Effects of Sulfhydryl Inhibitors on Nonlinear Membrane Currents in Frog Skeletal Muscle Fibers

ADOM GONZALEZ, PURA BOLAÑOS, and CARLO CAPUTO

From the Centro de Biofisica y Bioquimica, Instituto Venezolano de Investigaciones Cientificas, Caracas 1020A, Venezuela

ABSTRACT The effect of sulfhydryl reagents on nonlinear membrane currents of frog skeletal muscle fibers has been studied using the triple Vaseline gap voltage-clamp technique. These compounds, which are known to interfere with depolarization contraction coupling, also appear to diminish intramembranous charge movement recorded with fibers polarized to -100 mV (charge 1). This effect, however, is accompanied by changes in the fiber membrane conductance and in most cases by the appearance of an inwardly directed current in the potential range between -60 and $+20$ mV. This current is reduced by both cadmium and nifedipine and does not occur in Ca-free solution, suggesting that it is carried by calcium ions flowing through regular calcium channels that are more easily activated in the presence of SH reagent. These changes in the membrane electrical active and passive properties decrease the quality and reliability of the P/n pulse subtracting procedure normally used for charge movement measurements. These effects can be substantially reduced by cadmium ions (0.1 mM), which has no effect on charge movement. When SH reagents are applied in the presence of cadmium, no effects are observed, indicating that this cation may protect the membrane from the reagent effects. The effects of -SH reagents can be observed by applying them in the absence of cadmium, followed by addition of the cation. Under these conditions the conductance changes are reversed and the effects of the SH reagents on charge movement can be measured with a higher degree of confidence. Maximum charge is reduced by 32% in the presence of 1.5 mM PCMB and by 31% in the presence of 2 mM PHMPS. These effects do not occur in the presence of DTT and in some cases they may be reversed by this agent. Charge 2, recorded in depolarized muscle fibers, is also reduced by these agents.

INTRODUCTION

Functional modification of excitable membranes by chemical agents, known to interact with different amino acids, has been widely used to establish the role of

specific groups of membrane macromolecules in membrane function (Shrager, 1977; Rack and Woll, 1984; Drews and Rack, 1988; Meves, Rubly, and Stampfli, 1988). In this context, the importance of sulfhydryl groups for the function of excitable membranes has long been recognized by different authors (Smith, 1958; Huneeus-Cox, Fernández, and Smith, 1966; Shrager, 1977; Zuazaga, Steinacker, and del Castillo, 1984). In the case of muscle fibers, sulfhydryl reagents have been found to cause contractile failure (Kirsten and Kuperman, 1970), probably at the level of one of the steps in depolarization–contraction coupling (DCC) (Caputo, Bolaños, and Gonzalez, 1993). There is much evidence that intramembranous charge movement represents the electrical manifestation of the mechanism that provides voltage sensitivity for contractile activation (Schneider and Chandler, 1973; Chandler, Rakowski, and Schneider, 1976). In this work we have studied the effects of organic mercurial compounds, considered to be highly specific reagents for -SH groups (Means and Feeney, 1971; Torchinskii, 1974), on intramembranous charge movement signals in voltage-clamped skeletal muscle fibers.

Based on the effects of dihydropyridine (DHP) compounds on calcium currents and charge movement, it has recently been proposed that the molecular moiety that is the receptor for DHP may have a double function as calcium channel and as voltage sensor for DCC, whose operation is manifested by the charge movement signals (Rios and Brum, 1987; Tanabe, Takeshima, Mikami, Flockerzi, Takahashi, Kojima, Matsuo, Hirose, and Numa, 1987). This hypothesis has received strong support by the demonstration that in dysgenic mice myotubes, incorporation of complementary DNA encoding the DHP receptor may restore both contraction and calcium currents (Tanabe, Beam, Powell, and Numa, 1988). The results presented in this work demonstrate that while charge movement may be significantly reduced by sulfhydryl inhibitors, calcium currents are not adversely affected. Assuming that the action of the -SH reagents occurred directly at the level of the DHP receptor, this would indicate that its function as calcium channel is somewhat dissociated from its function as voltage sensor for DCC. Alternatively, one could assume that these agents directly interfere with the calcium release channels of the sarcoplasmic reticulum and that charge movement signals are reduced by some other mechanism. Recently, a feedback or allosteric mechanism between the voltage sensor molecule and calcium release channels (Rios, Karhanek, González, and Ma, 1992) has been proposed to participate in excitation–contraction coupling (ECC); however, this mechanism does not require reduction of maximum charge when calcium release is reduced.

A short report of this work has been presented to the Biophysical Society (Gonzalez, Bolaños, and Caputo, 1989).

METHODS

The experiments described in this work were carried out with muscle fibers dissected from lumbricalis IV digiti muscles from *Rana pipiens* and *Leptodactylus insularis*. Nonlinear membrane currents were measured using the triple Vaseline gap voltage-clamp technique (Hille and Campbell, 1976). The experimental chamber and the voltage-clamp circuit, as well as the experimental procedure were the same as already described in detail (Caputo and Bolaños, 1989). In brief, after dissection the fibers were exposed to the loading solution and mounted in the chamber. After sealing the gaps, the loading solution in pools E and C (the nomenclature of

Hille and Campbell [1976] is followed) was replaced by the internal solution and the fiber was cut. After a period of at least 30 min to allow for internal diffusion of EGTA, external solutions were replaced in pools A and B, first to characterize the sodium current and sodium inactivation property of the fiber (with a 40 mM sodium Ringer's solution), and later to measure charge movement. The output of the current amplifier was fed to a 15-bit A/D converter (model MP2735-1; Analogic, Wakefield, MA). The system for data acquisition, pulse generation, and analysis was kindly provided by Prof. F. Bezanilla (Department of Physiology, UCLA, Los Angeles, CA). It allows sampling signals as fast as 7 μs and allows 1,200 samples per record. Linear capacitive currents were reduced using a leak capacity subtracting circuit, and the remaining currents were subtracted using the $P/-4$ procedure, in which the four subtracting pulses are applied in the opposite direction to the test pulse (i.e., in the hyperpolarizing direction) and the resultant currents are added to the one obtained during the test pulse (Bezanilla, Taylor, and Fernandez, 1982). It is important to notice that with this procedure the subtracting pulses are applied before each test pulse. To diminish the contamination of nonlinear currents moved by the subtracting pulses, these are normally superimposed on either very negative, -150 mV, or very positive, $+50$ mV, subtracting holding potentials (Caputo and Bolaños, 1989); however, after exposure to the sulfhydryl reagents, the decreased membrane resistance often makes it difficult to apply this protocol, since problems in the subtraction procedure occur. Thus, in most cases, subtraction was carried out from the holding potential, as specified in the text. Furthermore, to improve membrane stability, many experiments were carried out in the presence of Cd^{2+}, which, as will be shown, appears to block unwanted ionic currents without much effect on charge movement. The charge was computed as the numerical time integral of the current trace. Usually, the integration time was 40 ms and was sufficient to reach saturation of the current integral value. Since many current traces showed pedestals or sloping baselines due to residual ionic currents, the analysis of these traces was done by first subtracting for a straight or sloping baseline. In the latter case this was accomplished by fitting a straight line in the interval between ~20 and 40 ms from the beginning of the pulse, most of the time forcing it to be a straight line without slope. This interval could vary slightly between different fibers, but was always the same for a given fiber. In cases in which the residual ionic inward currents were too prominent, this procedure could not be used since it gave raise to large errors in the determination of the transient time integrals, as shown in Fig. 1. The two columns of records in the figure show the results of fitting sloping baselines, using different time intervals, to the two upper records (A) that were obtained at -10 (record on the left) and $+30$ mV (record on the right), respectively, with the fiber bathed in normal solution. These two records correspond to the experiment illustrated in Fig. 3 (first column of records on the left). The other records (B–E) in Fig. 1 show the same records, after sloping baseline subtraction, carried out using different time intervals. The hyperbola-like smooth curves in the records represent the running integrals of the current transients obtained in each case with a 40-ms integration period. The four different cases correspond to trials in which the time intervals for sloping baseline subtraction were between 20 and 41 (B); 13 and 41 (C); 10 and 41 (D); and 20 and 50 ms (E), respectively. The coincidence of the initial baselines of the current and current-time integral, and the behavior of the running integral curve were chosen as criteria for discarding particular records. Thus, for the current record obtained at -10 mV, both criteria were met with the 20–41- and 20–50-ms time intervals; with the 13–41-ms interval the two baselines did not coincide well, while with the 10–41-ms interval, apart from a small baseline separation, the integral curve did not show a sustained plateau value. For the case of the record obtained at $+30$ mV, there was either a large separation between the initial baselines or a clear decrease or increase of the integral curve during the integration interval. Records of this type were not used for further analysis.

The internal solution had the following composition (in mM/liter): 120 Cs-aspartate, 10 EGTA-Tris, 4 $MgSO_4$, 4 Na_2ATP, and 10 HEPES. The external solution for measuring charge

movement had the following composition (in mM/liter): 60 TEA$_2$SO$_4$, 5 MgSO$_4$, 8 CaSO$_4$, 3 Rb$_2$SO$_4$, 10 MOPS-TEA, 20 glucose, and 300 nM TTX. The loading solution, in which the fibers were mounted in the chamber, had the following composition (in mM/liter): 120 K-aspartate, 5 MgCl$_2$, 1 CaCl$_2$, and 10 HEPES buffer, pH 7.2. Charge movement measurements were carried out at 10°C. The reagents para-chloromercuribenzoic acid (PCMB),
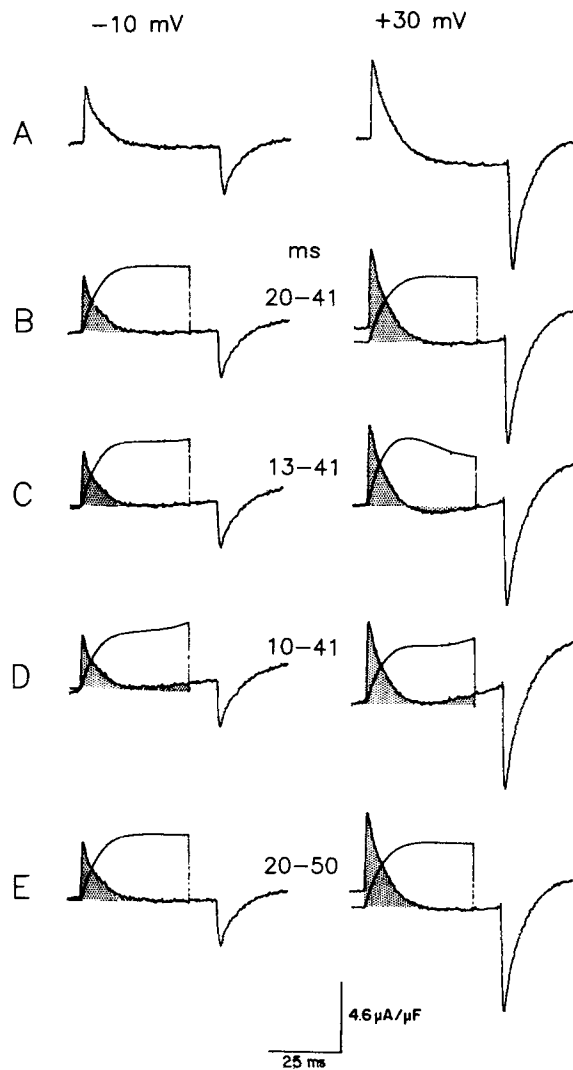

FIGURE 1. Sloping baseline subtraction examples for the case of two traces, one obtained at −10 mV (records on the left) and the other at +30 mV (records on the right). The sloping baseline subtraction was carried out using the time intervals shown between records. The values of the charge computed by using the running integral values shown in records B–E were the following (records at −10 mV): (B) 23.5, (C) 21.6, (D) 24.4, and (E) 24.0 nC/μF; (records at +30 mV): (B) 40.5, (C) 20.1, (D) 36.3, and (E) 42.0 nC/μF. In the first case the procedure worked and no large differences were caused by changing the interval used to fit the sloping baseline. In the second case, the inward residual current was too large, and the subtraction procedure was not reliable. Further details are given in the text.

para-hydroximercuri-phenyl-sulfonic acid (PHMPS), dithiothreitol (DTT), and nifedipine were purchased from Sigma Immunochemicals (St. Louis, MO). Stock solutions of PCMB were prepared in a tetraethylammonium solution at pH 7.8 to improve solubility when added to the experimental solutions. PHMPS and DTT were dissolved directly in the experimental solutions. A stock solution of nifedipine was prepared in ethanol. All these solutions were prepared immediately before use.

RESULTS

Charge Movement

Membrane resistance (R_m) and total membrane capacity (C_m) of all the cut fibers used in this work were measured in the external solution containing TEA-sulfate by applying hyperpolarizing pulses, usually 10–20 mV, and then measuring the steady current and the time integrals of the ON and OFF capacitive transients. For 36 fibers the values (mean ± SEM) for R_m and C_m were 2.1 ± 0.2 kΩ·cm^2 and 14.5 ± 0.4 μF/cm^2, respectively. These values are very similar to those previously reported for the fibers of the same species (Caputo and Bolaños, 1989). Both mercurial compounds used in this work, PHMPS and PCMB, were found to decrease these values, depending on their concentration and the exposure duration. For exposure durations up to 60 min and concentrations up to 1.5 mM the R_m decreased by a maximum of 17% and the C_m by a maximum of 10%. Besides affecting the membrane passive properties, these compounds also often facilitated the activation of an inward current, as shall be described later; this effect introduced serious problems in the measurement of intramembranous charge movement when the protocol normally used by us (Caputo and Bolaños, 1989) was employed. To reduce these problems the following precautions were taken: (*a*) charge movement was measured after a maximum of 50 min exposure to the mercurial compounds; (*b*) while under normal conditions charge movement is measured using the P + P/−4 procedure, with a subtracting holding potential value of −150 mV, after treatment of the fibers with mercurial compounds, subtraction was carried out from the normal holding potential of −100 mV; (*c*) Cd^{2+} at low concentration was used in many experiments; however, since Cd ions appeared to have an antagonistic effect, when the mercurial compounds were applied in the presence of this cation, special precautions had to be taken, as will be described later.

In a very few fibers, exposure to the mercurial compounds did not produce secondary effects of the type mentioned above. Fig. 2 illustrates the effect of PHMPS on one of these rare fibers. In Fig. 2 *A*, the records on the left were obtained in normal medium, while those on the right were obtained after a 30-min exposure to 2 mM PHMPS. All the records, obtained at different membrane potential values, show a clear reduction of the charge signals after treatment with the drug, as is better illustrated in Fig. 2 *B*. Fig. 2 *A* also shows that, under normal conditions, an inwardly directed current appeared when the membrane was depolarized to +20 mV; this current was not greatly enhanced after exposure to PHMPS; in fact, the records on the left show that the inward current appeared at 0 mV, and was not much increased at +20 mV. This behavior was quite unusual, since in most cases exposure to the mercurial compounds caused development of inward currents at less negative potentials, and enhancement of their amplitude in the potential range between 0 and +40 mV. Fig. 2 *B* shows plots of the ON (circles) and OFF (triangles) charges versus the membrane potential obtained before (open symbols) and after (filled symbols) treatment with PHMPS. Due to the presence of ionic currents in some of the traces, a sloping baseline subtraction procedure illustrated in Fig. 2 *C* was used. The records in Fig. 2 *C* are the same as those marked with an asterisk in Fig. 1 *A*, except that they are shown after baseline subtraction; the upper smooth line is the running integral of
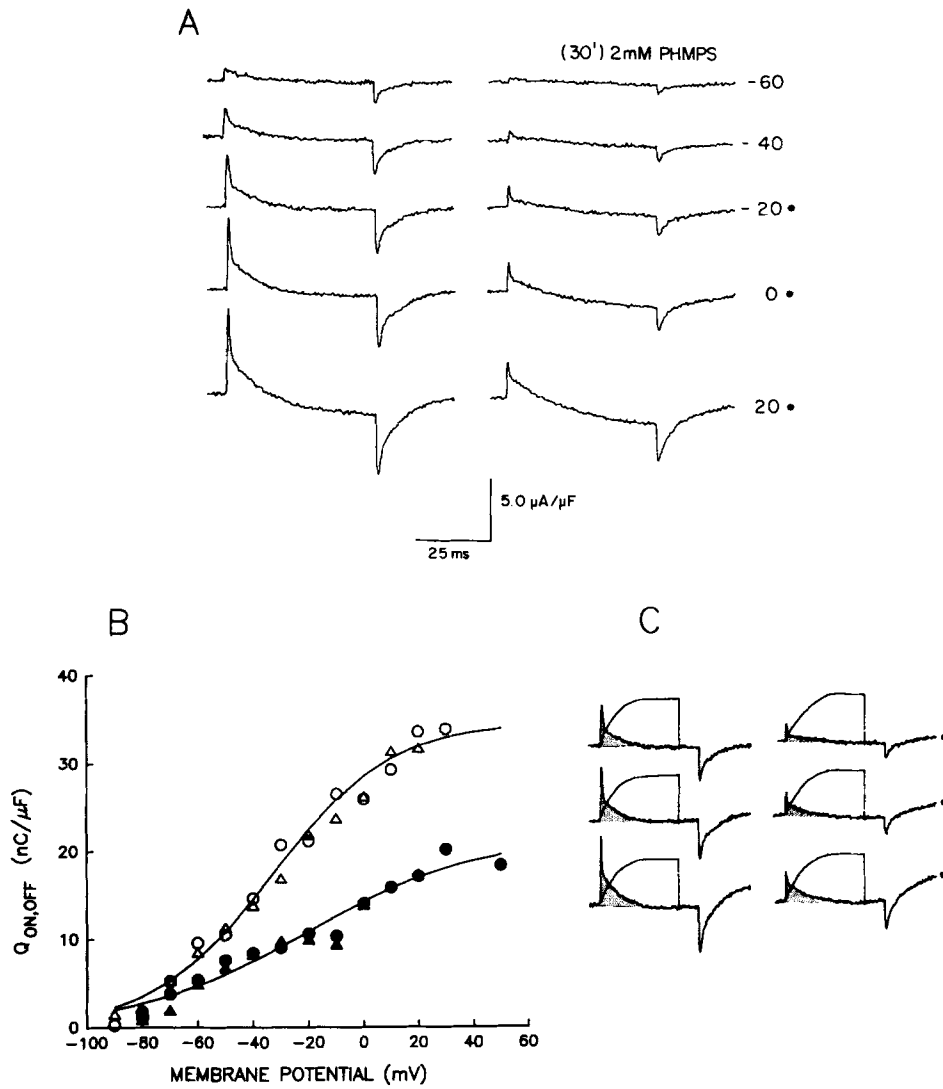
FIGURE 2.   Effect of 2 mM PHMPS on intramembranous charge movement. This was one of the few fibers that did not show large ionic currents after addition of the mercurial compounds. In A the records on the left were obtained in the normal external medium. Those on the right were obtained 30 min after adding PHMPS. B shows the voltage dependence of the charge movement signals for the same fiber. The open symbols show the results obtained in the normal medium and the filled symbols show the results obtained in the presence of PHMPS. The circles and triangles represent the ON and OFF values of the charge. At positive values only the ON transients could be measured. The continuous curves were calculated from Eq. 1 with the following parameters: control, $Q_{max} = 35$ nC/μF, $\overline{V} = -33$ mV, and $k = 21$ mV; PHMPS, $Q_{max} = 21$ nC/μF, $\overline{V} = -21$ mV, and $k = 31$ mV. (Fiber EN311B)

the ON transient. In this and all the other experiments of this work, the integration time was 40 ms. For this case the interval used to fit the sloping baseline was between 27 and 41 ms. The same method was used for the OFF transients, when possible. The baseline subtraction procedure was not used when the residual ionic currents were large (see Fig. 1), and in these cases it was preferable not to include the records in the corresponding graphs. Likewise, at positive potentials the OFF components of the signals were not considered for measurement of charge. In the graph of Fig. 1 B, the curves fitted to the experimental points were calculated using the equation for a two-state Boltzmann distribution model (Schneider and Chandler, 1973):

$$Q/Q_{max} = 1/[1 + \exp - (V_m - \overline{V})/k]$$

in which Q is the charge moved at a given potential V_m, Q_{max} is the maximum charge moved, $\overline{V}$ is the voltage at which 50% of the charge is moved, and $1/k$ is a steepness factor.

For the case of the fiber in Fig. 2, it appears that following treatment with PHMPS, Q_{max} was reduced from 34.7 to 21.4 nC/μF while $\overline{V}$ was changed from -33 to -21 mV and k from 21 to 31 mV. The mean values (mean $\pm$ SEM) of the charge movement parameters that describe the above relationship, obtained in 24 fibers under normal conditions, were $Q_{max} = 40 \pm 2$ nC/μF, $\overline{V} = -31 \pm 2$ mV, and $k = 18 \pm 1$ mV, which are in good agreement with previously reported values from our laboratory (Caputo and Bolaños, 1989). Fig. 3 shows the results obtained with another fiber treated with 1.5 mM PCMB. This fiber followed a more usual pattern, since the organic mercurial, besides causing a decrease in the charge signals, also induced an inward current at more negative membrane potential values. The figure shows that in the normal medium (records on the left) an inward current only appears at a potential of 0 mV; after exposure to PCMB (records in the middle) the inward current appears at -20 mV, indicating a substantial shift in the activation properties of this current. Finally, the records on the right show that the inwardly directed current component could be effectively abolished by Cd ions (0.2 mM), which, as will be shown later (Fig. 5), has no major effect on the charge movement signals. This point will receive further consideration when discussing Fig. 5. Fig. 3 B shows the charge–potential relationships obtained in this experiment. The open and filled circles represent the experimental points obtained in the normal solution and after addition of 1.5 mM PCMB and 0.2 mM Cd^{2+} (first and last columns of records), which were used for fitting of the respective theoretical curves, whose parameters appear in the figure legend. The curve representing the results obtained before adding PCMB was calculated without using the records obtained at $+10$ and $+30$ mV, since they showed prominent inward currents and presented difficulties for the analysis, as shown in Fig. 3 C. For the same reason the records shown in the middle column, obtained after addition of PCMB alone, were not used for further analysis. Fig. 3 C shows the procedure used for obtaining the time integral (integration time 40 ms) of the ON transients, after baseline correction of the records obtained at positive potentials. In this case, the interval used to fit the baseline was between 20 and 41 ms after the beginning of the pulse. For the case of the records obtained under normal conditions at $+10$ and $+30$ mV, which show rather large residual ionic currents, sloping baseline subtraction could not be performed, since errors of the
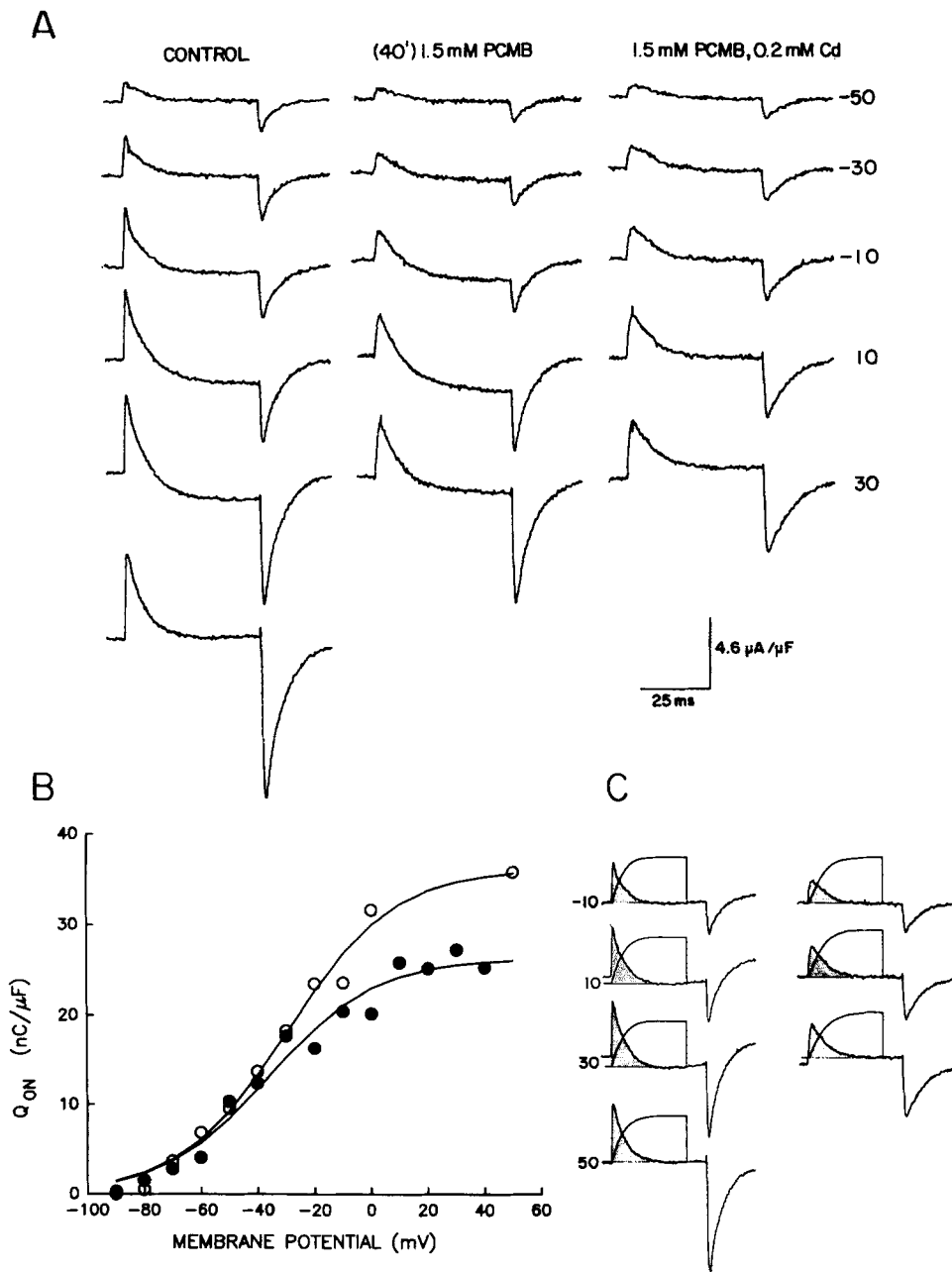
FIGURE 3.   Effect of 1.5 mM PCMB in the absence and in the presence of 2 mM Cd^{2+} on nonlinear membrane currents. In A, the records on the left show control charge movement signals recorded at different membrane potential values, which appear on the right side of the figure. The traces obtained at 10 and 30 mV show the development of an inwardly directed current after the falling phase of the ON charge signal. The records in the middle indicate that in the presence of 1.5 mM PCMB, charge movement signals are reduced at all potentials and

type shown in Fig. 1 were introduced. The ON transient of the record obtained at +50 mV could be used because the ionic current had already reversed at this potential value. The right records of Fig. 3 *C* show that after addition of PCMB and Cd^{2+} the problems were reduced but not eliminated, since in this case a pedestal-like outward component appeared in the current trace at the more positive potential (+30 mV). In this case the subtraction procedure was carried out assuming that it was time invariant. Since this problem appeared only at very positive potential values, no further precautions were taken to correct it.

Nature of the Inward Current

In most fibers, the currents, observed in the presence of the mercurial compounds, were so large that they made measurements of charge movement difficult (an example of this is shown in Fig. 7 *A*). Therefore, it was necessary to study the effect of mercurial compounds under conditions in which this current was blocked. The abolition of the inward current by Cd^{2+} strongly suggests that it is carried by Ca^{2+}; therefore, some other experiments, examples of which are shown in Fig. 4, were carried out to better identify its nature. Fig. 4 *A* shows that in the presence of 0.5 mM PHMPS the inward current, which is clearly visible at a membrane potential of 0 mV, could be abolished by 10 µM nifedipine. In this experiment the records on the left were obtained after a 30-min exposure to 0.5 mM PHMPS. Notice that in this case the effects on both the charge signal and the inward current were rather limited due to the low concentration and the short exposure time. The records on the right were obtained 10 min after adding 10 µM nifedipine; nifedipine causes a further reduction of the charge signal, but more importantly abolishes the inward current. Although the extra reduction of the charge signal, observed in the presence of nifedipine, could also be explained in terms of the more prolonged exposure to PCMB, in additional experiments it was found that nifedipine per se caused a sizable reduction of charge movement, in agreement with other authors (Lamb, 1987; Rios and Brum, 1987). The reduction by nifedipine of the inward current developed in the presence of mercurial compounds was confirmed in two other fibers. Fig. 4 *B* shows the effect of a calcium-free solution on this inward current. In the experiment, a fiber was treated with 2 mM PHMPS for 30 min before obtaining the records on the left. After this, the fiber was exposed to a solution containing the same amount of PHMPS, no Ca^{2+}, and 10 mM Mg^{2+}. It can be seen that with no calcium in the external medium

---

that inwardly directed currents appear to flow at a membrane potential value of −10 mV. The records on the right show that Cd^{2+} is effective in reducing the inwardly directed current without restoring the charge signals. *B* shows the membrane potential dependence of the charge movement signals shown in *A*. Due to the presence of ionic currents, only the ON components of charge were measured; thus, the symbols show the ON charge obtained under control conditions (*open circles*) and in the presence of PCMB + cadmium (*filled circles*). The continuous curves were calculated from Eq. 1 using the following values: *open circles*, $Q_{max} = 36$ nC/µF, $\bar{V} = -30$ mV, and $k = 19$ mV; *filled circles*, $Q_{max} = 26$ nC/µF, $\bar{V} = -36$ mV, and $k = 19$ mV. The missing open circles in the graph correspond to the traces obtained at +10, +20, and +30 mV, which showed problems for sloping baseline corrections that are better demonstrated in *C* and in Fig. 1. (Fiber NV120B)
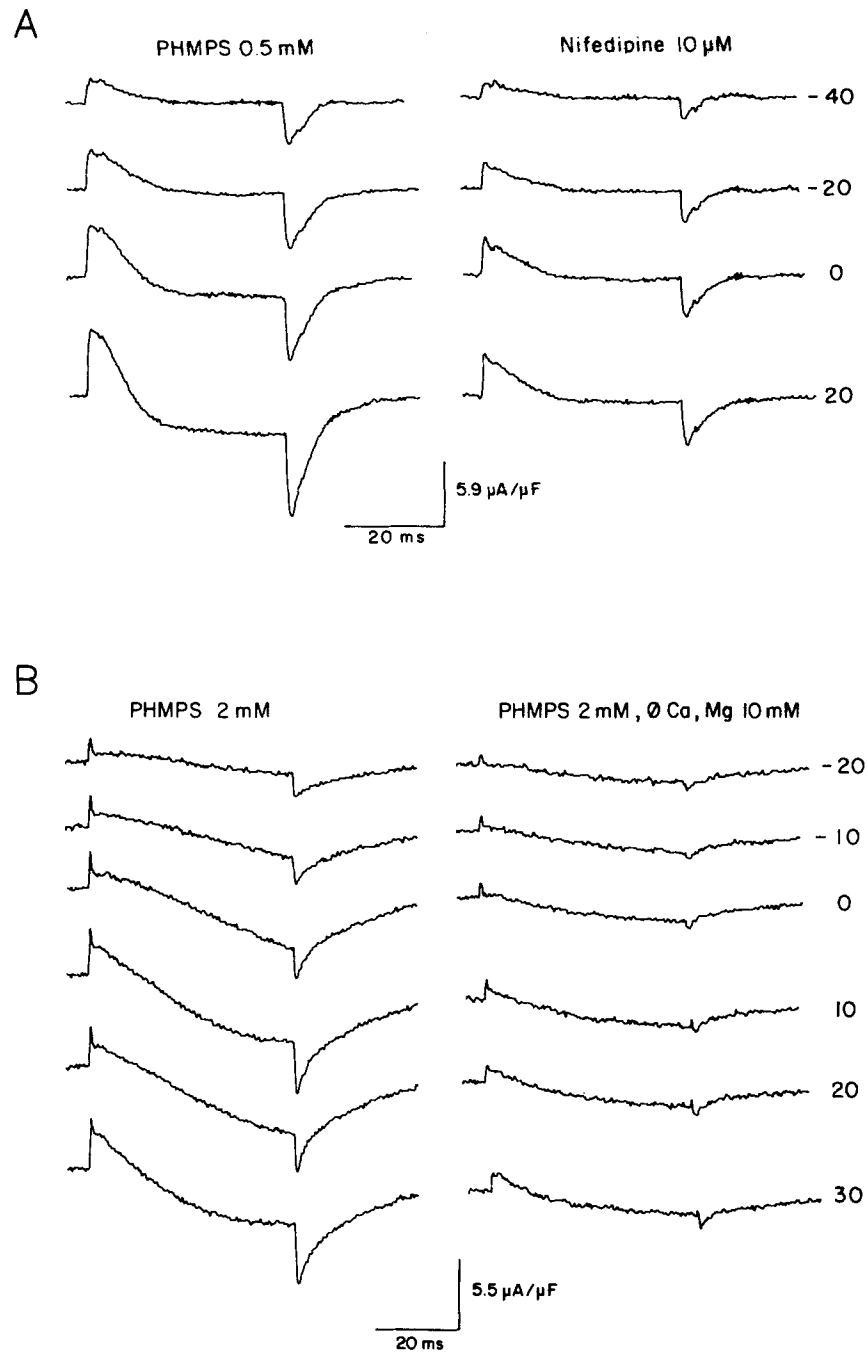
FIGURE 4. Blockade of the ionic current developed in the presence of mercurial compounds. (A) Effect of 10 μM nifedipine on nonlinear membrane currents in the presence of 0.5 mM PHMPS. The records on the left were obtained after a 30-min exposure to 0.5 mM PHMPS. Due to the low concentration, the ionic current was rather small and the charge signals were not greatly diminished; after exposure of the fiber to nifedipine (records on the right), the charge

the inward current disappeared, with a further reduction of the charge signal. However, the combination of calcium-free medium and mercurial compound treatment was found to be very deleterious and only two successful experiments of this type could be performed before the fibers were completely deteriorated. The reduction of the inward currents activated in the presence of mercurial compounds by two calcium channel blockers, cadmium and nifedipine, and by a low-calcium condition, strongly suggests a primary role for calcium channels in the genesis of the inward currents. These results give strong support to the idea that the mercurial compound facilitates the opening of a calcium conductance, which is normally activated at more positive potentials. This idea was confirmed in other unpublished experiments designed to study the effect of mercurial compounds on calcium currents: it was found that the main effect of these compounds was to change the activation characteristics of calcium currents without greatly affecting their maximal value (Gonzalez, A., and C. Caputo, unpublished experiments). The facilitation of inward currents by sulfhydryl reagents has also been reported to occur in crustacean muscle fibers, with evidence that calcium ions are also involved in this preparation (Lizardi, Garcia, Sanchez, and Zuazaga, 1989).

Since in most of the fibers tested with PHMPS or PCMB the development of calcium current renders difficult the quantitative measurements of the effects on intramembranous charge movement, it became necessary to study these effects after blockade of the calcium currents. Although the combination of calcium-free solutions and -SH reagents caused the fiber to deteriorate in a short time, two experiments could be carried out exposing the fibers to 1 mM PHMPS in a solution containing no added calcium and 10 mM Mg^{2+}. Fig. 5 shows one such experiment. In Fig. 5 *A*, the record on the left is superimposed traces of charge movement signals obtained at different membrane potential values before (darker traces) and after (lighter traces) adding 1 mM PHMPS, while the record on the right corresponds to the running time integrals of the currents shown on the left. Fig. 5 *B* shows the charge–membrane potential relationship obtained in this experiment. In this case it was found that Q_{max} was reduced from 34 to 25 nC/μF, $\overline{V}$ changed from -30 to -25 mV, and k changed from 16 to -18.4 mV. The other experiment gave similar results, but with a larger outward residual current.

Other experiments were carried out using cadmium as a blocker of the putative Ca current, since nifedipine could not be used due to its direct effect on charge movement.

The Effect of Cadmium Ions

Confirming the results of Fig. 3, Fig. 6 shows that 0.2 mM Cd^{2+} has no effect on the magnitude of intramembranous charge movement signals, and only affects its kinetics in agreement with recent results by Hui (1991); the experiment also

---

signals appear to be further reduced and the inward current abolished. (*B*) Effect of medium prepared with no added calcium and 10 mM magnesium ions on nonlinear membrane currents in the presence of 2 mM PHMPS. The records on the left were obtained after a 30-min exposure to PHMPS. The records on the right were obtained with no calcium in the external medium. In addition to a substantial decrease in the charge signal, it is clear that the inward current visible in the presence of calcium is abolished.
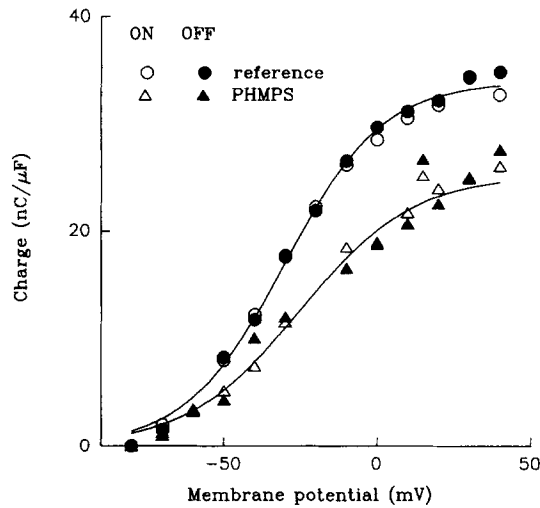
A
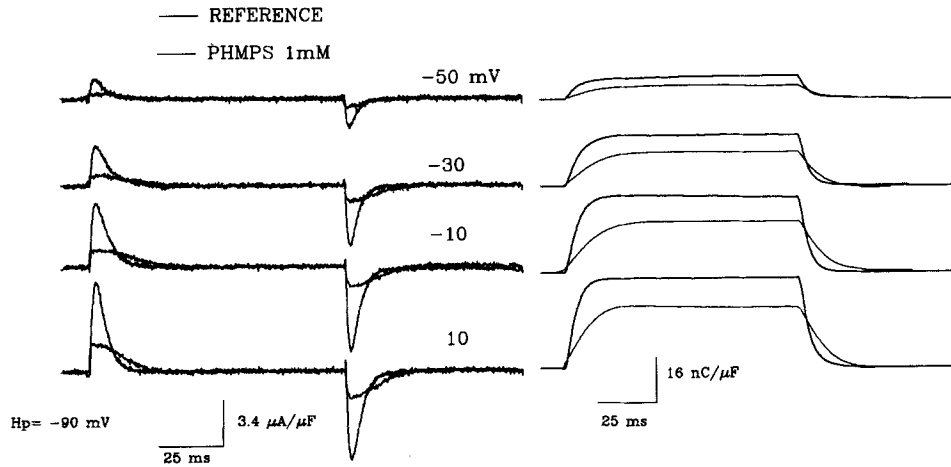
B

FIGURE 5. Effect of 1 mM PHMPS on intramembrane charge movement measured in the virtual absence of Ca^{2+}. The experiment was carried out with the fiber exposed to a solution containing no added Ca^{2+} and 10 mM Mg^{2+}. In this experiment the duration of the depolarizing pulses was 100 ms. A (left-hand side) shows superimposed traces of the charge signal obtained at different membrane potential values, shown on the right, before and after addition of 1 mM PHMPS. A (right-hand side) shows the corresponding running integrals of the current traces shown on the left. The darker and larger records correspond to the control situation. B shows the charge–potential relationships in the absence (circles) and presence (triangles) of PHMPS. The open and filled symbols correspond to the ON and OFF transients.

demonstrates that prolonging its duration and performing multiple solution changes does not cause major changes in the charge movement signals. This possibility is relevant for experiments to be discussed later. In Fig. 6 A, the first column of records shows charge movement signals measured under control conditions. The records of the second column were obtained 20 min after exposing the fiber to 0.2 mM Cd^{2+}. Those of the third column were obtained 30 min after washing out this cation; and
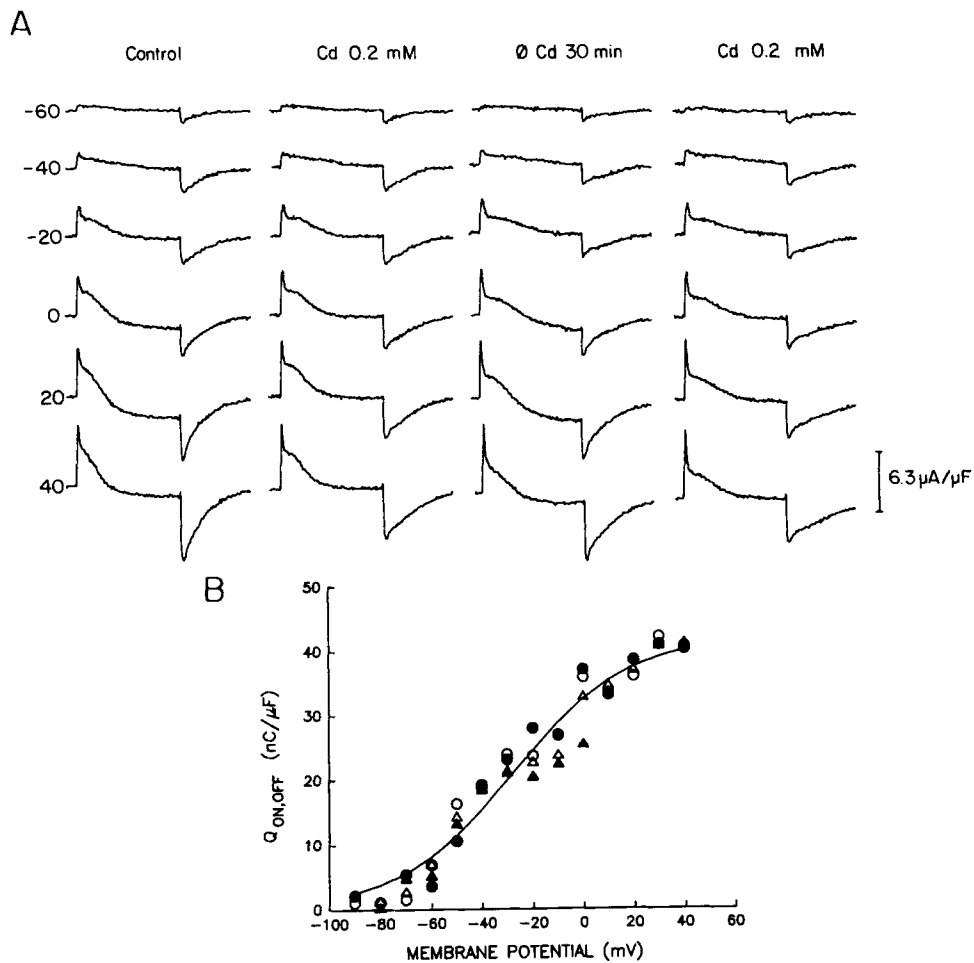
A

FIGURE 6.   Effect of 0.2 mM cadmium and of repeated solution changes on intramembrane charge movement signals. In *A*, the records of the first and third columns were obtained in the absence of Cd^{2+}, while those of the second and fourth columns were obtained in its presence. Only some effects can be appreciated to occur on the time course of the signals, mostly in the OFF component, without major effects on their magnitude. This is better shown in *B*, in which the means of the ON and OFF components of the charge from the records of the second and fourth columns have been plotted vs. the fiber membrane potential. The curve was calculated from Eq. 1 with the following parameters: $Q_{max} = 42$ nC/μF, $\bar{V} = -28$ mV, and $k = 23$ mV. (Fiber NV080A)

finally, the records in the last column were obtained 20 min after reexposing the fiber to Cd^{2+}. While the magnitude of the charge movement signal is not greatly affected, its kinetics, particularly those of the OFF component, appear to be slowed in a reversible way (compare the records of the second and third columns). Similar results were obtained in another experiment in which 2 mM Cd^{2+} was used. Fig. 6 *B* shows a graph in which the values of the ON (circles) and OFF (triangles) charges, obtained

from the second (open symbols) and fourth columns (filled symbols), have been plotted against the membrane potential. In the presence of Cd^{2+} the OFF transients at positive potentials became very slow and their corresponding values have not been included in the graph.

Fig. 7 shows an experiment designed to measure the effects of PCMB on charge movement, after treatment with Cd^{2+} to avoid the problems related with activation of calcium currents. The records on the left were obtained in the presence of 0.1 mM Cd, while those on the right were obtained 30 min after adding 2 mM PCMB to the Cd-containing medium. Although it is clear that in the presence of Cd, PCMB did not cause the usual activation of Ca currents, the effect on charge movement appeared to be much smaller than expected. Fig. 7 B shows the charge potential relationship obtained in this experiment. The same results were obtained with two other fibers. Table I summarizes the results obtained in this series of experiments. The results indicate that in the presence of Cd^{2+}, treatment with -SH reagents is without effect. (Paired t test statistics showed that the difference between charge parameters before and after treatment was not significant; for instance, for the case of charge, $T = 1.46$ with three degrees of freedom, and the two-tailed P value is 0.2397, which is not significant.) The lack of effect of mercurial compounds applied in the presence of cadmium suggests a protective action on the fiber membrane against the mercurial compounds. It is known that cadmium, as is also the case for mercury ions, may interact with one or two sulfhydryl groups (Bruce Jacobson and Turner, 1980). If this oxidation occurred here, it would do so without the same deleterious effects that occur when oxidation occurs through organic mercurials. Since in the experiments shown in Table I the concentration of PCMB was far greater than that of cadmium, the possibility of a direct interaction of cadmium with the reagents can be neglected.

The Effect of PHMPS and PCMB on Charge Movement

The next series of experiments was carried out with a group of fibers whose charge movement signals were measured sequentially in the following solutions: normal solution + 0.1 mM Cd, normal solution, normal solution + organic mercurial, and normal solution + organic mercurial + 0.1 mM Cd^{2+}. An example of such an experiment is given in Fig. 8 A. The records obtained in the first run, carried out under normal conditions and not shown in the figure, were practically identical to those shown in the second column in the figure. For the case of this fiber, the presence of a putative calcium current was evident in the absence of cadmium, causing larger than usual tail current contamination in the OFF component of the charge signal. These currents were effectively blocked by cadmium, as shown in the first column of records, and reappeared after washout of this cation, as shown in the

---

FIGURE 7. (*opposite*) Protective effect of Cd^{2+}. The figure shows that when added in the presence of 0.1 mM Cd ions, 2 mM PCMB has no effect on the nonlinear membrane currents. A shows the experimental records, while B shows the charge–voltage relationship obtained in the presence of 0.1 mM Cd before (*open symbols*) and after addition of 2 mM PCMB (*filled symbols*). The circles and triangles represent the ON and OFF transients, respectively. The charge parameters defining the curve are: $Q_{max} = 36$ nC/μF, $\bar{V} = -26$ mV, and $k = 24$ mV. (Fiber SP280A)

A   Cd 0.1 mM   Cd 0.1 mM + PCMB 2 mM

                                                                    −60

                                                                    −40

                                                                    −20

                                                                     0

                                                                     20

                                      4.7 µA/µF
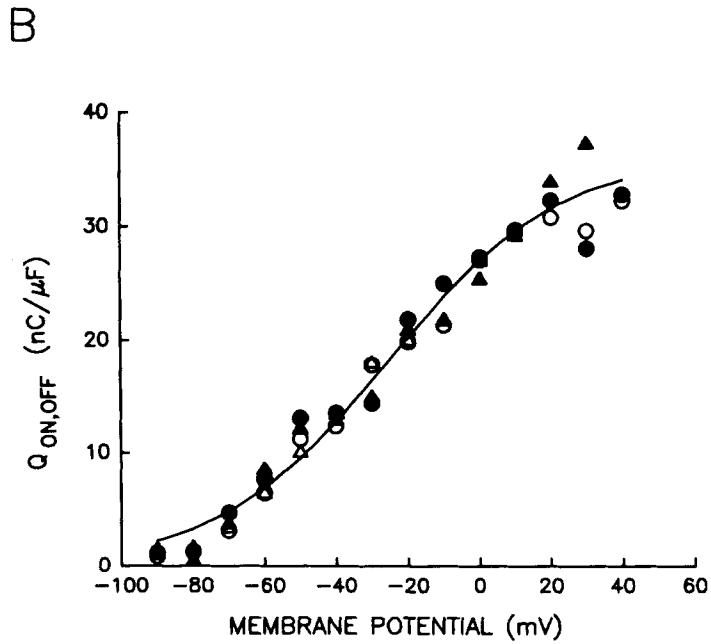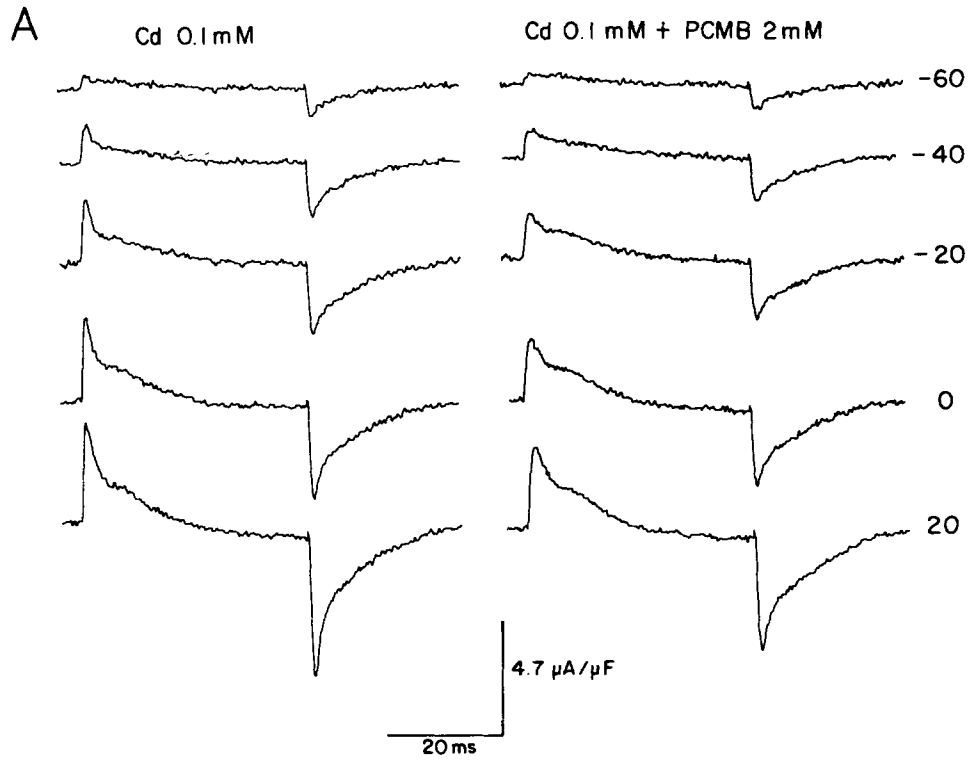
                                      20 ms

B

FIGURE 7

second column. Under these conditions, exposure to 1.5 mM PHMPS caused the activation of much larger currents of this type. The third column of records, obtained under these conditions, illustrates the impossibility of measuring the charge signals in the presence of such large currents. While measurement of the ON component is complicated by the activation of the inward current, the OFF component is practically masked by the presence of prominent tail currents. These difficulties, however, could be overcome by reexposure of the fiber to Cd^{2+} ions; under these conditions the signal contamination, caused by the presence of a large calcium conductance, was reduced, and the effects of PHMPS could be better appreciated. Therefore, in this and the other experiments summarized in Table II, comparison was made between the results obtained under the experimental conditions of the first and fourth columns of the figure. This comparison is made in Fig. 8 B, in which both the ON (circles) and OFF (triangles) charges are plotted in the absence (open symbols) and the presence (filled symbols) of PHMPS. Fig. 8 C illustrates the procedure followed for performing the integration of the ON transients. Baseline subtraction was not

TABLE I

Fiber No.	Cadmium					
	Control			-SH reagent		
	Q_{max}	$\overline{V}$	k	Q_{max}	$\overline{V}$	k
	$nC/\mu F$	mV	mV	$nC/\mu F$	mV	mV
JL100A*	47.0	−25.0	19.1	47.0	−25.0	19.1
JL100C*	43.6	−22.8	22.9	41.5	−32.6	23.3
SP200B‡	36.0	−25.7	23.6	36.0	−25.7	23.6
SP280A‡	26.9	−28.0	18.7	26.1	−40.2	13.4
Mean ± SE	38.4	−25.4	21.1	37.7	−30.9	19.9
	4.5	1.1	1.3	4.5	3.5	2.4

*PHMPS; ‡PCMB.

necessary for the records on the left, but was used for those on the right, which correspond to the last row of records in Fig. 8 A. For this fiber it appears that Q_{max} is decreased from 46.2 to 27.9 $nC/\mu F$, $\overline{V}$ changed from −41 to −40 mV, and k changed from 16 to 17 mV. The possibility that these effects on charge movement could be due to fiber deterioration caused by the repeated solution changes and not to an effect of the mercurial compounds could be discarded by several test experiments, one of which was shown in Fig. 6, in which the fibers were subjected to the same number of solution changes between Cd-free and Cd-containing media for the same total experiment duration. From several experiments of this type, we convinced ourselves that this experimental protocol did not necessarily cause fiber deterioration; in the cases in which deterioration occurred, the leakage current increased and measurement of charge movement could not be performed. To test the extent of error introduced by the subtraction procedure during these long experiments, in some cases we measured the effect of PHMPS both on charge movement with the subtraction procedure, and on total current without it. Fig. 9 illustrates one of these
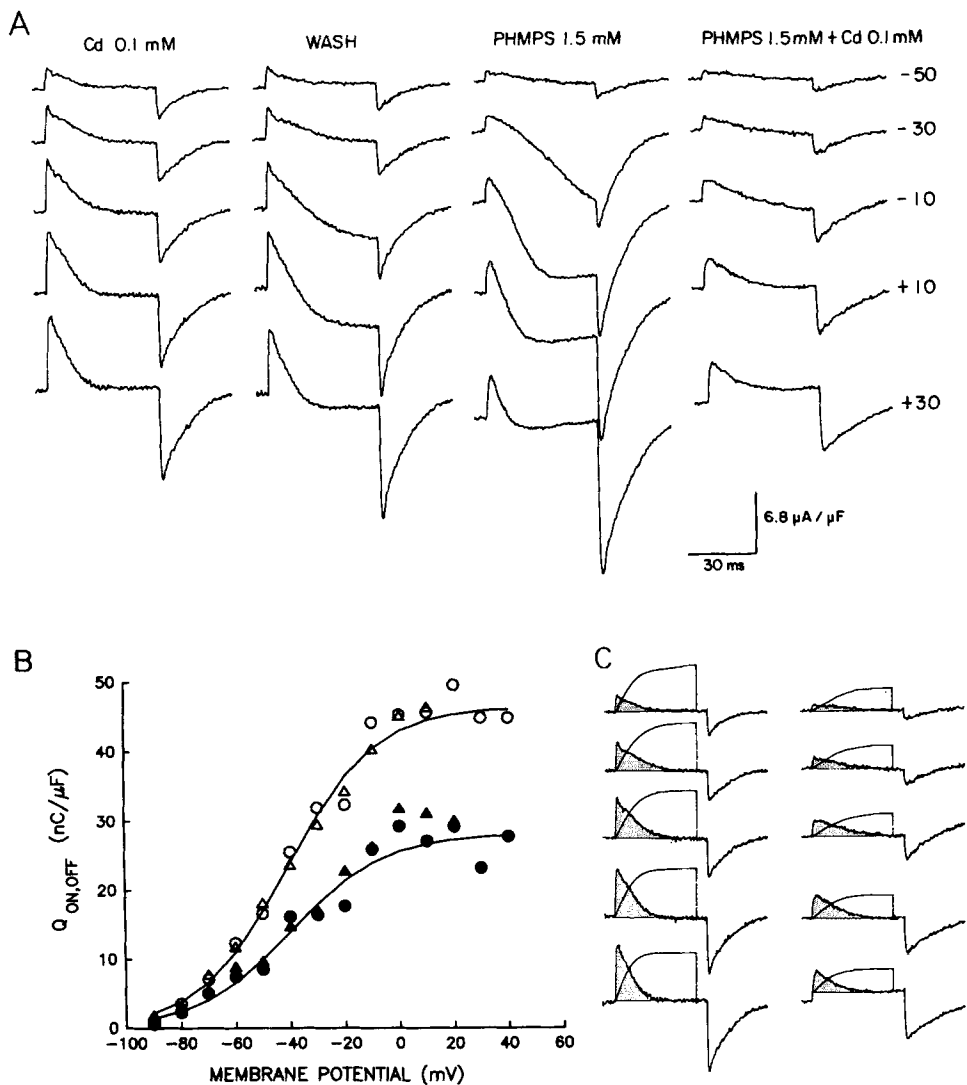
FIGURE 8. Effect of 1.5 mM PHMPS on charge movement in a fiber stabilized by 0.1 mM Cd ions. *A* shows the experimental records obtained in the absence and presence of 0.1 mM Cd and 1.5 mM PHMPS. In this experiment, as described in detail in the text, we first tested the effect of Cd ions on the membrane. The first two columns of records show the signals obtained in the presence and absence of this cation. Cadmium was later removed and the fiber was exposed to 1.5 mM PHMPS. In the presence of this agent, large possible changes in the charge movement signals are masked by the presence of large ionic currents, presumably carried by calcium. Addition of Cd blocks these currents and makes obvious the reduction of charge movement caused by PHMPS. *B* shows the charge–voltage relationship obtained in the runs shown in the first column on the left (*open symbols*) and last column on the right (*filled symbols*) of *A*. The circles and triangles represent the ON and OFF components of the charge signals, respectively. The continuous curves were obtained with the following charge parameters: Upper curve, $Q_{max} = 46$ nC/μF, $V = -41$ mV, and $k = 16$ mV. Lower curve, $Q_{max} = 27$ nC/μF, $\bar{V} = -41$ mV, and $k = 18$ mV. (Fiber NV280A)

experiments. To simplify the figure, only the nonlinear and total current records obtained in the presence of Cd^{2+} (0.2 mM) and in the presence of Cd^{2+} and PHMPS (1 mM) are shown in the upper portion of the figure (Fig. 9, A and C, respectively). Thus, Fig. 9 A shows basically the same results as the first and fourth columns of records of Fig. 7 A. Fig. 9 C shows total current moved in the range between +40 and −160 mV. These records were obtained without the leak-capacity subtracting circuit and without using the $P/−4$ pulse subtracting procedure, i.e., under the same conditions in which the fiber membrane capacity is measured. Fig. 9 B shows the nonlinear charge vs. potential relationship, while Fig. 9 D shows the total current vs. potential relationship. The open and filled symbols represent the results obtained in the absence and presence of PHMPS, respectively. The circles and triangles represent

TABLE II

Fiber No.	Control			PCMB		
	Q_{max}	$\overline{V}$	k	Q_{max}	$\overline{V}$	k
	$nC/\mu F$	mV	mV	$nC/\mu F$	mV	mV
SP250A	30.8	−34.2	13.7	13.8	−33.3	19.1
SP260B	44.4	−17.7	19.3	27.5	−37.1	16.3
NV090B	43.7	−30.4	20.2	31.3	−36.3	15.9
NV120B	36.1	−29.8	18.7	26.1	−36.2	18.5
NV130A	37.6	−31.9	22.7	30.7	−34.1	20.2
NV140A	37.8	−32.2	17.9	27.9	−37.0	18.2
Mean ± SE	38.4	−29.4	18.8	26.2	−35.7	18.0
	2.1	2.4	1.2	2.6	0.6	0.7
	Control			PHMPS		
NV150A	31.6	−36.3	24.1	20.3	−47.3	15.8
NV150B	41.9	−28.2	25.3	19.1	−42.9	12.4
NV280A	45.7	−40.7	16.5	27.4	−41.1	18.7
NV280B	42.3	−41.6	14.6	34.5	−39.3	23.6
NV290A	48.1	−35.2	18.4	40.0	−35.1	19.7
SP111A	42.8	−34.3	17.9	32.3	−41.3	16.3
Mean ± SE	41.9	−36.1	19.5	28.9	−41.2	17.8
	2.2	2.0	1.7	3.4	1.6	1.6

the ON and OFF transients, respectively. The dashed line shows the linear regression fitted to the open symbols in the range of potentials between −220 and −120 mV, and the cross represents the holding potential. The deviation of the points from the linear regression between −80 and 40 mV represents the contribution of intramembranous charge movement to total current. The decrease in total charge observed in the presence of PHMPS corresponds mostly to the decrease in charge movement illustrated in Fig. 9 B. However, PHMPS also causes a decrease in the remaining component of total current, which is clearly appreciated at the more negative membrane potentials. It is important to notice that the subtracting pulses fell in the range between −100 and −140 mV, in which the decrease in total charge is ∼10%; this value coincides with the decrease observed in all the other fibers, and it is taken
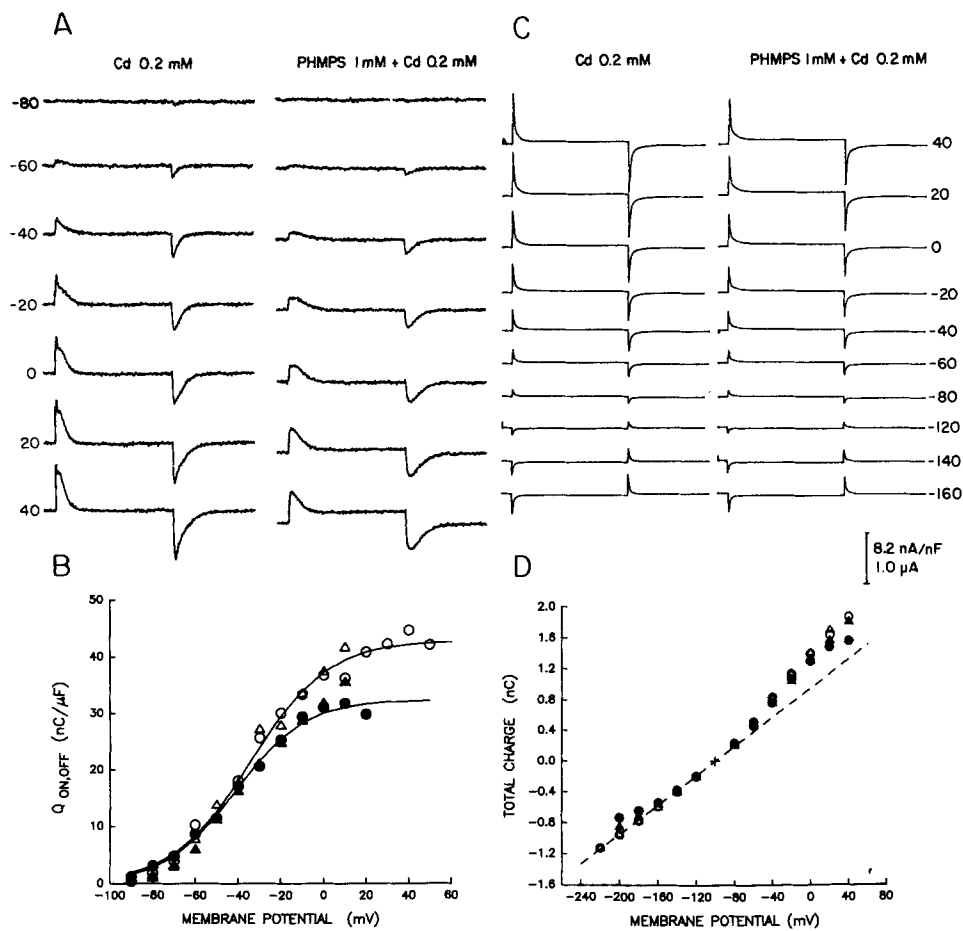
FIGURE 9. Effects of PHMPS on intramembrane charge movement and total charge in a fiber protected with 0.2 mM cadmium. The experiment was similar to that shown in Fig. 6, except that total charge was also measured; for this effect the P/−n procedure and the leak capacity subtractor were disabled. The circles and triangles in the graphs represent the charge moved during the ON and OFF transients, respectively. The open symbols were obtained in the absence, and the filled symbols in the presence, of 1 mM PHMPS. The fitted curves in *B* were obtained with the following charge parameters: upper curve, Q_{max} = 43 nC/μF, $\overline{V}$ = 34 mV, and k = 18 mV; lower curve, Q_{max} = 32 nC/μF, $\overline{V}$ = −41 mV, and k = 16 mV. (Fiber SP111A)

into account when the charge movement is normalized for the fiber membrane capacity. Table II summarizes the results obtained in several experiments of the type shown in Figs. 8 and 9, carried out either with PCMB or PHMPS. In this table the charge movement parameters were obtained under conditions similar to those shown in columns 1 and 4 of Fig. 8 *A*. Thus, both the control and experimental conditions included the presence of 0.1 mM Cd ions; however, this cation was washed out before exposing the fibers to the organic mercurial reagents, and was later readmitted to

allow charge movement measurement. In the case of the results obtained in the presence of 1.5 mM PCMB, Q_{max} was reduced by ~32%, while in the case of PHMPS the reduction amounted to 31%. In the case of the effect of PCMB on Q_{max}, paired t test statistics showed that $T = 7.31$ with five degrees of freedom, given the one-tailed P value of 0.0004, which is extremely significant. The effects on $\overline{V}$ and k were found to be marginally significant and not significant, respectively. In the case of the effect of PHMPS on Q_{max}, T was 5.39 with five degrees of freedom, giving a one-tailed P value of 0.0015, which is very significant. Likewise, the effects on $\overline{V}$ and k were found to be not significant.

Protective Effect of DTT

In the preceding paper (Caputo, Bolaños, and Gonzalez, 1993), it was shown that DTT was effective in protecting from, or partially reverting the effects of PCMB and PHMPS on the fiber contractile capacity. Fig. 10 shows that DTT may also protect a fiber from the effects of PHMPS. In this experiment, after measurement of charge movement under control conditions (records on the left), the fiber was exposed first to 2 mM DTT (records in the middle) without a major effect on the fiber behavior. Further exposure to 1 mM PHMPS in the presence of DTT failed to produce any noticeable effect (records on the right). In other experiments it was found that at higher concentrations (4 mM) and after prolonged exposure this compound itself appeared to be effective in reducing the charge movement signals, without any effect on inward currents. In two experiments DTT was added after treatment with PHMPS and was found to partially reverse the effect of this agent on charge movement. Therefore, DTT appears to behave differently from cadmium.

Effects on Charge 2

The work of Brum and Rios (1987) supports the idea that after sustained membrane depolarization the charge movement signal known as charge 1 is converted into charge 2. There is evidence indicating that some experimental procedures, for instance, exposing the fibers to low-calcium media (Brum, Fitts, Pizarro, and Rios, 1988), facilitate the transformation of charge 1 into charge 2. Therefore, some experiments were carried out to measure the effects of sulfhydryl inhibitors on charge 2 to test the possibility that these compounds might cause the interconversion of charge 1 into charge 2. Fig. 11 A shows the effect of 1 mM PHMPS on charge 2, produced in a fiber depolarized to 0 mV. The graph shown in Fig. 11 B was obtained by plotting the absolute values of the time integrals of the OFF transients of the intramembranous charge movement, since they had a negative value (Caputo and Bolaños, 1989). The curves obtained in the absence and presence of 1 mM PHMPS show clearly that charge 2 is greatly reduced by this substance, indicating that these compounds affect the charge movement molecule independently of whether the membrane is polarized or depolarized. Interestingly, the ON transient seems to be affected to a greater extent than the OFF one; we have no explanation for this and believe that further experiments need to be done to clarify the nature of this differential effect.
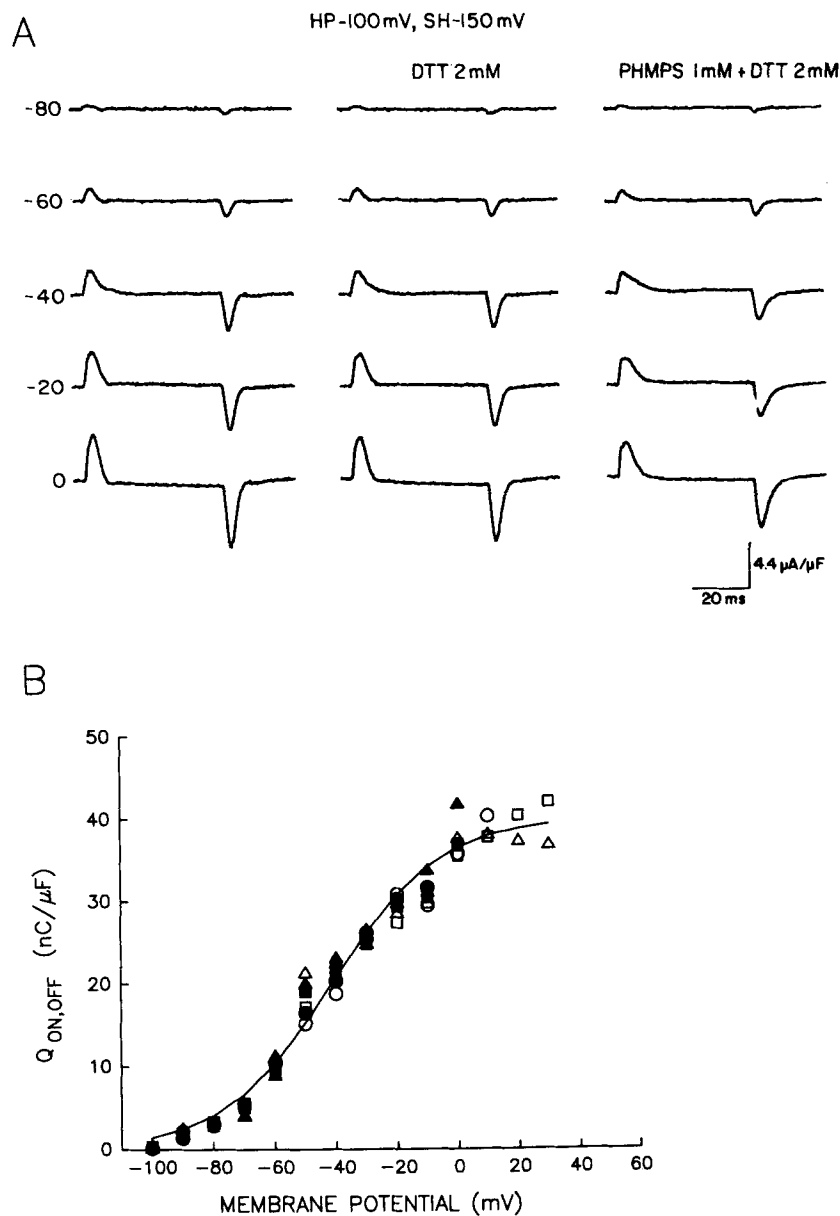
FIGURE 10. Protection by DTT from the effects of 1 mM PHMPS. *A* shows an experiment in which charge movement was measured before exposure (records on the left), after the fiber was exposed to 2 mM DTT (records in the middle), and finally, after exposure to 1 mM PHMPS (records on the right). DTT by itself has no appreciable effects on the charge signals, and prevents the effects normally caused by PHMPS. *B* shows the charge–potential relationship obtained from the records shown in *A*. The circles represent the results obtained in the normal solution (records on the left); the squares represent the results obtained in the presence of DTT alone (records in the center); and the triangles represent the results obtained after adding PHMPS (records on the right). The open and filled symbols represent the ON and OFF transients, respectively. The curve that fits the experimental points obtained under the different conditions was calculated using the following parameters: Q_{max} = 40 nC/μF, $\overline{V}$ = −41 mV, and k = 18 mV. (Fiber SP198B)
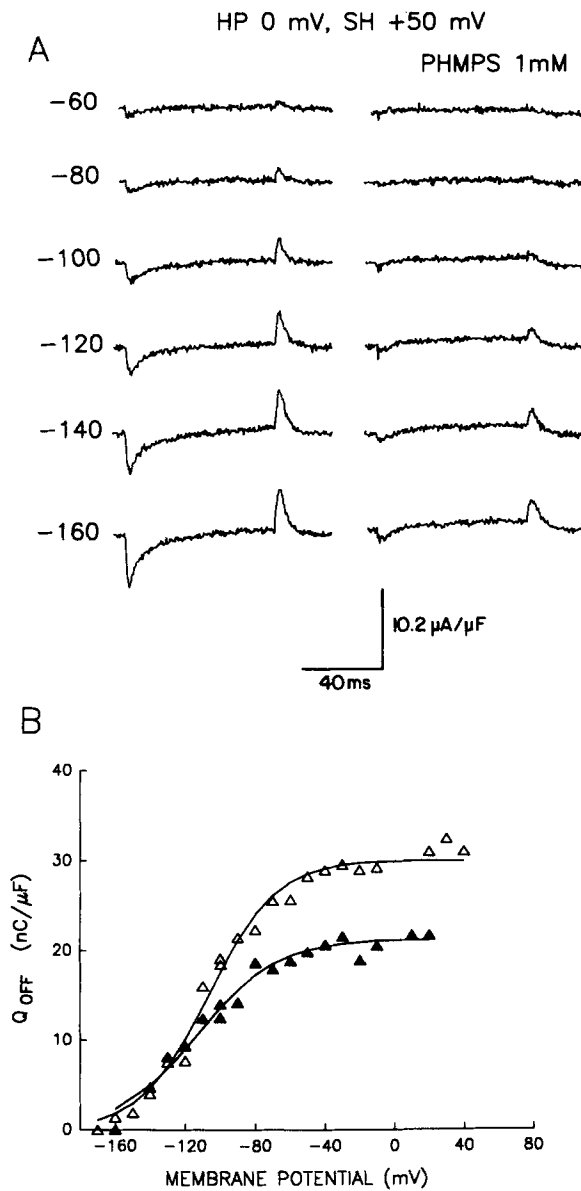
FIGURE 11. Effects of 1 mM PHMPS upon charge 2. (A) After obtaining control charge signals at -100 mV (records not shown), the fiber was depolarized to 0 mV and the records representing charge 2 (shown on the left) were obtained. The records on the right show the reduction in charge 2 caused by 1 mM PHMPS. (B) Voltage dependence of charge 2 in the absence (*open triangles*) and presence (*filled triangles*) of 1 mM PHMPS. The parameters used to calculate the respective curves were: open triangles, $Q_{max} = 30$ nC/μF, $\overline{V} = -107$ mV, and $k = 19$ mV; filled triangles, $Q_{max} = 21$ nC/μF, $\overline{V} = -113$ mV, and $k = 22$ mV. (Fiber AB269B)

DISCUSSION

In this work we have found that the sulfhydryl reagents PCMB and PHMPS, besides affecting intramembranous charge movement, also facilitate the activation of an ionic current, presumably carried by Ca^{2+}. This current is blocked by Cd^{2+} and nifedipine, and is not present when there is no calcium in the external medium. The activation of this current complicates the measurement of the effects of mercurial reagents on charge movement, and we have used cadmium ions to block it. A further complica-

tion has been the finding that intramembranous charge movement is not reduced by these organic mercurial reagents when they are applied in the presence of cadmium; however, if cadmium is added after the organic mercurial compounds have reacted with the membrane, the ionic current is appreciably reduced and a sizable decrease of charge movement of ~30% can be observed without much effect on its voltage sensitivity.

The protective effect of Cd^{2+} from the effects of PCMB and PHMPS on charge movement is completely different from the effect that Cd^{2+} ions have on calcium currents. In fact, it is known that these are blocked through interference of the divalent cation with the conductive path; in this action -SH groups are not necessarily involved, as is the case for the well-known interaction of cadmium with calmoduline (Cheung, 1988), which has no cysteine residues in its amino acid chain. On the other hand, it seems possible that the protective effect on the membrane is carried out through interaction with SH groups. Cadmium ions are known to react with these groups with high affinity (Bruce Jacobson and Turner, 1980). It is not necessary to suppose that these interactions occur with the same SH groups that bind the organic mercurials, since cadmium might interact with other SH groups (one or two), causing conformational changes in the sensor molecule that preclude the subsequent binding of PCMB or PHMPS to other -SH groups.

Maximum charge reduction of ~30%, observed in the experiments in which Cd^{2+} was used to stabilize the membrane, can be considered to represent a lower limit for the effect, since if a higher concentration or longer exposure could have been used the effect could have been greater. However, as has been explained, this could not be done to avoid membrane damage since the technique used for measuring charge movement is far more sensitive to changes in the membrane electrical properties than the one used to measure contractile responses (Caputo et al., 1993). Thus, it has not been possible to carry out reliable experiments in which charge movement was totally abolished and thus establish a quantitative comparison of the effects of these compounds on contractile responses and charge movement. While the loss of contractile ability appeared to follow a time course consistent with the reduction of the charge movement signal, the fact remains that under roughly similar experimental conditions, contractile activity could be completely abolished, while charge movement was reduced by only ~30%, perhaps indicating that only some fraction of the charge signal is involved in ECC.

Additional support for the idea of a causal relationship between the two effects is provided by the demonstration that mercurial compounds appear to block contractile activity by interfering with a step in DCC. In fact, in the previous work (Caputo et al., 1993) it has been shown that loss of contractility caused by these agents cannot be explained in terms of membrane depolarization, depletion of the sarcoplasmic reticulum calcium store, or failure of the contractile apparatus. This idea is also supported by the fact that DTT appears to protect both intramembranous charge movement and contractile responses from the effect of the organic mercurials.

A first possibility to consider is that these compounds interfere with DCC by direct interaction with the -SH groups of cysteine residues present in the voltage sensor molecule, interfering with its function. Recently, Etter (1990) has shown that phenylglyoxal, a reagent that interacts specifically with arginine residues, also interferes

with contractile activation, possibly by its action on intramembranous charge movement. However, the action of phenylglyoxal appears to differ from that of the SH reagents used in this work in at least two other aspects besides the target chemical group: (1) calcium currents are also diminished by this compound, with approximately the same time course as charge movement (Bolaños, 1991); and (2) phenylglyoxal preferentially affects charge 1 without much affecting charge 2 (Etter, 1990), while SH reagents appear to affect both types of charge. On the other hand, SH reagents appear to affect charge movement but not calcium currents. While charge movement is reduced or abolished by these compounds, inward currents are clearly activated under the experimental conditions used by us to measure charge movement, where they are not normally conspicuous. The results obtained with nifedipine and cadmium indicate that these currents flow through the slow type calcium channels, even though we cannot rule out that these channels may be somewhat modified by the sulfhydryl reagents. This differential effect has several important implications. One is that the mercurial-sensitive charge movement component, as it is measured in these experiments, is not involved in the gating of these calcium channels. Gating for these channels could be provided by the charge component not affected by the sulfhydryl reagents and more precisely by the nifedipine-sensitive component (Lamb, 1987; Rios and Brum, 1987). Another implication refers to the idea that the DHP receptor molecules may function both as calcium channels and as voltage sensors for DCC (Rios and Brum, 1987; Tanabe et al., 1987, 1988; Tanabe, Beam, Adams, Niidome, and Numa, 1990). Lamb (1991) has recently addressed the question of whether, assuming that there is only one type of molecule for both functions, these are carried out simultaneously, or whether the molecule may switch from one role to the other depending on some local factor. Along the same lines of thought, the results presented here might indicate that these two functions are associated either with moieties of the DHP receptor molecule under different physical states, or with different regions of the same molecule. Since most of these receptor molecules are not normally functioning as calcium channels (Schwartz, McCleskey, and Almers, 1985), it is possible that they may exist under two different configurations, one related to voltage sensing and the other to calcium conduction. If this were the case, one might speculate that sulfhydryl reagents favor the transformation from one configuration to the other by interfering with disulfide or even hydrogen bonding between different portions of the molecule.

The differential effect of PCMB and PHMPS on charge movement and calcium current discussed above might be considered difficult to reconcile with the view that the DHP receptor molecule may serve for both functions. In this respect it is worth mentioning that there is another agent, namely perchlorate, which modifies the function of the voltage sensor by shifting to the left the activation curves of contraction (Gomolla, Gottschalk, and Luttgau, 1983) and of charge movement (Luttgau, Gottschalk, Kovacs, and Fuxreiter, 1983), without affecting calcium channel characteristics (Feldmeyer and Luttgau, 1988). Moreover, sulfhydryl reagents also appear to differentially affect several ionic currents (Smith, 1958; Shrager, 1977; Zuazaga et al., 1984; Lizardi et al., 1989). Thus, while calcium currents are not adversely affected, sodium currents in muscle fibers (Gonzalez, A., and C. Caputo, unpublished experiments) and sodium and potassium currents in squid axons (Caputo, Perozo, and Bezanilla, 1990) are appreciably reduced.

Due to the large number of SH groups present in the DHP receptor sequence, it is not possible, at this time, to identify the specific groups that could be involved in the effects described above. Furthermore, it can be considered that, among the SH groups that are essential for its voltage-sensing function, some might be located on a putative active site of the receptor, others outside this active site but still important for maintaining its configuration, and others on or near allosteric or regulatory sites. Thus, the effects of the organomercurial compounds may not necessarily be due to inhibition of specific SH groups located on the active site, but rather to their interaction with SH groups located far away from the active site but important for maintaining its configuration.

A second possibility to explain the effect of sulfhydryl reagents on DCC could be based on the recent proposals that the voltage sensor and the calcium release process might be linked by feedback or allosteric mechanisms. Recent evidence suggests that a component of the charge movement signal, Q_γ, might be determined (Pizarro, Csernoch, Uribe, Rodriguez, and Rios, 1991) or affected in its kinetics (Pape, Jong, and Chandler, 1992) by the calcium released from the sarcoplasmic reticulum; thus, a direct effect of -SH reagents on calcium release could cause a change in the charge movement signal. However, as discussed in the preceding paper (Caputo et al., 1993), this would imply the existence of two types of release channels: one type activated by voltage and affected by -SH reagents, and the other activated by caffeine and not affected by these compounds. Also recently, Rios et al. (1992) have proposed an allosteric model of transmission in ECC based on the structural relationships between the DHP receptor (voltage sensors) in the tubular membrane and the ryanodyne receptors (calcium release channels) in the sarcoplasmic reticulum. In this model the voltage sensor is considered to be an allosteric ligand and the release channel an allosteric protein. According to this hypothesis, any effect caused by the -SH reagents at the level of the release channel, or at the level of the connecting structure between voltage sensor and release channel, could affect the voltage dependence of charge movement, but not maximum charge. Although this model would not explain the reduction of charge movement by -SH reagents, one could imagine that after modification of the calcium release channels by -SH reagents, the coupling between these two proteins is modified in such a way that the voltage sensor is locked in a configuration insensible to voltage. This possibility is in agreement with the reduction of both charge 1 and charge 2.

REFERENCES

Bezanilla, F., R. E. Taylor, and J. M. Fernandez. 1982. Distribution and kinetics of membrane dielectric polarization. I. Long-term inactivation of gating currents. *Journal of General Physiology.* 79:21–40.

Bolaños, P. 1991. Phenylglyoxal effects on calcium currents and charge movement. *Biophysical Journal.* 59:64a. (Abstr.)

Bruce Jacobson, K., and J. E. Turner. 1980. The interaction of cadmium and certain other metal ions with proteins and nucleic acids. *Toxicology.* 16:1–37.

Brum, G., R. Fitts, G. Pizarro, and E. Rios. 1988. Voltage sensor of the frog skeletal muscle membrane require calcium to function in excitation-contraction coupling. *Journal of Physiology.* 398:475–505.

Brum, G., and E. Rios. 1987. Intramembrane charge movement in frog skeletal muscle fibres: properties of charge 2. *Journal of Physiology.* 387:489–517.

Caputo, C., and P. Bolaños. 1989. Effects of D-600 on intramembrane charge movement of polarized and depolarized frog muscle fibers. *Journal of General Physiology.* 94:43–64.

Caputo, C., P. Bolaños, and A. Gonzalez. 1993. Effects of sulfhydryl inhibitors on depolarization–contraction coupling in frog skeletal muscle fibers. *Journal of General Physiology.* 101:411–424.

Caputo, C., E. Perozo, and F. Bezanilla. 1990. Chemical modification of squid axon K channels by p-hydroxymercuryphenilsulphonic acid (PHMPS). *Biophysical Journal.* 57:16a. (Abstr.)

Chandler, W. K., R. F. Rakowski, and M. F. Schneider. 1976. Effects of glycerol treatment and maintained depolarization on charge movement in skeletal muscle. *Journal of Physiology.* 254:285–316.

Cheung, W. Y. 1988. Calmodulin and its activation by cadmium ions. *Annals of the New York Academy of Sciences.* 522:74–87.

Drews, G., and M. Rack. 1988. Modification of sodium and gating currents by amino group specific cross-linking and monofunctional reagents. *Biophysical Journal.* 54:383–391.

Etter, E. F. 1990. The effect of phenylglyoxal on contraction and intramembrane charge movement in frog skeletal muscle. *Journal of Physiology.* 421:441–462.

Feldmeyer, D., and H. C. Luttgau. 1988. The effect of perchlorate on Ca currents and mechanical force in skeletal muscle fibres. *Pflügers Archiv.* 411:R190.

Gomolla, M., G. Gottschalk, and H. C. Luttgau. 1983. Perchlorate-induced alterations in electrical and mechanical parameters of frog skeletal muscle fibres. *Journal of Physiology.* 343:197–214.

Gonzalez, A., P. Bolaños, and C. Caputo. 1989. Effects of sulfhydryl reagents on charge movement and contractile responses in frog muscle fibers. *Biophysical Journal.* 55:87a. (Abstr.)

Hille, B., and D. T. Campbell. 1976. An improved vaseline gap voltage clamp for skeletal muscle fibers. *Journal of General Physiology.* 67:265–293.

Hui, C. S. 1991. Factors affecting the appearance of the hump charge movement in frog cut twitch fibers. *Journal of General Physiology.* 98:315–347.

Huneeus-Cox, F., H. L. Fernández, and B. H. Smith. 1966. Effects of redox and sulfhydryl reagents on the bioelectric properties of the giant axon of the squid. *Biophysical Journal.* 6:675–689.

Kirsten, E. B., and A. S. Kuperman. 1970. Effects of sulphydryl inhibitors on frog sartorius muscle; p-chloromercuribenzoic acid and n-chloromercuribenzenesulphonic acid. *British Journal of Pharmacology.* 40:814–826.

Lamb, G. D. 1987. Asymmetric charge movement in polarized and depolarized muscle fibres of the rabbit. *Journal of Physiology.* 383:349–367.

Lamb, G. D. 1991. Ca^{2+} channels or voltage sensors? *Nature.* 352:113.

Lizardi, M., M. C. Garcia, J. A. Sanchez, and C. Zuazaga. 1989. Induction of calcium currents and enhancement of mechanical activity in crustacean muscle by sulfhydryl reagents. *Biophysical Journal.* 55:304a. (Abstr.)

Lüttgau, C., G. Gottschalk, L. Kovacs, and M. Fuxreiter. 1983. How perchlorate improves excitation-contraction coupling in skeletal muscle fibers. *Biophysical Journal.* 45:637–641.

Means, G. E., and R. E. Feeney. 1971. Chemical Modification of Proteins. Holden-Day Inc., San Francisco. 254 pp.

Meves, H., N. Rubly, and R. Stampfli. 1988. The action of arginine-specific reagents on ionic and gating currents in frog myelinated nerve. *Biochimica et Biophysica Acta.* 943:1–12.

Pape, P. C., D.-S. Jong, and W. K. Chandler. 1992. Effects of sarcoplasmic reticulum (SR) calcium loading on intramembranous charge movement in frog cut muscle fibers. *Biophysical Journal.* 61:A130. (Abstr.)

Pizarro, G., L. Csernoch, I. Uribe, M. Rodriguez, and E. Rios. 1991. The relationship between Q_γ and Ca release from the sarcoplasmic reticulum in skeletal muscle. *Journal of General Physiology.* 97:913–947.

Rack, M., and K. H. Woll. 1984. Effects of chemical modification of carboxyl groups on the voltage-clamped nerve fiber of the frog. *Journal of Membrane Biology.* 82:41–48.

Rios, E., and G. Brum. 1987. Involvement of dihydropyridine receptors in excitation-contraction coupling in skeletal muscle. *Nature.* 325:717–720.

Rios, E., M. Karhanek, A. Gonzalez, and J. Ma. 1992. An allosteric model of transmission in E-C coupling. *Biophysical Journal.* 61:A131. (Abstr.)

Schneider, M. F., and W. K. Chandler. 1973. Voltage dependent charge movement in skeletal muscle: a possible step in excitation-contraction coupling. *Nature.* 242:244–246.

Schwartz, L. M., E. W. McCleskey, and W. Almers. 1985. Dihydropyridine receptors in muscle are voltage dependent but most are not functional calcium channels. *Nature.* 314:747–751.

Shrager, P. 1977. Slow sodium inactivation in nerve after exposure to sulfhydryl blocking reagents. *Journal of General Physiology.* 69:183–202.

Smith, H. M. 1958. Effects of sulfhydryl blockade on axonal function. *Journal of Cellular and Comparative Physiology.* 51:161–171.

Tanabe, T., K. G. Beam, B. A. Adams, T. Niidome, and S. Numa. 1990. Regions of the skeletal muscle dihydropyridine receptor critical for excitation-contraction coupling. *Nature.* 346:567–569.

Tanabe, T., K. G. Beam, J. A. Powell, and S. Numa. 1988. Restoration of excitation-contraction and slow calcium currents in dysgenic muscle by dihydropyridine receptor complementary DNA. *Nature.* 336:134–139.

Tanabe, T., H. Takeshima, A. Mikami, V. Flockerzi, H. Takahashi, M. Kojima, M. Matsuo, T. Hirose, and S. Numa. 1987. Primary structure of the receptor for calcium channel blockers from skeletal muscle. *Nature.* 328:313–318.

Torchinskii, Y. M. 1974. Sulfhydryl and disulfide groups of proteins. Studies in Soviet Science. Consultants Bureau, New York. 275 pp.

Zuazaga, C., A. Steinacker, and J. del Castillo. 1984. The role of sulfhydryl and disulfide groups of membrane proteins in electrical conduction and chemical transmission. *Puerto Rico Health Sciences Journal.* 3:125–139.