

# Caffeine and Excitation-Contraction Coupling in the Guinea Pig *Taenia Coli*

Y. ITO and H. KURIYAMA

From the Department of Physiology, Faculty of Dentistry, Kyushu University, Fukuoka, 812, Japan

**ABSTRACT** The effects of caffeine (0.2–10 mM) on the electrical and mechanical activities of guinea pig *taenia coli* were investigated with the double sucrose-gap method. Caffeine evoked a small tension with a latency of 20–30 sec, then phasic contraction developed and finally relaxation. The initial tension development also appeared in the Na-free solution without any marked changes in the membrane potential and membrane resistance. The phasic contraction disappeared in the Na-free solution. The relaxation in the presence of caffeine was accompanied by depolarization block of the spike generation. The minimum concentration of Ca ion needed to evoke the tension development by the caffeine was  $10^{-7}$  M. Caffeine also potentiated the twitch tension below a concentration of 5 mM either in the Na-free solution or at low temperature (5°C).  $\text{NO}_3^-$  and  $\text{Br}^-$  showed a similar response to caffeine on the potentiation of the twitch tension at low temperature.

## INTRODUCTION

Sandow (1965) reviewed excitation-contraction coupling in skeletal muscle and described the effects of caffeine on the mechanical properties of this muscle. In low concentrations, up to about 2–4 mM for frog muscle, its sole effect is potentiation of twitch contraction. In higher concentrations, greater than about 5 mM, its predominant effect is to cause contracture. The effects of caffeine on muscle vary widely with the species, mammalian muscle being especially resistant to contracture. The mechanism of the action of caffeine was investigated by Herz and Weber (1965) and they concluded that caffeine produces its effects by increasing the capacity of the sarcoplasmic reticulum to release Ca ion. Furthermore, in the skeletal muscle, both the mechanical changes and the electrochemical alterations are in general the same as those produced by anion substitution (Ritchie, 1954; Podolsky and Hubert, 1961; Sandow, Taylor, Isaacson, and Seguin, 1964). In the antral smooth muscle of guinea pig Sakamoto and Kuriyama (1971) investigated the actions of various anions on the electrical and mechanical properties, and they found that anions modified the electrical and mechanical thresh-

olds. For example,  $\text{NO}_3^-$  and  $\text{Br}^-$  ions hyperpolarized the membrane, reduced the membrane resistance, enhanced the spike amplitude, lowered the mechanical threshold, and enlarged the amplitude of the tension. In the skeletal muscle, anions do not significantly alter the action potential but do increase the membrane resistance. The differences found between the skeletal muscle and the smooth muscle might be due mainly to the morphological difference between them, since the structures of the sarcoplasmic reticulum and tubular system (T-system), as observed in the skeletal muscle, were poorly developed or nearly absent in the smooth muscle (Needham and Shoenberg, 1968).

The present experiments were carried out to investigate the effects of caffeine on the electrical and mechanical properties of the smooth muscle of guinea pig taenia coli. The main purpose of observing the effect of caffeine was to find out whether or not the sarcoplasmic membrane possesses the properties of the sarcoplasmic reticulum observed in the skeletal muscle. The results led to the conclusion that caffeine mobilized the bound Ca ion and increased the Na conductance. Increased membrane activity was caused by the secondary action of the mobilized bound Ca ion. The effects of caffeine on the mechanical property are directly related to the electrical property, and the caffeine potentiation of the mechanical property was masked by the increased Na conductance. These results also suggested that the sarcoplasmic membrane plays the role of the reticulum observed in the skeletal muscle.

#### METHODS

##### *Tissues and Apparatus for the Double Sucrose-Gap Experiments*

Guinea pigs, weighing 250–300 g, were stunned and bled. The taenia coli was dissected from the cecum and was 0.8–0.5 mm in diameter and 20–30 mm in length. The sucrose-gap apparatus used was almost the same as that described by Bülbbring and Tomita (1969) and Kuriyama and Tomita (1970), i.e. of the total muscle length (20–30 mm), a part less than 1 mm in the middle portion was exposed to test solution, while both ends were bathed in sucrose solution.

##### *Tissues and Apparatus for the Recording of the Mechanical Activity*

Muscle of total length 30–40 mm and width 1 mm was dissected from the cecum, and mounted in an organ bath 50 cc in volume through which solution flowed continuously. One end of the preparation was fixed to a glass hook and the other end was tied by silk thread to the hook of the tension recorder.

The isometric tension was recorded by a strain gauge (Nihon Kohden Ltd., Tokyo, Japan) in both types of experiment.

##### *Solutions*

Locke and Krebs solutions of the following compositions were used (mM): (a) Locke solution: NaCl, 154.0; KCl, 5.6;  $\text{CaCl}_2$ , 2.2;  $\text{NaHCO}_3$ , 1.8; and glucose, 5.6. (b)

Krebs solution: NaCl, 120.4; KCl, 5.9; CaCl<sub>2</sub>, 2.5; NaHCO<sub>3</sub>, 15.5; MgCl<sub>2</sub>, 1.2; NaH<sub>2</sub>PO<sub>4</sub>, 1.2; and glucose, 11.5. Locke solution was mainly used for these experiments, since the solution did not contain Mg ion and therefore, it was easy to control the concentration of Ca ion with GEDTA. Furthermore, the regular appearance of the spontaneous membrane activity was recorded more easily in Locke solution than in Krebs (Kuriyama and Tomita, 1970). For Na-free solution, NaHCO<sub>3</sub> as well as NaCl was omitted, and tris (hydroxymethyl) aminomethane was substituted for Na ion. The pH of the solution was adjusted to 7.1–7.2 with 1 N HCl. In order to prepare the various concentrations of Ca ion (10<sup>-4</sup>–10<sup>-8</sup> M), these concentrations were modified by GEDTA-buffer (glycoetherdiaminetetraacetic acid–buffer) using the following equation abbreviated by Imai and Takeda (1967) from the equation presented by Chabarek and Martell (1959).

$$pCa = 2pH - 7.28 + \log \left\{ \frac{[\text{GEDTA}] \text{ added}}{[\text{CaCl}_2] \text{ added}} - 1 \right\}$$

The pH was adjusted with Tris-maleic acid and NaHCO<sub>3</sub> eliminated from the Locke solution. When K<sub>2</sub>SO<sub>4</sub> solution was used to induce contracture of the muscle, NaCl and NaHCO<sub>3</sub> were completely replaced by K<sub>2</sub>SO<sub>4</sub> isotonicity and other ionic concentrations were not changed with Locke solution.

#### *Temperature Control*

When Krebs solution was used, most experiments were carried out at a temperature of 36°C using Coolnics (model CRT-1B Yamada, Tokyo, Japan), and when Locke solution was used, most experiments were carried out at a room temperature of 22°–26°C. When the temperature was cooled down to 5°C, the circulator required about 15 min to reach a steady state, although the rewarming of the bath solution up to 36°C required a much shorter time.

## RESULTS

### *Effects of Caffeine on the Electrical and Mechanical Properties of the Taenia Coli*

The effects of caffeine (5–10 mM) on the spontaneous mechanical activity could be classified into three stages; i.e., initial slow contraction, later phasic tension development, and relaxation phase.

Fig. 1 shows the effects of caffeine (10 mM) on the mechanical activity of the taenia coli in Locke solution at 26°C. The initial tension development was followed by phasic contraction and the amplitude of the phasic tension was gradually lowered and finally complete relaxation occurred. Often the initial phase was masked by the rapidly developed phasic tension. The initial tension appeared with a latency of 20–30 sec, and the relaxation of the tissue first appeared after 250–600 sec of perfusion in the caffeine-containing solution (10 mM).

Fig. 2 shows the effects of various concentrations of caffeine (1–8 mM) on the electrical and mechanical activity of the taenia coli in Locke solu-

tion at 22°C. The frequency of the spikes and the amplitude of the phasic tension increased in proportion to the increased concentration of caffeine. When the concentration of caffeine was increased to 3 mM, an increase in spike frequency appeared after a latency of about 5 sec (c). The spike activity then completely ceased with depolarization of the membrane (depolarization block of the spike) and relaxation occurred. The increased spike frequency

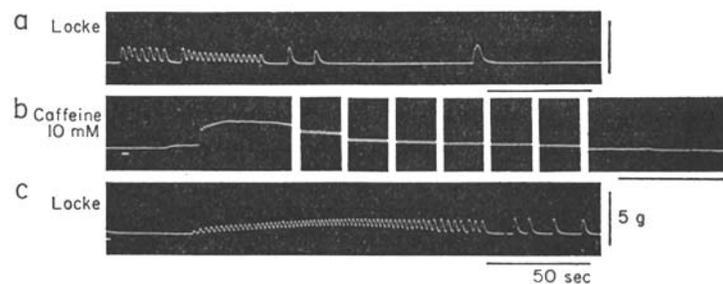


FIGURE 1. Effects of caffeine (10 mM) on the mechanical activity of the taenia coli. Intervals between records in b are 1 min. Horizontal bars on records indicate the application (b) and removal (c) of caffeine.

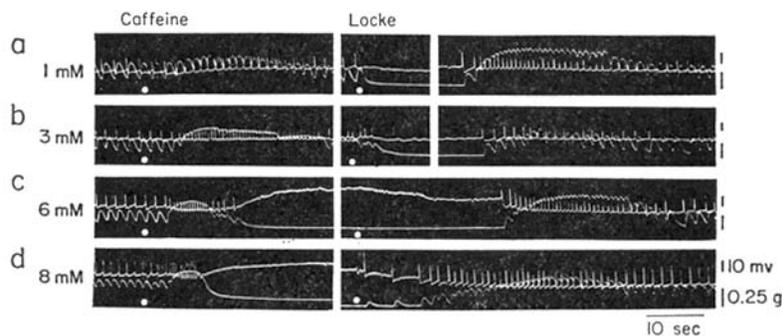


FIGURE 2. Effects of various concentrations of caffeine (1, 3, 6, and 8 mM) on the spontaneous electrical and mechanical activities of the taenia coli. Dots on the figure indicate application and removal of caffeine. When 6 mM (c) and 8 mM (d) of caffeine were applied to the tissue, the increased spike frequency caused markedly developed phasic tension thus artificially shifting the membrane potential level in the direction of hyperpolarization. Therefore, the measured values of the depolarization even after the tissue was relaxed may not be the true values of the depolarization.

and tension produced by caffeine corresponded to the second stage of the phasic tension development, and the stage of the depolarization block of the membrane for the spike generation corresponded to the relaxation stage of the tissue (third stage). The latencies of the initial tension increase and subsequent relaxation recorded with the tension-recording method were longer than the latencies recorded with the double sucrose-gap method. This is probably due to differences in thickness of the preparations.

*The Ions Involved in the Effects of Caffeine on the Electrical and Mechanical Activities*

When the tissue was perfused in the Ca-deficient solution, dissociation of the initial slow contraction and later phasic contraction became easier than in Locke solution.

Fig. 3 shows the effects of caffeine (1–10 mM) on the smooth muscle in Ca-deficient Locke solution ( $5 \times 10^{-4}$  M). When the concentration of caffeine was increased to more than 3 mM, the initial slow tension development could be observed separately from the later phasic contraction, but when the concentration was increased to 10 mM, the initial tension and later phasic tension developed successively.

In order to eliminate the later phasic tension development by treatment with caffeine, Tris was substituted for Na ion in Locke solution and the concentration of Ca ion was reduced to  $10^{-5}$  M. Fig. 4 shows the effects of caffeine (10 mM) in Na-free (Tris) with  $10^{-5}$  M Ca-Locke solution. In Locke solution, the outward current pulse triggered the spike with twitch contraction. When the tissue was perfused with the Ca-deficient and Na-free solution, the membrane was hyperpolarized (12 mv) and the membrane resistance was reduced (0.34 times the control). Treatment with caffeine (10 mM) slightly depolarized the membrane (4 mv) and increased the input resistance (1.1 times) compared with the results in the Na-free solution and produced marked tension development with a latency of 10 sec. The depolarization of the membrane on treatment with caffeine in the Na-free, Ca-deficient solution was not significant, since in the five specimens, the membrane was hyperpolarized to 12–16 mv in the Na-free, Ca-deficient solution and the maximum depolarization of the membrane by caffeine (10 mM) was only 5 mv. The input resistances of the membrane varied from 0.9 to 1.2 times the value obtained in the Na-free, Ca-deficient solution. These results might indicate that the initial tension development was not directly related to the ionic permeability across the cell membrane, but it might be due to release of bound Ca ion from the membrane. On the other hand, the second and third stages were closely related to increased Na conductance, since the membrane was depolarized and there was a decrease in membrane resistance when caffeine was applied in Locke solution.

Fig. 5 shows the effects of caffeine (10 mM) on the spikes and twitch tensions elicited by outward current pulses in Locke solution. At a concentration of 10 mM, caffeine strongly depolarized the membrane (12 mv) after 3 min. (b). At this time, the second stage of the spontaneous electrical activities had passed. After perfusion for 10 and 15 min (c) and (d), the membrane resistance, the spike amplitude, and twitch tension were decreased. Caffeine

produced depolarization block of the membrane activity elicited by electrical stimulation in addition to the spontaneous membrane activity. These

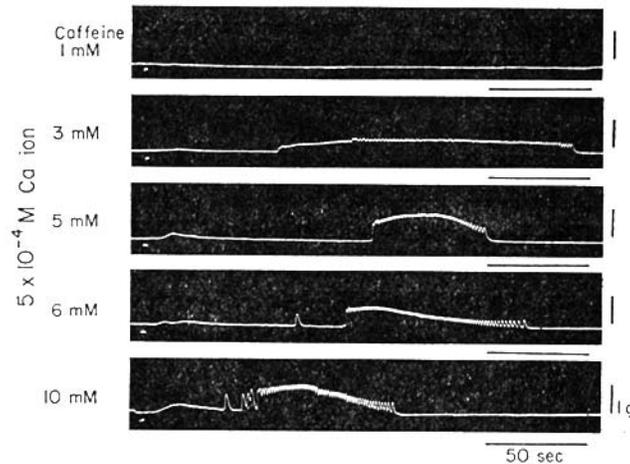


FIGURE 3. Effects of caffeine (1–10 mM) on the mechanical activity of the taenia coli in the Ca-deficient Locke solution ( $5 \times 10^{-4}$  M). Horizontal bars indicate the applications of caffeine to different tissues at the various concentrations. The tissues were perfused with the Ca-deficient Locke solution for 20–25 min before the applications of caffeine.

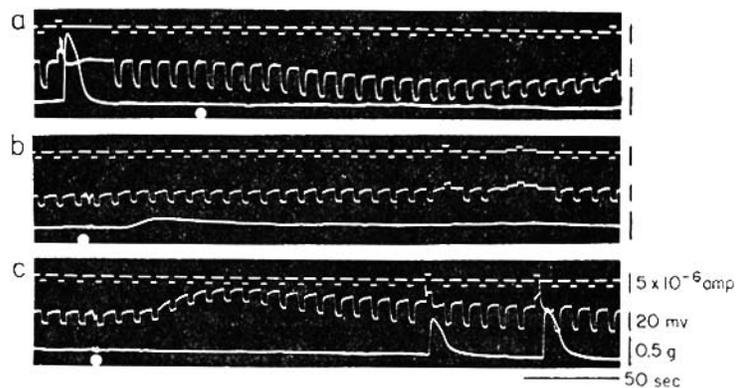


FIGURE 4. Effects of caffeine (10 mM) on the electrical and mechanical activities of the taenia coli in Na-free (Tris) with  $10^{-5}$  M Ca-Locke solution. Dot in a, replacement of Locke solution with Na-free solution. Dot in b, application of caffeine. Dot in c, replacement with Locke solution.

results might indicate that in Locke or Krebs solutions, caffeine continuously depolarized the membrane throughout the three stages due mainly to increase in the Na conductance.

*Minimum Concentration of Ca Ion Required to Induce the Tension Development by Caffeine*

Effects of caffeine (10 mM) on the mechanical activities at various concentrations of Ca ion were investigated. When external Ca ion was reduced to  $10^{-7}$  M with GEDTA buffer, caffeine produced a slight development of tension in the tissue.

Fig. 6 shows an example of the effects of caffeine at various concentrations of Ca ion ( $10^{-7}$ – $10^{-5}$  M) in Locke solution. Caffeine did not produce tension development when the concentration of Ca ion was  $10^{-8}$  M, and the minimum concentration required to produce tension development by caffeine was  $10^{-7}$  M Ca ion. The same concentration of Ca ion was also found to be necessary for “caffeine contracture” during  $K_2SO_4$ -induced contracture.

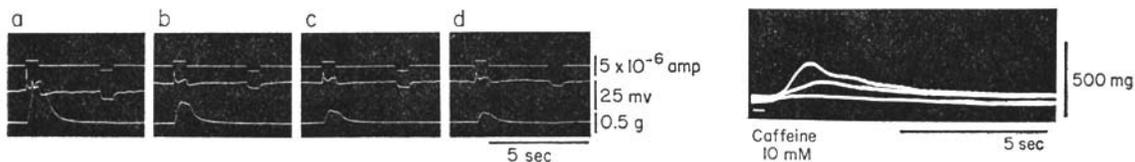


FIGURE 5

FIGURE 5. Effects of caffeine (10 mM) on the spike and twitch tension evoked by the electrical stimulation. a, control Locke solution; b, after 3 min; c, after 10 min; d, after 15 min.

FIGURE 6

FIGURE 6. Effects of caffeine (10 mM) on the mechanical activity of taenia coli in low concentrations of Ca ion. Ca ion in the solution was reduced to  $10^{-5}$ ,  $10^{-6}$ , and  $10^{-7}$  M (upper, middle, and lower contractions, respectively). Three contractions were superimposed photographically. Bar on the figure indicates the applications of caffeine.

Fig. 7 shows an example of the effects of caffeine on the taenia coli muscle in the  $K_2SO_4$ -Locke solution with various concentrations of Ca ion ( $10^{-7}$ – $10^{-5}$  M). When Locke solution was replaced with  $K_2SO_4$ -Locke solution, contracture of the tissue could be produced. The rapidly developed tension (called phasic response by Imai and Takeda, 1967) gradually declined to a sustained tonic tension (tonic response). When Ca ions were reduced in steps, the amplitudes of the initial rapidly developed tension were proportionally reduced in steps (b-d). After the tissue relaxed to a certain steady level 5 mM of caffeine was applied. At concentrations of Ca ion higher than  $10^{-7}$  M, caffeine again evoked the contracture (c). At a concentration of  $10^{-8}$  M Ca ion, the  $K_2SO_4$ -Locke solution evoked the contracture but there was no caffeine contracture after the K contracture.

The effects of simultaneous treatments with  $K_2SO_4$ -Locke solution and the various concentrations of caffeine (2–8 mM) were observed. As shown in Fig. 8, in the absence of caffeine, the  $K_2SO_4$ -Locke solution evoked the contracture

at 36°C which then gradually declined to a certain amplitude and was sustained for more than 10 min (tonic response). However, when caffeine (2 mM) was added to the K<sub>2</sub>SO<sub>4</sub> solution the sustained contracture was reduced in amplitude, and with more than 5 mM caffeine, the sustained contracture rapidly declined to the resting level within 2–3 min. In the presence or absence of caffeine, the amplitude of the initial contracture (phasic response) was nearly the same, but caffeine suppressed the tonic response of the contracture. These observations agreed with those of Sunano (1966) on the rat intestine. The tonic response of the K<sub>2</sub>SO<sub>4</sub>-induced contracture was also suppressed when the tissue was kept at low temperature.

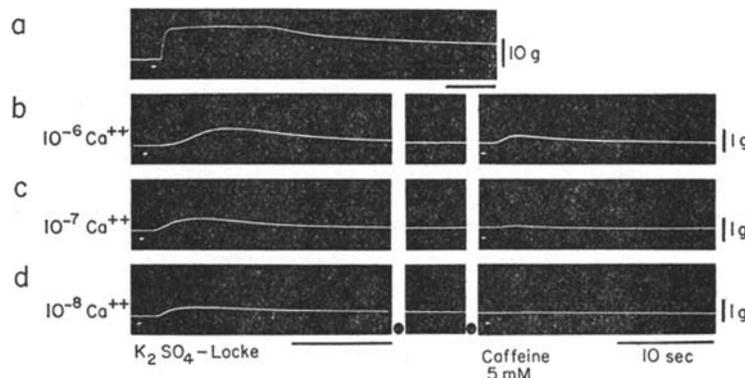


FIGURE 7. Effects of caffeine (5 mM) on the mechanical activity of the taenia coli during the K contracture at various concentrations of Ca ion ( $10^{-6}$  M in b,  $10^{-7}$  M in c, and  $10^{-8}$  M in d). The Ca concentration was reduced throughout each record. a, K contracture in K<sub>2</sub>SO<sub>4</sub>-Locke solution. Dots on the figures at left side indicate the application of K<sub>2</sub>SO<sub>4</sub>-Locke. Dots at right side indicate the treatment with caffeine. Interval between records in b, c, and d is 5 min.

Fig. 9 shows the effects of caffeine (0.5–15 mM) on the isotonic K<sub>2</sub>SO<sub>4</sub>-induced contracture at low temperature. When the tissue was kept at low temperature (5°C), the contracture of the tissue evoked by isotonic K<sub>2</sub>SO<sub>4</sub> lasted for only a few minutes and relaxed rapidly to a level just above that before the treatment. On treatment with caffeine (2–15 mM) the relaxed tissue developed tension but the amplitude was small. In a concentration of 0.5 mM, caffeine did not evoke the development of tension. The diphasic action of caffeine on the K<sub>2</sub>SO<sub>4</sub>-induced contracture depended on the temperature, and caffeine mainly influenced the tonic response.

An effect of caffeine similar to that on the suppression of the tonic response of the K<sub>2</sub>SO<sub>4</sub>-induced contracture was also observed on treatment with dinitrophenol (DNP). DNP is known to depolarize the membrane and increase the spike frequency but to reduce the tension development in the taenia coli (Axelsson and Bülbring, 1961).

Fig. 10 shows the effects of DNP ( $2 \times 10^{-5} M$ ) on the mechanical activity in  $K_2SO_4$ -Locke solution. The  $K_2SO_4$ -induced contracture was rapidly reduced in amplitude when DNP was applied with a latency of 2-4 min.

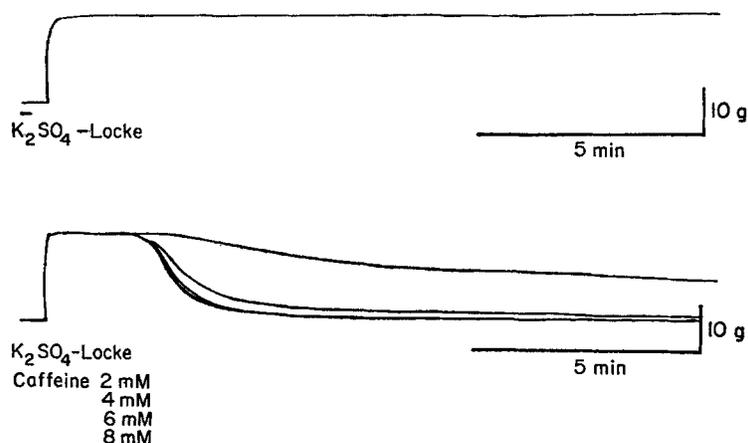


FIGURE 8. Effects of  $K_2SO_4$ -Locke solution with caffeine (2, 4, 6, and 8 mM) on the mechanical activity of the taenia coli. Bars indicate applications of isotonic  $K_2SO_4$  (upper trace) and of isotonic  $K_2SO_4$  with caffeine (lower trace). Four different experiments are superimposed in the lower trace. With more than 6 mM of caffeine, the course of the relaxation completely overlapped.

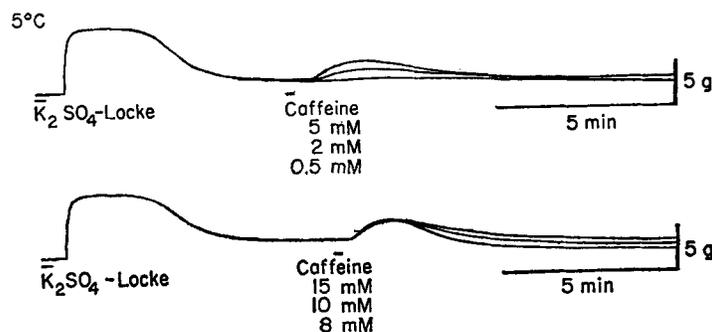


FIGURE 9. Effects of caffeine (0.5-15 mM) after treatment with isotonic  $K_2SO_4$  at  $5^\circ C$ . Upper record, 0.5, 2, and 5 mM of caffeine after treatment with  $K_2SO_4$ . Lower record, 8, 10, and 15 mM of caffeine after treatment with  $K_2SO_4$ . 0.5 mM of caffeine produced no response. Rise time and velocity of the caffeine-induced tension development remained the same when the concentration was increased beyond 8 mM but relaxation time was increased proportionally with the concentration of caffeine.

The effect of caffeine (5 mM) was also observed (Fig. 10) after treatment with  $K_2SO_4$ -Locke solution and DNP ( $2 \times 10^{-5} M$ ). Even after treatment with  $K_2SO_4$ -Locke solution and DNP, caffeine was still able to produce the contracture, but its amplitude and duration were much smaller.

*Caffeine Potentiation of Twitch Tension*

It is a well-known fact that caffeine potentiates the phasic contraction recorded from the frog striated muscle at low concentrations (below 5 mM) without enhancement of the spike amplitude (Sandow, 1965). In normal Locke and Krebs solutions at 36°C, caffeine did not potentiate the contraction of the taenia coli.

Fig. 11 shows the effects of various concentrations of caffeine (2–9 mM) on the membrane resistance, the spike amplitude, and contraction in the Na-free (Tris) Locke solution at 36°C. When Na ion was replaced with Tris, the membrane was hyperpolarized (6–12 mv,  $n = 6$ ) and the input resistance was reduced (0.48–0.72) times the control value,  $n = 6$ ). Spikes could

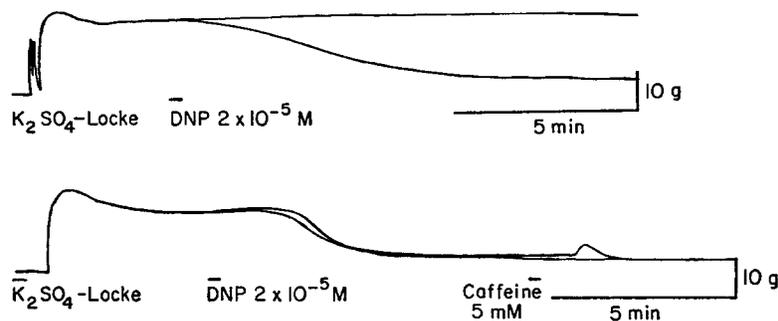


FIGURE 10. Effects of DNP ( $2 \times 10^{-5}$  M) on the K-induced contracture of the taenia coli. Upper record, application of DNP is indicated as a bar, and the effect of DNP on the contracture is superimposed on the contracture without DNP. Lower record, effects of DNP ( $2 \times 10^{-5}$  M) and caffeine (5 mM) on the K-induced contracture. Bars indicate the application of  $K_2SO_4$ -Locke solution, DNP, and caffeine successively. The two records with and without caffeine are superimposed.

be elicited by application of current pulses of stronger intensity than those applied to the control. The amplitude of the tension was either unchanged or was enhanced. Similar results were observed in the stomach muscle by Sakamoto and Kuriyama (1971). When concentrations of 2–5 mM caffeine were applied, the amplitude of the phasic tension was slightly enhanced (a-c). With increased concentrations of caffeine, the amplitude of the phasic contraction was again reduced (d). Lower concentrations of caffeine (0.5–1.5 mM) had no effect on the tension development. These results showed that the low concentrations of caffeine (2–5 mM) in the Na-free solution potentiated the twitch tension development without any marked change in the membrane potential and the membrane resistance.

When the temperature was reduced from 36° to 5°C, the membrane was slightly depolarized (3–5 mv). The spike and twitch tension could be elicited

by electrical stimulation. The durations of the spike and twitch tension were markedly prolonged. The amplitude of the twitch tension was reduced and the duration prolonged more significantly than the reduction of the spike amplitude.

Fig. 12 shows the effect of caffeine (0.5 mM) in Krebs solution on the taenia

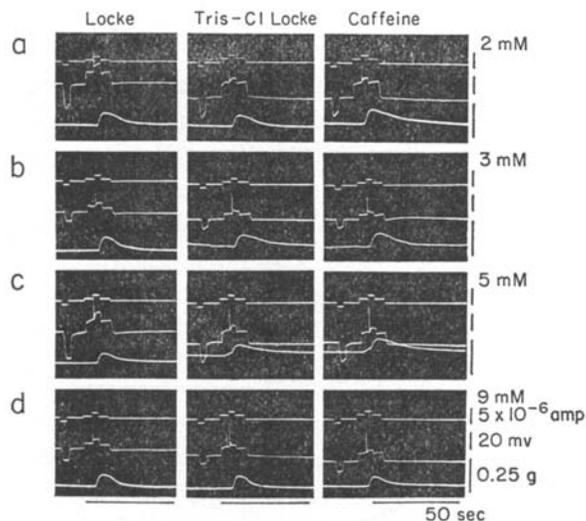


FIGURE 11. Effects of caffeine (2, 3, 5, and 9 mM) in the Na-free (Tris) solution on the electrical and mechanical activities evoked by outward current, and the changes of the membrane resistance. The records in the Na-free solution were taken after 20–30 min of exposure and the records in the Na-free solution with caffeine were taken after about 5 min of exposure.

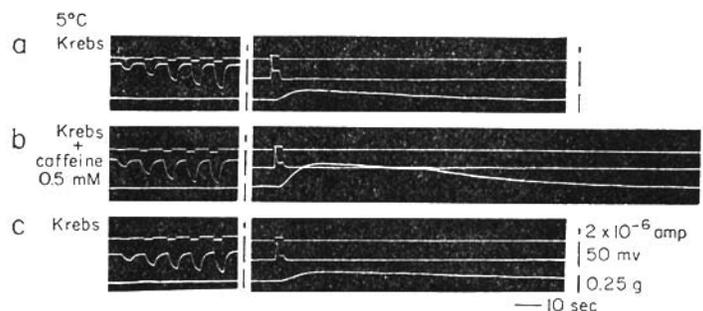


FIGURE 12. Effects of caffeine (15 mM) on the membrane resistance, spike, and contraction of the taenia coli at 5°C.

coli at low temperature (5°C). 0.5 mM caffeine modified neither the membrane potential nor the spike amplitude but markedly enhanced the amplitude and duration of the twitch tension. The membrane resistance was also not changed by treatment with caffeine (0.5 mM). The potentiation of the twitch tension could be observed up to concentrations of 2 mM, and further increase

in concentration of caffeine then reduced the amplitude of the twitch tension, and the membrane was depolarized. The critical concentration of caffeine between potentiation or inhibition of the twitch tension development was 2 mM at the low temperature (5°C) and it was 5 mM in the Na-free solution (36°C).

The effects of caffeine (0.5 mM) on the electrical and mechanical activities of the taenia coli in  $\text{NO}_3^-$  and Br-Krebs at 5°C were also observed, since replacement of the Cl ion with  $\text{NO}_3^-$  or Br ion hyperpolarized the membrane, reduced the membrane resistance, and enhanced the electrical and mechanical activities of the stomach smooth muscle (Sakamoto and Kuriyama, 1971). Fig. 13 shows the effects of caffeine (0.5 mM) on the electrical and mechanical activities of the taenia coli in  $\text{NO}_3^-$ -Krebs solution at 5°C. When Cl ion was replaced with  $\text{NO}_3^-$  ion at 36°C, the membrane was slightly

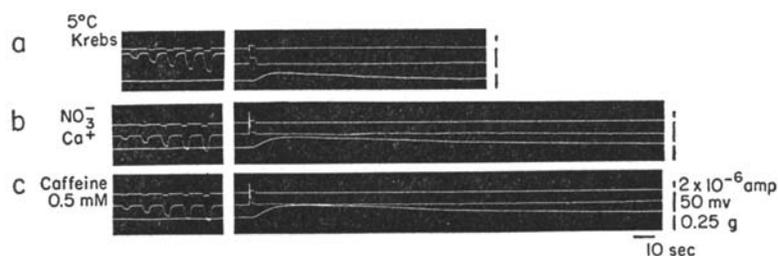


FIGURE 13. Effects of caffeine (0.5 mM) on the membrane resistance, spike, and contraction of the taenia coli in the Cl-deficient ( $\text{NO}_3^-$ ) solution of 5°C. 4.4 mM of Cl ion still remained in the Krebs solution. a, control; b,  $\text{NO}_3^-$ -Krebs; c,  $\text{NO}_3^-$ -Krebs with 0.5 mM caffeine.

hyperpolarized (3–8 mv,  $n = 5$ ) and the input resistance was decreased (0.86–0.71 times the control). At 5°C, the  $\text{NO}_3^-$  ion still reduced the membrane resistance compared with that in Krebs at 5°C (0.89 times the control value at 5°C,  $n = 3$ ), but no change of the membrane potential was observed. The amplitude and duration of the phasic tension were enhanced. When 0.5 mM caffeine was applied in  $\text{NO}_3^-$ -Krebs, the amplitude and duration of the phasic contraction were not changed further (c). Similar effects of caffeine were also observed in Br-Krebs. In the presence of Br ion, caffeine (0.5 mM) had no effect on the amplitude and duration of the twitch tension. These effects of  $\text{NO}_3^-$  and Br-Krebs solutions with no subsequent effect of caffeine might suggest that the anions, Br and  $\text{NO}_3^-$ , may act at the site where caffeine potentiates the phasic twitch contraction. However, even in the presence of  $\text{NO}_3^-$  and Br ions, increased concentration of caffeine up to 10 mM reduced the amplitude of the twitch tension.

## DISCUSSION

The discussion is based on the assumption that the mechanical contraction is triggered by calcium ion and the minimum concentration of calcium ion needed to evoke the tension is around  $10^{-6}$  M taken from the estimation made in frog myoplasm ( $2 \times 10^{-6}$  M [Ebashi and Endo, 1968]), and in crustacean muscle fiber ( $1.6-3.2 \times 10^{-6}$  M [Hagiwara and Nakajima, 1966]). Free calcium ion concentration of the smooth muscle in the resting state is also assumed to be around  $10^{-7}$  M as estimated in the frog muscle (Ebashi and Endo, 1968).

Calcium ion shows multiple action in the smooth muscle cell, namely stabilizing the membrane by reduction of the Na permeability in the resting state, carrying the current during the active state of the membrane, and triggering the contraction (Bülbring and Kuriyama, 1963; Nonomura, Hotta, and Ohashi, 1966; Brading, Bülbring, and Tomita, 1969; Sakamoto and Kuriyama, 1971). Influx of Ca ion during the active state of the membrane is probably enough to activate the chemomechanocoupling directly, since calculations based on the membrane capacity ( $5 \mu\text{F}/\text{cm}^2$ ), the maximum rate of rise of the spike (10 v/sec), and the large volume: surface ratio ( $12 \times 10^{-10}$  cm<sup>3</sup>:  $12 \times 10^{-6}$  cm<sup>2</sup>) indicate that the Ca influx (about  $1.2 \times 10^{-6}$  M) is sufficient to trigger the contraction (Kuriyama and Tomita, 1965; Tomita, 1966; 1970). This influx of Ca ions, however, may not lead directly to contraction following the spike. Sakamoto and Kuriyama (1971) postulated that the Ca ion which flows into the muscle fiber of the stomach as the current carrier may release at least a portion of the Ca ion bound to the membrane and this may activate the contractile protein, since even when the membrane potential and the spike amplitude remained the same in various ionic environments, the amplitude of the phasic tension was not uniform. They thought that the amount of calcium bound to the membrane might be modified by the various ions, namely Na and Cl ions. Moreover, Bülbring and Tomita (1970) and Kuriyama and Tomita (1970) thought that Ca ion was bound or adsorbed onto the muscle membrane competitively with Na ion. Reduction or absence of Na ion enhanced the amplitude of the spike and phasic tension when Cl was present. When Cl ion was replaced with NO<sub>3</sub> or Br ion, the electrical and mechanical activities were further enhanced in Na-free solution (Sakamoto and Kuriyama, 1971). The effects of caffeine on the taenia coli in various ionic environments further support the above postulate.

The results obtained from the present experiments suggest that caffeine mobilizes the bound Ca ion from the membrane, and as a result increases the Na permeability, thus modifying the electrical activity. In the Na-free

solution, a low concentration of caffeine potentiated the amplitude of the twitch tension without any changes in the membrane activity although the same concentration reduced the tension in Krebs or Locke solution.

Dissociation of the processes underlying the mobilization of Ca ion and the increased Na permeability could be observed at the low temperature (5°C); i.e., caffeine produced the contracture and potentiation of the phasic contraction without any remarkable changes in the membrane potential, membrane resistance, and the spike amplitude. At normal temperatures (36°C), the increased Na permeability suppressed the potentiation induced by caffeine as shown in the experiment in the Na-free solution. The depolarization block of the spike which appeared after treatment with caffeine (the third stage) was related to increased Na permeability in Locke solution and led to relaxation of the muscle due to the inactivation of the contraction mechanism. Presumably, the presence of Na ion might competitively act either on the same site with Ca ion at the membrane (Kuriyama and Tomita, 1970; Bülbiring and Tomita, 1970; Sakamoto and Kuriyama, 1971) or on the Ca-releasing site in the muscle cells (Ebashi and Endo, 1968), since in the Na-free solution, the contracture had a much longer duration than in the presence of Na ion.

On the role of Ca ion during the generation of the contracture, Urakawa and Holland (1964) proposed that in the phasic contraction of the guinea pig taenia coli, sufficient Ca ion was released from a cellular site to initiate contraction, whereas in the tonic contraction enough Ca ion crossed the membrane to initiate contraction. On the other hand, Imai and Takeda (1967) postulated that the tonic response of the contracture in the taenia coli was thought to be due to release of bound Ca ion from the smooth muscle.

In the present experiments, the tonic response of the contracture evoked by the isotonic K-Locke solution was suppressed by caffeine, low temperature, and DNP, although caffeine evoked the contracture of the relaxed tissue after treatment with the isotonic K-Locke solution at low temperature. Axelsson and Högberg (1967) also observed that caffeine relaxed contracture of depolarized taenia reversibly without change in transmembrane potential. Caffeine might accelerate the release of all bound Ca ion in the early stage so rapidly at 36°C, that the tissue would then relax without generation of the tonic response, and might also accelerate the release of bound Ca ion, which had been suppressed, thus evoking the contraction again at 5°C.

The effects of caffeine on the smooth muscle had some similarity to those of anions. When Cl ion was replaced with NO<sub>3</sub> ion in steps, the amplitude and duration of the tension of the taenia coli were enhanced in steps at 5 and 38°C. In the presence of NO<sub>3</sub> or Br ion, caffeine no longer potentiated the phasic tension even at low temperature (5°C).

In the skeletal muscle, caffeine and anions had only a small effect on the membrane activity although in the smooth muscle both markedly modified this activity. For example, when Cl ion was replaced with other anions, the membrane conductance was always reduced in the skeletal muscle (Hutter and Padsha, 1959). On the other hand, in the smooth muscle of taenia coli and stomach the membrane conductance was increased when Cl ion was replaced with anions of smaller hydration size, but a decrease was observed when Cl ion was replaced with larger anions (Brading et al., 1969; Sakamoto and Kuriyama, 1971). If the smooth muscle membrane possesses the properties of the reticulum, effects of caffeine and various anions on the membrane activity and tension development might be explained by the same mechanism, i.e. the low concentration of caffeine and the anions ( $\text{NO}_3$  and Br ion) enhanced the amplitude and prolonged the duration of contraction, presumably due to increased free Ca ion in the myoplasm caused either by the inhibition of the reabsorption of Ca ion on to the muscle membrane (Ebashi, 1965) or by acceleration of the mobilization of Ca ion.

The amount of bound Ca ion might be related to the external concentration of Ca ion, since when  $[\text{Ca}]_o$  was reduced to below  $10^{-7}$  M, no contracture was evoked on treatment with caffeine. The amplitude of the caffeine-induced contracture increased proportionally with  $[\text{Ca}]_o$ . Imai and Takeda (1967) found that the minimum concentration of Ca ion for the  $\text{K}_2\text{SO}_4$ -induced phasic response was  $10^{-7}$  M as we also observed, and the amplitude of the tonic response, which is thought to be due to the release of bound Ca ion, was changed proportionally with the external Ca ion. They postulated that these effects of the external Ca ion concentration on the  $\text{K}_2\text{SO}_4$ -induced tonic response were due mainly to the inactivation of the releasing mechanism for Ca ion bound in the muscle.

It is postulated that the sarcoplasmic membrane of the smooth muscle of the taenia coli possesses the properties of the sarcoplasmic reticulum which are distributed in the striated muscle cell. Presumably, Ca ion bound to the sarcoplasmic membrane is released by the influx of Ca ion in electrogenesis.

As a conclusion, from the effects of caffeine in the various ionic environments, it is proposed that the effects of caffeine on the smooth muscle are fundamentally the same as those on the striated muscle, but the mobilization of Ca ion from the smooth muscle membrane by caffeine modifies the ionic permeability of the membrane, thus causing the responses different from those of the striated muscle.

*Received for publication 9 September 1970.*

#### REFERENCES

- AXELSSON, J., and E. BÜLBRING. 1961. Metabolic factors affecting the electrical activity of intestinal smooth muscle. *J. Physiol. (London)*. 156:344.

- AXELSSON, J., and S. G. R. HÖGBERG. 1967. Effect of caffeine on electrical and mechanical activity of smooth muscle. *Acta Pharmacol. Toxicol.* **25**(Suppl. 4): 53.
- BRADING, A., E. BÜLBRING, and T. TOMITA. 1969. The effect of temperature on the membrane conductance of the smooth muscle of the guinea-pig taenia coli. *J. Physiol. (London)*. **200**:621.
- BÜLBRING, E., and H. KURIYAMA. 1963. Effects of changes in the external sodium and calcium concentrations on spontaneous electrical activity in smooth muscle of guinea-pig taenia coli. *J. Physiol. (London)*. **166**:29.
- BÜLBRING, E., and T. TOMITA. 1969. Increase of membrane conductance by adrenaline in the smooth muscle of guinea-pig taenia coli. *Proc. Roy. Soc. Ser. B. Biol. Sci.* **172**:89.
- BÜLBRING, E., and T. TOMITA. 1970. Effects of Ca removal on the smooth muscle of the guinea-pig taenia coli. *J. Physiol. (London)*. **210**:217.
- CHABAREK, S., and A. E. MARTELL. 1959. *Organic Sequestering Agents*. John Wiley and Sons, Inc., New York.
- EBASHI, S. 1965. The sarcoplasmic reticulum and excitation-contraction coupling. In *Molecular Biology of Muscular Contraction*. S. Ebashi, F. Oosawa, T. Sekine, and Y. Tonomura, editors. *Biochimica and Biophysica Acta Library*. **9**:197. Igaku-shoin, Tokyo, and Elsevier Publishing Co., Amsterdam.
- EBASHI, S., and M. ENDO. 1968. Calcium ion and muscle contraction. *Progr. Biophys. Mol. Biol.* **5**:125.
- HAGIWARA, S., and S. NAKAJIMA. 1966. Effects of the intracellular Ca ion concentration upon the excitability of the muscle fiber membrane of a barnacle. *J. Gen. Physiol.* **40**:807.
- HERZ, R., and A. WEBER. 1965. Caffeine inhibition of Ca uptake by muscle reticulum. *Fed. Proc.* **24**:208.
- HUTTER, O. F., and S. M. PADSHA. 1959. Effect of nitrate and other anions on the membrane resistance of frog skeletal muscle. *J. Physiol. (London)*. **146**:117.
- IMAI, S., and K. TAKEDA. 1967. Action of calcium and certain multivalent cations on potassium contracture of guinea-pig's taenia coli. *J. Physiol. (London)*. **190**:155.
- KURIYAMA, H., and T. TOMITA. 1965. The responses of single smooth muscle cells of guinea-pig taenia coli to intracellularly applied currents, and their effect on the spontaneous electrical activity. *J. Physiol. (London)*. **178**:270.
- KURIYAMA, H., and T. TOMITA. 1970. The action potential in the smooth muscle of the guinea-pig taenia coli and ureter studied by the double sucrose-gap method. *J. Gen. Physiol.* **55**:147.
- NEEDHAM, D. M., and C. F. SHOENBERG. 1968. Proteins of the contractile mechanism in vertebrate smooth muscle. In *Handbook of Physiology*. Sect. 6. Alimentary canal. C. F. Code, editor. American Physiological Society, Washington. **4**:1793.
- NONOMURA, Y., Y. HOTTA, and H. OHASHI. 1966. Tetrodotoxin and manganese ions; effect on electrical activity and tension in taenia coli of guinea-pig. *Science (Washington)*. **152**:97.
- PODOLSKY, R. J., and C. E. HUBERT. 1961. Activation of the contractile mechanism in isolated myofibrils. *Fed. Proc.* **20**:301.
- RITCHIE, J. M. 1954. The effect of nitrate on the active state of muscle. *J. Physiol. (London)*. **126**:155.
- SAKAMOTO, Y., and H. KURIYAMA. 1971. The relationship between the electrical and mechanical activity of the guinea-pig stomach. *Jap. J. Physiol.* **20**:640.
- SANDOW, A. 1965. Excitation-contraction coupling in skeletal muscle. *Pharmacol. Rev.* **17**:265.
- SANDOW, A., S. R. TAYLOR, A. ISAACSON, and J. J. SEGUIN. 1964. Electromechanical coupling in potentiation of muscular contraction. *Science (Washington)*. **143**:577.
- SUNANO, S. 1966. Caffeine contracture of the intestinal smooth muscle of rat (in Japanese). *Sapporo Med. J.* **29**:98.
- TOMITA, T. 1966. Membrane capacity and resistance in mammalian smooth muscle. *J. Theor. Biol.* **12**:216.
- TOMITA, T. 1970. Electrical properties of mammalian smooth muscle. In *Smooth Muscle*. E. Bülbiring, A. Brading, A. Jones, and T. Tomita, editors. Edward Arnold (Publishers), Ltd., London. Pp. 198-243.
- URAKAWA, N., and W. C. HOLLAND. 1964. Ca<sup>45</sup> uptake and tissue calcium in K-induced phasic and tonic contraction in taenia coli. *Amer. J. Physiol.* **207**:873.