169. **Sarcotubular Regenerative Response Induced by EDTA in Propionate Solution**

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In a fluoride-rich solution, the sarcotubular system of frog muscle fibers exhibits the regenerative response,\(^1\) which is blocked by picrotoxin\(^2\) and augmented by ethylenediaminetetraacetic acid disodium salt (EDTA).\(^3\) In the experiments reported here an attempt has been made to determine whether the generation of the sarcotubular response is confined to the use of fluoride in a surrounding medium or the response represents a more fundamental property of the sarcotubular membrane. The results demonstrate that, although the sarcotubular response is normally absent in a propionate solution, addition of EDTA or ethyleneglycoldiethyltherdiaminetetraacetic acid (EGTA) makes its generation capable.

The materials, *i.e.*, surface fibers of the sartorius muscle of *Rana catesbiana*, and general experimental procedures used were the same as those in the previous papers.\(^1,3\) The point-voltage clamp method with two microelectrodes was used. Membrane potentials were held at around \(-100\) mV except for the duration of 1 sec clamping step. The propionate solution used contained 116.3 mM Na, 2 mM Ca, 2.5 mM K, 6.5 mM Cl, 111.5 mM propionate and phosphate buffer. EDTA was simply added to the solution, while pH was adjusted with NaOH to a value between 6.5 and 7.0 when EGTA was added. Tetrodotoxin (1 \(\mu\)g/ml) was added to all solutions. The experiments were performed at room temperature (21–23°C).

Muscle fibers equilibrated in the propionate solution maintained normal resting potentials. On depolarization, these fibers did not exhibit the sarcotubular regenerative response. Only anomalous rectification for small depolarization and delayed rectification for further depolarization, which was not inactivated during a 1 sec depolarization step, were seen before the electrical recording was disturbed by contraction (Fig. 1).

When 4 mM EDTA was added to the propionate solution, however, resting potentials of the fibers were reduced considerably (up to about \(-30\) mV). On depolarization from a holding potential of around \(-100\) mV, all of these fibers examined gave rise to the regen-
erative response with features similar to those previously observed in the F-rich solution (Fig. 2). Total current measured in the fibers at the peak of the response was inward and smaller than the value obtained in the F-rich solution with 1 mM EDTA. Both the inward current during the depolarizing step and the inward current tail observed upon its termination disappeared within 5 min after the addition of picrotoxin (3 mg/ml). Thus the regenerative response generated in the propionate solution was also blocked by picrotoxin.

Glycerol-treated fibers in which the transverse tubular system was disrupted showed reduced resting potentials in the 4 mM EDTA added propionate solution. On depolarization from a holding potential of around $-100 \text{ mV}$, none of these fibers tested exhibited the regenerative response. For small depolarization, the current-voltage

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**Fig. 1.** Current-voltage relations in a point-voltage clamped normal fiber after 7 hr immersion in propionate solution with tetrodotoxin. Ordinate, total current (outward current upward); abscissa, membrane potential. Open circles, at 100 msec (omitted if identical with, or very close to, the value at 1 sec); filled circles, at 1 sec; triangles, current tail at 75 msec after termination of polarizing step. Resting potential, $-90 \text{ mV}$; holding potential, $-102 \text{ mV}$. 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).
relation measured at 1 sec from the onset of pulses was linear (Fig. 3). For depolarizations above about -50 mV, the fibers exhibited delayed rectification which was considerably inactivated, for larger depolarizations, during a 1 sec pulse. Depolarizing anomalous rectification was not noticed in these fibers as in the glycerol-treated fibers in the F-rich solution, but more data are required before concluding that the difference is solution dependent. It is clear from the results presented that the regenerative response observed

Fig. 2. Current-voltage relations recorded from a normal fiber after 3 hr immersion in 4 mM EDTA containing propionate solution with tetrodotoxin. Open circles, at response peak (360 msec at minimum); filled circles, at 1 sec; triangles, inward current tail at 75 msec after termination of depolarizing step. Resting potential, -32 mV; holding potential, -100 mV. Inset record, plotted at point labelled a in the relations, shows regenerative response. For further explanation see legend in Fig. 1.
in the propionate solution with EDTA was generated in the sarco-
tubular system.

When 10 mM EGTA, instead of EDTA, was added to the pro-
pionate solution, resting potentials of the fibers were likewise re-
duced. On depolarization from a holding potential of around $-100$ mV, these fibers exhibited the regenerative response. Vigorous contraction occurred in the fibers on depolarization above about $-50$ mV, which was not seen in the propionate solution with EDTA.

Present results demonstrate that the sarcotubular membrane, and not the surface membrane, of the muscle fibers is capable of generating the regenerative response even in the propionate solution, if treated with EDTA or EGTA. Thus it is concluded that calcium is involved in the regenerative response and that the Ca-related response represents a specific character of the sarcotubular membrane. Similar prolonged responses have been observed previously in solutions with Ca-binding anion. Present results suggest that the sarcotubular system is involved also in these responses. The possibility should be examined that the Ca-related sarcotubular permeability underlying the regenerative response is involved in excitation-contraction coupling; since the regenerative response is inhibited by picrotoxin, twitch is potentiated by picrotoxin and a sarcotubular conductance contributes to action potentials as revealed by the plateau formation by picrotoxin.

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References