

# Tonic Contraction and the Control of Relaxation in a Chemically Skinned Molluscan Smooth Muscle

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**ABSTRACT** The same functional states that characterize the living anterior byssus retractor muscle (ABRM) from *Mytilus edulis* can be initiated in the saponin-treated (chemically skinned) muscle preparation under controlled biochemical conditions. A tonic contraction was induced if the concentration of free  $\text{Ca}^{2+}$  was above  $\sim 10^{-7}$  M in the presence of  $\text{Mg}^{2+}$  and ATP. Maximum tension development was achieved at a  $\text{Ca}^{2+}$  concentration of  $\sim 10^{-4}$  M. Within these  $\text{Ca}^{2+}$  concentrations tension was always associated with the presence of "active state," as indicated by a high recovery of tension after a quick release in muscle length. Tonic tension, and the associated active state, was maintained for hours during these conditions irrespective of variations in both ionic strength and pH. Reduction of the  $\text{Ca}^{2+}$  concentration to below threshold for tension initiation during a tonic contraction immediately switched off the active state and relaxation of the muscle preparation resulted. However, the rate of relaxation was extremely low, leaving a substantial fraction of tension in the absence of active state. Both 5-hydroxytryptamine (5-HT) and cAMP accelerated this slow relaxation in the absence of  $\text{Ca}^{2+}$ . Thus, this state was considered equivalent to the "catch state" in the living ABRM. In the presence of  $\text{Ca}^{2+}$  concentrations above  $10^{-7}$  M, cAMP did not affect either the maximum tension developed or the  $\text{Ca}^{2+}$  sensitivity of the chemically skinned muscle preparation.

## INTRODUCTION

A chemically skinned smooth muscle preparation from the mollusk *Mytilus edulis*, the anterior byssus retractor muscle (ABRM), has recently been described (Cornelius, 1980). This preparation is very suitable for measuring the effects of ionic composition in the interfilamentous space on contractility. It is the purpose of this study to investigate whether similar functional states that characterize the living ABRM can be induced in this preparation by variation in the concentration of free  $\text{Ca}^{2+}$  and  $\text{Mg}^{2+}$ , ionic strength, or pH.

In the living ABRM, the tonic contraction induced by stimulation with acetylcholine (ACh) is characterized as follows: first, in the presence of ACh, tension and active state can be maintained for a very long period of time. Second, after removing ACh, the active state disappears quickly but the

tension decreases extremely slowly. This state, characterized by the presence of a high but slowly decreasing tension in the absence of the active state, has been termed the "fused state" (Jewell, 1959) or the "catch state." The slow relaxation induced after removing ACh in a tonic contraction can be selectively accelerated if 5-hydroxytryptamine (5-HT) is applied (Twarog, 1954). This effect is probably mediated via membrane receptors for 5-HT, resulting in intracellular release of cAMP (Cole and Twarog, 1972). 5-HT and cAMP have been demonstrated to relax a catch-like state in EDTA- or Triton X-100-treated ABRM under specified conditions of ionic strength and pH (Marchand-Dumont and Baguet, 1975). In the present study I investigated whether 5-HT and cAMP had similar effects on the rate of relaxation in the chemically skinned ABRM preparation as in the living muscle and which factors determine their mode of action.

Thus, comparing this chemically skinned ABRM preparation with the living ABRM, two questions will be addressed: (a) is it possible with the skinned ABRM to reproduce the active, the relaxed, and the so-called catch states? and (b) what specifically determines the initiation and termination of these various states?

#### METHODS

Specimens of *Mytilus edulis* with shell lengths smaller than ~20 mm were used in this study. The ABRM from these animals were isolated, trimmed, and mounted on the myograph as previously described (Cornelius, 1980). The ABRM was isolated with one end still attached to a piece of shell and split longitudinally until it had a maximum thickness of ~100  $\mu\text{m}$ . The shell was clamped into the muscle chamber base and the other end of the muscle was tied to a force transducer (DSC-6; Kistler-Morse Corp., Bellevue, WA). The length of the ABRM could be varied by moving the force transducer via a micrometer arrangement. The muscle chamber was perfused with sea water containing 25  $\mu\text{M}$  5-HT to relax the preparation completely, and the length of the ABRM was adjusted to the resting length  $L_0$  described by Cornelius and Lowy (1978). All experiments were performed at 22°C.

#### Solutions

Essentially, four standard solutions were used: wash solution (W), relaxing solution (R), contracting solution (C), and chemical skinning solution (S). All experimental solutions contained Mg-ATP (3.5 mM),  $\text{Mg}^{2+}$  (4.0 mM), piperazine-*N*, *N'*-bis-(2-ethanesulfonic acid) (PIPES,  $\text{pK} = 6.8$  at 20°C; 20 mM), ethylene glycol-*bis* ( $\beta$ -aminoethyl ether) *N*, *N'*-tetraacetic acid (EGTA; 2 mM and in some cases 20 mM). Potassium methane sulfonate was used to adjust the ionic strength (*I*) to 0.14 M. In some experiments ionic strengths of 0.07 and 0.28 M were used. The pH was 6.8 in all solutions unless otherwise stated. The wash solution and relaxing solution contained no calcium. The skinning solution (S) was prepared by adding saponin (0.05% wt/vol) to W or R solution. The total concentrations of calcium, magnesium, and ATP needed to produce the desired concentrations of free  $\text{Ca}^{2+}$  in the contracting solution was calculated on a minicomputer (PRIME 300; Prime Computer Inc., Framingham, MA) by using the following logarithmic apparent stability constants at pH = 6.8 (Cornelius, 1980):

$$\text{p}K'_{\text{Ca-EGTA}} = 6.29, \text{p}K'_{\text{Mg-EGTA}} = 1.38, \text{p}K'_{\text{Ca-ATP}} = 3.90, \text{and} \\ \text{p}K'_{\text{Mg-ATP}} = 4.23.$$

In experiments where the phosphocreatine-creatine phosphokinase system was used to regenerate the ATP, they were added to a concentration of 15 mM and 50 IU/ml, respectively. Phosphocreatine, creatine phosphokinase, cAMP, and 5-HT were obtained from Sigma Chemical Co., St. Louis, MO.

### *Chemical Skinning*

The general method for disrupting the ABRM muscle cell membranes by using the nonionic detergent saponin has already been reported (Cornelius, 1980). The muscle preparation was first contracted by applying ACh (55  $\mu$ M) in seawater and relaxed with 5-HT containing seawater to indicate maximum tension reference and zero tension reference, respectively. Then the muscle preparation was exposed to skinning

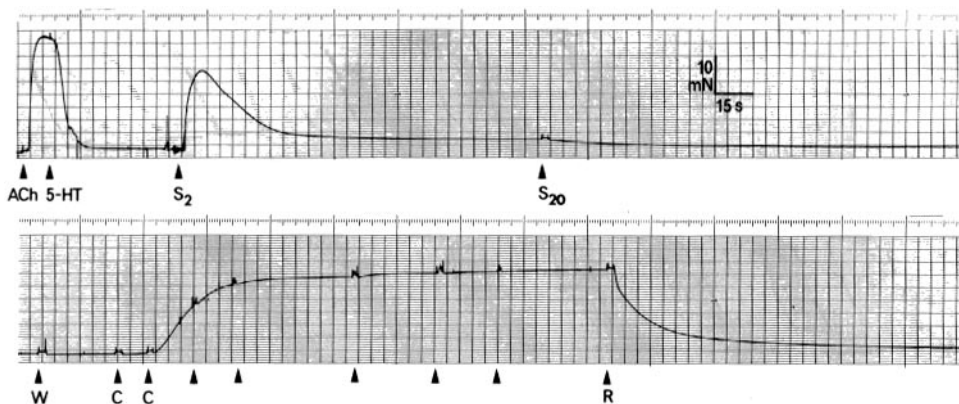


FIGURE 1. Chemical skinning procedure applied to ABRM fiber preparation under isometric conditions at 22°C as described in the text. A tonic contraction is induced in the living ABRM by applying ACh ( $5.5 \times 10^{-5}$  M) and complete relaxation is ensured by washing in the presence of 5-HT ( $10^{-5}$  M). Saponin (0.05% wt/vol) is added in a high potassium solution with first 2 mM EGTA ( $S_2$ ) and then 20 mM ( $S_{20}$ ). After washing (W), contraction-relaxation cycles can be initiated by the addition of a high  $Ca^{2+}$  solution (C,  $pCa \cong 4.5$ ), followed by relaxing solution (R,  $pCa \cong 8.6$ ). The relaxing solution contained  $10^{-5}$  M cAMP in the experiment shown to obtain rapid relaxation.

solution for 10–15 min, followed by a washing (W solution). Finally, a contraction-relaxation cycle was initiated by applying contracting solution with the optimum concentration of free  $Ca^{2+}$  ( $\sim 10^{-4}$  M) and subsequently adding the relaxing solution with high free EGTA concentration. The skinning procedure is illustrated in Fig. 1.

### RESULTS

Using the nonionic detergent saponin, it is possible to obtain a chemically skinned ABRM preparation that maintains almost the same maximum force-producing capacity as the living ABRM. Fig. 1 shows how a living ABRM preparation  $\sim 100 \mu$ m thick develops a tonic contraction in the presence of ACh and relaxes when ACh is removed and seawater containing 5-HT is added. Disruption of the muscle membrane and removal of  $Ca^{2+}$  is achieved by exposing the muscle to saponin during a potassium contracture (S solution).

After washing the preparation, contraction-relaxation cycles can be initiated by simply varying the concentration of free  $\text{Ca}^{2+}$  in the presence of  $\text{Mg}^{2+}$  and ATP. To maintain a prolonged contraction, it is necessary either to change the contracting solution frequently, as shown in Fig. 1, or alternatively to regenerate the ATP broken down during contraction using the phosphocreatine-creatine phosphokinase system.

In the living ABRM, ACh induces a tonic contraction where tension and

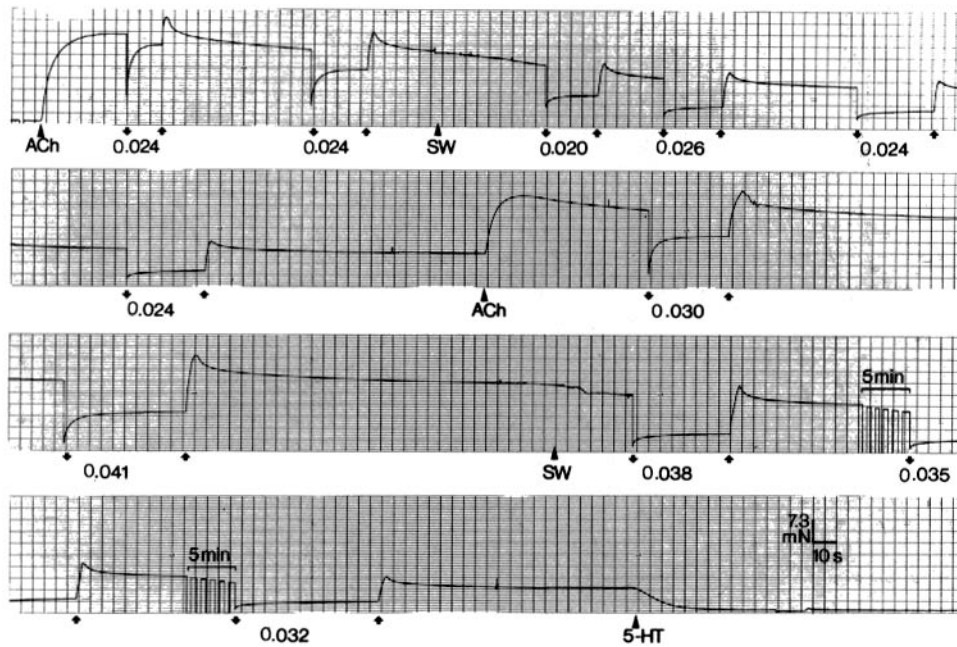


FIGURE 2. Tension record of a living ABRM demonstrating the catch state. After ACh is added a tonic contraction is developed and quick release of  $\sim 3\%$  of  $L_o$  (the relative amount of release is indicated by the number within the arrows) demonstrates the presence of active state. Readjusting of the muscle length and washout of ACh does not change the rate of tension decay. However, in the absence of ACh tension recovery is minimal when the muscle is released. Thus catch has developed. Adding ACh again re-establishes the tension as well as the active state. The sequence of events can be repeated, and the figure further demonstrates the ability of 5-HT to switch off catch by accelerating the rate of relaxation.

the active state persist as long as ACh is present. Removing ACh switches off the active state, whereas tension decreases extremely slowly (Fig. 2). This state, where a high but slowly decaying tension is maintained in the absence of the active state, is termed the fused state (Jewell, 1959) or catch state. Application of 5-HT greatly accelerates the rate of tension decay after an ACh response and is thus said to switch off the catch state.

In the chemically skinned ABRM preparation, contraction is induced when the concentration of free  $\text{Ca}^{2+}$  is increased from  $\sim 0.1 \mu\text{M}$  ( $\text{pCa} \sim 7$ ) to  $\sim 30$

$\mu\text{M}$  ( $\text{pCa} \sim 4.5$ ) in the presence of  $\text{Mg}^{2+}$  and ATP. Fig. 3 shows that as long as  $\text{Ca}^{2+}$  is kept high, maximum tension is maintained and tension recovery after a quick length release is considerable, which indicates the presence of the active state. Decreasing the  $\text{Ca}^{2+}$  concentration to  $\sim 1 \text{ nM}$  after a contraction relaxes the muscle preparation, although extremely slowly. During this phase, tension recovery is poor after a quick release, although a high tension is maintained when the muscle length is readjusted. The situation is fully reversible: applying  $\text{Ca}^{2+}$  again re-establishes both the original tension level

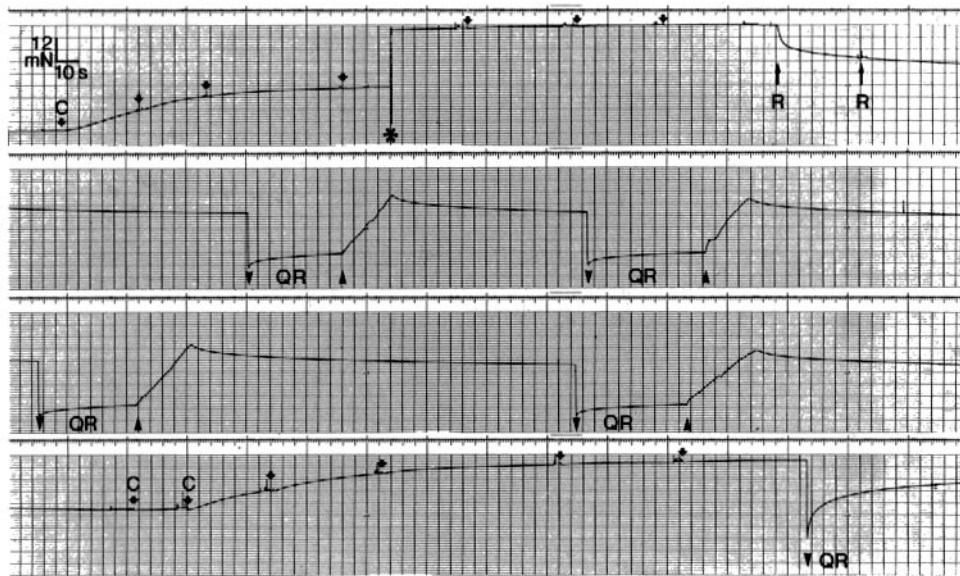


FIGURE 3. Tension record of chemically skinned ABRM showing the same characteristics as living ABRM in the catch state. The muscle is contracted by application of contracting solution (C). When  $\text{Ca}^{2+}$  is subsequently removed by application of relaxing solution (R), tension declines only very slowly. However, the active state disappears, as indicated by the poor tension recovery after a quick release (QR) of  $\sim 3\%$  of  $L_o$ . If the  $\text{Ca}^{2+}$  concentration is again raised, the tension level is re-established and the active state reappears. The asterisk indicates change in recorder sensitivity.

as well as the active state. It turns out, therefore, that varying the  $[\text{Ca}^{2+}]$  alone can induce the state in which a high tension is maintained in the absence of the active state, i.e., the catch state. In a series of experiments, the effect of 5-HT and cAMP on the rate of relaxation was studied under different conditions of ionic strength and pH. Fig. 4 demonstrates the effect of applying 5-HT ( $10^{-5} \text{ M}$ ) on the rate of relaxation at three different ionic strengths. The muscle is skinned at the appropriate ionic strength and carried through a few contraction-relaxation cycles at that particular ionic strength. Relaxing solution with 5-HT is then applied and the rates of relaxation are compared. As seen from the figures, 5-HT is able to accelerate relaxation in all the conditions

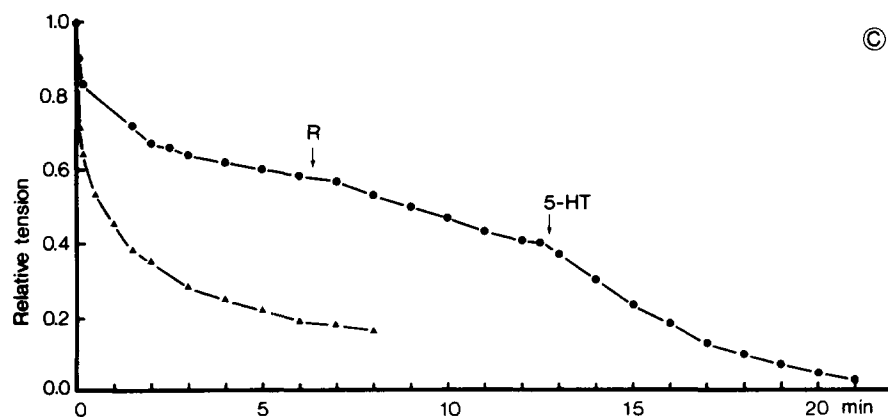
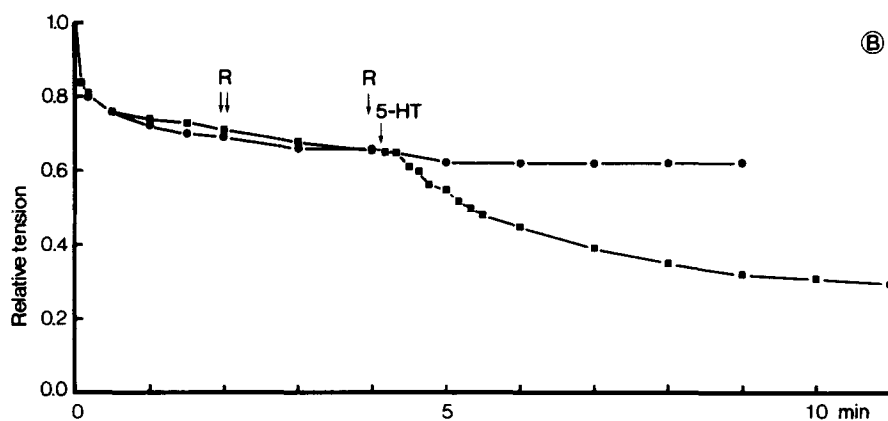
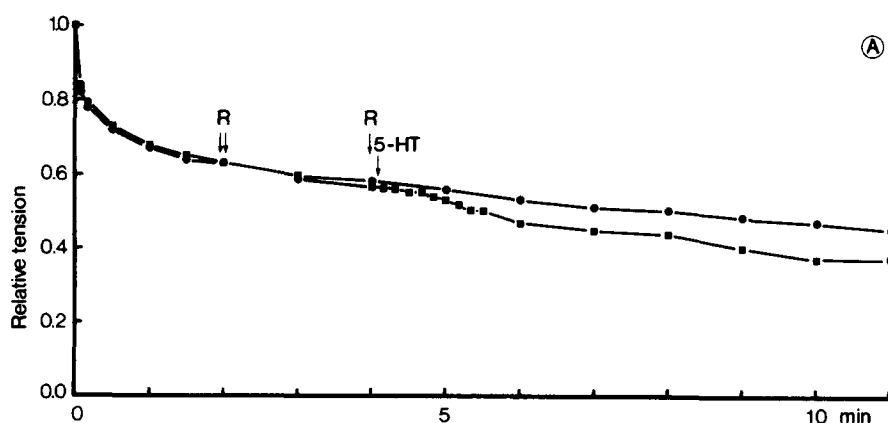


FIGURE 4. Effect of 5-HT on the rate of relaxation in a saponin-treated ABRM at three different ionic strengths: 0.07 M (A), 0.14 M (B), and 0.28 M (C). At zero time, relaxing solution (R) was applied to a maximum contracted ABRM (●). After a while this solution was replaced with relaxing solution containing  $10^{-5}$  M 5-HT (■). In panel C, this solution containing 5-HT was applied at zero time (▲).

of ionic strength studied. However, the effect of 5-HT is very small at ionic strength  $I = 0.07$  M. It was also noted that a certain delay was encountered from the moment of application of 5-HT until the accelerating effect became obvious. This delay was usually in the range of a few seconds. At all three ionic strengths studied, the time course of relaxation did not follow a single exponential decay irrespective of the presence or absence of 5-HT.

Fig. 5 shows that cAMP ( $10^{-5}$  M) has a more pronounced accelerating effect on relaxation than 5-HT, and cAMP is found to be equally effective at all three ionic strengths studied.

When  $\text{Ca}^{2+}$  is removed from a contracted ABRM, the time course of relaxation is not monoexponential. If the tension decay after removal of  $\text{Ca}^{2+}$  is plotted in a semilogarithmic diagram, the curve can be resolved into three straight lines with fairly good accuracy. From the slopes of the straight lines, the time constants of relaxation can be calculated. Although the results can vary considerably, the time constants given by these three lines are always in the range of 5–10, 25–50, and 600–800 s (Fig. 6A). Addition of cAMP ( $10^{-5}$  M) to the relaxing solution results in faster relaxation and if the time course of relaxation is analyzed in a semilogarithmic plot as before, only two straight lines are needed to resolve the relaxation curve (Fig. 6B). The time constants of relaxation with cAMP present are found to lie in the range of 5–10 and 25–50 s.

If 5-HT or cAMP is added while the preparation is in the contracted state, i.e., with  $\text{Ca}^{2+}$  present in concentrations higher than  $10^{-7}$  M, no effect is observed either on the force-producing capacity or on the active state of the preparation.

In an attempt to see whether cAMP had an effect on the  $\text{Ca}^{2+}$  affinity of the contractile apparatus at the concentration known to accelerate relaxation,  $\text{Ca}^{2+}$  dose-response curves with and without  $10^{-5}$  M cAMP present were obtained. As shown in Fig. 7, the dose-response curve in the presence and absence of cAMP is not different.

$\text{Mg}^{2+}$ , which in a previous study was found to influence  $\text{Ca}^{2+}$  sensitivity (Cornelius, 1980), had only a small effect on the rate of relaxation. Varying the  $\text{Mg}^{2+}$  concentration in the range of 0.4–8.0 mM produced a slightly increased rate of relaxation at higher  $\text{Mg}^{2+}$  concentrations (data not shown).

#### DISCUSSION

Using chemically skinned smooth muscle preparations, it has proven very difficult to preserve maximum force-producing capacity and maximum rate of tension development at the same time. In the ABRM, glycerol treatment has been demonstrated to affect the  $\text{Ca}^{2+}$ -dependent regulation of the contraction-relaxation cycle and catch state (Baguet and Marchand-Dumont, 1975), which makes such preparations unsuitable for the study of Ca regulation (cf. Baguet, 1973; Tanaka and Tanaka, 1979). Using saponin for chemically skinning, these deleterious effects are avoided (Endo et al., 1977; Cornelius, 1980).

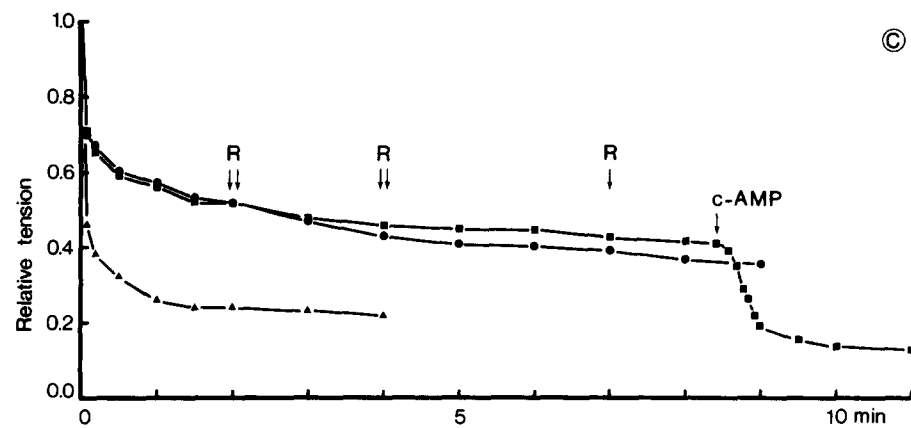
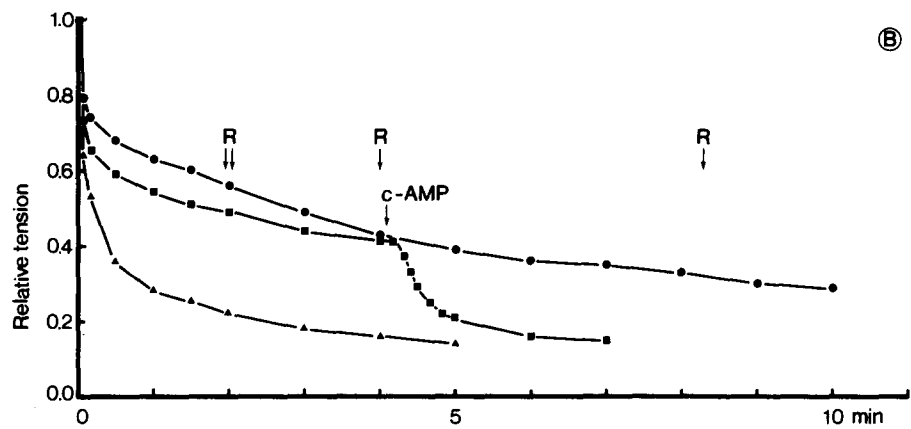
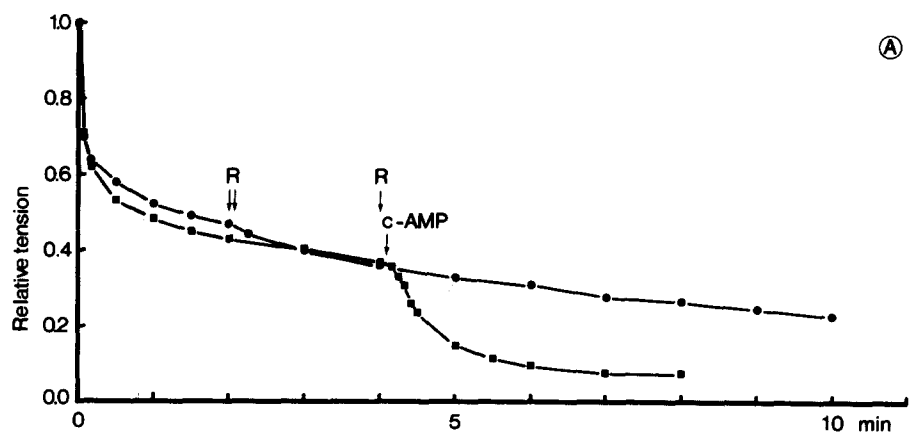


FIGURE 5. Effect of cAMP on the rate of relaxation at three different ionic strengths. A: 0.07 M; B: 0.14 M; and C: 0.28 M. Circles (●) indicate the relative tension present in control ABRM with standard relaxing solution added at zero time. cAMP ( $10^{-5}$  M) was added either a certain time after normal relaxation was induced (■) or initially (▲).



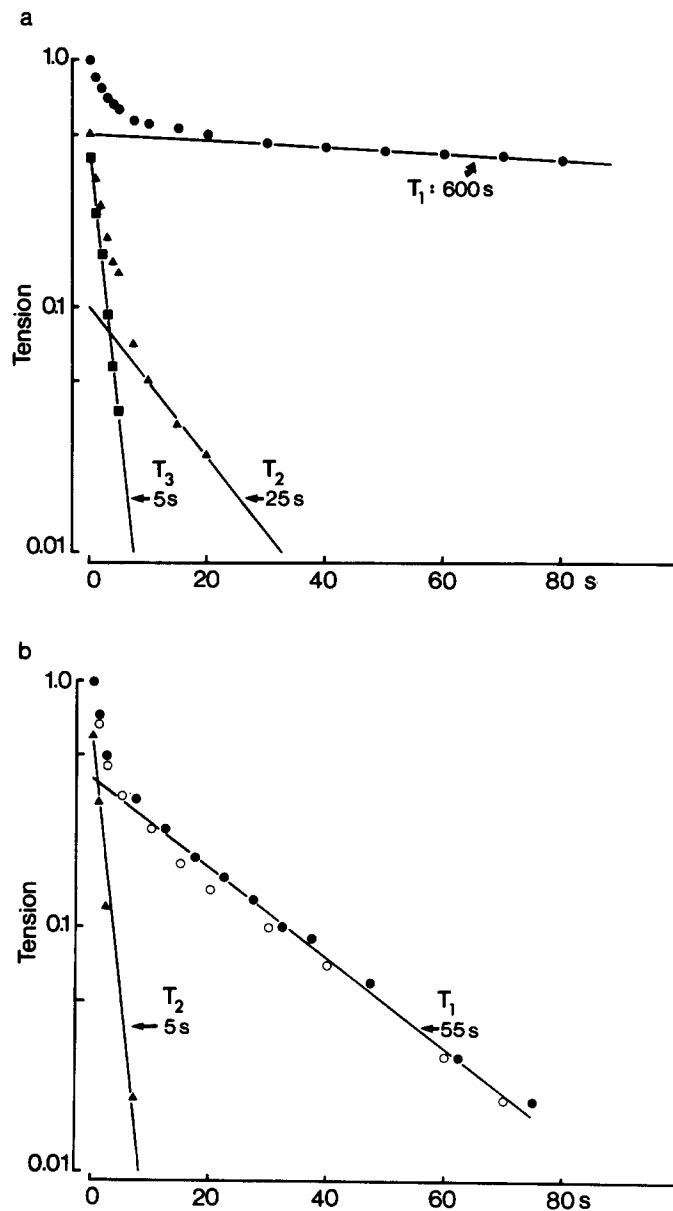


FIGURE 6. Isometric tension decay in the saponin-treated ABRM in the presence and absence of cAMP. The upper panel (a) shows control ABRM relaxed with standard relaxing solution. The lower panel (b) shows ABRM relaxed with relaxing solution containing  $10^{-5}\text{ M}$  cAMP. In both cases the free EGTA concentration was 20 mM and pH 6.8. In the control experiment tension decay ( $\bullet$ ) is apparently triply exponential, i.e., the relative tension can be resolved into three straight lines in the semilogarithmic plot. The slope of the straight lines corresponds to the time constants ( $\tau$ ) of tension decay as indicated on the figure. With cAMP present the tension decay is doubly exponential. Two experiments using the same ABRM are shown ( $\bullet$ ,  $\circ$ ). The large fraction of  $\sim 50\%$  that decays with a very large time constant of  $\sim 600$  has apparently disappeared with cAMP present.

*Tonic Contraction*

In the saponin-treated ABRM, contraction is induced when the concentration of free  $\text{Ca}^{2+}$  is increased above  $10^{-7}$  M in the presence of ATP and  $\text{Mg}^{2+}$  (see Fig. 1). Using an optimal  $\text{Ca}^{2+}$  concentration of  $\sim 10^{-4}$  M tension can be maintained for hours and, provided sufficient ATP is present, the active state

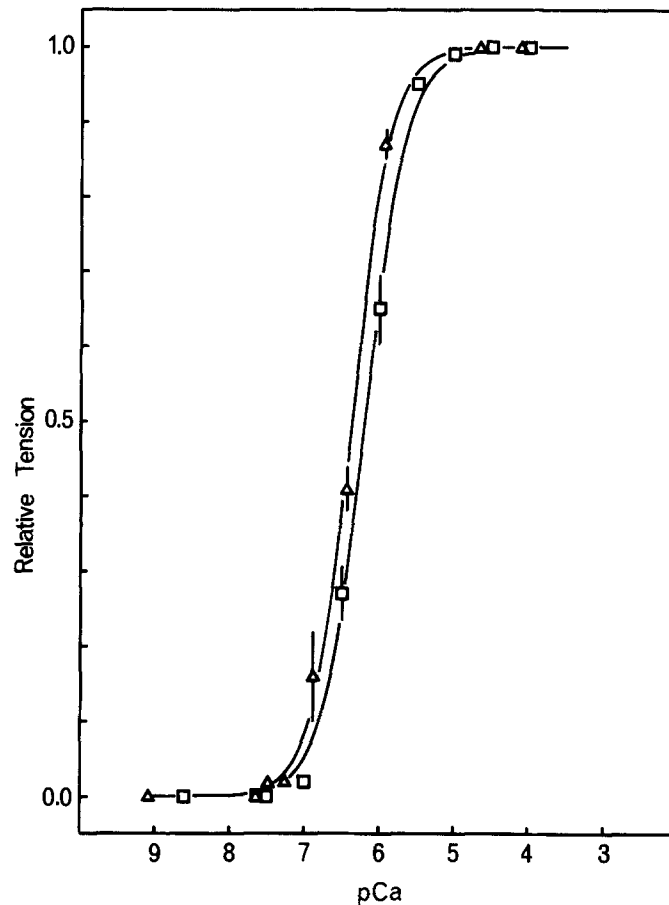


FIGURE 7. Relationship between relative tension and concentration of free  $\text{Ca}^{2+}$  (pCa). Means of data from control experiments ( $\square$ ) and experiments with  $10^{-5}$  M cAMP present in the contracting solutions ( $\Delta$ ) are shown. Each point in the two curves represents means of six experiments with SEM ( $n = 6$ ) indicated as vertical bars.

will persist. This state is therefore similar to the state generated when a living ABRM is stimulated with ACh, i.e., it is equivalent to a tonic contraction.

The maintenance of tension and the active state was dependent primarily on the high concentration of  $\text{Ca}^{2+}$  and the presence of Mg-ATP: removing ATP in the presence of  $\text{Ca}^{2+}$  caused the preparation to develop a state equivalent to the rigor state in the ATP-depleted living ABRM (see Cornelius,

1980, Fig. 4). Removing  $\text{Ca}^{2+}$  in the presence of ATP resulted in the decay of tension.

Variations of ionic strength in the range of 0.07–0.28 M and in pH between 6.5 and 7.0 did not affect the characteristics of the tonic contraction in the saponin-treated ABRM. The same kind of response was obtained using the contracting solution with pH 6.5,  $I = 0.07$  M, or pH 7.0,  $I = 0.28$  M as with the standard contracting solution of pH 6.8,  $I = 0.14$  M.

These results are at variance with results obtained by Baguet and Marchand-Dumont (1975) using Triton X-100- and EDTA-treated ABRM preparations. Using a contracting solution of ionic strength 0.28 M, pH = 7.0, they found that a steady tension could be maintained, whereas the active state disappeared progressively over a 8–10 min period. However, no ATP-regenerating system was included in these experiments and it is not indicated how often the contracting solution containing 5 mM ATP was changed during contraction. It is possible that the inability of the EDTA-treated ABRM preparation used by these authors to maintain the active state in the presence of high  $\text{Ca}^{2+}$  could be explained by an insufficient concentration of ATP in the myofibrillar space.

#### *Relaxation and Catch*

When the concentration of free  $\text{Ca}^{2+}$  is decreased to  $<10^{-7}$  M during contraction in a saponin-treated ABRM by application of relaxing solution with a sufficiently high free EGTA concentration, the tonic contraction is terminated. However, although the active state immediately disappears upon lowering the  $\text{Ca}^{2+}$  concentration, the tension decreases extremely slowly (see Fig. 3). Even when relaxing solution with 20 mM EGTA is applied, tension decay can last for hours. This is the same sequence of events as seen after a tonic contraction in the living ABRM: when stimulation ends and ACh is washed away, the active state disappears but tension decreases very slowly. In the living ABRM this state is called the catch state (see Fig. 2).

In the living ABRM, tryptaminergic nerves release 5-HT, which specifically terminates the catch state. The primary effect of 5-HT is to increase the concentration of intracellular cAMP via binding at specific relaxing receptors in the membrane (Cole and Twarog, 1972; Achazi et al., 1974).

In the saponin-treated ABRM, both 5-HT and cAMP are found to accelerate relaxation (Figs. 4 and 5). With respect to 5-HT, this must mean that the 5-HT receptors are at least partially intact even after the detergent treatment. 5-HT was least effective in speeding up relaxation at ionic strength  $I = 0.07$  M, whereas cAMP was equally effective at all three ionic strengths studied (see Fig. 5). The explanation for the lower effectiveness of 5-HT at  $I = 0.07$  M could be that here fewer functional relaxing receptors are present either as a direct consequence of ionic strength or because the saponin treatment is more effective in disrupting the membrane at the lower ionic strength.

In contrast to the results reported here, Marchand-Dumont and Baguet (1975) found both 5-HT and cAMP to be ineffective in speeding up relaxation at low ionic strength using EDTA-treated ABRM. Since 5-HT does release

catch in the living ABRM, it was concluded that low ionic strength and pH could not be the explanation of catch as originally proposed by Johnson et al. (1959).

In the saponin-treated ABRM, variation of the ionic strength in the range of 0.07–0.28 M did not change the rate of relaxation, and variation of pH between 6.5 and 7.0 was also without effect on relaxation or active state. Thus the experiments reported here do not point to ionic strength or pH as important factors in the initiation of catch.

Increasing the free concentration of  $Mg^{2+}$  in the relaxing solution in the range of 0.4–8.0 mM had a slight increasing effect on the rate of relaxation. This small effect can conceivably be explained by competition between  $Mg^{2+}$  and  $Ca^{2+}$  (Cornelius, 1980): increasing the  $Mg^{2+}$  concentration would tend to increase the rate of  $Ca^{2+}$  dissociation from the functional force-producing units and thereby enhance  $Ca^{2+}$  sequestration by the EGTA present.

5-HT and cAMP are only effective in speeding up relaxation if the  $Ca^{2+}$  concentration is below threshold for tension initiation. Thus the activating  $Ca^{2+}$  must somehow block the 5-HT/cAMP relaxing mechanism. Since EGTA is present in a sufficiently high concentration to eliminate the effects of  $Ca^{2+}$ -accumulating structures, the action of 5-HT/cAMP must be directly on the contractile system, as also suggested by Marchand-Dumont and Baguet (1975). In the living ABRM, cAMP could have an additional effect on  $Ca^{2+}$ -accumulating structures or on the membrane. However, Achazi et al. (1974) were unable to demonstrate any effect of cAMP on the  $Ca^{2+}$  flux in vesicle fractions from the ABRM.

Similar tension responses to contracting solution with increasing  $Ca^{2+}$ -concentration are found in control experiments and in experiments where  $10^{-5}$  M cAMP is present. Thus the present study demonstrates no effect of cAMP on the  $Ca^{2+}$  affinity of the contractile apparatus in this muscle. Provided that an increase of intracellular cAMP level is the mechanism of action of 5-HT, these results do not support the hypothesis that 5-HT switches off catch by reducing the  $Ca^{2+}$  affinity of the contractile proteins (Marchand-Dumont and Baguet, 1975).

Similar results have been obtained with mechanically skinned fibers from the caudofemoralis of the cat (Fabiato and Fabiato, 1978). There the relaxing effect of cAMP could be attributed to an effect on the sarcoplasmic reticulum: in the presence of cAMP the pumping capacity of the sarcoplasmic reticulum is enhanced, thereby decreasing the free  $Ca^{2+}$  concentration and causing relaxation.

The results illustrated in Fig. 6 indicate that cAMP decreases the time constant for the slower of several dissociation steps leading to relaxation. Thus without cAMP there is a large fraction of ~50% that decays with a time constant of ~600 s. This fraction disappears when cAMP is present. It is tempting to interpret this as representing a fraction of cross-bridges with a slow turn-over that is somehow accelerated by cAMP. No evidence has been found that a phosphorylation of the regulatory light chains of myosin is required for the regulation of myosin-actin interaction in mollusks. However,

recent reports have indicated that paramyosin is phosphorylated and that this phosphorylation is taking place via a cAMP-dependent enzyme (Cooley et al., 1979; Achazi, 1979). It is possible that a phosphorylation of the paramyosin backbone in the thick filaments could modify the contractile protein interaction, resulting in an acceleration of the cross-bridge cycling rate, without affecting the  $\text{Ca}^{2+}$  sensitivity of the contractile proteins. Such a mechanism would resemble one operating in vascular smooth muscle where cAMP-dependent phosphorylation of the 20,000 mol wt myosin light chain regulates the shortening velocity (Aksoy et al., 1980). However, the data are insufficient to prove any detailed mechanism for the action of cAMP. Among the factors that might have contributed to the rather large variance in measurements of the rate of relaxation are differences in the extent to which limiting effects from diffusion barriers are present. Delayed diffusion could be avoided by using much thinner fiber bundle preparations. However, such preparations are difficult to prepare and deteriorate easily.

In conclusion, the saponin-treated ABRM can be made to produce the same functional states that characterize the living ABRM. A tonic contraction is induced when the  $\text{Ca}^{2+}$  concentration is raised above  $10^{-7}$  M in the presence of  $\text{Mg}^{2+}$  and ATP. Maximum tension is generated at a  $\text{Ca}^{2+}$  concentration of  $\sim 10^{-4}$  M. During such contraction the muscle is in the active state. Removing  $\text{Ca}^{2+}$  switches off the active state. However, tension decay can either be fast or very slow, as in the catch state. To induce a fast relaxation, 5-HT or cAMP has to be included after the  $\text{Ca}^{2+}$  concentration has been lowered below threshold for tension initiation, whereas in the absence of these drugs the catch state develops. This is in accordance with the explanation on catch given by Twarog (1979) that activating  $\text{Ca}^{2+}$  blocks the relaxing system. The mechanism of cAMP remains in doubt, although certain negative conclusions can be drawn. In the saponin-treated ABRM, cAMP must act directly on the contractile apparatus and not via  $\text{Ca}^{2+}$ -accumulating structures. However, its mode of action does not affect the  $\text{Ca}^{2+}$  sensitivity directly. The results suggest that in the living ABRM it is the removal of  $\text{Ca}^{2+}$  from the myofibrillar space that induces catch, i.e., the switching off of the active state and the fast turnover of cross-bridges. This is in contrast to previous explanations of catch on the basis of a sustained high intracellular  $\text{Ca}^{2+}$  concentration concomitant with a change in  $\text{Ca}^{2+}$  sensitivity (for references see Twarog, 1977). In accordance with Twarog (1979), it is suggested that cAMP speeds up cross-bridge turnover, thereby converting the slow relaxation into a fast relaxation at low  $\text{Ca}^{2+}$  concentrations.

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