs between the output of the tape recorder and the command input of the clamp circuit (A3). The twitches resulting from these fast (A2) and slow (A3) action potentials were not markedly different.

In B is shown another example. In this case the simulated AP was slowed down more drastically by inserting an RC filter with a time constant of 120 μs, (B2). But the twitch was not noticeably affected by this procedure. These results show that the slight slowing down of action potential due to the imperfectness of clamping has negligible effects on the magnitude of twitch elicited.

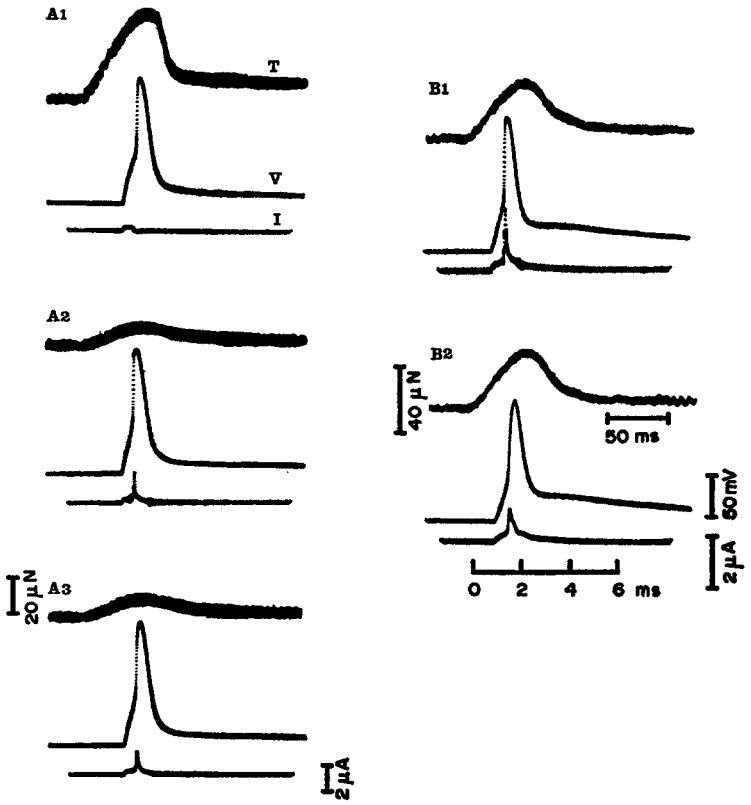

FIGURE 7. The upper beam of each photograph is the twitch on a slow time base (*T*). The middle beam is potential on a fast time base (*V*). The lowest beam is current on a fast time base (*I*). A1: Real action potential, current and twitch in normal Ringer. Height and maximum rate of rise of the action potenital are 147 mV and 1,330 V/s, respectively. A2: Simulated action potential, current, and twitch in TTX (2.5×10^{-8} g/ml). Height and maximum rate of rise of action potential are 146 mV and 1,450 V/s. A3: Same as in A2, except the rate of rise of the simulated AP was slowed down by an RC circuit with a time constant of 30 μs, which was inserted between the output of tape recorder and the command input. This simulated AP has a height and a rate of rise of 144 mV and 1,070 V/s, respectively. Fiber diameter 158 μm. Nodal length 200 μm. Bath temperature 23.5°C. B1 and B2: Another fiber. B1: Simulated action potential, current, and twitch in TTX (2.5×10^{-8} g/ml). The height and the maximum rate of rise were 158 mV and 1,430 V/s. B2: The same as in B1 except the rate of rise of the action potential was slowed down further by an RC filter with a time constant of 120 μs. The height of the action potential became 142 mV and the maximum rate of rise 550 V/s. But the twitch did not noticeably differ from B1. Fiber diameter 159 μm. Nodal length 154 μm. Bath temperature 22.5°C.

VOLTAGE-CLAMP CIRCUIT We suspected that imposing the voltage clamping itself might have some effects on the contractile mechanisms of the node. For example, if there had occurred a high-frequency oscillation that escaped detection, the oscillating current might have affected the contractile

mechanisms. This possibility was eliminated by several experiments in which simulated action potentials were imposed by voltage clamping to the node in *normal Ringer solution* (Fig. 8). The twitch produced under these conditions (B), was nearly the same as the twitch produced by a real action potential under the current-clamping condition (A). The result indicates that imposing voltage clamping itself does not influence the mechanical output of the node.

END EFFECTS The nodal length was determined optically. As discussed in the previous paper, it might or might not correspond to the real (or effective) nodal length. During the simulated action potential the optically determined nodal length was the same as that during the real action potential (legend of Table I). But it might still be argued that the end effects are different between the two conditions, and the difference in twitch force arose because a greater portion of muscle might have been activated by the normal action potential than by the simulated AP.

In order to examine this possibility we performed experiments in which

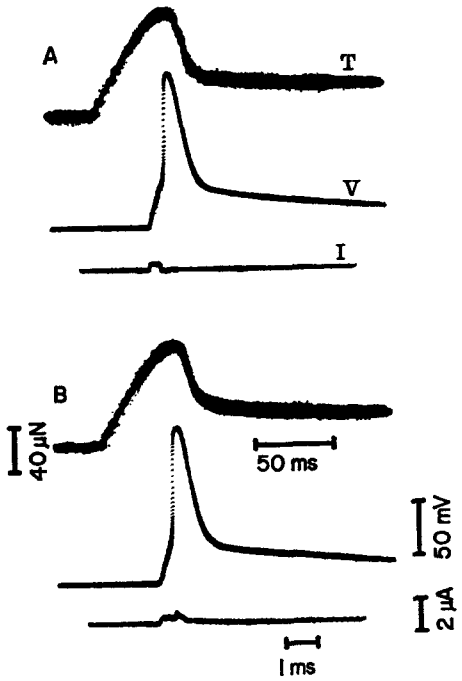

FIGURE 8. The upper beam of each photograph is a twitch on a slow time base (*T*). The middle beam is potential on a fast time base (*V*). The lowest beam is current on a fast time base (*I*). (A) Real action potential in normal Ringer solution under current-clamping condition. (B) The action potential was produced under voltage-clamping condition in normal Ringer solution, by sending the wave form of the action potential stored on tape into the command input. Fiber diameter 118 μm. Nodal length 338 μm. Bath temperature 22.5°C.

twitch height was measured at varied nodal lengths in the same fiber (Fig. 9). The filled symbols in the graph of Fig. 9 show experiments on three fibers in which twitches produced by real AP's were recorded with varied nodal lengths. Fig. 9 A1 and A2 are sample records of the experiments. As seen from the figure, the twitch height became smaller as the nodal length became shorter. A regression line was drawn through these points, and the extrapolated value of the nodal length at zero twitch height was determined. This will be referred to as the end effect, or the difference between optically determined length and the physiologically effective length. If the extrapolated point fell across to the right side of the ordinate of Fig. 9, a negative sign was assigned to the value. For example, in the case of the fiber represented by the

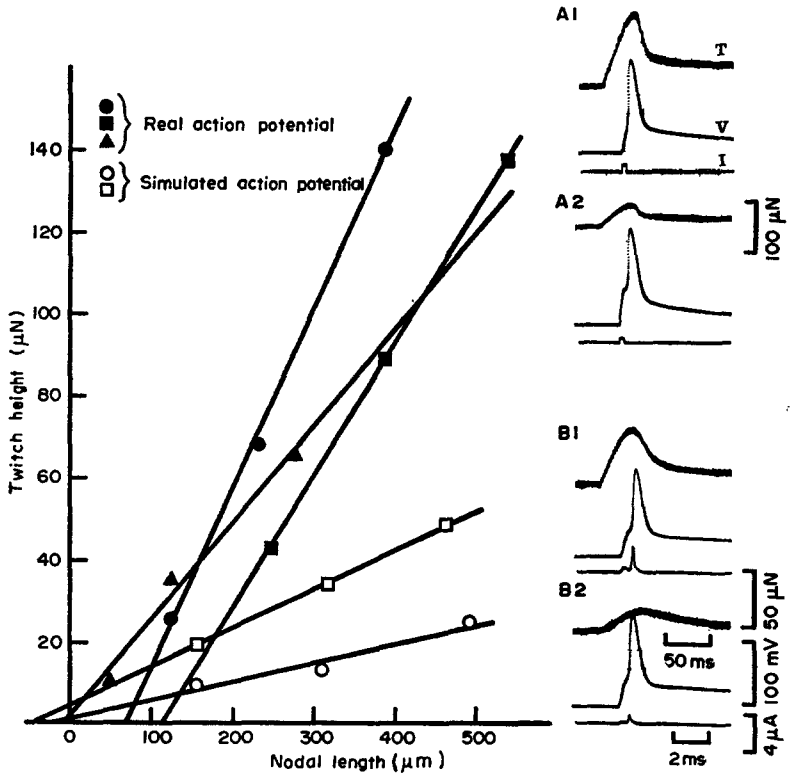

FIGURE 9. End effect of sucrose gap. ● ■ ▲: three different fibers in which twitches were induced by real action potentials. Twitch heights were plotted on the ordinate against the nodal length on the abscissa, ○ □: Two different fibers, in which twitches were produced by simulated action potentials in TTX (2.5×10^{-8} g/ml). A1 and A2: Sample records of real action potential (V), current (I), and twitches (T). Nodal length was 537 μm in A1 and 246 μm in A2. Fiber diameter 102 μm. B1 and B2: Sample photographs of simulated action potential (V), current (I), and twitches (T). The sucrose-gap hyperpolarization was not bucked up. Nodal length was 461 μm in B1 and 154 μm in B2. Fiber diameter is 120 μm. Temperature 21.5–23.5°C.

filled circles in Fig. 9, the end effect was $-69\ \mu m$, indicating that the effective nodal length was smaller than the optically determined nodal length. The hollow symbols of Fig. 9 represent the results of experiments in which twitches were produced by simulated AP's, and B1 and B2 are sample records.

Table II summarizes the values of the end effects obtained. As seen from Table II A, in the case of the real action potential the end effect averages $-22\ \mu m$, ranging from -113 to $125\ \mu m$. On the other hand, the end effects of the simulated AP varied more widely from 29 to $456\ \mu m$ (Table II B1). This is

TABLE II
END EFFECT

(A) Fiber reference	End effect, twitch by real AP	(B) Fiber reference	(1) End effect, twitch by simulated AP	(2) End effect, simulated AP electrical quantity
	μm		μm	μm
X129	+21	X133	+456	+38
X131	+125	X136	+165	+119
X132	−67	X138	+45	+34
X134	−69	X140	+119	+9
X137	+7	X179	+45	+34
X184	−59	X180	+49	+33
X186	−113	X181	+334	+142
		X182	+29	+15
Mean ± SEM	−22±30	Mean ± SEM	155±56	53±17

End effects were calculated from regression lines as in Fig. 9. The negative value was assigned when the extrapolated value came to the right side of the ordinate of Fig. 9. Temperature was 21.5–23.5°C.

probably due to the difference in the easiness of the experiments. In the case of the real AP the only procedure to be performed was to narrow the nodal length, while the node was periodically stimulated. On the other hand, in the experiments with simulated AP's, we had to loosen and then tighten the voltage clamp each time the nodal length was changed. It is likely that in Fiber X133 and X181, the node was deteriorated when the nodal length became very narrow, and started to produce larger twitches.[1]

That this is probably the case was inferred by analyzing the electrical quantities flowing during the simulated AP at different nodal lengths. As seen in B1 and B2 of Fig. 9 the currents (the lowest beam of each record) flowing during simulated AP became less as the nodal length was made smaller. The area comprising the current records (electrical quantity) would

[1] When the fiber deteriorated, we frequently encountered a situation in which twitches became larger than when healthy. Depolarization also produced an increase in twitch. This phenomenon will be discussed later.

be another indication of the nodal area. Similar plots as in Fig. 9 have been made using the electrical quantities rather than the twitch height. Table II B2, which lists the end effect thus obtained for the electrical quantities, shows that the mean end effect is 53 μm. Particularly noteworthy are the cases of X133 and X181, in which large discrepancies existed in the values of end effect determined by the two procedures. Thus, a likely conclusion is that in these two fibers deterioration of the node produced larger contractions at the narrow nodal length.

Although the data of the experiments are not as satisfactory as one wished, it is clear that the smaller twitches produced by the simulated AP's are not due to a smaller area being activated. Rather the difference in the end effects, if there were one at all, is in the wrong direction to explain the smaller twitches produced by the simulated AP's, and indicates a possible overestimation of the twitch in response to the simulated AP.

SIZE DIFFERENCE OF ACTION POTENTIALS As discussed in the previous paper, because of the short-circuiting factor of the sucrose gap, about 98% of the real magnitude of action potential is recorded. However, since the short-circuiting factors ought to be nearly the same during the real action potential and simulated AP, the real magnitude of action potential in the node should be the same so long as the recordable potentials are the same.

Another problem involves the cable property of the node. In the case of real action potential, the magnitude of the potential changes over the short length of the node should be the same, whereas in the case of simulated AP the potential is not uniform, the potential changes near the I-side pool being larger than those near the V-side pool. Thus, the average spike height of the simulated AP along the node would be a little larger than the recorded potential. The potential distribution in the simulated AP can be analyzed, as a first approximation, by the short-cable theory,

When a steady-state sinusoidal current is applied to the left end of the node, the potential changes at a point along the node with distance x from the left end relative to that at the right end is given by:

$$\cosh \{\gamma(L - x)\}, \tag{1}$$

in which γ is the propagation constant and L is the length of the node. We constructed an electrical model of the node using capacitors and resistors, and applied a wave form of action potential from the tape recorder. It was found that potential decrement along the model was approximately the same as that of sinusoidal wave of 900 Hz. So, we analyzed the potential distribution of a sinusoidal wave of 900 Hz using Eq. 1.

Assuming that at 900 Hz, all the membrane admittance is due to capacity (3.2 μF/cm^2, 1 kHz; Nakajima and Bastian, in preparation), then in the sample of Table I A the average value of L/λ_{AC} was 0.7 ranging from 0.36 to

0.85. (λ_{AC} = length constant determined by AC, which is the reciprocal of the real part of γ, Falk and Fatt, 1964; Eisenberg and Johnson, 1970). We have computed the potential distribution of 21 equally spaced points along the node with the value of $L/\lambda_{AC} = 0.7$. The average value of the magnitudes of potential at these 21 points turned out to be larger than the magnitude at $x = L$ by only 1.6%. In order to estimate how these potentials contribute to the total contraction, we needed to know the relationship between twitch height and action potential height. Similar experiments as in Fig. 7, except using larger RC values to achieve larger reduction of simulated AP, were conducted on three fibers. The results revealed that when action potential height was changed from 195 mV to 150 mV the twitch decreased on the average by only 15% without obvious nonlinearity. Thus, we assumed a linear relationship between action potential and twitch height over this range of action potential, and this relationship was used to convert the changes of action potential into the change of twitch. In the case of $L/\lambda_{AC} = 0.7$; the average AP height was 1.6% larger than the recorded AP, and this means that the average twitch height would be only 0.9% greater than the case where there was no decrement of potential.

The above calculation is an underestimate of the situation. In actuality, when the simulated action potential approached the peak, the potassium conductance would have been increased. Although the data for muscle fiber are not readily available, from Fig. 17 of Hodgkin and Huxley's (1952) paper, it could be that at the peak of action potential the potassium conductance is about 4 mmho/cm^2. This corresponds to an extra admittance of about one-fourth of the high-frequency capacity, and this does not affect the above estimation of the error considerably. In summary, although there is some uncertainty in adopting the linear analysis to this system, it seems that the errors from the short cable property of the node are less than 2%, and thus if there had not been a cable property, the twitches caused by the simulated AP's would have been slightly smaller than the values reported here.

CHANGES IN RESTING POTENTIAL The resting membrane potential has a distinct effect on twitch of muscle. Fig. 10 illustrates the relationship between resting potential and twitch height. The twitch was produced by a real action potential (filled circles and the sample records A, B, C), and the membrane potential had been set by conditioning hyper- or depolarizations. It can be seen that twitches became larger by conditioning depolarizations and smaller by conditioning hyperpolarizations. Since each record of Fig. 10 was obtained at an interval of 16.4 s, the conditioning polarizations would have been applied for about 10 s before an action potential was elicited. In the experiment of Fig. 10, there was no deterioration during the long period of the experiment and the hyper- or depolarizations were applied many times in random sequences without resulting in "hysteresis." The same kind of experiment on

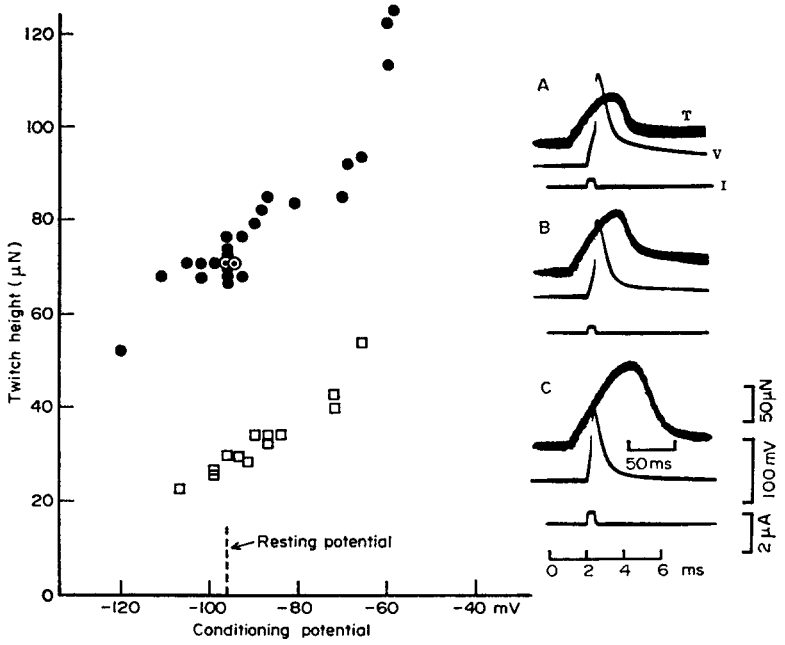

FIGURE 10. Graph: Relationship between conditioning potentials and the twitch heights. ●: Twitches produced by real action potentials. The double circles indicate coincidence of two data. □: Twitches produced by simulated AP. Inset photographs: Twitches were produced by real action potentials. The conditioning membrane potentials were −96 mV in A, −69 mV in B and −58 mV in C. The resting potentials were estimated on the assumption that the peak level of the normal action potential has an overshoot of 46 mV (Table III of Nakajima and Bastian, 1974). The estimated levels of the peak of the action potentials are 46 mV in A, 49 mV in B, and 47 mV in C. Fiber diameter 115 μm. Experiment at room temperature.

four fibers revealed that a depolarization from −100 mV to −90 mV produced an average increase in twitch height of 17% ranging from 5 to 26%.

A similar relation between the conditioning potentials and the twitch height was obtained when the twitches were elicited by simulated AP (open squares of Fig. 10). One experiment was also conducted in which a depolarizing test pulse of 2.5 ms in duration was superimposed on the conditioning polarizations, keeping the potential level at the peak of test pulse constant (−16 mV). Again the conditioning depolarization enhanced the contraction.

We did not expect these results, since Hodgkin and Horowicz (1960) and Heistracher and Hunt (1969) showed that in the steady state the degree of restoration of the contractile system was less when the membrane was depolarized (inactivation of contracture). But, the fact that an increase in external K concentration enhanced the twitch tension (Sandow and Kahn, 1952; Mashima and Matsumura, 1962) is in keeping with the present finding. Perhaps this phenomenon is related to the fact that in the present case the condi-

tioning depolarizations were applied *before* and *after* the application of test pulse, and this *after-depolarization*, although, if applied by itself, is subthreshold for contraction, produced the augmentation of twitches. This kind of effect can be expected from the result by Adrian, Chandler, and Hodgkin (Fig. 4 of their paper, 1969). Dr. C. Caputo and Dr. R. DiPolo have also told us that they recently observed a similar phenomenon in barnacle muscle (personal communication).

Turning back to the experiments summarized in Table I A, the resting potential during simulated AP was maintained at the same level as that during the control period. Therefore, no error would arise in estimating twitches during this period. However, during the washing period of TTX, when the recovery of twitch was attained, there occurred on the average a depolarization of 3.1 mV. Therefore, the twitch after the recovery from TTX could have been overestimated by a few percent.

DISCUSSION

Presence of Excitation in the T System

One of the main conclusions from this study is: *when a normal action potential occurs in the surface membrane of Xenopus muscle bathed in the normal Ringer, an Na-dependent action potential occurs in the T system.* This conclusion is in agreement, and is to be compared with that of Costantin (1970), and Costantin and Taylor (1971), who showed that: *a regenerative action potential was triggered in the T system when the surface membrane was depolarized by rectangular pulses in 50% $[Na]_o$ solution.* The idea of the existence of regenerative activity in the T system has also been strengthened by recent experiments by Bezanilla, et al. (1972), Nakajima and Bastian (1972), Peachey and Adrian (1973), and Caputo and DiPolo (1973).

Necessity of Excitability in the T System

Furthermore, the present study showed that the action potential of the T system is *necessary* to produce normal twitch or normal tetanus. It was estimated that, if it were not for the action potential in the T system, the magnitude of the isotonic twitch shortening would be reduced to about 30% of that of the normal twitch in a fiber of 116 μm in diameter at room temperature. In the case of short-duration isotonic tetanus, when the magnitude of shortening is not very large, again the mechanical output would be reduced to about 30%, if the T-system excitability is inhibited. Either way, under the present conditions the T-system excitability contributes to roughly 70% of the magnitude of the mechanical output. However, in order to relate the present results with the "degree of activation" or "active state," further experiments are necessary.

Finally, it was shown that at about 10°C, the action potential in the T sys-

tem became less necessary in the production of normal twitch, although the excitability of the T system still probably existed. The contribution of the T-system AP at 10°C is only 15% of the total magnitude of the contraction. Thus, it is very likely that the necessity of the T-system excitability becomes negligibly small at a temperature near 0°C. The T-system excitability contributes only to quick muscular contraction near room temperatures.

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