Effects of Sulfhydryl Inhibitors on Depolarization–Contraction Coupling in Frog Skeletal Muscle Fibers

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ABSTRACT We have studied the effects of the sulfhydryl reagents on contractile responses, using either electrically stimulated single muscle fibers or short muscle fibers that were voltage-clamped with a two-microelectrode voltage-clamp technique that allows the fiber tension in response to membrane depolarization to be recorded. The sulfhydryl inhibitors para-chloromercuribenzoic acid (PCMB) and parahydroximercuriphenyl sulfonic acid (PHMPS), at concentrations from 0.5 to 2 mM, cause loss of the contractile ability; however, before this effect is completed, they change the fiber contractile behavior in a complex way. After relatively short exposure to the compounds, <20 min, before the fibers lose their contractile capacity, secondary tension responses may appear after electrically elicited twitches or tetani. After losing their ability to contract in response to electrical stimulation, the fibers maintain their capacity to develop caffeine contractures, even after prolonged periods (120 min) of exposure to PHMPS. In fibers under voltage-clamp conditions, contractility is also lost; however, before this happens, long-lasting (i.e., minutes) episodes of spontaneous contractile activity may occur with the membrane polarized at -100 mV. After more prolonged exposure (>30 min), the responses to membrane depolarization are reduced and eventually disappear. The agent DTT at a concentration of 2 mM appears to protect the fibers from the effects of PCMB and PHMPS. Furthermore, after loss of the contractile responses by the action of PCMB or PHMPS, addition of 2 mM DTT causes recovery of tension development capacity.

INTRODUCTION

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; Zuazaga, Steinacker, and del Castillo, 1984). In the case of muscle fibers, it is known that sulfhydryl reagents may interact directly with the sarcoplasmic reticulum calcium release channels, inducing calcium release (Abramson, Trimm, Weden, and Salama, 1983; Oba and Hotta, 1985; Palade, 1987; Brunder, Dettbarn, and Palade, 1988; Abramson and Salama, 1989). This effect could explain the

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spontaneous contractile activity observed in the presence of sulfhydryl inhibitors by Okamoto and Kuperman (1966). Kirsten and Kuperman (1970) also reported that prolonged exposure to these reagents caused membrane depolarization and contractile failure. While it is possible that contractile failure might have been due either to loss of excitability or to the onset of contractile inactivation caused by membrane depolarization (Hodgkin and Horowicz, 1960; Caputo and Bolaños, 1987), or to inhibition of the contractile proteins (Wilson, dosRemedios, Stephenson, and Williams, 1991), a direct effect of these compounds on some step of the depolarization–contraction coupling (DCC) mechanism could not be discarded. In this work we have studied the effects of some sulfhydryl reacting compounds on contractile responses in skeletal muscle fibers that were electrically stimulated with external electrodes, or in voltage-clamped short muscle fibers. The compounds used in this work were the organic mercurials, para-chloromercuribenzoic acid (PCMB) and parahydroximercuriphenylsulfonic acid (monosodium salt; PHMPS), considered to be highly specific reagents for -SH groups (Means and Feeney, 1971; Torchinskii, 1974).

A short report of this work has been previously presented elsewhere (Gonzalez, Bolaños, and Caputo, 1989).

METHODS

The experiments described in this work were carried out with muscle fibers dissected from semitendinosus or lumbricalis IV digiti muscles from *Rana pipiens* and *Leptodactylus insularis*.

The experiments under voltage-clamp conditions were carried out using bundles of 5–20 fibers dissected from the lumbricalis muscles of *Rana pipiens*. These fibers have a short length, ~1.5 mm, and can be voltage-clamped with a two-microelectrode technique, following the procedure already described (Caputo, Bezanilla, and Horowicz, 1984). A few experiments were carried out with single fibers dissected from the semitendinosus muscle to study the effect of PHMPS on contractile responses induced by direct electrical stimulation. These fibers were mounted in a chamber that allowed the change of the bathing solutions, and could be stimulated with rectangular current pulses (0.2 ms duration) between two platinum plate electrodes placed symmetrically 4 mm apart on either side of the fiber. The stimulus strength was usually 15–20% above threshold.

Tension was recorded with a force transducer (series 400; Cambridge Research & Instruments, Cambridge, MA) and displayed on an oscillograph (model 220; Gould Inc., Glen Burnie, MD). The basic solution used in these experiments was a normal Ringer's (NR) that had the following composition (mM): 115 NaCl, 2.5 KCl, 1.8 $CaCl_2$, and 10 mM Tris buffer, pH 7.4; tetrodotoxin was added at a concentration of 10^{-6} g/liter, 240 mosM. The reagents PCMB, PHMPS, and DTT 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 solution. PHMPS and DTT were dissolved directly in the experimental solutions. All these solutions were prepared immediately before use. Temperature was between 20 and 22°C.

RESULTS

Contractile Activity

In agreement with the results obtained by Kirsten and Kuperman (1970), we have found that exposure of muscle fibers to different sulfhydryl reagents leads to the loss

of contractile responses. Fig. 1 shows the effect of PHMPS at two concentrations on the twitch and tetanic responses of two different fibers. In the experiment of Fig. 1 *A*, after obtaining two twitches and one tetanus, shown in the upper row, the fiber was exposed to 0.5 mM PHMPS, and after 25 min both twitch and tetanic tension appeared to be potentiated by 83 and 14%, respectively. Interestingly, after the fiber had completely relaxed from the tetanus, a secondary tension response appeared

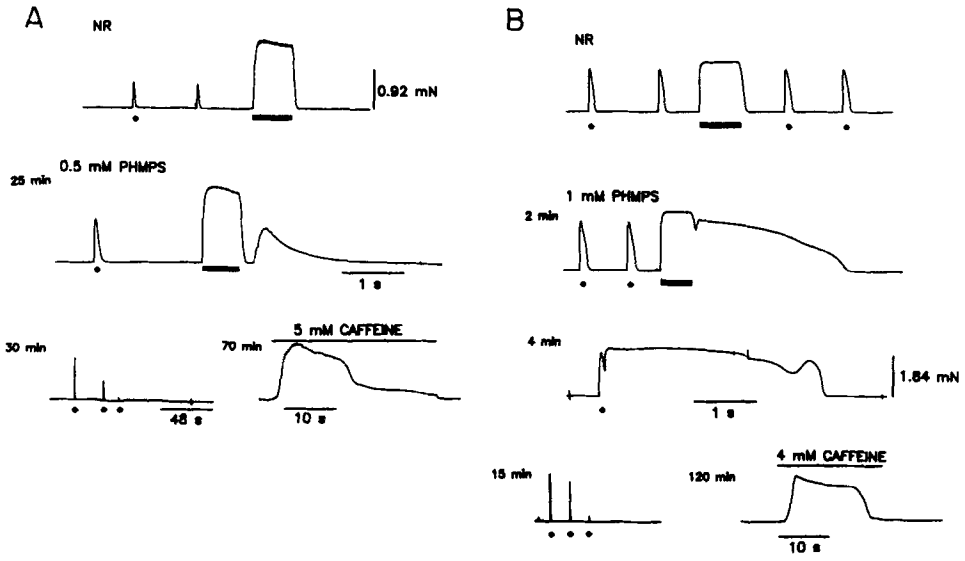

FIGURE 1. Effect of PHMPS on the contractile responses of two different single muscle fibers. In *A*, the upper record shows two twitches and a tetanus obtained under control conditions; the marks below the responses indicate the stimuli. Circles and bars were used for twitches and tetani, respectively. The record in the middle was obtained 25 min after exposing the fiber to 0.5 mM PHMPS. A potentiation of both twitch and tetanus is observed. The tetanus is followed by a spontaneous response occurring after the relaxation phase of the tetanus was completed. The bottom record on the left shows that after 5 min more of exposure to the compounds the twitching ability was lost in few minutes. The last record shows that after 40 min additional exposure, the fiber still maintains its ability to develop a caffeine (5 mM) contracture. *B* shows a similar experiment in which a fiber was exposed to 1 mM PHMPS. After a 2-min exposure, twitch potentiation and prolonged secondary response following a tetanus are visible; after 4 min the twitch potentiation is reversed and a secondary response following the twitch is observed. Finally, after 15 min the fiber twitching ability is almost abolished, while even after 120 min exposure to PHMPS caffeine contracture is unaffected.

showing slower tension buildup and relaxation phases than the tetanus. 5 min later the twitch responses were much diminished, and after few stimuli they were completely abolished, as shown in the bottom panel on the left (notice the different time scale). The last record shows that 70 min after exposing the fiber to PHMPS, caffeine at 5 mM was able to induce a contracture with a peak tension equivalent to 83% of the initial tetanic tension, which means that the fiber capacity to produce

maximal caffeine contractures was not affected (see Lüttgau and Oetliker, 1968; Caputo, 1976). In the experiment of Fig. 1 *B*, carried out with another fiber after the responses shown in the upper trace were obtained, the fiber was exposed to 1 mM PHMPS. The second trace was obtained 2 min after adding the drug and the potentiation of the twitch and tetanus were already evident, amounting to 18 and 20%, respectively. In this case, however, a secondary contractile response started during the relaxation phase of the tetanus and showed both a higher tension and a more prolonged time course than the case shown in Fig. 1 *A*. The third trace was obtained 4 min later and shows that even after a single twitch there is a secondary tension response with a prolonged and complex time course. After ~15 min the twitch response decreased rapidly and was completely abolished after three stimuli. In this case the fiber was left in the presence of 1 mM PHMPS for 120 min before inducing a caffeine contracture whose peak tension amounted to 91% of the initial tetanic tension. Similar results were obtained with four other fibers. Besides confirming the earlier findings of Okamoto and Kuperman (1966) and of Kirsten and Kuperman (1970), these results show that before loss of the contractile responses, PHMPS causes a potentiation of both the twitch and the tetanus and induces a secondary tension response after a tetanus or a twitch. More importantly, the results demonstrate that after prolonged exposure (up to 120 min) of intact muscle fibers to PHMPS at 0.5 or 1 mM, the fiber contractile capacity is not affected since caffeine contractures of normal size could still be elicited.

The next series of experiments was carried out under voltage-clamp conditions, to avoid problems related with membrane depolarization and loss of excitability caused by these mercurial agents. In agreement with the findings of Okamoto and Kuperman (1966), and with the results shown in Fig. 1, it was found that mercurial agents may induce spontaneous contractile activity, even in the absence of membrane depolarization as it is shown in Fig. 2. In the upper two cases, the fibers were exposed to 0.3 mM PCMB, while in the lower experiment 1 mM PHMPS was used. It can be seen that in all cases spontaneous contractile activity occurred a few seconds after a response to depolarizing pulses, but its occurrence did not impair the response to a successive depolarization. Visual observation of the fibers under the microscope confirmed that only the voltage-clamped fiber contracted spontaneously in the presence of these compounds. The other fibers in the bundle did not appear to contract. This observation is further confirmed by the size of the spontaneous responses that correspond to those of a single fiber. In the upper run (Fig. 2 *A*), the fiber was depolarized to −45 mV, and after a few seconds a prolonged period (notice the change in the time scale) of contractile activity ensued, which ended by abrupt spontaneous relaxation. A second test depolarization was effective in producing a contractile response. A similar result is shown in Fig. 2 *B*. In the experiment shown in Fig. 2 *C*, obtained with a fiber exposed to 1 mM PHMPS, spontaneous contractile activity starts during the relaxation phase of an incomplete tetanus-like response. These results show that spontaneous contractile activity occurs even though the fiber is polarized at −100 mV, and that the contractile activity appears to be oscillatory, with small fluctuations occurring on top of larger tension oscillations. After short exposures the occurrence of these spontaneous responses did not impair the depolarization-induced responses, strongly suggesting the presence of two separate modalities of calcium release, one occurring under control of the membrane

potential and the other independent of it. These results suggest that the spontaneous responses are associated with asynchronous bursts of calcium release episodes, possibly due to a direct, caffeine-like action of these compounds at the level of the sarcoplasmic reticulum membrane (Abramson et al., 1983; Palade, 1987). This observation would then indicate that both these compounds are cell permeant.

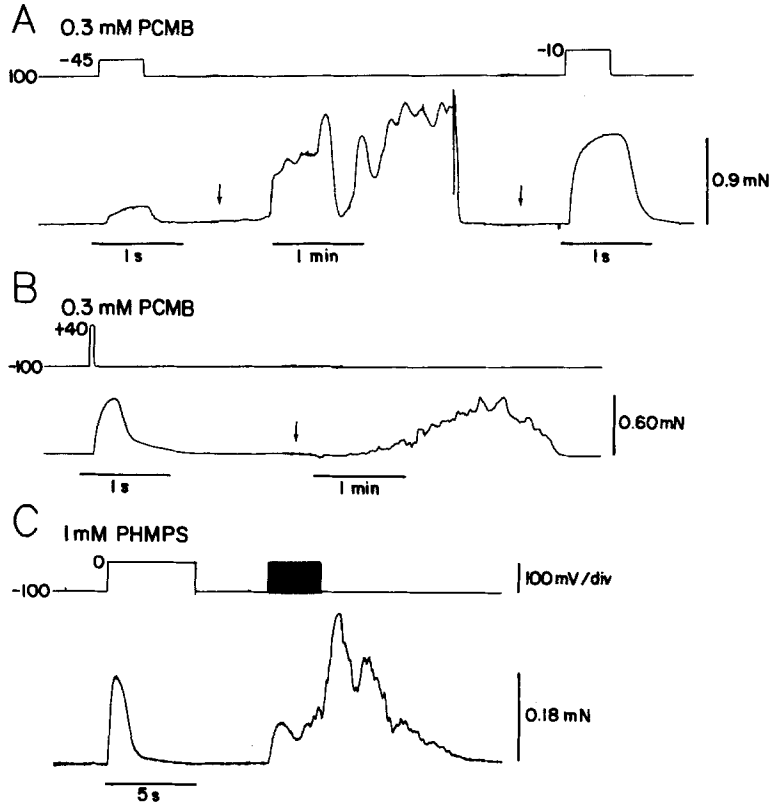

FIGURE 2. Effects of sulfhydryl reagents on the contractile responses of three different fibers (*A*, *B*, and *C*), voltage-clamped with the two-microelectrode technique. In each record the upper trace represents membrane potential and the lower trace tension. *A* and *B* were obtained in the presence of PCMB (0.3 mM), while *C* was obtained in the presence of 1 mM PHMPS. After a relatively brief exposure to these compounds, <20 min, contractile responses could still be elicited by membrane depolarization, and episodes of spontaneous contractile activity, lasting minutes, occurred with the fibers polarized at −100 mV. Observe the change in the time scale in *A* and *B* as indicated by arrows. After an episode of spontaneous contractile activity (A), membrane depolarization could still elicit a contractile response.

Fig. 3 shows that prolonged exposure to 0.4 mM PCMB leads to complete contractile failure. The upper series of records was obtained after a 17-min exposure, and although the fiber maximal tension output was not affected, changes in its contractile capacity are evidenced by the fact that during a prolonged depolarization (second record) the contracture showed no plateau, and during repetitive stimulation

(third record) the tetanus-like response was not sustained but decayed promptly. The second row of records was obtained 7 min later, and tension development was clearly reduced. In this case a secondary tension development occurs, similar to those described in Fig. 1. Finally, the last row shows that 2 min later the fiber contractile ability was completely lost.

Fig. 4 shows examples of the tension–potential relationship obtained with different pulse durations in the absence and in the presence of PCMB before complete abolition of contractility. In the graphs the normalized tension is plotted against the fiber membrane potential. The graph on the left summarizes the results obtained by depolarizing two different fibers with 50-ms (triangles) or 500-ms (circles) pulses. The

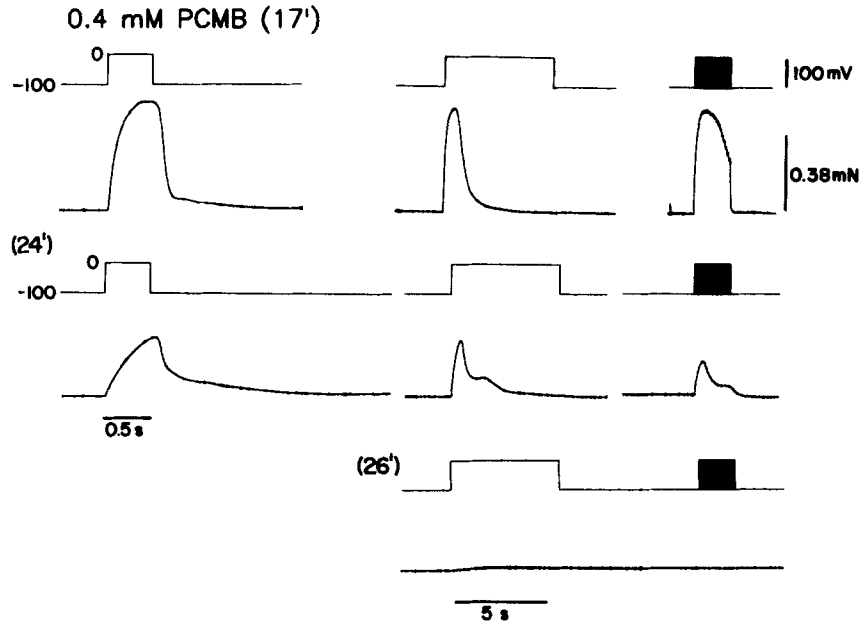

FIGURE 3. Effect of prolonged exposure to 0.4 mM PCMB on the contractile responses of a muscle fiber to membrane depolarization. The upper records were obtained after a 17-min exposure to the drug. The lower records show that after 24 min the responses were clearly reduced and that their time course was substantially modified, with a secondary component visible in the relaxation phase.

results obtained with one fiber exposed to normal Ringer's solution are expressed with the empty symbols, while the filled symbols represent the results obtained with the second fiber after a 7-min exposure to a solution containing 0.3 mM PCMB. The absolute peak tension values obtained in the two cases, with the 500-ms pulses, were very similar, indicating that after this relatively short exposure period peak tension in response to a long pulse (500 ms) is not affected, while the tension responses to shorter pulses (50 ms) are diminished. The curves in the figure were fitted assuming a Boltzmann-like function of the type:

$$T = T_{max}/[1 + \exp(V_m - V)/k]$$

The upper curve in the graph was fitted taking into account the points represented by the open triangles and circles and by the filled circles. The lower curve was fitted to the filled triangles. It appears that besides a decrease in peak tension, the activation curve obtained with the shorter pulses in the presence of PCMB shows a midpoint value shift of ~5 mV toward more positive potentials. This shift appears to be caused by a change in the steepness of the curve rather than a change in the contractile threshold. The graph on the right illustrates the results obtained in

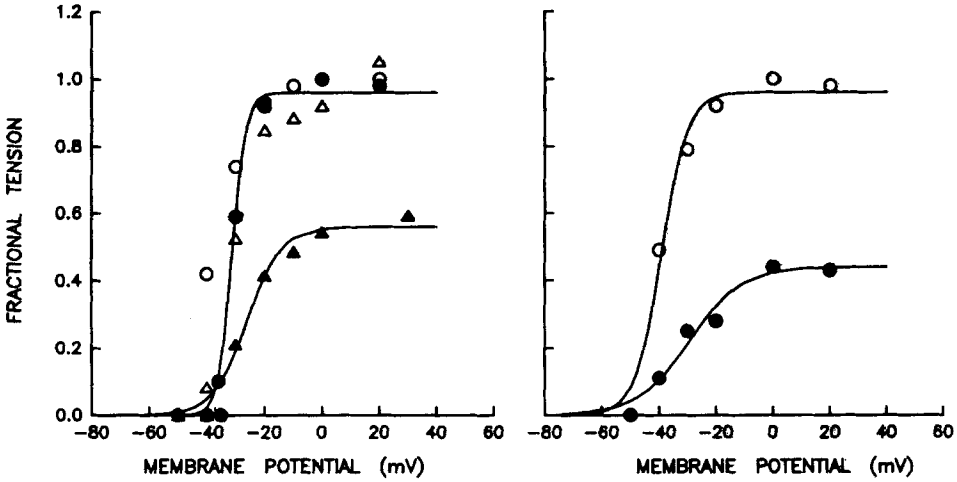

FIGURE 4. Effect of PCMB on the tension–potential relationship of different fibers. In the graphs the normalized tension is plotted against the fiber membrane potential. In the graph on the left the results obtained with two fibers are shown. One fiber, represented by the open symbols, was bathed in normal Ringer's and depolarized with a 50-ms (*triangles*) or 500-ms (*circles*) depolarizing pulse; in this case no substantial difference was observed. The other fiber, represented by the filled symbols, was exposed to 0.3 mM PCMB for 7 min. In this case the responses to the short pulses were clearly diminished, while those to long pulses were not affected. The curves were fitted by a Boltzmann-type function with the following parameters: upper curve, T_{max} = 0.96, midpoint value (V_m) = −31 mV, steepness factor = 2.4 mV; lower curve, T_{max} = 0.56, midpoint value = −26 mV, steepness factor = 5.9. The graph on the right shows the results obtained with another fiber before (*open circles*) and after a 10-min exposure to 0.4 mM PCMB (*filled circles*) and depolarized with 500-ms pulses. The parameters for the two curves were as follows: upper curve, T_{max} = 0.96, midpoint value = −39.2, steepness factor = 4.6 mV; lower curve, T_{max} = 0.44, midpoint value = −29.4, steepness factor = 9.1 mV.

another experiment in which the tension–potential relationship (with a 500-ms pulse duration) was obtained in the same fiber before and 10 min after adding 0.4 mM PCMB. Also in this case, besides a decrease in peak tension, there is a shift of the midpoint of the activation curve of ~10 mV toward more positive potentials. Unfortunately, it was not possible to obtain complete sets of these curves in the same fiber before and after adding the SH reagents; however, it seems clear that the responses to short depolarizing pulses are affected to a greater extent than those to long ones. These results also seem to indicate that after PCMB has greatly reduced

contractile output, the voltage dependency of contractile activation is affected by a change in the steepness factor rather than a change in the contractile threshold. It is interesting to notice that in the case of dantrolene as well (50 μM), used under similar experimental conditions, its effectiveness in blocking contraction has been reported to be greater with shorter depolarizing pulses, although with this agent the tension–potential relationship showed a change both in steepness and in the contractile threshold (Caputo and Bolaños, 1987).

Fig. 5 *A* shows that the blocking effects of mercurial reagents on contractile activation can be prevented by protecting the muscle fiber with DTT. The upper row of Fig. 5 *A* shows that after a 26-min exposure to 1 mM PHMPS in the presence of 2 mM DTT, the fiber contractile capacity was well sustained during contracture- and tetanus-like responses, and showed relatively little decay during repetitive stimulation with short pulses. The lower row of records was obtained 4 min after washing out DTT. It is clear that contractile output had already been affected, as shown by the first contracture-like response; repetitive stimulation caused a much greater tension decay than before washing out DTT, which is also the case for the tetanus-like response. Fig. 5 *B* shows another experiment in which a fiber was initially exposed to 0.4 mM PCMB and 2 mM DTT; after 33 min the upper row of records was obtained showing that contractile responses were maintained; the records in the middle were obtained 14 min after washing DTT, and a clear reduction in the responses is observed. Finally, 4 min later contractility was totally abolished. Besides protecting the fibers from the effects of PHMPS and PCMB, DTT can restore contractility after the action of these reagents has occurred. The experiment of Fig. 6 shows that after abolition of contractile capacity by exposure to 1 mM PCMB for 80 min, addition of 2 mM DTT can restore contractile ability in a relatively short time. This result, which seems to be crucial for establishing that the loss of contractile ability is not caused by deterioration of the fiber, was obtained in other similar experiments, summarized in Table I. In the table, the recovery of twitches (responses to short depolarizing pulses), contractures (responses to long depolarizing pulses), and tetani (responses to repetitive stimulation with short pulses), is shown. Thus, it seems safe to conclude that the loss of contractile ability induced by these agents is not due to generalized and nonspecific fiber deterioration.

DISCUSSION

In this work we demonstrate that the sulfhydryl reagents PCMB and PHMPS affect the normal function of skeletal muscle fibers by multiple effects action.

FIGURE 5. (*opposite*) (*A*) Protective action of 2 mM DTT on the contractile responses of a fiber exposed to 1 mM PHMPS. The upper record shows that after a 26-min exposure to both compounds, normal-looking contractile responses could still be obtained. The lower record was obtained 4 min after washing out DTT, and a clear reduction in the fiber contractile capacity can be appreciated. (*B*) Protective action of 2 mM DTT on the contractile responses of a fiber exposed to 0.4 mM PCMB. The upper row of records was obtained 33 min after exposing the fiber to the two agents, the records in the middle were obtained 14 min after withdrawal of DTT, and those in the bottom were obtained 4 min later, by which time contraction was totally abolished.

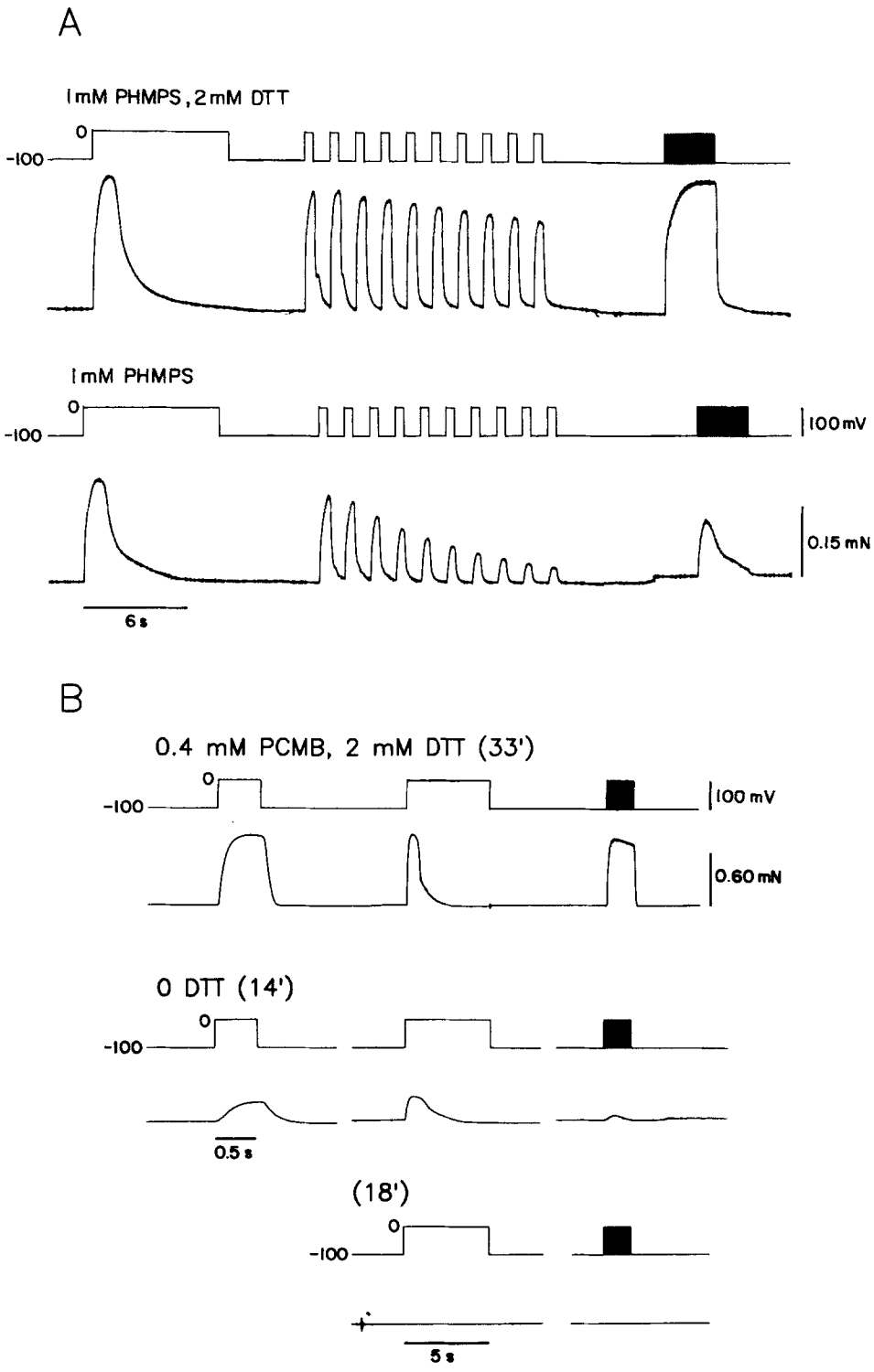
A
1mM PHMPS, 2mM DTT
0
-100
1mM PHMPS
100mV
0.15 mN
6 s
B
0.4 mM PCMB, 2 mM DTT (33')
100 mV
0.60 mN
0 DTT (14')
0.5 s
(18')
5 s

FIGURE 5.

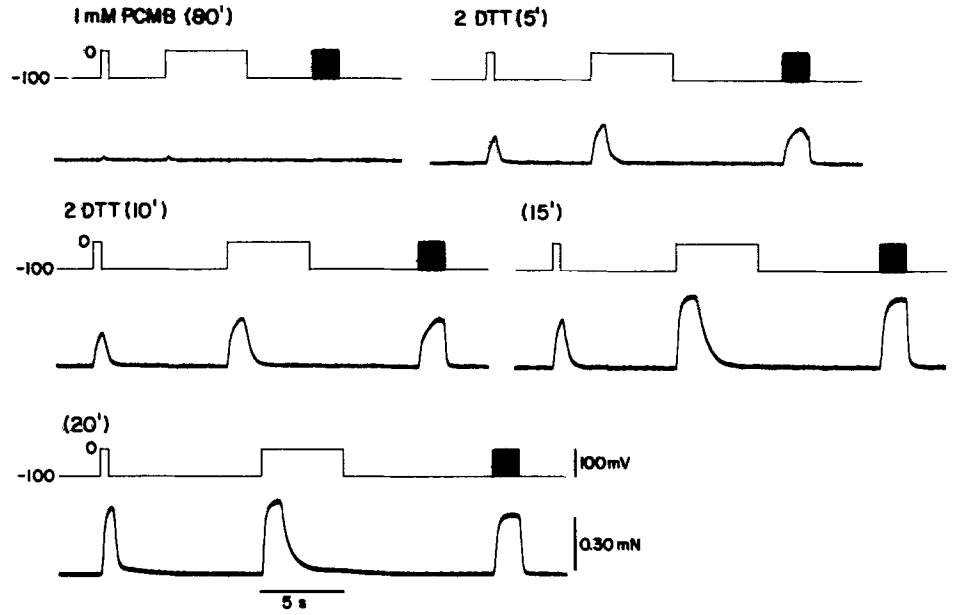

FIGURE 6. Restoration by DTT of contractility lost after prolonged exposure to PCMB. In this experiment the fiber had been previously exposed to 1 mM PCMB for a period of 80 min, and its contractile capacity had been practically abolished, as shown in the first record in the upper row. The second record in the same row shows that contractile responses were restored after exposing the fiber to 2 mM DTT. The other records in the figure show the progressive increase of the responses in the presence of DTT.

(*1*) At a relatively low concentration and in <20 min they may cause spontaneous release of calcium from the sarcoplasmic reticulum in polarized fibers under voltage-clamp conditions, or secondary tension responses after tetani or twitches induced by direct electrical stimulation. The spontaneous calcium release occurring

TABLE I

Contraction loss			Contraction recovery by exposure to 2 mM DTT			
Fiber No.	-SH reagent	Treatment length	Exposure length	Tension A	Tension B	Tension C
	mM	*min*	*min*		*mN*	
04191088	PCMB 1	80	4	0.13	0.18	0.17
			15	0.23	0.33	0.31
01290988	PCMB 0.4	20	4	0.28	0.29	0.26
05141088	PCMB 0.5	40	4	0.13	0.20	0.13
17141088	PHMPS 1	37	4	0.10	0.17	0.12

A, twitch; B, contracture; C, tetanus.

at this stage is not directly associated with membrane depolarization and could be considered to be caffeine-like (Palade, 1987). (*2*) After more prolonged exposure, the fiber ability to contract in response to membrane depolarization is diminished and then lost. This effect develops without apparent changes in the contractile threshold, although the steepness of the activation curve is diminished, does not occur in the presence of DTT, and may even be reversed by this compound. (*3*) Caffeine contractures of normal amplitude can still be induced after very prolonged exposure to these reagents, indicating that (*a*) the contractile machinery itself is not adversely affected by sulfhydryl reagents under the experimental conditions used here, and (*b*) the sarcoplasmic reticulum capacity to release calcium, albeit in a voltage-independent manner, is not lost.

The mercurial compounds of the type used in this work are among the most specific reagents for protein -SH groups since their affinity for these groups is much larger than that for any other reactive groups in proteins (Means and Feeney, 1971; Torchinskii, 1974). They react rapidly and in a very selective way with exposed -SH groups of proteins according to the scheme:

$$\text{P-SH} + \text{Hg-R} \rightarrow \text{P-SHg-R} + \text{H}^+$$

Once the reaction is completed, the possibility of disulfide and hydrogen bonding (Gregoret, Rader, Fletterick, and Cohen, 1991), as well as other chemical reactions of SH groups, is lost and this may cause changes in protein structures that can lead to changes in their biological activity. However, the ubiquitous distribution of SH groups in cellular structures complicates the interpretation of the effects caused by these reagents. For the case of muscle fibers, the most important effect described in this work, the loss of contractile activity caused by organic mercurials, could be due to at least the following different mechanisms: (*a*) membrane depolarization leading to loss of excitability and/or to contractile inactivation; (*b*) interference with the ECC coupling mechanism; (*c*) interference at the level of the contractile proteins; (*d*) depletion of the calcium store in the sarcoplasmic reticulum; and finally, (*e*) nonspecific general deterioration of the fibers.

Possibility *a* can be discarded in view of the results obtained under voltage-clamp conditions, while possibility *c* is disproved by the demonstration that after a very long exposure to PHMPS, normal-sized caffeine contractures can still be obtained. With regard to this point, Wilson et al. (1991) have reported that the sulfhydryl oxidizing agent 5,5′-dithiobis(2-nitrobenzoic acid) (DTNB) at 10 mM and pH 8.6 may reduce contractile force in skinned muscle fibers. The difference with our results is possibly due to the different experimental conditions (skinned vs. whole fiber, concentration, pH, and reactivity of the agent). The maintenance of caffeine contractures after prolonged exposure to the agents also argues against possibilities *d* and *e*. The latter is also disproved by the action of DTT that reverses the effects caused by these mercurial agents.

Possibility *b* seems the most probable; however, one could suppose that the blocking action of -SH reagents on contractile activation could occur either at the level of the calcium release channels of the SR, at the level of the voltage sensor for DCC, or at the level of the coupling mechanism or structure between voltage sensors and calcium release channels.

There is ample evidence in the literature indicating that sulfhydryl reagents,

including PCMB and heavy metal ions, cause release of calcium from isolated sarcoplasmic reticulum (Okamoto and Kuperman, 1966; Abramson et al., 1983; Oba and Hotta, 1985; Palade, 1987; Brunder et al., 1988; Abramson and Salama, 1989). Palade (1987) has proposed that this release from isolated SR vesicles occurs through activation of Ca^{2+}-induced Ca^{2+} release channels that are blocked by polyamines. More recently, Brunder, Gyorke, Dettbarn, and Palade (1992) have obtained pharmacological evidence indicating that the isolated SR "calcium-induced calcium release channel," which is activated by -SH reagents and caffeine and blocked by polyamines, corresponds to the ryanodine receptor that is considered to be the "calcium release channel for DCC." Thus, it appears that calcium release from isolated sarcoplasmic reticulum preparations is not blocked but activated by -SH reagents. In agreement with this view and confirming the observation of Okamoto and Kuperman (1966) and of Kirsten and Kuperman (1970), we have found that in intact fibers the sulfhydryl inhibitors PHMPS and PCMB may cause early spontaneous contractile episodes in fully polarized fibers, possibly due to a direct interaction of these compounds with calcium release channels (Palade, 1987). Our results also demonstrate that the episodes of calcium release are not directly associated with membrane depolarization, although their occurrence may be conditioned or at least favored by a previous depolarization; in some cases they may even occur as prolonged or secondary contractile responses, after depolarization-induced primary responses. The possibility that such episodes of spontaneous and secondary release may occur through a mechanism of calcium-induced calcium release has been brought to our attention during the reviewing process of this paper and agrees with some of the results of Palade (1987). The results presented in this paper clearly indicate that after prolonged exposure to -SH reagents, when the release in response to membrane depolarization is blocked, calcium release in response to caffeine is not affected.

Thus, unless we assume the existence of two types of release channels, one voltage dependent, which is blocked after prolonged exposure to the -SH reagents, and the other voltage independent, whose activation by caffeine is not affected by these compounds, which may even activate it, a direct action of -SH reagents on the calcium release channels of the SR could be discarded. Nevertheless, although the possibility of two types of channels is not usually considered (Fleischer and Inui, 1989; Brunder et al., 1992), some structural and pharmacological arguments supporting it have been made (Rios and Pizarro, 1991), and therefore it should not be dismissed completely.

Assuming the existence of only one type of release channel, the most plausible explanation for the effects of -SH reagents is based on a direct or indirect (via a coupling structure) action on the voltage sensor for DCC. The effect of organic mercurials on charge movement reported in the following paper (Gonzalez, Bolaños, and Caputo, 1993) is compatible with this possibility. In fact, the compounds used in this work appear to cause a reduction in the maximum charge of $\sim 30\%$. This reduction can be considered to be a lower estimate for the effect since if a higher concentration or longer exposure had been used the effect could have been greater; however, this could not be done to avoid membrane damage. Nevertheless, the loss of contractile ability appears to follow a time course consistent with the reduction of

the charge movement signal, giving some support to the idea of a causal relationship between the two effects. Therefore, one could suppose that these compounds may interact with -SH groups of cysteine residues present in the voltage sensor molecule interfering with its function. This idea is supported by the fact that DTT appears to protect both intramembrane charge movement and contractile responses from the effect of the organic mercurials.

Alternatively, the voltage sensor could be affected in an indirect way, through one of the different feedback pathways that have been described to exist between intramembranous charge movement and calcium release. For instance, reconsidering the already discussed possibility of a direct effect on the DCC release channel, one might consider that a reduction in the amount of released calcium could affect the charge signal, either affecting the Q_γ component (Pizarro, Csernoch, Uribe, Rodríguez, and Ríos, 1991) or changing its kinetic properties (Pape, Jong, and Chandler, 1992). Also pertinent to this possibility is the recent proposal of an allosteric model for transmission in ECC recently made by Rios, Karhanek, Gonzalez, and Ma (1992), which considers the mechanical interaction between the dihydropyridine and ryanodine receptors. Considering these proposals, sulfhydryl reagents could act at or near the ryanodine receptor, indirectly affecting the charge movement signals.

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