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Potentiating and Depressing Actions of Caffeine on Twitch Tension in the Isolated Atrial Muscle of the Bullfrog

Xiao-Liang CHEN* and Moto MATSUMURA

Department of Physiology, Kawasaki Medical School, Kurashiki 701-01, Japan

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ABSTRACT. Caffeine shows the dual actions on the twitch tension of isolated atrial muscles of bullfrogs. When the preparation, which is repetitively stimulated at the rate of 6/min, is exposed to Ringer solutions containing caffeine of 1, 2, 5 and 10 mM, the twitch tension first increases in 0.5-2 min and then decreases exponentially with the time constants of 4.5—6.2 min. Namely, the actions of caffeine are biphasic; the initial potentiation and the following depression. The time constants are independent of concentrations of either caffeine or of external calcium. The initial potentiation is explained from the increased sensitivity of troponin to Ca and the following slow decay is explained from the depletion of stored Ca inside the sarcoplasmic reticulum. After washout of caffeine solution, the twitch tension further decreases transiently and then recovers slowly to the control tension. These tension changes during the recovery seem to be a mirror image of the tension changes observed during caffeine exposure. The 90% recovery time, however, is prolonged by an increase in caffeine concentration and is shortened by an increase in calcium concentration. The explanations are that the transient tension drop is produced by the removal of the potentiating action and that the slow tension recovery is produced by the reaccumulation of calcium into the sarcoplasmic reticulum.

Key words : Cardiac muscle — Muscle contraction — Caffeine — Excitation-contraction coupling

Caffeine is well known as a potent cardiac alkaloid and has ever been used as a tool for the purpose to elucidate the excitation-contraction coupling or the mechanical activation in the cardiac as well as in the skeletal muscles. The actions of caffeine are complicated and multisided. First, caffeine releases Ca from the sarcoplasmic reticulum (SR) or inhibits apparent uptake of Ca by SR.[1—3] Although caffeine does not change significantly size and shape of the action potential,[4—7] it may increase the slow inward current carried by Ca^{2+} [5—6] and lowers the mechanical threshold potential of frog skeletal muscle.[8] Caffeine also increases the Ca-sensitivity to troponin.[9—11] It inhibits also the phosphodiesterase activity, or increases Ca extrusion through Na-Ca exchange mechanism.[12]

The common feature of the actions of caffeine on the isolated cardiac muscle is, as is reviewed by Chapman,[3] to increase a twitch tension or a shortening velocity. The active state measured from the capacity to bear a

陳　孝良，松村幹郎

*Visiting Research Associate from Beijing Second Medical College under the Mutual Exchange Fellowship supported by Kawasaki Medical School.

Present address : Department of Physiology, Beijing Second Medical College, Beijing, People's Republic of China

load and capacity to shorten is prolonged and intensified.[13–15] Caffeine also produces a contracture both in mammals[16] and in frogs.[4,7,17,18] On the other hand, further investigations have revealed that caffeine, if its action is long-lasting or its concentration is relatively high, depresses the beating tension of muscles isolated from mammalian ventricle[10,16,19] or from frog atrium.[4,7,20] To explain the depressing action, Hess & Wier[10] shows that in Purkinje fibers caffeine decreases the free myoplasmic $[Ca^{2+}]$ measured by aequorin. Therefore, the actions of caffeine seem to be biphasic, potentiation and depression. The present work will describe the time course, dose-dependency and the modification by external $[Ca^{2+}]$ of the dual actions of caffeine in bullfrog atrial muscle. Some of the results have been already presented.[21]

METHODS

The trabecular muscles are prepared from the atrium of bullfrog, *Rana catesbeiana*, and the strips are 0.5–1 mm in diameter and 5–7 mm long. The pacemaker tissues are dissected away so that the spontaneous beating can be excluded. Small stainless steel wire hooks are tied to both ends of the muscle preparation, and one end is fixed and another end is connected to the strain-gauge tension transducer (Showa Instrument, 5356A). The muscle chamber of volume of $65 \times 6 \times 4$ mm is placed inside an another chamber which is perfused by water regulated at constant temperature of 25°C. The standard Ringer solution and caffeine-Ringer solutions are also kept at constant temperature of 25°C. The solution in the muscle chamber is quickly exchanged by flushing a test solution of 50 ml from a syringe within 10 seconds. The standard Ringer solution contains (mM) NaCl 117, KCl 2, $CaCl_2$ 1.8, glucose 4 and HEPES buffer 3 being adjusted to pH of 7.2. Caffeine Ringer solution is prepared by adding powder of caffeine anhydrate (Wako Pure Chemicals) at the final concentrations of 1, 2, 5 and 10 mM. Solutions of different Ca concentrations are prepared by adding different volumes of 1M $CaCl_2$ stock solution to Ca-free Ringer solution. The small changes either in tonicity or in concentration of other ions than Ca are neglected.

Before starting the tension recording, the preparations are placed in the refrigerator for 1–5 hours at 4°C. By this time, the hypodynamic state have been established,[22] and thereafter the constant tensions are obtained for at least 2 hours. Then the preparation is immersed in the muscle chamber containing Ringer solution of 25°C bubbled with 100% oxygen. It is stimulated transversely through the massive Ag–AgCl electrodes placed 6 mm apart. The pulses are 3 msec duration, $2\times$ supramaximal strength and are delivered at the rate of 6/min. The tension is recorded on the pen-recorder (Rikadenki Kogyo, R102), which has frequency characteristics of 100% at 0.5 Hz, 99% at 1 Hz and 97% at 2 Hz. Special care is taken to obtain the optimum muscle length, since the tension is greatly dependent on muscle length. At first, the muscle length where the maximum tension develops (L_{max}) is determined in the standard Ringer solution. Then it is slightly slackened so that the developed tension may be 0.9 of the maximum, and is kept at this length, $L_{0.9\ max}$, throughout the experiments.

RESULTS

1. Potentiation and depression of twich tension

The muscle is kept at $L_{0.9\ max}$ and it is stimulated at the rate of 6/min. The beating tension increases as the increase in rates from 1/min to 12/min and decreases as the further increase in rates up to 30/min. In this study, the beating rate is always 6/min instead of 12/min, because tensions at 6/min are maintained for longer time than those at 12/min.

A few minutes after starting the beating, the tension attains the steady amplitude. At this stage, the Ringer solutions containing 1, 2, 5 and 10 mM caffeine is perfused into the muscle chamber. Figure 1 shows the tension changes during application and after washout of caffeine. Exposure of the preparation to caffeine-Ringer solution always produces the initial potentiation of twitches. This potentiation reaches the maximum in a minute and is followed by the gradual tension decay. At 10 mM caffeine, the small sustained contracture tension development is observed during the later decay phase of twitch tension. This tension decay approximates to a single exponential and reaches the steady level in 20–30 min. When the caffeine concentrations is as high as 5 or 10 mM, the potentiating action is rapid and remarkable but the steady tension after decay is smaller than the pre-caffeine tension.

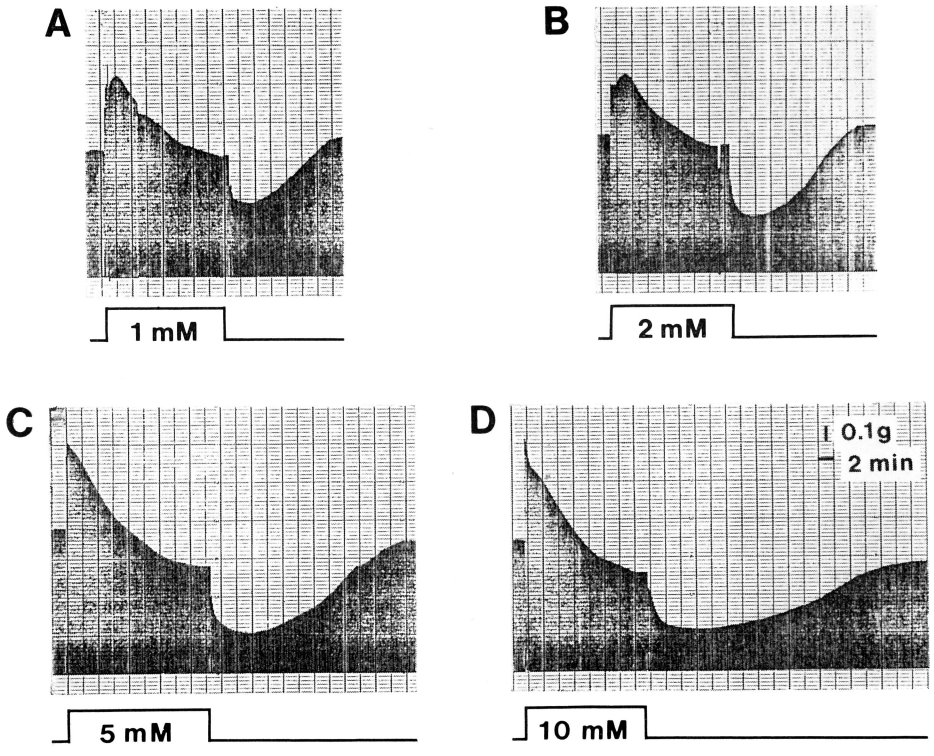

Fig 1. Isometric twitch tensions before, during and after perfusion of caffeine-Ringer solution. Caffeine concentration is 1 mM in A, 2 mM in B, 5 mM in C and 10 mM in D. The solution is exchanged within 10 sec and the period of caffeine perfusion is indicated below each record. All the records are obtained from the same preparation. Calcium concentration is 1.8 mM. Stimulation rate is 6/min, 25°C.

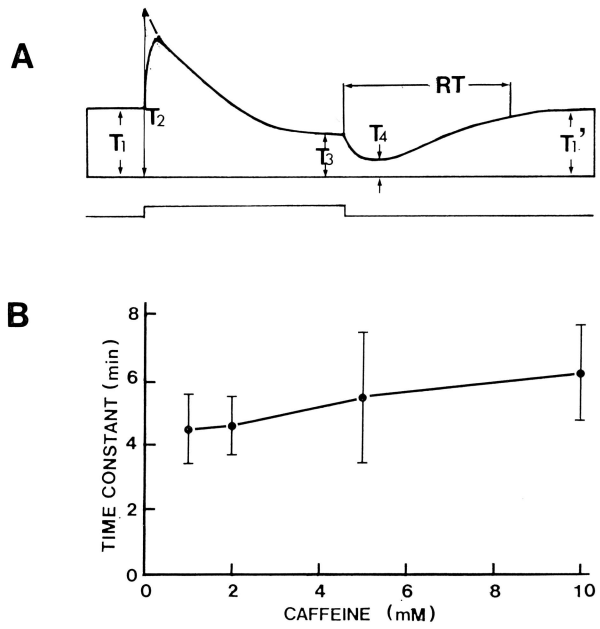

Fig. 2. A : The general feature of tension changes produced by caffeine. T_1 : control tension, T_2 : expected tension at time=0 obtained by extrapolation of the exponential decay, T_3 : steady state tension during caffeine exposure, T_4 : minimum tension after washout of caffeine, and T_1' : tension after recovery. RT is the time required for tension to attain 90% T_1' measured from the time of washout of caffeine. B : Relationship between decay time constant and caffeine concentrations. Values are indicated by the mean ±S.E. Calcium concentration is 1.8 mM. Stimulation rate is 6/min, 25°C.

2. The tension decay in caffeine solution

The time courses of tension decay can be fitted to the single exponential relation. Let the tensions during potentiation, decay and recovery be T_1 through T_4, as shown in Figure 2A, then the tension during decay, T(t), is written as

$$T(t) = (T_2 - T_3)\, e^{-t/\alpha} + T_3$$

$$\text{or } T(t) - T_3 = (T_2 - T_3)\, e^{-t/\alpha}$$

where α is the time constant. Figure 2B shows the decay time constant against caffeine concentration. At first, the tension decay is expected to be accelerated by an increase in caffeine concentrations, but it is rather slightly slower at 5 or 10 mM caffeine than that 1 or 2 mM. At 1.8 mM Ca, the time constants of the tension decay are 4.5±1.1 min (mean ±S.E.) at 1 mM, 4.6±0.9 min at 2 mM, 5.5±2.0 min at 5 mM and 6.2±1.5 min at 10 mM caffeine, indicating that the time constants are not considerably affected by caffeine concentrations. When external $[Ca^{2+}]$ is increased from 1.8 to 4.5 or 9.0 mM, the beating tension increaes by 25% of the control. Application of caffeine to Ca rich-Ringer solution causes additional potentiation and the following tension decay (Fig. 3). Potentiation and depression after application of caffeine are observed in 0.72 mM Ca solution, where the twitch tension is reduced. The results from 5

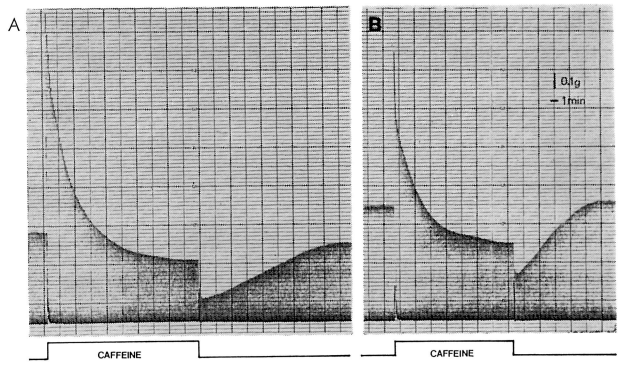

Fig. 3. Effects of calcium concentration on the potentiating and depressing actions of caffeine. Two records are obtained from the same preparation. The caffeine concentration is 5 mM both in A and in B and the period of caffeine perfusion is indicated by a bar below each record. Calcium concentration is 1.8 mM in A and 4.5 mM in B. Stimulation rate is 6/min, 25°C.

preparations are shown in Figure 4 and the time constants are found to be almost constant between 0.72 mM and 9.0 mM Ca. In summary, the decay

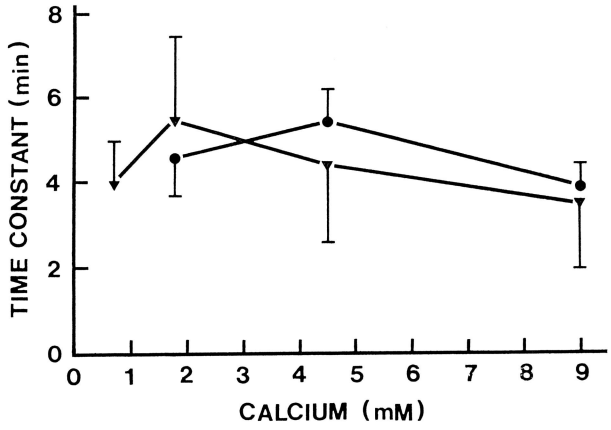

Fig. 4. The decay time constant against Ca concentrations at two sets of caffeine concentrations of 2 mM (circles) and 5 mM (triangles). Vertical bars show + or −S.E. 25°C.

time constants are nearly independent of either caffeine concentration or of external [Ca] up to 9 mM.

The general feature of the initial potentiation and the following tension decay are observed even if repetitive stimulation is interrupted. Figure 5A shows the twitch tensions of muscle preparation regularly stimulated at the rate of 6/min in 5 mM caffeine Ringer solution, and Figure 5B shows the twitch tensions when the stimulation is discontinued during the tension decay phase. The tensions after the quiescent period in Figure 5B, except the initial several beats, are the same as those observed during regular repetitive stimulation shown

in Figure 5A. The result indicates the tension decay is produced not by the changes related to repetitive contractions but by the direct action of caffeine.

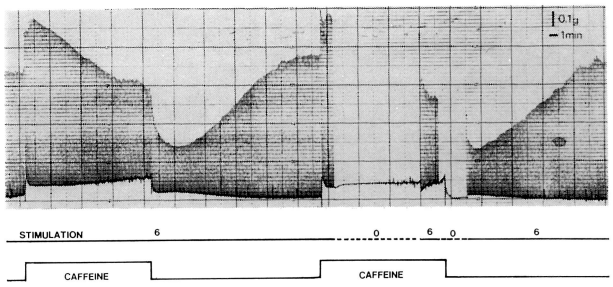

Fig 5. Tension changes under the influence of caffeine actions on twitch tension of muscle stimulated regularly at the rate of 6/min (left) and imposed by interruption of stimulation (right). Periods of regular stimulation and of caffeine perfusion are indicated below each record. Caffeine concentration is 5 mM and Ca concentration is 1.8 mM. 25°C.

3. Recovery of tension after washout of caffeine

As is illustrated in Figures 1, 3 and 5, when the caffeine Ringer solution is replaced with the standard caffeine-free Ringer solution, the peak twitch tension once decreases and then recovers slowly to the amplitude before caffeine treatment. The effects of washout of caffeine are also biphasic ; initial depression and following slow recovery. At 1 or 2 mM caffeine, the recovery is almost complete even though the exposure time to caffeine is as long as 20 or 30 min. But, the final tension, T_1', is smaller by about 20% than the control tension T_1, if caffeine concentration is 10 mM.

The ratio of the minimum tension, T_4, to the final tension, T_1', during recovery is decreased as an increase in caffeine concentration, as shown in Figure 6. The 90% recovery time is taken for convenience as the index of recovery rate, because the time course of the changes in peak tension during recovery is linear rather than exponential. The relationship between the 90%

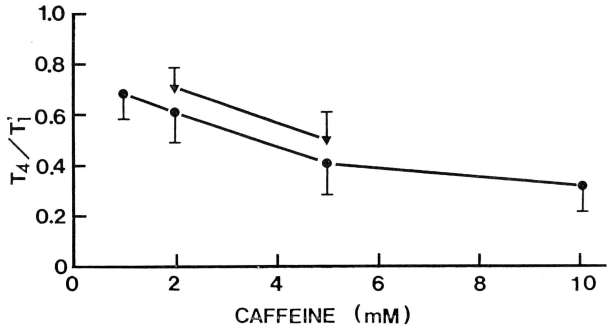

Fig. 6. Relationship between the minimum tension after washout and caffeine concentration, examined at different Ca concentrations. The minimum tension is shown as a relative value to tension after recovery, T_4/T_1'. Vertical bars show + or −S.E. Ca concentrations are 1.8 mM (circles) and 9.0 mM (triangles).

recovery time and caffeine concentration is shown in Figure 7. They are, 9.7, 11.5, 18.2 and 20.5 min at 1, 2, 5 and 10 mM caffeine and at 1.8 mM Ca. Contrary to the tension decay during caffeine exposure, the time course of the tension recovery after the washout does not fit to the single exponential relation, neither does it fit to the three compartment model of Chapman & Miller.[20] Moreover, it delays as the caffeine concentrations increase, suggesting that the recovery is not necessarily the reversal phenomenon of the tension decay in caffeine solution. The parameters of tension recovery are also modified by external [Ca]. As is shown in Figures 6 and 7, the minimum twitch tension, T_4,

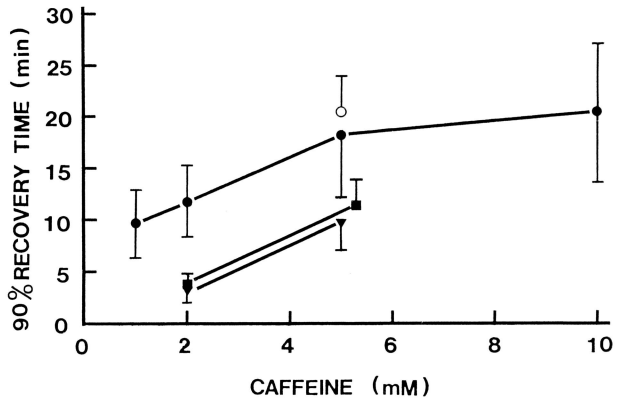

Fig. 7. Relationship between time required for 90% tension recovery and caffeine concentration, examined at different Ca concentrations. Ca concentrations are 0.72 mM (hollow circle); 1.8 mM (filled circles), 4.5 mM (squares) and 9.0 mM (triangles). Vertical bars show + or − S.E. 25°C.

is larger in 9.0 mM Ca than that in 1.8 mM Ca, and the 90% recovery times are shortened by an increase in external [Ca]. These times after removal of 5 mM caffeine are 20.7, 17.2, 10.8 and 10.0 min at [Ca] of 0.72, 1.8, 4.5 and 9 mM. Although the recovery after washout looks a mirror image of the tension decay during exposure to caffeine, the properties of recovery are different from those of decay process in the sense that the time course of recovery is not exponential and that it is dependent on the concentrations both of caffeine and of Ca.

DISCUSSION

Several previous authors[4,7,10,20] have reported the depressing action of caffeine on twitch tension, but they describe only briefly about the recovery after washout. Among them, Chapman & Miller[20] have pointed out the biphasic recovery and Niedergerke & Page[7] shows more rapid recovery process. The present work illustrates the dual actions of caffeine both during application and after removal. On application of caffeine, one of the dual actions is the potentiation which appears rapidly and another is the following depression which develops slowly. It shows that, upon removal of caffeine, the tension is also biphasic ; further transient decrease and the slow recovery towards the control level. These

tension changes during the recovery are qualitatively symmetrical to those during caffeine exposure.

Potentiation in twitch in caffeine solution is rapid with the peak time of a few tens of seconds. Our atrial preparations have the diameter of at most 1.0 mm. If the preparation is assumed to be a cylinder with 0.3 mm radius and the diffusion constant of caffeine in myoplasm is 2.66×10^{-6} cm^2/sec, nearly the same as in free solution,[23] then the half saturation time in myoplasmic caffeine concentration is calculated to be 21 sec, from the equation of $D' \cdot t_{\frac{1}{2}}/R^2 = 0.063$, where D' is the apparent diffusion constant and R is the radius.[24] Since our preparation composed of several thinner bundles, the diffusion of caffeine into the muscle fiber should be faster than the expected value from this calculation. Therefore, at the time of the peak tension the myoplasmic caffeine concentration is in a state of the equilibrium with the external solution.

There are several possibilities regarding to the nature of the initial potentiation. First, caffeine enhances Ca release from SR,[1,2] which results in a rise in background intracellular $[Ca^{2+}]$ and subsequent rise in $[Ca^{2+}]$ in association with the action potential. Secondly, the site of caffeine action is sarcolemma.[25] This is supported by the finding on the increase in slow inward current examined by Kimoto *et al.*[5] and by Yatani *et al.*[6a] with the voltage clamp method. In mammalian Purkinje fibers, however, caffeine decreases the slow inward current and also reduces the peak of the Ca-aequorin transient, both L_1 and L_2 components denoted by Hess & Wier.[10] According to them, troponin sensitivity to Ca^{2+} is increased by caffeine, which will be the most plausible nature for potentiation.

After attaining the peak, the tension starts to decrease gradually. The time course of the main part of tension decay can be fitted to the single exponential relation with the time constants of several minutes. The interruption of stimulation does not modify the time course of this tension decay. This result is contradictory to that reported by Niedergerke & Page,[7] who showed that the decay phase depended not on the time of caffeine treatment but on the twitch number. The probable explanation for tension decay is the consumption of Ca store within SR[7,9,10,22,24] and resulting decrease in the quantity of released Ca. If the loss of Ca^{2+} from SR were compensated by Ca^{2+} entered from the extracellular space through the membrane in association with an action potential, the interruption of stimulation would accelerate the tension decay. But, this is not true. The Ca inflow during an action potential does not play a role in Ca supply to SR. It is plausible that the myoplasmic $[Ca^{2+}]$ is regulated mainly by the Na-Ca exchange mechanism,[7,26,27] and $[Ca^{2+}]_i$ at the relaxed state between each contraction is maintained identically whether the beatings are continued or interrupted. Slight prolongation of decay time course at 5 or 10 mM caffeine solution suggests that the amount of Ca^{2+} that are released from SR exceeds to the amount of Ca^{2+} that are extruded through the Na-Ca exchange mechanism. And accordingly, myoplasmic $[Ca^{2+}]$ will be increased, which results in a small contracture as well as prolongation of the tension decay. The characteristic property of the tension decay except its tail is independent of either caffeine concentration or of external $[Ca^{2+}]$, although the final steady tension is small at high concentration of caffeine. These results are consistent to those of Niedergerke & Page.[7] The independence for the time constants of

caffeine concentrations is simply explained, if it is assumed that the number of reactive sites of SR to caffeine is not so few as to be saturated by 1 mM caffeine but not so many as to be saturated by 5–10 mM caffeine and that the rate of release of stored Ca is proportional to the content of Ca inside SR. This idea is reasonable, because the fragmented SR experiments indicate that the Ca efflux from SR to myoplasm is inhibited if the intravesicular Ca is reduced.[28]

Further decrease in tension after washout of caffeine is the reversal phenomenon of initial potentiation in caffeine solution. At this time, the potentiating action has been eliminated while the Ca store has not been reestablished. When caffeine concentration is high, the residual [Ca] inside SR is small so T_4 is decreased. On the other hand, the time course of the slow tension recovery will reflect the reaccumulation of Ca into SR. The myoplasmic $[Ca^{2+}]$ has been reduced after washout of caffeine, and this, in turn, depresses the Na–Ca exchange mechanism or enhances Ca inflow during an action potential, and consequently brings myoplasmic $[Ca^{2+}]$ back to the standard level. Thus, these two processes will restore Ca within SR and result in tension recovery. When Ca is high, the two processes are augmented and so the tension recovery is accelerated. The result, however, that the time course of tension recovery does not fit to the single exponential suggests that the Ca reaccumulation is not a sole factor of determining the time course of tension recovery. Other factors such as cAMP, ADP and especially $[Mg^{2+}]$ may be cosidered.

Acknowledgments

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