241. *Dual Hyperpolarizing Conductance Increases in Frog Muscle Fibers*

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In frog sartorius muscle fibers a slow increase in the amplitude of the electrotonic potential during a large constant hyperpolarizing current pulse has been previously reported.[1] This early hyperpolarizing conductance increase was attributed to a voltage-dependent change in K conductance. The work reported here reveals a second but delayed hyperpolarizing conductance increase. These early and delayed increases in conductance respond differently to changes in pH and to picrotoxin.

The experiments were performed on sartorius muscle fibers of *Rana catesbiana* using a conventional two-intracellular-microelectrode method. Constant current pulses of one second duration were applied through one electrode and electrotonic potentials measured with the other. Phosphate buffer was used for physiological solutions of different pH values, and 10^{-6} g/ml of tetrodotoxin was routinely added in the solutions to eliminate any effects of the membrane's Na channel. Only fibers which showed resting potentials greater than -80 mV after dual penetration were used.

At pH 7 or pH 7.5, newly penetrated fibers showed only the early conductance increase for hyperpolarizations up to membrane potentials of -200 mV. After long periods of penetration, fibers tended to show, in addition, a delayed decrease in membrane resistance, which was apparently similar to the delayed hyperpolarizing conductance increase described below. Such deteriorated or low resting potential fibers, which usually showed an overshoot potential after the cessation of a hyperpolarizing current pulse, were discarded.

At pH 5.6, newly penetrated fibers showed the early conductance increase alone for relatively small hyperpolarizations (Fig. 1, A_1, A_2). The time course of these electrotonic potentials was essentially similar to that in neutral or alkaline solution except for an increase in their amplitude. The results indicate that pH does not affect the early hyperpolarizing conductance increase appreciably but affects a time-invariant conductance for small hyperpolarizations. The latter acid-inactivated (alkaline-activated) conductance contributes to the rest-

ing membrane conductance and has been ascribed to Cl conductance.[2)]

For membrane potentials greater than about −165 mV at pH 5.6, however, the early conductance increase was followed by a delayed increase in conductance, and no post-pulse overshoot was noticed (Fig. 1, A_3–A_5). The delayed hyperpolarizing conductance increase is ascribed to the effect of acid solution and not to deterioration. This is demonstrated by its absence at comparable membrane potentials when the same fiber was transferred to pH 7. Instead the fiber

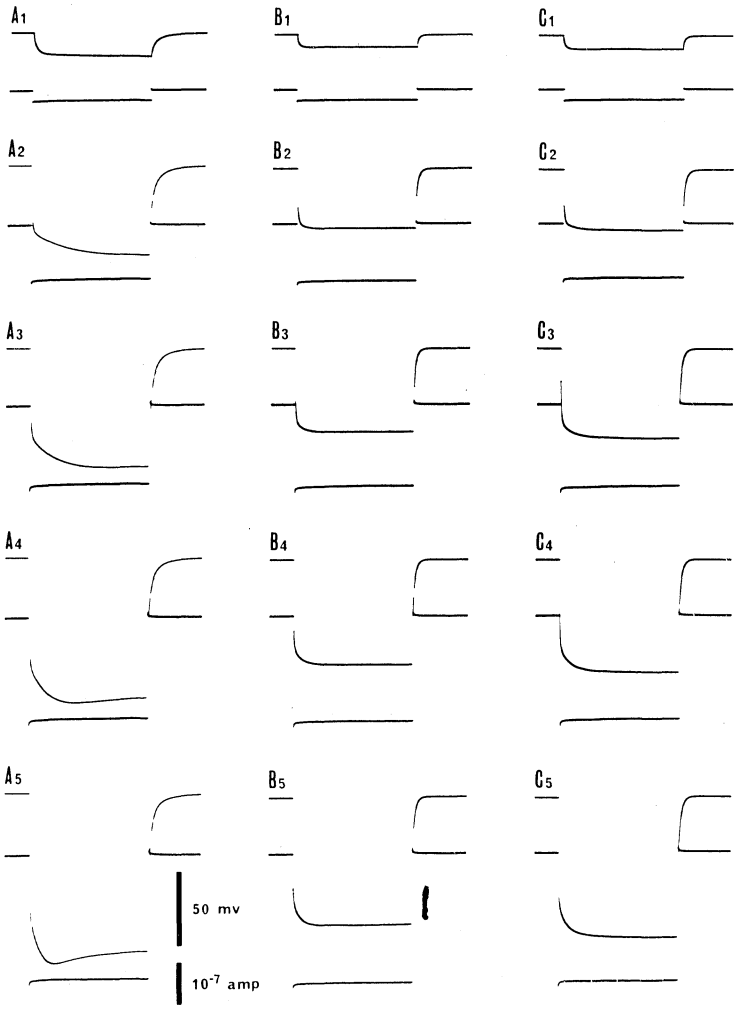


Fig. 1. Records of electrotonic potentials in a single muscle fiber of the frog sartorius in tetrodotoxin-containing Ringer solution (upper traces), produced by constant hyperpolarizing current pulses of 1 sec duration (lower traces). Photographs taken, in order, at pH 5.6 (A_1-A_5), 15 min after transfer to pH 7.5 (B_1-B_5) and 20 min after transfer to pH 7 (C_1-C_5). Resting potential of the fiber, −83 mV.

showed the slow increase in the amplitude of electrotonic potentials at pH 7 (Fig. 1, C_3–C_5). Note that the weak or absent slow potential increase in this fiber at pH 7 or pH 7.5 is due to its deterioration in the later stages of the experiment.

With increasing hyperpolarization, both early and late current-voltage (I–V) relations deviate from linear resting slopes more in acid than in alkaline solution, indicating the increase in conductance. As a result the difference in membrane conductances at various pH

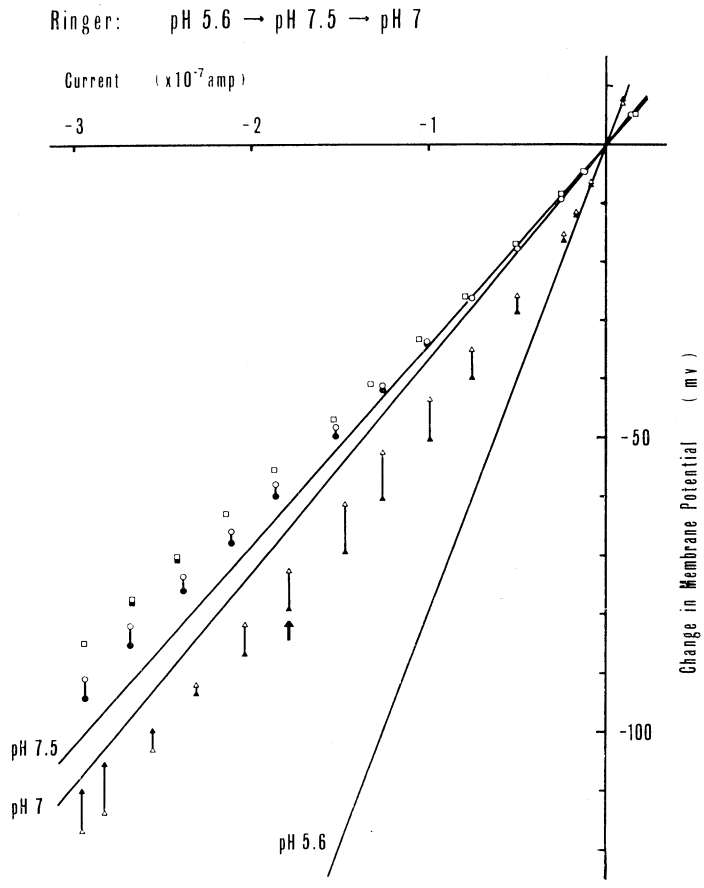
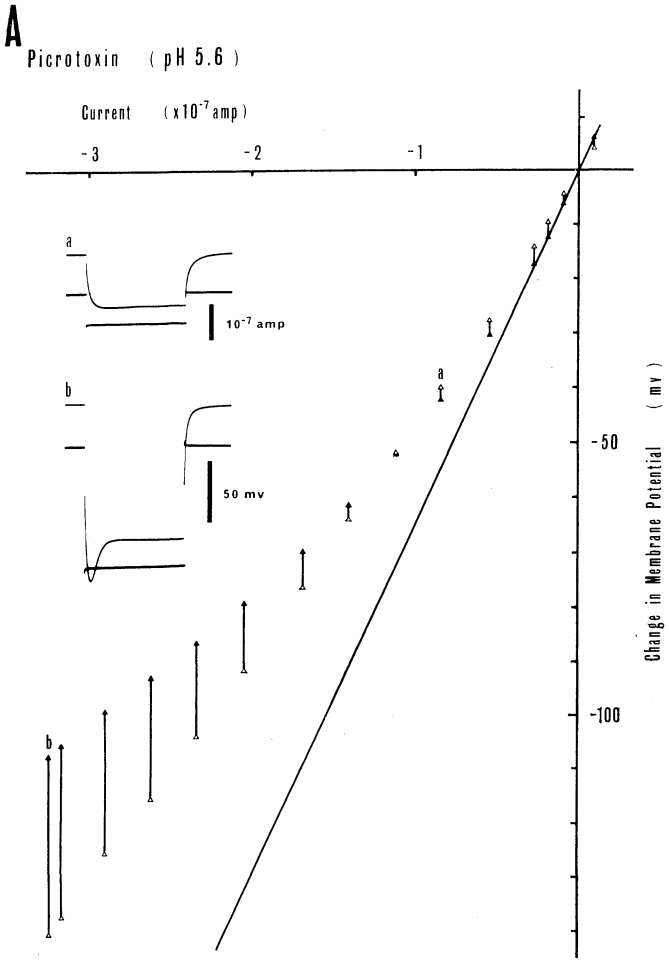


Fig. 2. Early (open symbols; 190 msec from onset of current) and late (filled symbols; at 1 sec) I-V relations. Data from the experiment in Fig. 1. Ordinate, change in membrane potential; abscissa, current intensity. Triangles, at pH 5.6; squares, at pH 7.5; circles, at pH 7. Absence of filled symbols indicates their coincidence with open symbols. The three straight lines represent late resting slope conductances as extrapolated from values determined near the resting level at pH values indicated. Arrow indicates appearance of delayed hyperpolarizing conductance increase, as shown in Fig. 1, A_3.

values is greatly reduced (Fig. 2). These results indicate the presence of an acid-activated conductance increase for large hyperpolarizations, which may or may not cause the delayed conductance increase.

The addition of 10^{-3} g/ml of picrotoxin, which is considered to inhibit Cl activation,[3] selectively blocked the early hyperpolarizing conductance increase in the acid, neutral and alkaline solutions. In addition at all three pH levels a delayed hyperpolarizing conductance increase which was not accompanied by post-pulse overshoot was seen for both small and large hyperpolarizations (Fig. 3, A, B). It remains to be determined whether the channel responsible for the delayed conductance increase is same as what is activated with large hyperpolarizations in the acid solution.



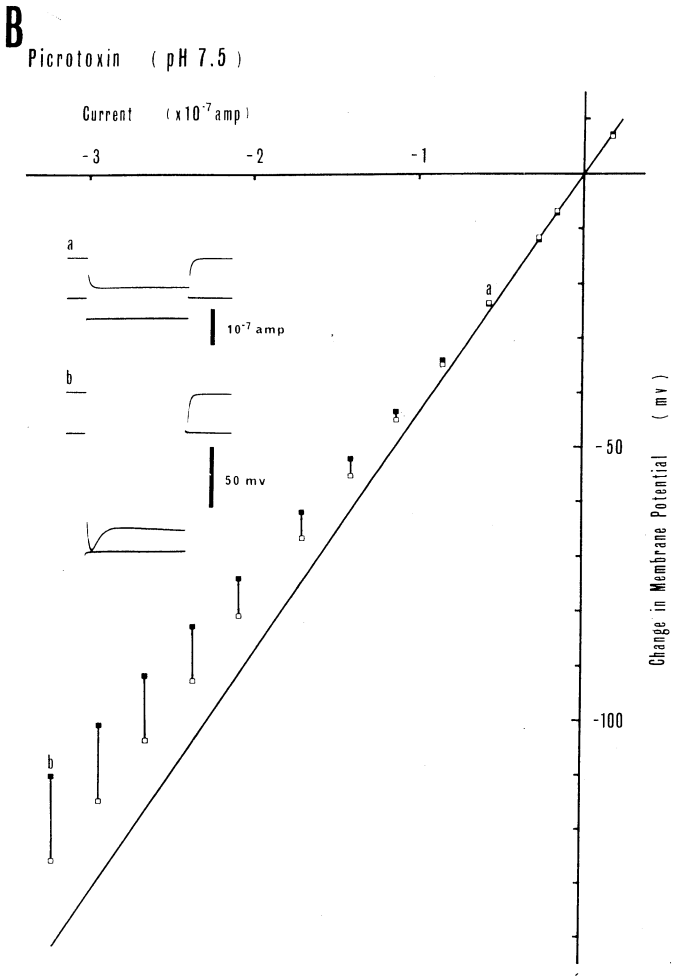


Fig. 3. Early (open symbols; at 90 msec in A, at 60 msec in B) and late (filled symbols; at 1 sec) I-V relations. Ordinate, change in membrane potential; abscissa, current intensity. The straight line represents late resting slope conductances as in Fig. 2. a and b in the graph refer to inset records (upper traces, potential; lower traces, current; current pulse duration, 1 sec). A, at pH 5.6, 50 min after addition of picrotoxin. Resting potential, −81 mV. B, another fiber at pH 7.5, 110 min after picrotoxin. Resting potential, −85 mV.

Often, for large hyperpolarizations a slow increase in the amplitude of electrotonic potential was noticed after the maximum delayed hyperpolarizing conductance increase (Fig. 3B, inset b). It might represent a time-dependent reduction in the delayed conductance increase, or merely an effect of the remaining early conductance increase.

The addition of picrotoxin at different pH values caused only a slight decrease in the resting membrane conductance of the fibers tested. Therefore, picrotoxin has no marked effects on the alkaline-activated, time-invariant conductance. For large hyperpolarizations, however, amplitudes of the electrotonic potentails produced in good fibers by current pulses of a given size were larger after picrotoxin than before not only early in the pulses but also at the end of the pulses (*i.e.*, after delayed conductance increase). Often, the inhibition of the early hyperpolarizing conductance increase by picrotoxin was so complete that a linear resting slope for early I–V relation was maintained up to large hyperpolarizations at pH 7.

More than one-half hour was needed to obtain maximum effects of picrotoxin, in contrast to less than ten minutes in the case of pH changes.[2] A probable interpretation of this difference would be that the alkaline-activated, picrotoxin-insensitive and time-invariant Cl conductance is located in the sarcolemma, and the picrotoxin-blocked early hyperpolarizing conductance increase is located in some internal membrane system. An assumption that Cl conductance is responsible for the latter is consistent with the idea of an internally located Cl-permeable membrane,[4] and might be related to the picrotoxin-blocked anomalous rectification.[5] However more data are required for final conclusions.

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