162. *Enhancement by EDTA of Sarcotubular Regenerative Response Produced in F-Rich Solution*

By Kimihisa TAKEDA and Yutaka OOMURA
Department of Physiology, Faculty of Medicine,
Kanazawa University, Kanazawa

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The sarcotubular system[1] of frog muscle fibers generates a regenerative depolarizing response in a F-rich solution, provided normal resting potentials are maintained with injected current.[2] In these experiments bicarbonate buffer was used in the solution. The work reported here reveals that the regenerative response is depressed by the use of phosphate buffer, and that the addition of 1 mM ethylenediaminetetraacetic acid disodium salt (EDTA) greatly enhances the response. The materials, *i.e.*, surface fibers of the sartorius muscle of *Rana catesbiana*, and general experimental procedures used were the same as those previously reported,[2] except that the F-rich solution in the present study contained normal Na, no Ca, 2.5 mM KCl and phosphate buffer. The point-voltage clamp method with two microelectrodes was used. Membrane potentials were held at around −100 mV except for the duration of one second clamping step. Tetrodotoxin (10^{-6} g/ml) was added to all solutions. The experiments were performed at room temperature (21–23°C).

The inward current component due to the regenerative response in the sarcotubular system was generally small and the negative slope region of the current-voltage (I-V) relation was minor, if any, when phosphate buffer was used in the F-rich solution. In the fiber shown in Fig. 1, as in many other fibers, outward current simply decreased after the peak during step depolarizations (Fig. 1, inset records), and a negative slope region was not noticeable in the I-V relation at 180 msec (Fig. 1). These features of the fiber rather resembled those of glycerol-treated fibers in which the regenerative response is absent.[2] Presence of the regenerative response in the fiber, however, is evidenced by the inward current tails observed upon termination of depolarizing steps (Fig. 1; also inset records), which are absent in glycerol-treated fibers.

It is assumed, then, that EDTA may affect the regenerative response, if the response involves the interaction of F ions with Ca. In fact, the addition of 1 mM EDTA to the solution greatly enhanced the regenerative response. Threshold potentials and the time to the

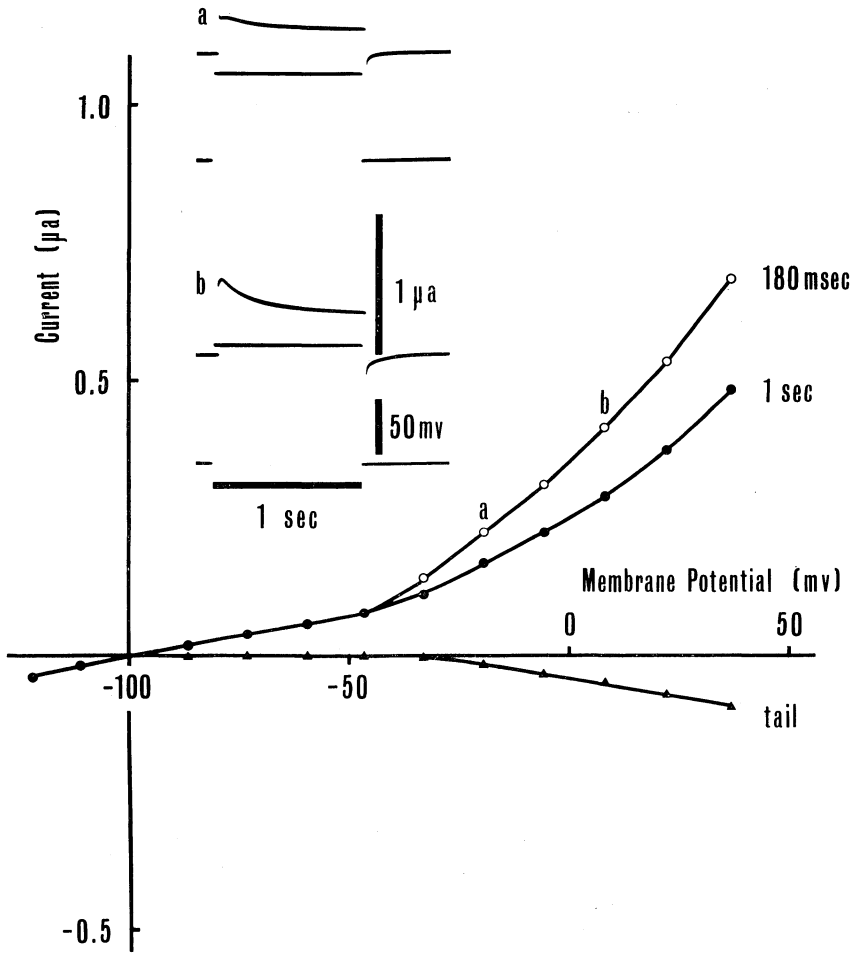

Fig. 1. Total current-voltage relations in a point-voltage clamped fiber bathed in phosphate buffered F-rich solution containing tetrodotoxin. Open circles, at 180 msec; filled circles, at 1 sec; triangles, inward current tails after termination of depolarizing steps. Resting potential, −38 mV; maintained holding potential, −100 mV. Inset records, plotted at points labelled a and b in the relations, show total current (upper trace, outward current upward) produced by a 1 sec voltage step (lower trace, depolarization upward).

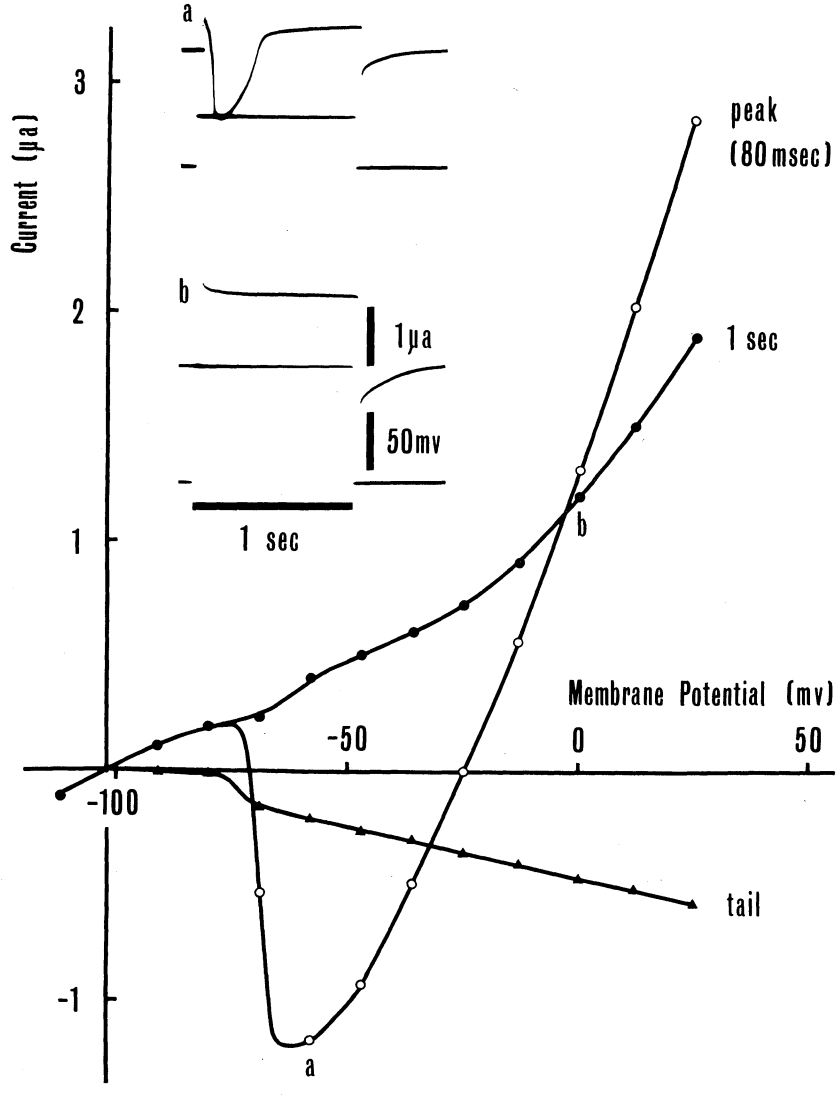

Fig. 2. Total current-voltage relations recorded from a fiber 120 min after transfer to 1 mM EDTA containing phosphate buffered F-rich solution with tetrodotoxin. Open circles, at the response peak (80 msec); filled circles, at 1 sec; triangles, inward current tails. Resting potential, −42 mV; maintained holding potential, −102 mV. For further explanation see legend in Fig. 1.

peak of the response were reduced. Total current at the peak of the response was large and inward. Inward current tails were correspondingly large. The duration of the main response, however, was several hundred milliseconds and comparable to that occurring in the absence of EDTA. Fig. 2 shows an example. The threshold potential of the response in this fiber was about –70 mV. The time to the peak of the response was 80 msec. Total current at the peak of the response was inward and 1.2 μA.

Present results indicate that the regenerative depolarizing response in the sarcotubular system is influenced by the pH buffer of the F-rich solution. The great enhancement of the regenerative response by EDTA demonstrates that EDTA affects the sarcotubular system, and that the alkali-earth cations (possibly Ca) in the sarcotubular system are involved in the response. These results suggest that the regenerative response in the absence of EDTA involves the interaction of F ions with, possibly, Ca in the sarcotubular system.

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After preparation of this manuscript a paper by S. Winegrad[3] appeared reporting that EDTA caused high permeability to Ca in cardiac muscle membrane.

References

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