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Contraction of detergent-treated smooth muscle

(chemical skinning/taenia coli/contractile regulation)

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ABSTRACT **After exposure of segments of rabbit taenia coli to the nonionic detergent Triton X-100, tension could be induced by increasing the $[Ca^{2+}]$ in the micromolar range. In the presence of a saturating $[Ca^{2+}]$, this preparation developed nearly 100% of the control tetanus tension recorded from the intact muscle prior to the detergent treatment. In addition, tension could be induced by increasing the $[Mg^{2+}]$, in the virtual absence of Ca^{2+}. Mg^{2+} seems to inhibit the Ca^{2+}-induced tension in a predominantly competitive manner.**

Studies of single cells of skeletal and cardiac muscle from which the sarcolemma has been mechanically removed ("skinned fibers") have provided insights into the processes of activation of the contractile system (1–6). The removal of the sarcolemma greatly increases the accessibility of the contractile myofilaments to solutions containing ions and substrates. In this way, the effects of ions and substrates on the tension recorded from the skinned muscle fibers can be used to measure their interactions with the contractile proteins.

A similar preparation of smooth muscle cells has not been adequately developed. The small size of the smooth muscle cells and the large amount of connective tissue surrounding the cells have prohibited the application of the mechanical skinning techniques used for striated muscle fibers. Two other chemical methods which have been successfully utilized to remove the sarcolemma of striated muscle (7, 8) have failed to yield satisfactory results when applied to smooth muscle. One of these methods relies on exposure of the muscle to 50% glycerol (9), a procedure that can modify the sensitivity of the contractile proteins to Ca^{2+} (10–12). A second procedure involves short-term exposure to EDTA [(ethylenedinitrilo)tetraacetic acid] to greatly increase membrane permeability to ions (13). When exposed to a saturating free $[Ca^{2+}]$, the EDTA-treated muscle, like the glycerinated muscle, develops only 5–10% of the maximal tension produced by the intact muscle.

In the present report, I describe a new method of "chemical skinning" of smooth muscle consisting of a relatively brief exposure of the muscle bundle to the nonionic detergent Triton X-100. Preliminary accounts of this method have been presented (14–18). Under suitable ionic conditions, the Triton-treated muscle can develop, in response to Ca^{2+}, force of up to 100% of the maximal tension obtained in the intact muscle prior to skinning. This appears to be a better result than obtained in striated muscle, because skinned striated muscle fibers can develop only about 50% of the tension developed by the intact fibers (1–6).

METHODS

Female rabbits (albino, New Zealand), weighing 1.5–2.0 kg, were sacrificed by cervical fracture. The taeniae coli were isolated from the cecum with care taken to avoid stretching the muscle segment. Strips of taeniae approximately 5 × 2 × 0.2 mm were mounted horizontally between two Lucite forceps in a muscle chamber containing oxygenated Krebs solution. One forcep was attached to a force-transducer (modified strain gauge, from Kistler-Morse, DSK-3) and the other, to a micrometer device for measuring and adjusting muscle length. The muscles were allowed to equilibrate for 2 hr at room temperature (23°–25°) under a 2-g load.

After the equilibration period, the muscles were stretched to their optimal length for tension development and tetanically stimulated by means of a 12-V (rms) 60-Hz electric current passed between two transverse platinum/platinum chloride electrodes (19). The Krebs solution was continually gassed with 95% O_2/5% CO_2 and had the following composition (mM): NaCl, 118; KCl, 4.7; $MgSO_4$, 1.18; KH_2PO_4, 1.2; $CaCl_2$, 2.5; $NaHCO_3$, 25.0; glucose, 11. The pH was 7.4.

The following protocol was adopted to obtain the skinned smooth muscle preparation. After the equilibration and control tetanus responses were obtained, the muscle segments were exposed to the various solutions, described below, while remaining attached in their respective chambers at room temperature. Tension was recorded continuously. Additionally, the solutions within the chambers were well stirred to avoid diffusion problems due to large unstirred layers adjacent to the muscle bundle. This protocol allowed direct comparisons of the tension developed by the skinned muscle segments to the tetanic tension elicited from the intact muscle prior to skinning.

The Krebs solution was replaced by a "presoak" solution [5 mM ethylene glycol-bis(β-aminoethyl ether)-*N,N'*-tetraacetic acid (EGTA)/20 mM morpholinopropanesulfonic acid (Mops)/50 mM KCl/150 mM sucrose, at $10^{-7.5}$ M Ca^{2+}]. After 30 min, the presoak solution was replaced by the "skinning" solution which was the presoak solution with the addition of Triton X-100 (final concentration, 1 ml/100 ml) and dithioerythritol or dithiothreitol (final concentration, 0.5 mM). The muscles were exposed to this skinning solution for approximately 16 hr (overnight). In some experiments, one of the following modifications was made: (*i*) the exposure time to the detergent was shortened to 2 hr; (*ii*) EDTA was substituted for EGTA; (*iii*) Brij-58 was substituted for Triton X-100. The muscle was then exposed to a detergent-free and sucrose-free solution for 2 hr to facilitate removal of the detergent from the muscle bundle. Contractions were induced by means of solutions having the following composition: 5.0 mM EGTA + CaEGTA (ratio of CaEGTA to EGTA adjusted to attain the desired $[Ca^{2+}]$ in the range 10^{-9}–$10^{-4.6}$ while keeping the total EGTA concentration constant), 20 mM Mops, variable Mg acetate to maintain $[Mg^{2+}]$ at 1.0 or 6.9 mM, 1.0 mM $MgATP^{2-}$,

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Abbreviations: EDTA, (ethylenedinitrilo)tetraacetic acid; EGTA, ethylene glycol-bis(β-aminoethyl ether)-*N,N'*-tetraacetic acid; Mops, morpholinopropanesulfonic acid.

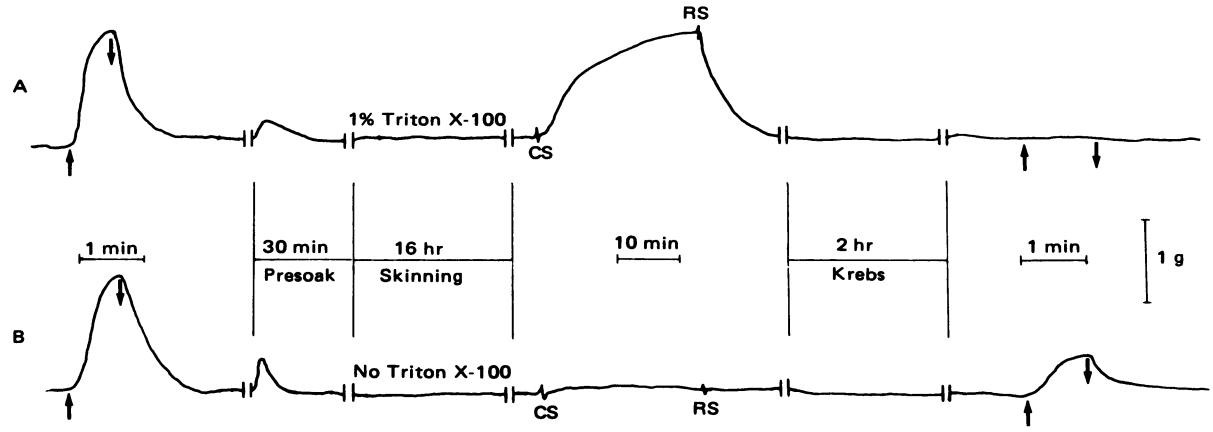

FIG. 1. Experiment comparing the contractile responses of muscle exposed to 1% Triton X-100 for 16 hr (*A*) to muscle treated in an otherwise identical manner except for the omission of the detergent (*B*). EGTA is the ligand in this experiment. Initially, the muscles were bathed in Krebs' solution and stimulated, tetanically, with alternating current (upward-pointing arrows). The stimulation was terminated at the downward-pointing arrow. Not shown in the record is a 2-hr exposure to detergent- and sucrose-free solutions after the 16-hr exposure to the skinning solution. After this, the muscles were exposed to a contracting solution (CS) which consisted of 5 mM CaEGTA ($[Ca^{2+}]$, $10^{-4.6}$ M)/20 mM Mops/6.9 mM Mg^{2+}/1.0 mM $MgATP^{2-}$/0.5 mM dithioerythritol/5 mM phospho*enol*pyruvate/50 units of pyruvate kinase per ml/KCl to make I = 0.13. The pH was 7.0. After a steady-state tension was reached in the muscle in *A*, the solution was replaced by a relaxing solution (RS) which was identical to the previous solution except that 5 mM EGTA replaced the CaEGTA so that the $[Ca^{2+}]$ was about 10^{-9} M. After reexposing the muscles to oxygenated Krebs solution for 2 hr, tetanic stimulation was applied as indicated by the arrows.

5.0 mM phospho*enol*pyruvate, 50 units of pyruvate kinase per ml, 0.5 mM dithioerythritol or dithiothreitol, and variable amounts of KCl to maintain the ionic strength at 0.13. All solutions were made from isoionic-strength stock solutions. With exception of the KCl and Mg acetate stock solutions, all stock solutions were titrated to pH 7.0 with KOH. Additionally, the experimental solutions were adjusted to pH 7.0 with KOH when necessary. The MgATP and CaEGTA stock solutions were made up by mixing equimolar amounts of MgO with Na_2H_2ATP and of $CaCO_3$ with H_4EGTA and titrating to pH 7.0 with KOH.

The composition of the experimental solutions was determined by means of a computer program (modified version of one supplied by M. Kawai) to attain the desired $[Ca^{2+}]$, $[MgATP^{2-}]$, and $[Mg^{2+}]$. The binding constants for EGTA were obtained from Schwartzenbach *et al.* (20) and for ATP, from Sillen and Martell (21).

RESULTS

Fig. 1 compares the tension responses of a muscle that was exposed for 16 hr to Triton X-100 to a muscle that was treated in an otherwise identical manner except for the omission of the detergent. Control tetanic tensions were obtained in both muscles for comparison purposes. The transient contracture observed after exposure of the muscle to the presoak is due to K^+ depolarization (K^+ is the major cation in the presoak and subsequent solutions). The detergent-treated muscle developed force when the $[Ca^{2+}]$ was increased to $10^{-4.6}$ M, the $[Mg^{2+}]$ to 1.0 mM, and the $[MgATP^{2-}]$ to 1.0 mM which was comparable to the control tetanic tension. The tension response was reversible because decreasing the $[Ca^{2+}]$ to 10^{-9} M caused complete relaxation. In contrast, the muscle that was not exposed to the detergent failed to develop a Ca-induced tension response. However, after reexposure of both muscles to oxygenated Krebs' solution, tetanic stimulation produced a tension response in the muscle that was not exposed to the detergent, of nearly 50% of the control tetanic response. The detergent-treated muscle failed to respond to tetanic stimulation after exposure to Krebs' solution, showing that the effects of the detergent were irreversible. Thus, Triton increased the permeability of the muscle bundle to ions and substrates, an effect that could be mediated by disruption of the plasma membranes.

In other experiments, decreasing the duration of exposure to Triton X-100 to 2 hr or substituting EDTA for EGTA ($[Ca^{2+}]$, $10^{-7.5}$ M) during the skinning produced preparations that gave Ca-induced tension responses of only 50% of the control tetanic tension. Substitution of Brij-58 for Triton X-100 led to a preparation with a poor Ca sensitivity.

The Ca sensitivity of the Triton-treated muscle is shown in Fig. 2. After the control tetanus and the skinning procedure, the muscle was exposed to a series of solutions containing Mg^{2+}, $MgATP^{2-}$, and incrementally increasing $[Ca^{2+}]$. In the virtual absence of Ca^{2+} ($[Ca^{2+}]$, 10^{-9} M) a tension response was induced by increasing the $[Mg^{2+}]$ from 1.0 to 6.9 mM. Although it is not shown in the record, the Mg-induced tension response saturated in the range of 6.0 to 8.9 mM Mg^{2+} and averaged 30 ± 2% of the control tetanic tension (mean ± SE, n = 16). Stepwise increases in the $[Ca^{2+}]$ caused graded increases in the tension developed by the skinned muscle. The first detectable Ca-induced tension response in the presence of 6.9 mM Mg^{2+} was observed at $[Ca^{2+}] = 10^{-6.2}$ M and the maximal response (81% of control tetanus in this muscle), at $[Ca] = 10^{-5.4}$ M. The tension responses were reversible as shown by the relaxation when the $[Ca^{2+}]$ and $[Mg^{2+}]$ were decreased to 10^{-9} M and 1.0 mM, respectively.

The experiment is continued in the lower tracing of Fig. 2. The tension responses were obtained at different Ca concentrations while the $[Mg^{2+}]$ was maintained at 1.0 mM. The large tension response seen when the $[Ca^{2+}]$ was increased from 10^{-9} M to $10^{-6.4}$ M indicated that the threshold for the Ca-induced tension response was decreased by the decrease in $[Mg^{2+}]$. The maximal Ca-induced tension response also occurred at a lower $[Ca^{2+}]$, which was $10^{-5.6}$ for this muscle. Increasing the $[Mg^{2+}]$ to 6.9 mM while maintaining a saturating $[Ca^{2+}]$ caused no further increase in tension. The maximal tension developed in response to saturating $[Ca^{2+}]$ averaged for six muscles was 94 ± 4% (mean ± SE) of the control tetanic tension.

Fig. 3 summarizes the results of six experiments of the type shown in Fig. 2. The tension data are normalized as the fractional response to Ca^{2+}, so that the Mg-induced tension response is not shown. A decrease in the $[Mg^{2+}]$ from 6.9 to 1.0 mM

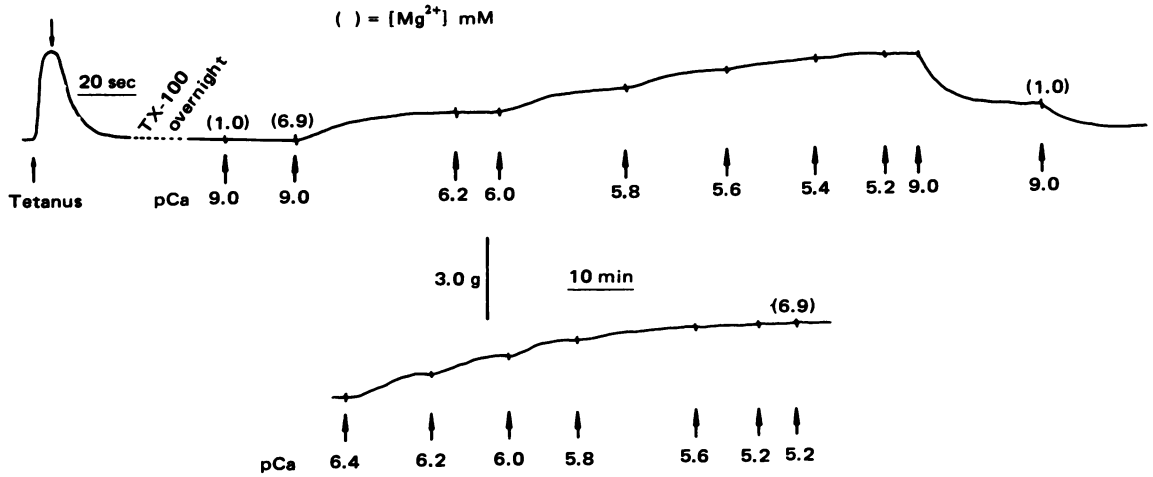

FIG. 2. Tension responses of skinned smooth muscle. The baseline is the passive tension of the muscle. The lower tracing is a continuation of the experiment represented in the upper record. The control tetanic tension was 3.7 g. EGTA was used throughout as the ligand. The $[Ca^{2+}]$ of each solution is given by pCa ($-\log [Ca^{2+}]$).

caused a nearly parallel shift to the left of the tension–pCa relationship.

These data can be described by $f(Ca) = (1 + (K/[Ca])^n)^{-1}$, in which f(Ca) is the Ca-induced tension, as shown by the curves plotted through the data points; the equation was generated by a nonlinear least squares curve-fitting procedure (22, 23). The parameters n and K calculated from the curve fitting procedure are shown in the figure legend. The shift in the tension–pCa relationship is quantitated by the small but significant ($P < 0.001$) change in pK ($-\log_{10} K$).

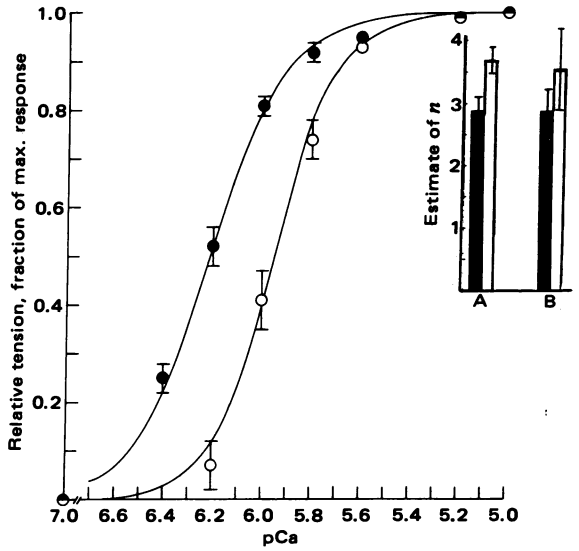

FIG. 3. Relationship between tension and $[Ca^{2+}]$. The Ca-induced tension responses are plotted as fractions of the maximal tension at 1.0 mM Mg^{2+} (●) and 6.9 mM Mg^{2+} (○). The tension induced by Mg^{2+} is not shown in this graph. The data are obtained from six experiments of the type shown in Fig. 2 and are grouped according to the pCa at each $[Mg^{2+}]$; they are shown as means ± SEM. The two sets of pooled data were fitted by the equation $f(Ca) = (1 + (K/[Ca])^{n-1}$ (drawn as the smooth curve through the data), in which f(Ca) is the Ca-induced tension. The estimates of the parameters n and K calculated from the data shown are: for $[Mg^{2+}] = 1.0$, $n = 2.87 \pm 0.14$ and p$K = 6.21 \pm 0.01$; for $[Mg^{2+}] = 6.9$, $n = 3.53 \pm 0.26$ and p$K = 5.95 \pm 0.01$. (*Inset*) The 95% confidence limits of the estimates of n are represented by the vertical bars in panel B. Panel A shows the means ± SEM of the estimates of n when calculated from the data from each muscle at each $[Mg^{2+}]$. The difference between the estimates in panel A was analyzed by the paired t test and analysis of variance and was statistically significant ($P < 0.02$).

The slight increase in steepness of the tension–pCa relationship at high $[Mg^{2+}]$ is reflected by an increase in the parameter n. The *inset* shows that the two estimates of n at each $[Mg^{2+}]$, based on the pooled data plotted in the figure, do not overlap the calculated 95% confidence limits. If the parameter n is calculated from the data from each muscle, then comparisons of the effect of $[Mg^{2+}]$ show slightly greater significance when compared by the analysis of variance or the paired t test.

DISCUSSION

The present study describes a new skinned smooth muscle preparation capable of developing a force in response to Ca^{2+}, in the presence of Mg^{2+} and ATP, that is nearly equal to the tetanic tension measured in the intact muscle. Thus, the limitations of poor tension development previously observed in skinned (13) and glycerinated (9) smooth muscle have been overcome. The tension-generating ability of the detergent-treated smooth muscle suggests that the intercellular connections and the attachment sites of the contractile proteins, as well as the contractile proteins themselves, are not damaged by the detergent. It has been suggested that the dense bodies, both attached to the plasma membrane and free-floating in the cytoplasm, are the attachment sites of the thin filaments (24–27). This speculation remains to be tested by electron microscopy although preliminary data suggest that the dense bodies that lie near the cell surface are unaffected by the detergent treatment.

The Ca^{2+} sensitivity of the detergent-treated smooth muscle preparation at low $[Mg^{2+}]$ is similar to that of skinned fibers of skeletal (4, 28) and cardiac (6) muscle as well as smooth muscle actomyosin (29–31). Thus, the similarities of the Ca^{2+} sensitivity of the Triton X-100-treated muscle to other muscle preparations together with the tension-generating ability suggest that the 16-hr detergent treatment in the presence of EGTA had no deleterious effects on the contractile and regulatory proteins.

The decreases in force due to modifications of the protocol are probably due to different mechanisms. Only 50% of the tension developed by the intact muscle could be developed by muscles that were skinned for only 2 hr or when EDTA was substituted for EGTA. The 2-hr skinning is probably not long enough to disrupt the membranes sufficiently to allow free access of ions and substrates to the contractile proteins, whereas the effect of EDTA may be related to Mg^{2+} chelation. Pre-

liminary experiments (unpublished data) have indicated that overnight exposure of the smooth muscle to Triton X-100 and EGTA does not remove all of the bound Mg and that 5–10 mmol/kg wet weight remain in the tissue. Overnight Triton X-100 treatment with EDTA substituted for EGTA decreased the Mg content to zero (as measured by atomic absorption spectrophotometry). In this regard, Mg may promote the aggregation of myosin (32) as well as the polymerization of actin (33, 34). Thus, if Mg is removed by EDTA, the structures of the actin and myosin may be adversely affected insofar as force development is concerned.

Substitution of Brij-58 for Triton X-100 also decreased the tension responses to Ca^{2+}. This result was surprising because Orentlicher *et al.* (35) reported that Brij-58 caused no functional or antomical alterations of the contractile apparatus of skinned crayfish skeletal muscles. Furthermore, Julian (36) noted that Lubrol-WX, a nonionic detergent similar to Brij-58 (37), was better than Triton X-100 or Tween 80. Additionally, it has been found that both Brij-58 and Triton X-100, at a concentration of 1%, solubilize about the same maximal amounts of protein and lipid from mycoplasma membranes but that the kinds of proteins extracted are different (38). Thus, the difference in the effects of Brij and Triton on smooth muscle may be related to the kind of proteins extracted by these detergents.

It has been suggested (9) that glycerinated smooth muscle requires 10 mM Mg^{2+} to permit a Ca-induced tension response. However, the present study demonstrates that a lower [Mg^{2+}] is compatible with full Ca^{2+} sensitivity. The presence of a Ca-independent tension response has been taken to indicate loss of regulation of the contractile proteins (10). This possibility is excluded by the presence of a Ca-induced tension response virtually identical to the tetanic tension developed by the intact muscle. The Mg-induced tension response may be related to competition by Mg^{2+} for the Ca^{2+}-activating sites, as suggested by the nearly parallel shift in the tension–pCa relationship. This sort of competition has been observed in skinned fibers of skeletal (28) and cardiac (6) muscle. However, Mg^{2+} may also increase the steepness of the tension–pCa relationship, an effect that cannot be ascribed to competition. More data are needed to clarify this finding and to determine the extent to which it contributes to the Mg^{2+} effects.

In these experiments, the muscles were skinned under rigor conditions (no added MgATP) and in the presence of subthreshold [Ca^{2+}]. During such treatment, no force development was observed, in support of the findings of Butler *et al.* (39). Thus, the development of rigor in smooth muscle need not be associated with the development of force as was proposed by Bose (40).

The detergent-treated smooth muscle preparation presented in this report is superior to other smooth muscle preparations. It offers a new opportunity to study the regulation of tension-generating processes in a relatively unequivocal manner. Although the Ca^{2+}-sensitivity of this preparation is similar to that of striated muscles, the presence of a Mg-induced tension response and the absence of a rigor contracture suggest that the mechanisms governing contractile regulation in these two muscle types are different. Additionally, M. Endo has recently developed a similar preparation using saponin as the skinning agent (S. Ebashi, personal communication).

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