EFFECTS OF CONDITIONING MEMBRANE POTENTIALS ON CALCIUM TRANSIENTS IN SKELETAL MUSCLE FIBERS

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Using single muscle fibers of the frog, Hodgkin and Horowicz (9) studied the effect of conditioning depolarizations on the contraction that is subsequently elicited by a test depolarization and found that the contraction was diminished. This inhibitory effect of conditioning depolarization is referred to as contractile inactivation (5, 7, 8, 10).

On the other hand, there are several reports that describe an opposite effect of membrane potential on contractile responses. Bezanilla *et al.* (3) found that subthreshold conditioning depolarizations of 100 msec enhanced contractions produced by a subsequent short (10 msec) test pulse. Bastian and Nakajima (1) reported that conditioning depolarizations of long duration (approximately 10 sec) enhanced muscle twitch produced either by action potentials or by short test pulses [cf. Caputo and DiPolo (6) for the case of barnacle muscle]. Possible interpretations of this effect are that it may result from a more efficient interaction between the activator (calcium) and the contractile machinery (namely, at some step after the calcium is released from the sarcoplasmic reticulum) or that it may result from a more effective coupling between surface depolarizations and the release of calcium (for example, through a better electrotonic propagation in the transverse tubular (T) system).

We have investigated the effects of conditioning membrane polarizations on the calcium release in voltage-clamped frog skeletal muscle fibers loaded with arsenazo III (4, 11) as a calcium indicator. It will be shown that the

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test pulse. Have you done anything like that—a prepulse and then start each test pulse from the same initial potential?

Nakajima: That would reveal the exact time-course of this hypothetical process. It could be 5 msec, say. But even if we got that result—5 msec—we still cannot say whether it coincides with the T system transient. My own feeling is that the T system transient is too fast. That is, that the T-system transient effect might be too small to account for everything. So, in that sense, I agree with Dr. Schneider's comment.

above described effects of conditioning potentials reside in the mechanisms that lead to the release of calcium from the sarcoplasmic reticulum.

Figure 1A shows a light absorption change initiated by a short pulse depolarization (5 msec). The absorption was measured at a wavelength of 652 nm and was recorded from a region of muscle fiber injected with arsenazo III and treated with tetrodotoxin (10^{-6} g/ml). It has been shown by Miledi *et al.* (11) and Baylor *et al.* (2) that the absorption change obtained at this wavelength probably represents a change in Ca^{2+} concentration inside muscle fibers. As shown in record B, when a conditioning subthreshold depolarization of 30 msec preceded the test pulse, the calcium transient became twice as large as that without the conditioning pulse. This enhancing effect of conditioning depolarization is short-lived. As shown in record C, when the

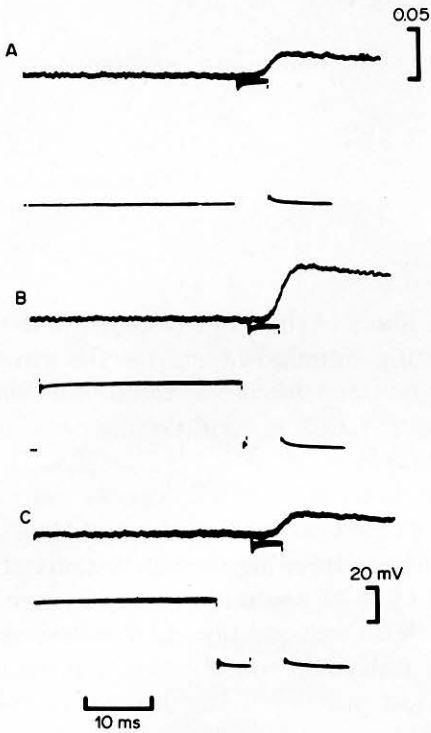

Figure 1. Calcium transients produced by depolarizing pulses in tetrodotoxin (10^{-6} g/ml) treated muscle fibers. A sheet of muscle fibers was prepared from the sartorius of *Rana temporaria*. Arsenazo III was electrophoretically injected (11). The calcium transient (upper beams) was measured as absorption changes at the wavelength of 652 nm from the injected part of muscle fiber. Increase of absorption upward. The light coming from a fiber area of 82 × 100 μm was collected. (At 537 nm, only small absorption decreases were observed, and at 720 nm the records showed essentially flat responses.) The membrane potential (lower beams) was clamped at −99 mV, and depolarizing pulses were applied under voltage-clamp. (A) Test pulse only. (B) Conditioning pulse and test pulse in succession. (C) Conditioning pulse and test pulse were applied with a time gap of 5 msec between them. The calibration (0.05) refers to $\Delta A/A_{652}$ (absorption changes over the resting absorption at 652 nm). Temperature: 20°C.

DISCUSSION

Caputo: If the duration of the prepulse is increased to very long values, you get inactivation. In the barnacle we showed that there is, at the beginning, potentiation of the response . . . then the potentiation decreases with the duration of the pulse (*J. Gen. Physiol.*, 1978, **71:** 467–488). And I think that the same thing happens in skeletal muscle in frog. With relatively short pulses—100 msec to 1 sec—you can get potentiation followed by inactivation. In the experiments of Hodgkin and Horowicz [(*J. Physiol. (London)*, 1960, **153:** 386–403], they had first one contracture—a test contracture—then a recovery contracture, and the membrane potential during recovery determined its peak tension. The inactivation process on long-term exposure is better seen, perhaps, in the Frankenhaeuser and Länngergren experiments in which they started at a different membrane potential (*Acta Physiol. Scand.*, 1967, **69:** 242–254). With long depolarization, there is a decrease in the response to the test contracture. So perhaps, the two phenomena in frog and in barnacle are similar for short pulses. For long pulses you certainly see inactivation. The time-course of decay of potentiation, observed with short prepulses, when you separate the pulses, is certainly very fast.

Nakajima: There are two processes going on. One is the phenomenon I described, and the other is inactivation. If the conditioning pulse is not very large, the inactivation will not occur. On the other hand, this process I showed can become a very big effect, if the pulse becomes short. In our experiment, the conditioning depolarization was not very big, therefore our results are not contaminated much by the inactivation.

Vergara: Why did you decide to use a 1 msec time constant for the T system?

Nakajima: The T system transients were calculated based on the Adrian, Chandler, and Hodgkin model [*J. Physiol. (London)*, 1969, **204:** 207–230], and it turned out that at $R = 0.7$, the center of gravity, the half-time of the rise was less than 0.5 msec.

Vergara: Now we have evidence that it might be a little bit slower.

Bezanilla: What kind of fibers are you using and what are their diameters?

Nakajima: Exactly the same as used by Adrian, Chandler, and Hodgkin. Every parameter is the same. It is an 80-μm-diameter fiber (model used for calculation).

Bezanilla: I remember that the test pulse duration was 10 msec for the experiment you described. We were concerned about cable properties, and at that time, we were not quite sure whether the effects of radial or longitudinal cable properties were important in our short fibers. We did the following experiment: We extended the test pulse, and when it was 50 msec long instead of 10, we could not potentiate the response with the first pulse (unpublished results).

Nakajima: What I am saying is that there is some slow process building up—with a time-constant in the range of 5 to 10 msec—and this process is activating the calcium release. The problem is, what is this process? One possibility is the cable property of the T system. It could be something else.

Schneider: I have tried to show that the "something else" might be charge movement. In our case, we used test pulses that were not quite as large and short as those used by Dr. Nakajima. Our test pulses were to about 10 to 20 mV above threshold for calcium release (Schneider and colleagues, this volume). I think it was pretty clear that for such pulses, the time shift of the calcium signal produced by a subthreshold prepulse corresponded with the time shift that one would expect on the basis of charge movement. Of course, both the charge movement and the calcium transient are in response to the actual time-course of change in membrane potential and were thus both influenced to an unknown extent by any delay in charging the T system membrane capacitance.

Rakowski: Since the time-constant of the inactivation process is so different from the time-constant of tubular charging, it would be very easy to test whether tubular cable delay was the correct explanation, just by hyperpolarizing to the same potential each time you do the

conditioning pulse (of the same duration) was started earlier so that there was a gap of about 5 msec between the end of the conditioning pulse and the beginning of the test pulse, the calcium transient became almost the same as in the control.

When the conditioning depolarization was very long (> 10 sec), we still observed a similar enhancing effect so long as the test pulse was short (5 msec). However, when the test pulse was long (say, 100 msec) we could not observe a consistent enhancing effect.

In summary, the calcium transient is enhanced either by short or by long conditioning depolarizations so long as the test pulse is short and follows the end of the conditioning pulse after a brief interval. One of the explanations is to attribute this to the way the surface membrane potential spreads electrotonically through the T system. Thus, if a short test pulse starts from a depolarized level, the potential transients at the T system would reach the final level more rapidly and would stay near the final level longer than when the test pulse starts from a hyperpolarized level, even if the T system membrane constants are not potential-dependent. When the test pulse is long, this small transient effect will be overshadowed by the much larger calcium release. Another explanation is that the same kind of transient behavior occurs at the coupling process between the T-system depolarization and the calcium release (such as the charge movement or the putative potential changes in sarcoplasmic reticulum membrane). Our experiments do not allow us to decide clearly between the two possibilities. Nevertheless, they show that the facilitating effect of conditioning depolarization is accompanied by a larger calcium transient. Therefore the mechanism of this type of facilitation does not reside in the steps that follow the rise in the level of ionized calcium.

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