The Role of Sodium Current in the Radial Spread of Contraction in Frog Muscle Fibers

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ABSTRACT The membrane potential of isolated muscle fibers was controlled with a two-electrode voltage clamp, and the radial extent of contraction elicited by depolarizing pulses of increasing magnitude was observed microscopically. Depolarizations of the fiber surface only 1–2 mv greater than the contraction threshold produced shortening throughout the entire cross-section of the muscle fiber. The radial spread of contraction was less effective in fibers exposed to tetrodotoxin or to a bathing medium with a greatly reduced sodium concentration. The results provide evidence that depolarization of a muscle fiber produces an increase in sodium conductance in the T tubule membrane and that the resultant sodium current contributes to the spread of depolarization along the T system.

Although it is generally accepted that the T system of twitch muscle fibers transmits the influence of surface depolarization radially, the mode of transmission within the T system has not been established. Huxley and Taylor (1958) found only a graded inward spread of contraction with increasing depolarization of local sites on the surface membrane, while the results of Gonzales-Serratos (1966) on the temperature dependence of the radial spread of activation were more compatible with active propagation within the T system. Recently Adrian, Costantin, and Peachey[1] (1969) reported that the radial spread of contraction with controlled surface depolarizations was not entirely consistent with a passive electrotonic spread of depolarization along the T system; they suggested that delayed rectification, an increase in the potassium conductance with depolarization, might be present in the T tubules. The experiments of ACP were performed on fibers exposed to tetrodotoxin (TTX), so that the presence of a regenerative increase in sodium conductance within the T tubules of a normal muscle fiber could not be ruled out.

[1] The abbreviation ACP will be employed to refer to this article in the present manuscript.

In the present study, the experimental approach utilized by ACP was applied to fibers bathed in a TTX-free Ringer solution. Since application of a long depolarizing step to a muscle fiber in normal Ringer solution will result in a large active sodium current and a propagated action potential before the contraction threshold is reached (Costantin, 1968), part of the sodium in the bathing solution was replaced by choline, decreasing the magnitude of the sodium current sufficiently to permit depolarizations to the contraction threshold without a propagated action potential. It was hoped that, if an active sodium current was present in the T tubules, it would be sufficient in magnitude, despite the lowered sodium concentration, to produce more effective inward spread of contraction than was observed by ACP in TTX-treated fibers.

METHODS

Single fibers dissected from the semitendinosus muscle of *Rana pipiens* were studied by the voltage clamp method described by ACP. A section of the fiber was immobilized by resting it on two vaseline-coated Lucite pedestals separated by 2–4 mm. The region of the fiber chosen for study was located between these two pedestals and thus was completely suspended in bathing solution.

The single fiber was impaled by two microelectrodes; one served to record the membrane potential and the second, inserted diametrically opposite the first, served to pass current from an appropriate feedback circuit. Since the space constant of a muscle fiber is greater than 1 mm, the electrotonic decrement of potential 20 μ from the site where the electrodes were inserted was less than 2%; thus, the method permitted the establishment of a relatively uniform depolarization over a short length of the fiber about the site of electrode insertion. Although some distortion of membrane potential is produced by electrode impalement itself, the effect is localized to a region of a few microns about the electrode site (see below). The voltage-clamped region was observed with polarization optics. A large illuminating aperture made it possible to obtain an optical section passing through the center of the fiber and thus to evaluate the depth of the fiber which contracted in response to depolarizing pulses of increasing magnitude. Contractions were recorded on 16 mm cinefilm at 16 or 32 frames/sec. The magnification of the specimen in the film plane was 60 times except for two fibers in which the magnification was 45 times. The feedback circuit and the optical equipment were similar to those described by ACP.

All experiments were performed at room temperature (20–26°C).

Determination of Contraction Thresholds

Fibers were clamped at a holding potential of −90 mv (inside potential minus outside potential). Thresholds were measured as a positive displacement of the inside of the cell from the holding potential. Contraction thresholds were determined by review of the cinefilms. Where there was some uncertainty as to which of two pulses should be taken as a threshold, the value was recorded as the mean of the two pulses.

The first sign of contraction when depolarizing steps of increasing magnitude were

applied was a small local contraction at the site of impalement with the current-passing electrode (Fig. 1). ACP have attributed this to a local distortion of the membrane potential caused by the high current density in this region. This local response was ignored, and the threshold for contraction of superficial myofibrils was determined on the side of the fiber diametrically opposite to the current electrode. The first shortening to develop in this region usually involved 10 or more sarcomeres, and the magnitude of this depolarization step was taken as the contraction threshold.

On occasion, a localized contraction was also seen at the voltage-recording electrode, presumably as a result of a low resistance leak in the membrane due to electrode impalement. Since this localized response only involved the one or two sarcomeres immediately adjacent to the electrode, it was readily distinguishable from the more generalized shortening which was taken as the contraction threshold. In most experiments, care was taken to retain both electrodes within the plane of focus (Fig. 2) to ascertain that the shortening observed in axial myofibrils was not a result of an extensive local contraction at one or the other electrode.

Determination of the Threshold for Net Inward Current

The steady-state relation between the clamping current (I_o) during a depolarizing pulse (V) and the membrane current density (I_m) at the site of the recording electrode is given by Cole's theorem, $I_m \propto I_o\ (dI_o/dV)$ (Cole, 1961). Thus a net inward membrane current at the site of application of a depolarizing pulse should result in a negative slope in the plot of steady-state electrode current vs. depolarization. Because of the relatively long capacitive transient in muscle fibers, inactivation of inward current occurs before a steady level of electrode current is reached during a depolarizing pulse (see Costantin, 1968, Fig. 5). Since capacitive current increases with increasing depolarization, however, a negative value of dI_o/dV determined at any time during an applied depolarization step will still reflect a net inward ionic current. Accordingly the threshold for the appearance of net inward current was determined by comparing current records of progressively increasing depolarizing pulses; the threshold was taken to be that pulse in which the current at some time during the pulse was less than the level reached at the same time during the previous pulse.

Solutions

All fibers were routinely isolated in normal Ringer solution containing 115 mM NaCl, 2.5 mM KCl, 1.8 mM $CaCl_2$, 2.15 mM Na_2HPO_4, and 0.85 mM NaH_2PO_4. The solution was then changed to one with a lowered sodium concentration by serial dilution of the bathing solution with a modified Ringer solution in which part of the NaCl had been replaced by an equimolar amount of choline chloride (obtained from Eastman Organic Chemicals, Rochester, N. Y.). The final sodium concentration achieved by this procedure was determined by flame photometry at the end of the experiment.

At least 20 min elapsed between the initial application of the choline-containing solution and impalement of the muscle fiber. The mean membrane potential (±SE of mean) of the 22 fibers studied was -91.4 ± 0.8 mv.

In those fibers exposed to TTX-containing solution, the TTX concentration was about 0.8 μg/ml.

RESULTS

Inward Spread of Activation with Long Depolarizing Pulses

50% SODIUM RINGER Fibers bathed in a Ringer solution in which the sodium concentration was lowered to approximately 50% of normal were examined with depolarizing steps 200 msec in duration. Pulses were applied every 15 sec, and the magnitude of each successive step was increased by 1 mv until extensive shortening occurred throughout the fiber cross-section. Fig. 1 shows a fiber in which the sodium concentration in the bathing medium has been reduced to 53 mM. In panel B, with a just threshold depolarization, there is slight shortening of the superficial myofibrils with no change in sarcomere length along the axis of the fiber. In panel C, when the depolarizing pulse is 1 mv larger than the threshold for surface contraction, both superficial and axial myofibrils shorten to approximately the same extent. Further depolarization (panel D) produced more extensive shortening of both superficial and axial myofibrils. This result is quite different from that reported by ACP in TTX-treated fibers. In their study, "the superficial myofibrils shortened more extensively than the central ones so that a bowing of the striation pattern usually developed" with suprathreshold depolarizations.

In four fibers the superficial myofibrils contracted in response to a depolarizing step which was 1–2.5 mv less than was required to produce shortening throughout the entire fiber cross-section. In four other fibers, there was no detectable difference between the threshold for surface contraction (V_s) and the threshold for activation of the entire fiber cross-section (V_c); with the application of a just threshold depolarization, both superficial and axial myofibrils appeared to shorten simultaneously. These results are summarized in Table I in the column headed $V_c - V_s$.

If it is assumed that each myofibril is activated whenever the depolarization of its adjacent T tubule reaches a definite threshold value for a given fiber, the depolarization V_s required to just activate the superficial myofibrils will be a measure of this T tubule threshold. Thus, when a depolarization of magnitude V_c is applied to the fiber surface, the T tubules at the center of the fiber (where a just threshold contraction is present) will be depolarized by an amount V_s, and the ratio V_s/V_c can be taken as an index of the effectiveness of the T system in transmitting a surface depolarization inward. This ratio is recorded in the penultimate column of Table I. In most of the fibers studied, the depolarization of the T tubules at the axis of the fiber appeared to be within 3% of the surface membrane depolarization. If the T system is assumed to be a network of tubules with linear cable properties, the space constant (λ_T) for electrotonic spread in the T system can be calculated from the ratio V_s/V_c (see ACP, equation 3). The values of λ_T are given in the last

column of Table I. Both V_s/V_c and λ_T for the fibers in the present experiments are consistently larger than those reported for TTX-treated fibers by ACP, and it would appear that the inward spread of activation is much more effective when TTX is omitted from the solution bathing the fiber.

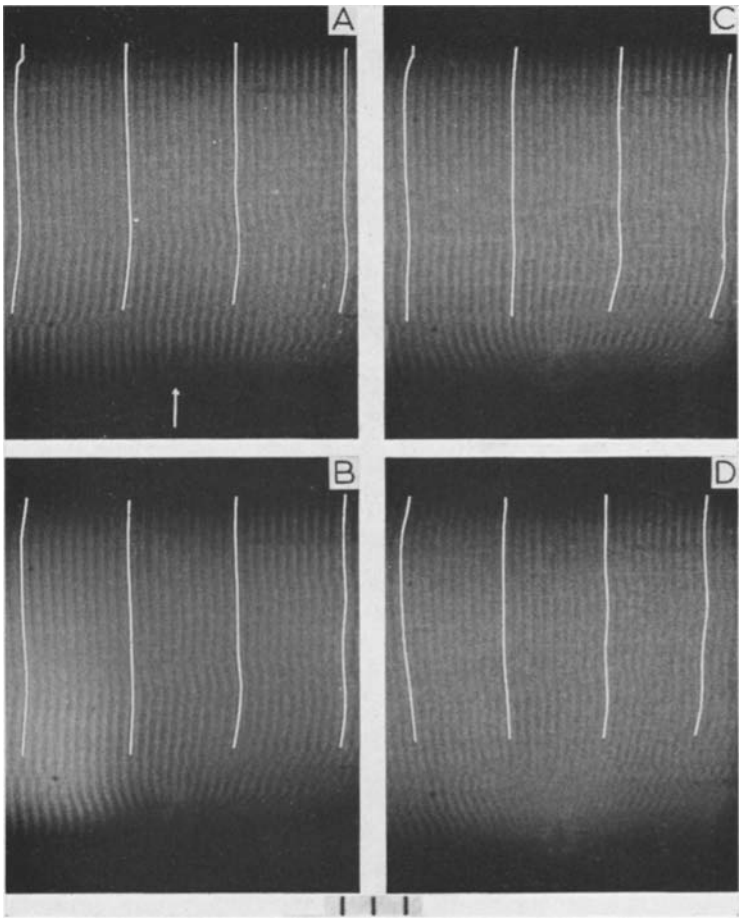

FIGURE 1. Frames from cinefilm of a relaxed muscle fiber (A) and of the maximum contraction elicited by 200 msec pulses of increasing magnitude (B, C, and D). A, holding potential = −90 mv. Sarcomere length = 3.38 μ. Arrow indicates site of insertion of current-passing electrode. The voltage-recording electrode has been inserted on the opposite side of the fiber out of the plane of focus. B, 30 mv depolarizing step. The superficial sarcomeres at the upper edge of the fiber have shortened to 3.33 μ. Axial sarcomere length = 3.38 μ. C, 31 mv depolarizing step. Superficial sarcomere length 3.30 μ. Axial sarcomere length = 3.34 μ. D, 32 mv depolarizing step. Superficial sarcomere length = 3.12 μ. Axial sarcomere length 3.20 μ. An intense localized contraction can be seen in the region of the current electrode in panels B, C, and D. Bathing solution, 53 mM sodium Ringer. Grid spacing 10 μ. The white lines in each frame mark every tenth sarcomere. The sarcomere length was determined as the mean of 20 sarcomeres.

EFFECT OF TTX IN 50% SODIUM RINGER Since the experiments of ACP on TTX-treated fibers were carried out in a normal Ringer solution, it was thought advisable to repeat their observations on TTX-treated fibers bathed in a low sodium Ringer solution. Three fibers were studied in a 50% sodium Ringer solution, and the results are shown in Table I. The ratio V_s/V_c and the calculated λ_T are similar to those reported by ACP for fibers of comparable diameter in normal Ringer solution.

TABLE I

INWARD SPREAD OF DEPOLARIZATION WITH 200 MSEC DEPOLARIZING PULSES

	Fiber	Sodium concentration	Radius	Contraction threshold	$V_c - V_s$	V_s/V_c	λ_T
		mM	μ	mv	mv		μ
50% sodium Ringer	Jan. 22	71	30.5	31	<1.0	>0.97	>87
	Mar. 7	48	38	26	<1.0	>0.96	>98
	May 27	64	38	29.5	1.5	0.95	86
	Feb. 6	56	39.5	31	1.0	0.97	113
	June 4	59	40	31	<1.0	>0.97	>114
	Feb. 5	64	44.5	32	<1.0	>0.97	>129
	Apr. 25	53	52	30	1.0	0.97	146
	June 3	60	58	32.5	2.5	0.93	105
50% sodium Ringer + TTX	June 5	56	29	32	3.0	0.91	48
	Feb. 12	52	31	34	2.0	0.94	65
	Feb. 19	66	47	36	3.0	0.92	81
Normal Ringer	May 1	120	42	31*	—	—	—
25% sodium Ringer	Mar. 18	32	39	29	2.5	0.92	66

* Propagated action potential and twitch. No localized contraction was obtained.

EFFECT OF VARYING SODIUM CONCENTRATIONS One fiber was examined in normal Ringer solution (120 mM Na) with 200 msec depolarizing pulses; a propagated action potential and a twitch developed before any sign of local contraction was seen.

If an active sodium current in the T tubules does play a role in the radial spread of activation, one would expect that, as the sodium concentration is reduced, V_s/V_c and λ_T would approach the values seen in TTX-treated fibers. The response of the one fiber studied in 32 mM Na Ringer (Table I) suggests that this is the case. The question was explored further when the response to brief depolarizing pulses was examined (see below).

Inward Spread of Activation with Brief Depolarizing Pulses

50% SODIUM RINGER If radial spread of activation occurs by passive electrotonic spread along the T tubules, greater attenuation of an applied

surface depolarization should occur with brief pulses than with pulses of long duration. This appears to be the case in TTX-treated fibers, since ACP found that V_s/V_c was consistently smaller for 3 msec pulses than for 200 msec pulses. It was therefore of interest to examine the radial spread of contraction with brief pulses in the present experiments. In order to elicit a sustained tetanus, a train of 10 pulses with a pulse interval of 10 msec was applied. The pulse duration was usually 3 msec, although 2 or 5 msec pulses were sometimes employed. The magnitude of the depolarizing pulses was increased in 1 mv steps. 11 fibers were examined in 50% sodium Ringer, and the results are summarized in Table II. The first sign of contraction in three fibers was a twitch which dislodged the microelectrodes. In two other fibers, it was possible to elicit a contraction of the superficial myofibrils alone, but a propagated action potential and a twitch developed with 2–3 mv further depolarization. In the remaining six fibers, local shortening of both superficial and axial myofibrils could be obtained with a controlled surface mem-

TABLE II

INWARD SPREAD OF DEPOLARIZATION WITH 100 MSEC TRAINS OF BRIEF DEPOLARIZING PULSES

	Fiber	Sodium concentration	Radius	Pulse duration	Contraction threshold	$V_c - V_s$	V_s/V_c
		mM	*μ*	*msec*	*mv*	*mv*	
50% sodium Ringer	May 2	59	35	3	47‡	—	—
	Mar. 6	58	37.5	3	39‡	—	—
	Mar. 7*	48	38	3	39.5	<0.0	>1.00
	May 27*	64	38	3	43	>2.0‡	<0.96
	June 4*	59	40	3	39.5	<0.0	>1.00
	May 7	54	42	3	42.5	<0.0	>1.00
	Feb. 5*	64	44.5	5	37	<1.0	>0.97
	Mar. 12	48	45	3	35‡	—	—
	Apr. 25*	53	52	3	42	>2.0‡	<0.95
	June 3*	60	58	3	41.5	<0.0	>1.00
	Jan. 29	72	68.5	5	22	<0.0	>1.00
				2	28	<0.0	>1.00
50% sodium Ringer + TTX	June 5*	56	29	3	48.5	12.0	0.80
	Feb. 12*	52	31	3	54	5.0	0.92
	Feb. 19*	66	47	3	51	10.0	0.84
Normal Ringer	Apr. 30	120	32.5	3	38	<1.0	>0.97
25% sodium Ringer	Mar. 19	28	34.5	3	47	7.5	0.86
	June 12	26	37	3	44.5	3.0	0.94
	Mar. 18*	32	39	3	49	4.0	0.92
	June 13	28	42	3	41.5	4.0	0.91

* Same fiber as in Table I.

‡ Propagated action potential and twitch.

brane depolarization. In one of these fibers (Feb. 5) both superficial and axial myofibrils appeared to shorten simultaneously, a result quite similar to that seen with long depolarizing pulses in this fiber. In the remaining five fibers, however, the axial myofibrils appeared to shorten more vigorously than the more superficial ones; the difference between center and surface threshold has been indicated as negative for these fibers in Table II. Fig. 2 is taken from the cinerecord of one of these fibers. The first detectable shortening, except for a small localized contraction at the site of the current-passing electrode, appeared to be confined to the axial myofibrils (panel B). Further

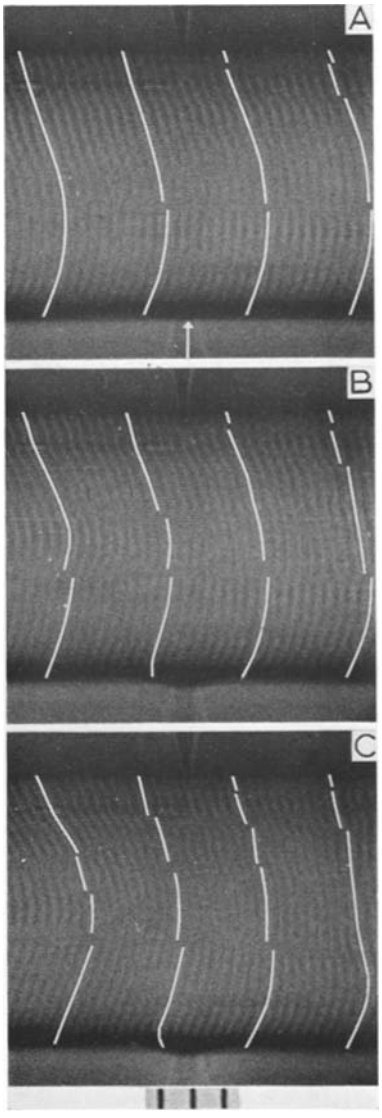

FIGURE 2. Frames from cinefilm of a relaxed muscle fiber (A) and of the maximum contraction elicited by a train of 3 msec depolarizing pulses (B and C). A, holding potential = −90 mv. Sarcomere length = 3.57 μ. Arrow indicates site of insertion of current-passing electrode. The voltage-recording electrode can be seen on the opposite side of the fiber. B, 43 mv depolarizing pulses. The axial sarcomeres have shortened to 3.34 μ. Superficial sarcomere length = 3.57 μ. C, 44 mv depolarizing pulses. Axial sarcomere length 3.12 μ. Superficial sarcomere length = 3.40 μ. A small localized contraction can be seen in the region of the current electrode in panels B and C. Bathing solution, 54 mM sodium Ringer. Grid spacing 10 μ. The white lines in each frame mark every ninth sarcomere. The sarcomere length was determined as the mean of 20 sarcomeres.

depolarization (panel C) produced shortening throughout the entire cross-section of the fiber, but the axial myofibrils shortened more extensively than the more superficial ones.

EFFECT OF TTX IN 50% SODIUM RINGER The three fibers in which the 50% sodium Ringer contained TTX were also examined with trains of 3 msec depolarizing pulses. In all three, contraction occurred first in the superficial myofibrils and spread radially as the surface depolarization was increased. The values of $V_c - V_s$ and V_s/V_c for these fibers are recorded in Table II. V_s/V_c is somewhat less in each instance than the value obtained with 200 msec depolarizing pulses in the same fiber, a result consistent with a time-dependent attenuation of a brief surface depolarization along the T system as reported by ACP.

EFFECT OF VARYING SODIUM CONCENTRATION One fiber was examined in normal Ringer solution with a train of 3 msec depolarizing pulses. A local contraction was elicited which appeared to involve the entire fiber cross-section. With a 1 mv increase in the magnitude of the depolarizing pulse, a propagated action potential was produced.

Four fibers were studied in a 25% sodium Ringer solution. In all four, the response to brief depolarizing pulses qualitatively resembled the response of a TTX-treated fiber, that is, shortening occurred first in the superficial myofibrils and spread radially as the surface depolarization was increased. As can be seen in Table II, the values of V_s/V_c approach those of TTX-treated fibers.

Net Inward Current in Low Sodium Ringer

With 200 msec depolarizing pulses, a net inward current was seen in only two fibers, although depolarization was sometimes carried up to 7 mv beyond the contraction threshold. With brief depolarizations a net inward current appeared within 1 mv of the contraction threshold in all fibers studied in TTX-free 50% sodium Ringer. With a depolarization of 2–4 mv beyond the contraction threshold, a propagated action potential usually developed. The one exception was the fiber of Jan. 29 in which depolarizations to 13 mv beyond the contraction threshold with 2 msec pulses failed to elicit a propagated action potential. In fibers in 25% sodium Ringer, a net inward current appeared with a depolarization of 1–7 mv beyond the contraction threshold. No evidence of inward current was found in TTX-treated fibers.

DISCUSSION

The present experiments have shown that a surface depolarization which is only slightly greater than the contraction threshold can produce shortening throughout the entire cross-section of a muscle fiber, and that the radial spread

of contraction can be reduced either by lowering the extracellular sodium concentration or by the addition of TTX to the bathing medium. Since TTX produces a specific block of the increase in sodium conductance with depolarization in a wide variety of tissues, the most straightforward explanation for the present results is that depolarization can produce an increase in sodium conductance in the T tubule membrane and that the resultant inward sodium current contributes to the spread of depolarization along the T system.

Reversal of the Potential Gradient in the T System

The observation that, in some fibers, shortening was greater in axial myofibrils than in more superficial ones (Fig. 2) suggests that in these preparations the T tubules were more depolarized in the center of the fiber than at the fiber surface. This reversal of the gradient of depolarization along the T system implies that a net inward membrane current was present throughout the entire T system in the region of the fiber involved in the contraction. That this net inward current was not sufficient to generate a propagated action potential along the length of the muscle fiber may be a result of the known differences in the ionic conductances of the surface and T system membranes. Since most of the chloride conductance of the muscle fiber resides in the surface membrane (Hodgkin and Horowicz, 1960 *a*; Eisenberg and Gage, 1969) the outward current in the surface membrane (carried by Cl^- and K^+) would be much larger than the outward current in the T system membrane (carried almost entirely by K^+) at the same depolarization. Furthermore, the marked fall in potassium conductance with depolarization (ingoing or anomalous rectification) would further serve to decrease the outward ionic current in the T system, and it seems likely that activation of only a very small inward sodium current would be required to produce a net inward current in the T tubules.

Another factor which might contribute to the apparent reversal of the depolarization gradient along the T system without the development of a propagated action potential along the length of the muscle fiber is the difference in geometry of these two systems. A localized depolarization applied to a cable will no longer decrement with distance when the membrane area near the site of depolarization produces an inward current larger than that required to balance the outward current generated by more distant areas of the membrane (Noble and Stein, 1966). In the present experiments, the portions of the fiber which extend laterally from the electrode site can be approximated by a linearly oriented, infinitely long cable. This is not true of the T system immediately underlying the site of electrode impalement, however; as a direct consequence of the radial convergence of the T tubules, the area of membrane at a distance from the fiber surface is inversely pro-

portional to the distance from the surface. Thus the area of membrane available to generate outward current is less than in a linearly oriented, infinitely long cable, and the depolarization required for the development of a reversed potential gradient will be correspondingly lower.

The absence of an "all-or-none" response when a steady depolarization is applied to the mouth of a single T tubule (Huxley and Taylor, 1958) might also be a consequence of the geometry of the T system. With local depolarization of the tubular network, the number of tubules would increase as the distance from the site of depolarization increased, and this would be expected to raise the threshold for a propagated depolarization. A similar explanation has been advanced by Noble (1962) to account for the absence of a regenerative response with point polarization of a sheet of cardiac muscle.

Contraction Threshold

Hodgkin and Horowicz (1960 *b*) have reported that replacement of sodium by choline produces a shift of about 5 mv in the relation between membrane potential and contracture tension in frog semitendinosus muscle. When the TTX-treated fibers in 50% sodium Ringer from the present study are compared with the results of ACP on TTX-treated fibers in normal Ringer (Table III), the thresholds are quite similar for both 200 and 3 msec depolarizing pulses, and no effect of choline is apparent. This may simply be the result of a species difference in contraction threshold in the two studies, however, since ACP's experiments were performed on *Rana temporaria*, while *Rana pipiens* was employed in the present experiments. A more surprising finding was that the contraction threshold was consistently lower in TTX-free bathing media; the threshold depolarization for 3 msec pulses was 41.3 ± 0.6 mv in TTX-free 50% sodium Ringer and 51.2 ± 1.6 mv in 50% sodium Ringer + TTX (Table III). One possible explanation for this result is that, in a TTX-free medium, the reversed potential gradient within the T system brings the axial T tubules beyond the contraction threshold while the super-

TABLE III

THRESHOLD FOR LOCAL CONTRACTION

Bathing medium	200 msec depolarization	3 msec depolarization
	mv	*mv*
50% sodium Ringer	30.4 ± 0.7	41.3 ± 0.6
25% sodium Ringer	—	45.5 ± 1.6
50% sodium Ringer + TTX	34.0 ± 1.2	51.2 ± 1.6
Normal Ringer + TTX (ACP)	33.0 ± 0.7	48.9 ± 1.7*

Mean ± SE of mean.

* Adrian, Costantin, and Peachey, unpublished data

ficial T tubules and the surface membrane are still below threshold. Thus the surface depolarization when active contraction is present only in axial myofibrils will be an underestimate of the true contraction threshold within the T system. This explanation, however, cannot account for the lowering of the contraction threshold with 3 msec pulses in 25% sodium Ringer or with 200 msec pulses in 50% sodium Ringer (Table III); the initial contraction in these two groups of fibers involved the superficial myofibrils, and the depolarization recorded by the microelectrode was presumably an accurate reflection of the depolarization of the T tubules immediately adjacent to the surface membrane. Moreover, the two fibers in 50% sodium Ringer (May 27 and Apr. 25) in which contraction in response to brief pulses involved only superficial myofibrils also gave low thresholds (43 and 42 mv, respectively, Table II). It is, of course, possible that both TTX and a variation of choline or sodium concentration act to shift the contraction threshold. One other possibility can be raised, however. The progression from 50% sodium Ringer to 25% sodium Ringer to TTX-Ringer can be regarded as a progression of increasing inhibition of the capacity of a biological membrane exposed to these solutions to produce an active sodium current and a regenerative depolarization. If the ultimate site at which membrane depolarization acts to release calcium is the sarcoplasmic reticulum (SR), and if the SR is itself capable of producing a sodium-dependent depolarization in response to depolarization of the T tubule, then the SR could serve to amplify the depolarization of the T tubule. In this case, the depolarization threshold in the T system of a TTX-free fiber would be, not the threshold for calcium release, but the threshold for activation of a regenerative depolarization in the SR membranes. An active mechanism for depolarization of the SR has also been suggested by Costantin and Podolsky (1967). For the present, the evidence for this is indirect and hardly conclusive, but the possibility must be seriously considered. If the SR is capable of a regenerative depolarization, large potential changes might be expected to occur across the membranes of the SR during the action potential, and these potential changes could act as the ultimate trigger for calcium release to the contractile mechanism.

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