INHIBITORY EFFECTS OF CAFFEINE ON DEVELOPED TENSION AND CALCIUM MOVEMENT IN GUINEA PIG TAENIA COLI IN HIGH-K MEDIUM

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Abstract—Since the correlation between the K-induced contracture and the Ca movement during the contracture in guinea-pig taenia coli has been clarified, the effects of caffeine were deemed worthy of investigation. The tonic contraction induced by 40 mM K disappeared within 5 min in the presence of caffeine above 7 mM. The inhibitory effect of caffeine was reversed by a high concentration of Ca added to the external medium. High-K added to the medium increased tissue Ca, Ca uptake and the size of cellular Ca fraction that did not exchange within 4 min (tightly bound fraction, TBF), and it decreased Ca efflux. An application of 7 mM caffeine to the muscle treated with high-K for 30 min restored all parameters to the control level. Further, caffeine had no effect on the developed tension of the glycerinated taenia induced by Ca, Mg and ATP. It was observed that 14C-caffeine entered the cell in the presence of high-K.

From these data, it is suggested that caffeine inhibits high-K induced contracture by inhibiting Ca influx into the contractile mechanism.

It has been reported that caffeine produces a transient contraction accompanied by a slight depolarization (1) by release of Ca ions from its binding site in the smooth muscle cell of guinea-pig taenia coli (2), and that it also abolishes the potassium-induced tonic response in taenia coli (1). However, there is no clear evidence on the correlation of the caffeine inhibition to the K-induced contracture with the Ca movement in taenia coli soaked in high-K medium with caffeine.

The present study was undertaken to clarify the effect of caffeine on the high-K induced contracture in relation to the Ca movement.

MATERIALS AND METHODS

Strips of taenia coli were isolated from male Hartley strain guinea pigs weighing about 500 g, and immersed in Tyrode solution bubbled with gas mixture (95% O₂ and 5% CO₂) at 37°C. The Tyrode solution contained (mM): NaCl 136.8, KCl 2.7, CaCl₂ 2.5, MgCl₂ 1.0, NaH₂PO₄ 0.4, NaHCO₃ 11.9 and glucose 5.5. 40 mM K (high-K) medium was made by subtracting 40 mM NaCl from normal Tyrode solution and adding 40 mM KCl.

Tension: Contractile responses were recorded isometrically by a strain-gauge transducer (Nihon Kohden).
Tissue Ca content, \( { }^{45}\text{Ca} \) uptake, \( { }^{45}\text{Ca} \) efflux and TBF: The measurement of tissue Ca content, \( { }^{45}\text{Ca} \) uptake and efflux, and the size of cellular Ca fraction which did not exchange within 4 min (tightly bound fraction, TBF) were carried out as described previously (3, 4).

Glycerinated muscle: The strips of taenia coli were placed in a glycerol solution and stored at 2°C. The glycerol solution which was kept at pH 6.8 contained 50% (V/V) glycerol, 50 mM KCl and 20 mM tris (hydroxymethyl)-aminomethane (Tris). After 24 hr the strips were placed in a fresh glycerol solution and stored at -15°C for 30 days. The glycerinated muscles were soaked in a solution containing 4 mM EGTA, 50 mM KCl and 20 mM Tris (pH 6.8) and the tension change was recorded isometrically. The concentration of free Ca ions was obtained by using an EGTA-Ca EGTA buffer system (5).

\( { }^{14}\text{C} \)-caffeine uptake: Muscle strips were incubated in the medium with \( { }^{14}\text{C} \)-caffeine plus high-K for periods of time up to 30 min. After the incubation period, muscle strips were treated with solubilizer (Soluene TM-100, Packard Co) and radioactivity was counted using a liquid-scintillation spectrometer (Packard type 2311). A tissue/medium (T/M) ratio of caffeine was calculated by dividing the total tissue radioactivity per gram of muscle by the radioactivity per ml of the Tyrode solution.

\( { }^{14}\text{C} \)-caffeine-1-methyl-\( { }^{14}\text{C} \) was obtained from New England Nuclear.

RESULTS

Tension

Effect of caffeine in various concentrations on the high-K induced contracture: Fig. 1 illustrates the inhibition of the high-K induced contracture by caffeine in various concentrations. When the muscle was treated with high-K, muscle tension developed then stayed at a steady level of about 10 g. After strips were incubated in the high-K medium for

![Graph](https://via.placeholder.com/150)

**Fig. 1.** Upper figure: Effects of caffeine in various concentrations on the tonic contraction induced by high-K (40 mM, isotonic). Lower figure: Effects of pretreatment with caffeine in various concentrations on the high-K contracture.
30 min caffeine was applied. One mM caffeine had a slight effect on the tonic contraction in the high-K medium. However, the drug above 7 mM gradually depressed the tonic contraction within 5 min. In another series of experiments, strips were incubated with caffeine for 15 min prior to the application of high-K. The pretreatment with 1 mM caffeine had a slight effect on the high-K induced contracture. However, high-K exhibited only a phasic contraction by the pretreatment with 7 mM caffeine. The pretreatment with 10 mM caffeine completely inhibited the effect of high-K (Fig. 1).

**Effect of external Ca on the caffeine inhibition to the high-K-induced contracture:** After 30 min of the high-K treatment, 7 mM caffeine was applied to the muscle. Moreover, 30 min after the caffeine application 10 mM Ca was added to the medium. The addition of external Ca reversely affected the caffeine inhibition.

![Diagram](image)

**Fig. 2.** Effect of external Ca on the response of caffeine in high-K medium.

![Graph](image)

**Fig. 3.** Correlation of tension change between external Ca concentration and high-K or high-K plus caffeine. The responses are plotted as % of maximal tension induced by high-K ([Ca], 2.5 mM).

- ● High-K, ○ High-K + 7 mM caffeine, ◯ High-K + 14 mM caffeine
of 10 mM Ca restored the high-K contracture which had been inhibited by caffeine. The restored contracture was again inhibited by bubbling of N₂ gas mixture (95% N₂ and 5% CO₂) (Fig. 2). The tension development restored by the higher concentration of Ca in high-K medium containing 7 mM caffeine increased according to the increase of Ca concentrations in the medium (Fig. 3). The high-K induced contracture in the high external Ca concentration (12.5 mM) was not completely inhibited by 7 mM caffeine. However, the N₂ gas bubbling inhibited the contracture (Fig. 2).

The glucose-depletion and/or the N₂ gas bubbling in the medium 30th min after the high-K application also showed a gradual decline of tension in the muscle. In the case of the inhibition to the high-K induced contracture by the metabolic inhibiting factors, the addition of Ca to the external medium did not produce any tension (Fig. 4).

These observations led to the view that the inhibitory action of caffeine may be due to the inhibitory effect on the process of transmembrane transport of Ca rather than the action on metabolic process.

Effect of caffeine on the Ca movement during the high-K induced contracture

It has been reported that the high-K induced contracture was accompanied by a significant change in the Ca movement; the increase in tissue Ca content, Ca net uptake and TBF and the decrease in Ca efflux (6, 7). In the present experiment we examined the effect of caffeine on the Ca movement after 30 min of the high-K induced contracture.

In the presence of high-K, tissue Ca increased gradually to 30 min and remained at the high level for 60 min. When caffeine was applied to the muscle 30 min after the addition of high-K, the increased muscle tension declined to the original level within 5 min and the tissue Ca decreased to control level within 15 min. Accompanying the abolition of the high-K induced contracture by caffeine, Ca uptake and TBF decreased to the control level (Fig. 5).

The change in ⁴⁵Ca efflux following the application of high-K to muscle was examined in the presence of 7 mM caffeine. Muscle strips were loaded with ⁴⁵Ca for 30 min in the Tyrode solution with high-K. After loading, the muscle strips were washed with a non-radioactive high-K Tyrode solution. This efflux curve was clearly slowed compared with the control curve which was obtained from the muscle loaded with ⁴⁵Ca for 30 min in normal
Fig. 5. Effects of caffeine on tissue Ca, Ca uptake and TBF during high-K induced contracture. High-K solution was added at time 0 and 7 mM caffeine was added time 30.

| Tissue Ca | Ca uptake | TBF |
|-----------|-----------|-----|
| Control   | □         | ■   |
| High-K    | □         | ○   | △   |
| High-K + Caffeine | □ | ○ | △ |

The tissue Ca content was determined by atomic absorption spectrophotometry.

Fig. 6. Effect of caffeine on the $^{46}$Ca efflux. Muscle strips were loaded with $^{46}$Ca for 30 min in Tyrode solution with high-K (40 mM, isotonic) solution. After loading, the muscle strips were washed with non-radioactive high-K Tyrode solution with or without 7 mM caffeine.

- High-K
- High K + 7 mM caffeine
- Tissue Ca content after 30 min in high-K solution

The tissue Ca content was determined by atomic absorption spectrophotometry.
Tyrode solution and washing with non-radioactive Tyrode solution without high-K (7). When the muscle strips were washed in the high-K medium with 7 mM caffeine, the $^{45}$Ca efflux was increased compared with muscles washed in high-K solution without caffeine (Fig. 6).

Thus, the abolition of the high-K induced contracture by caffeine correlated with facts that the increased tissue Ca, $^{45}$Ca uptake and TBF of the muscle in the high-K medium were decreased to control levels and the decreased $^{45}$Ca efflux was increased under the caffeine treatment.

**Effect of caffeine on the glycerinated taenia**

The effect of caffeine on the contractile proteins was observed. The glycerinated taenia caused the tension development in the presence of 5 mM Mg, 5 mM ATP and $1 \times 10^{-6}$ M Ca. Thirty min after the contraction, 7 mM caffeine was applied. Caffeine did not noticeably influence the tension of the glycerinated taenia (Fig. 7).

$^{14}$C-Caffeine uptake

Muscle strips were incubated with $^{14}$C-caffeine for periods of time up to 30 min. The T/M ratio was 0.46, 0.60, 0.67, 0.80 and 0.87 at the 1, 2, 5, 15 and 30 min after the incubation, respectively (Fig. 8). $^{14}$C-caffeine already entered the cell over the extracellular space measured by $^{14}$C-sorbitol (3) at the first 1 min. Moreover, $^{14}$C-caffeine also entered the

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**Fig. 7.** Effect of caffeine on tension of the glycerinated taenia coli.

**Fig. 8.** $^{14}$C-caffeine uptake by taenia coli in the presence of high K. High-K and $^{14}$C-caffeine plus 7 mM caffeine was added at time 0.

- 7 mM caffeine
- High K + 7 mM caffeine
cell in the presence of 40 mM K, in a similar manner to that in the normal medium.

DISCUSSION

Caffeine reportedly produces a decrease in the tension of taenia coli while having no effect on the depolarization induced by a high concentration of K (1), and in the present work the drug had no effect on the tension of the glycerinated taenia induced by Ca, Mg and ATP. These observations led to the hypothesis that the inhibitory effect of caffeine on the high-K induced contracture may not be due to inhibition of membrane excitability or by direct action on contractile proteins.

The inhibitory action of caffeine on high-K induced contracture was reversed by high external Ca. In the treatment with metabolic inhibitory factors, glucose depletion and/or N₂ gas bubbling, the addition of Ca did not restore the tension. Bülbirg and Kuriyama (8) reported that after prolonged exposure to glucose-free solution excess, Ca no longer restored the membrane activity but still restored the tension development. In the present experiments, glucose was removed from the external medium during K-induced contracture, therefore the energy source to maintain contraction may be decreased faster. The inhibitory effect of caffeine on high-K induced contracture was antagonized according to the increase of Ca concentration in the external medium; which suggests that caffeine acted on the Ca movement during high-K induced contracture.

It has been reported that the high-K induced contracture is accompanied by a significant change in the Ca movement; the increase in the tissue Ca content, Ca net uptake and slowly exchanging Ca fraction in the cell (TBF) and the decrease in Ca efflux (6, 7). From these data, it was proposed that the increased intracellular ionized Ca, resulting from the increased Ca uptake, interacts with contractile proteins to induce contraction, from which the Ca ion is taken up continuously at the accumulating site (6, 7). When the effect of caffeine on the Ca movement after 30 min of high-K induced contracture was examined, the increased tissue Ca, Ca uptake and TBF in high-K solution were decreased to the control level and the decreased Ca efflux was increased. These results indicate that caffeine may principally inhibit Ca uptake resulting in a decrease in the increased tissue Ca and TBF of the muscle in the high-K solution. Further, a marked increase in ⁴⁵Ca efflux by caffeine may be due to a decreased tissue Ca and TBF. It is reasonable to assume that caffeine lowers the increased intracellular Ca ions concentrations during the high-K treatment resulting in the inhibition of K-induced contracture.

Pfaffmann and McFarland (9) noted the possibility that the inhibitory effect of theophylline on high-K induced contracture of taenia coli may be partially mediated through an increase in intracellular level of cyclic AMP. On the other hand, Tsuda et al. (10) reported that papaverine abolished the high-K induced tonic response in taenia coli and inhibited the increased ⁴⁵Ca uptake, but the elevated tissue Ca content remained in the high-K solution. Papaverine also had no effect on ⁴⁵Ca efflux. Papaverine (11, 12) as well as caffeine (13), increases the level of cyclic AMP in the cell. Both papaverine and caffeine inhibited the high-K induced tonic response in taenia coli. However, these two
drugs acted on the Ca movement in the smooth muscle not entirely in the same way. Thus, we hesitate to rule out the possibility that the site of both drugs on the Ca movement in the muscle is related to a change in cyclic AMP level which in turn may play an essential role in the regulation of Ca movement in the plasma membrane in smooth muscle.

14C-caffeine entered the sartorius muscle in frog rapidly (14), and 14C-caffeine also entered the smooth muscle cell of guinea-pig taenia coli in the presence or absence of high-K. Whether or not the site of action of caffeine is located in the membrane or cytoplasm in muscle cell has yet to be elucidated.

In conclusion, our experiments support the data that caffeine inhibits high-K induced contracture by inhibiting Ca influx into the contractile mechanism while having no preventive effect on the membrane excitability (1) or the contractile proteins.

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