LOCAL ANESTHETICS AND EXCITATION-CONTRACTION COUPLING IN SKELETAL MUSCLE

EFFECTS ON A Ca^{++}-CHANNEL

W. ALMERS, *Department of Physiology and Biophysics, University of Washington, Seattle, Washington 98195*

In a skeletal muscle fiber the sarcoplasmic reticulum (SR) determines the intracellular Ca^{++} concentration $[Ca_i]^{++}$ and, thereby, contraction. More precisely, $[Ca]_i^{++}$ depends on the balance between Ca^{++} uptake and release. Ca^{++} uptake occurs via a Ca^{++} pump in the SR membrane and may soon be understood in molecular detail. Ca^{++} release occurs during muscle activity due to an increase in Ca^{++} efflux from the SR, probably via a Ca^{++} channel in the SR membrane. Little is known about this Ca^{++} channel other than that it is influenced by the membrane potential of the transverse tubular system (TT). The channel opens upon TT membrane depolarization and closes upon repolarization.

Local anesthetics are of interest in the study of this channel because, being membrane-permeant, they have ready access to the interior of intact muscle fibers (1). They also partition strongly into membranes and block ionic channels. In skeletal muscle, tertiary amine local anesthetics are generally known to inhibit Ca^{++} uptake by the SR (2) and there are suggestions that at least one of them, tetracaine, also blocks the Ca^{++} channel there (3). If local anesthetics can inhibit both uptake and release, one might expect them to potentiate or antagonize contractile activation by cell membrane depolarization, depending on which is inhibited most. We have investigated the action of various local anesthetics at neutral external pH (4–5°C) on the contractile response to cell membrane depolarization, using a voltage-clamp method (4) to record strength-duration curves for contractile activation. Two parameters of these curves were of particular interest: the threshold potential for activation with long (0.1–1 s) pulses, called rheobase, and the threshold duration of a strong depolarization to 50 or 100 mV, called minimum stimulus duration (MSD). At rheobase, Ca^{++} uptake and release must be equal; if a drug inhibited uptake more or less than release, one might expect the rheobase to shift in the negative or positive direction, respectively. At positive potentials, Ca^{++} release will ordinarily exceed uptake, and if the MSD gives an estimate of the time needed by the SR to release a threshold amount of Ca^{++}, then a drug-induced inhibition of release might show up as a lengthening of the MSD. On this basis, the effect of several local anesthetics can be summarized and tentatively interpreted as below. Lidocaine (10 mM) makes the rheobase potential 11 mV more negative and shortens or does not change the MSD. Probably the drug inhibits Ca^{++} uptake with little effect on release. QX 314 (10 mM), a quaternary analogue of lidocaine, has no appreciable effect within 1–1.5 h of application. Probably the drug does not readily cross the cell membrane and cannot reach its intracellular site of action.

Procaine (40 mM), like lidocaine, makes the rheobase potential more negative (13 mV) but nearly doubles the MSD. Probably the drug inhibits both uptake and release, the effect on uptake being the larger. It facilitates activation by long, weak depolarizations but inhibits activation by short, large stimuli. Tetracaine (0.5–2 mM) shifts the rheobase to more positive potentials (11 mV, 13 mV) and lengthens the MSD 7–10-fold (0.5 mM) or 30–45-fold (2 mM). Probably, block of Ca^{++}-channels exceeds inhibition of uptake for stimuli of all durations and amplitudes. Dibucaine (1 mM) entirely blocks the contractile response; even strong depolarizations (to +100 mV) lasting up to 1 s fail to elicit contraction. As with tetracaine, it can be suggested that block of Ca^{++}-channels by dibucaine is more pronounced than inhibition of Ca^{++} uptake.

Since our main interest is in Ca^{++} release through the hypothetical Ca^{++} channel of the SR, tetracaine was singled out for more detailed study (5, 6). Working on "skinned" muscle fibres, we find that tetracaine (2 mM) blocks caffeine-induced Ca^{++} release from the SR, but does not diminish contraction when Ca^{++} is applied directly to the contractile proteins. Sensitivity of contractile proteins to Ca^{++} also remains undiminished. When blocking contraction of intact fibres, tetracaine must, therefore, act by inhibiting Ca^{++} release.

Next we investigated the effect of tetracaine on asymmetric displacement currents of intact fibers. These currents were discovered by Schneider and Chandler (7), who also suggested that they might represent structural or orientational changes in the TT membrane of charged groups of molecules ("voltage sensors") from which the Ca^{++} channels in the SR derive their dependence on the cell membrane potential. If this view is correct, then tetracaine can block the Ca^{++} channel without interfering with the channel's voltage sensor, since tetracaine (2 mM) affects, within experimental error, neither size nor kinetics of the asymmetric displacement currents.

The strength-duration curves in the presence and absence of tetracaine, though characteristically different in shape, are consistent with the operation of a single unaltered gating mechanism for the Ca^{++} channel under both conditions. The observed difference in shape is predicted if one assumes release to be proportional to the number of open Ca^{++} channels in the SR, and the number of channels to be proportional to the amount of charge carried by asymmetrical displacement currents. In conclusion, I suggest that many local anesthetics block Ca^{++} channels in the SR, and, more specifically, that tetracaine blocks without significantly affecting the mechanism from which the channel derives its voltage dependence. From the strength-duration curves it is estimated that 0.5 and 2 mM tetracaine block 82% and 96% of all Ca^{++} channels, respectively.

This work was supported by U.S. Public Health Service grant AM-17803.

REFERENCES

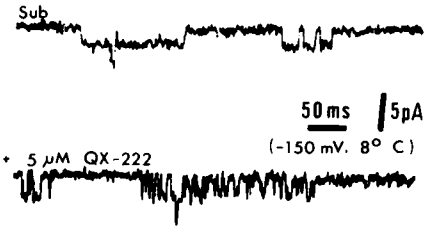
1. BIANCHI, C. P., and T. C. BOLTON. 1967. Action of local anesthetics on coupling systems in muscle. *J. Pharmacol. Exp. Ther.* **157**:388.
2. JOHNSON, P. N., and G. INESI. 1969. The effect of methylxanthines and local anesthetics on fragmented sarcoplasmic reticulum. *J. Pharmacol. Exp. Ther.* **169**:308.

3. LÜTTGAU, H. C., and H. OETLIKER. 1968. The action of caffeine on the activation of the contractile mechanism in striated muscle fibers. *J. Physiol. (Lond.).* **194**:51.
4. ADRIAN, R. H., W. K. CHANDLER, and A. L. HODGKIN. 1969. The kinetics of mechanical activation in frog muscle. *J. Physiol. (Lond.).* **204**:207.
5. ALMERS, W., and P. M. BEST. 1976. Effects of tetracaine on contraction and "gating currents" in frog skeletal muscle. *Biophys. J.* **16**:152*a*. (Abstr.).
6. ALMERS, W. 1976. The differential effects of tetracaine on delayed potassium channels and displacement currents in frog skeletal muscle. *J. Physiol. (Lond.).* **262**:613.
7. SCHNEIDER, M. F., and W. K. CHANDLER. 1973. Voltage dependent charge movement in skeletal muscle: a possible step in excitation-contraction coupling. *Nature (Lond.).* **242**:244.

LOCAL ANESTHETIC MOLECULES TRANSIENTLY BLOCK CURRENTS THROUGH INDIVIDUAL OPEN ACETYLCHOLINE RECEPTOR CHANNELS

J. H. STEINBACH, *Department of Neurobiology, Salk Institute, San Diego, California 92112*

Local anesthetics modify the time-course of end plate currents (EPCs) and miniature (M) EPC's and alter the spectra of acetylcholine (ACh)-induced EPC's, but the mechanism of local anesthetic action is not known. After studies of MEPC's and spectra of ACh noise, Ruff (1976) found that a sequential kinetic scheme described the effect of QX-222: $(nA + R \rightleftharpoons AnR) \rightleftharpoons AnR^* \overset{Q_G}{\underset{F}{\rightleftharpoons}} A_nR'Q$ where R is a receptor, R^* is an open receptor, R' is an open receptor with a greatly reduced conductance, A is an agonist molecule, and Q is a local anesthetic molecule. Erwin Neher and I have used the extracellular patch clamp (Neher and Sakmann, 1976) to examine the effect of QX-222 on currents through single, open ACh receptor channels in the extrajunctional regions of denervated frog cutaneous pectoris muscles. Receptors were activated by suberyldicholine in the absence or presence of QX-222 (5-50 μM). Typical records are shown below:



Qualitatively, the effect of QX-222 is consistent with the sequential scheme. Quantitative analysis of the data confirmed this impression, and allowed us to extract the fol-