EFFECT OF CAFFEINE ON CONTRACTILE ACTIVITY AND CALCIUM MOVEMENT IN GUINEA PIG TAENIA COLI

Tetsuyuki NASU* and Norimoto URAKAWA

Department of Veterinary Pharmacology, Faculty of Agriculture,
University of Tokyo, Bunkyo-ku, Tokyo, Japan

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* Present address: Department of Chemical Pharmacology, Faculty of Pharmaceutical Sciences, University of Tokushima, Shomachi, Tokushima, Japan.

Abstract—The effect of caffeine on contractile activity and Ca movement was investigated in guinea pig taenia coli. Caffeine at a concentration of 7 mM produced a contraction for approx. 7 min and increased $^{45}$Ca influx and efflux without a change in Ca content in the tissue. The effect of caffeine on Ca movement was the same as that of carbachol in the early phase of the contractile response. The caffeine-contraction was not potentiated by an addition of Ca up to four times that of normal concentration, however, the carbachol-contraction was potentiated. When the muscle was soaked in a Ca-free solution, it showed "Ca-free contraction" and a loss of the tissue Ca content. When caffeine was added immediately after the "Ca-free contraction" was relaxed, caffeine produced a transient contraction and increased the loss of the tissue Ca content. Under identical circumstances, carbachol did not induce a contraction or affect the Ca content of the tissue. In conclusion, caffeine was demonstrated to increase Ca exchangeability in smooth muscle cells as did carbachol, however, the results suggested the possibility that caffeine releases a bound Ca in the cell for contraction whereas carbachol induces a contraction by an increase in a permeability of the cell membrane to Ca ions.

It is generally accepted that caffeine produces a contracture and does not alter the resting membrane potential of the frog skeletal muscle (1) and the mechanism of action on the cellular level is also well known (2).

In the smooth muscle of guinea pig taenia coli, caffeine produces a transient contraction accompanied by slight depolarization (3). There is no clear evidence however concerning Ca movement during a contraction induced by caffeine in taenia coli.

The present study was undertaken to elucidate the effect of caffeine on contractile activity and Ca movement in guinea pig taenia coli.

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 95% O₂ and 5% CO₂ at 37°C. The 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.

A Ca-free Tyrode solution was identical to the normal except that CaCl₂ was not added. Another Ca-free solution contained 4 mM EGTA. All solutions were adjusted to pH 7.2.

* Present address: Department of Chemical Pharmacology, Faculty of Pharmaceutical Sciences, University of Tokushima, Shomachi, Tokushima, Japan.
Tension: Contractile responses were recorded isometrically by a strain-gauge transducer (Nikon-Kohden).

Tissue Ca content, \(^{45}\)Ca uptake and efflux: The measurement of tissue Ca content, \(^{45}\)Ca uptake and efflux were carried out as described previously (4, 5).

Loss of total Ca content from taenia coli incubated in the Ca-free solution: A tissue Ca content of taenia coli after a transfer from the normal solution to the Ca-free solution was determined. The strips were exposed to the Ca-free solution with or without EGTA and were then treated with caffeine or carbachol at 0 to 5 or from 30 to 45 min after the depletion of external Ca from the medium.

RESULTS

Tension change: Typical recordings of mechanical response observed after an application of caffeine are seen in Fig. 1. When 1 mM caffeine was applied to the muscle, the drug enhanced a spontaneous contraction or induced small rhythmic contractions in the resting muscle. Caffeine increased the size of contraction with increasing concentration to 10 mM. Muscle exposed 7 mM caffeine maintained a contraction for about 7 min. Moreover, the contractile activity of 7 mM caffeine was not affected by \(3 \times 10^{-6}\) M tetrodotoxin.

![Fig. 1. Tension change curves of taenia coli treated with various concentrations of caffeine.](image)

Ca movement during a caffeine-induced contraction: Ca content of the muscle in normal Tyrode solution was 4.6 ± 0.2 mEq/kg wet wt. of the tissue (8). The Ca content was not affected by the application of 7 mM caffeine for 30 min. \(^{46}\)Ca uptake of the muscle increased when \(^{46}\)Ca was added simultaneously with 7 mM caffeine. When \(^{46}\)Ca was added 15 min after the addition of caffeine, \(^{46}\)Ca uptake of the muscle did not differ from the control value (Fig. 2). In the experiment of \(^{45}\)Ca efflux, the muscle was loaded with \(^{45}\)Ca for 60 min, then it was washed out with a non-radioactive normal solution at an interval of 4 min. The rate of \(^{45}\)Ca efflux was calculated from the radioactivity of effluent in each test tube and was plotted as a fraction of the initial rate. The amount of \(^{45}\)Ca remaining in the strips was also plotted. When the muscle was treated with 7 mM caffeine for 4 min after the first 14 min of the washout period, the efflux curve exhibited an increase in \(^{45}\)Ca efflux (Fig. 3).

During the 7 mM caffeine-induced contraction, the Ca exchange in the smooth muscle cell increased without a change in Ca content in the tissue.
Fig. 2. Changes in tissue Ca and \(^{45}\)Ca uptake of taenia coli in the presence of 7 mM caffeine.

| Conditions | Tissue Ca | \(^{45}\)Ca uptake |
|------------|-----------|------------------|
| Control    | ■         | ●                |
| Caffeine   | □         | ○                |

Fig. 3. Effect of caffeine on \(^{45}\)Ca efflux from taenia coli.
- Upper curve: Radioactivity in the muscle (left ordinate).
- Lower curve: Rate of \(^{45}\)Ca efflux (right ordinate).

Seven mM caffeine was added at the point between two arrows.

Analysis of the contractile response to caffeine: In the present experiments, the contractile response to caffeine coincided with the period when the Ca exchange was increasing and the muscle relaxed at a time when the Ca exchange did not differ from the control value. On the other hand, \(1 \times 10^{-6}\) M carbachol was reported to increase the Ca exchange in the early phase of the contractile response in taenia coli (5). Accordingly, the effect of caffeine on Ca movement was the same as that of carbachol in the early phase.

The following experiments were performed to elucidate the source of Ca ions in the initiation of contractions induced by caffeine and carbachol.

(a) Effect of various Ca concentrations in medium on the drug responses: Maximum tensions during contractions induced by 7 mM caffeine or \(1 \times 10^{-6}\) M carbachol were measured in solutions which varied from 1 to 10 mM in Ca concentration. The external Ca concentration above 2.5 mM had no effect on the response to 7 mM caffeine as shown in Fig. 4. However, maximum was potentiated in the presence of \(1 \times 10^{-6}\) M carbachol by external Ca above 2.5 mM. The maximum tension of the contraction by 7 mM caffeine did not depend on the external Ca concentration above 2.5 mM while that by \(1 \times 10^{-6}\) M carbachol did reveal dependence.
(b) Effect of drugs on the contractile activity of the muscle in the Ca-depleted medium:

The muscle strips had been previously incubated in normal solution (Ca 2.5 mM) for 60 min, after which the bathing medium was exchanged with a Ca-free solution containing 4 mM EGTA. The transfer from normal solution to Ca-free solution caused a transient contraction in the muscle, which was termed as the "Ca-free contraction". Seven mM caffeine or $1 \times 10^{-6}$ M carbachol was added immediately after the "Ca-free contraction" was relaxed. Seven mM caffeine produced a transient contraction, having 1.5 g of muscle tension for about 2 min, however, $1 \times 10^{-6}$ M carbachol did not induce any response.

Fig. 4. Maximum tension induced by 7 mM caffeine or $1 \times 10^{-6}$ M carbachol in various external Ca concentrations.

![Graph showing maximum tension induced by caffeine and carbachol at different Ca concentrations.]

Fig. 5. Effect of 7 mM caffeine or $1 \times 10^{-6}$ M carbachol on the contractile activity of the muscle in the Ca-depleted medium. In one series of experiments, 7 mM caffeine was added immediately after the "Ca-free contraction" relaxed. In another series, different muscle was equilibrated for 30 min in Ca-free solution after which the muscle was treated with caffeine. The upper half of the figure demonstrates results of two series of experiments drawn together.

In the lower figure, $1 \times 10^{-6}$ M carbachol was added following the same schedule as used for application of caffeine.
(Fig. 5). In the next experiments, muscle strips were equilibrated for 30 min in Ca-free solution containing 4 mM EGTA, then the muscles were treated with 7 mM caffeine or $1 \times 10^{-6}$ M carbachol. There was no contractile response with either caffeine or carbachol.

(c) Effect of the drugs on Ca content in the tissue in the Ca depleted medium: Transfer of the muscle from the normal solution to the Ca-free solution with or without 4 mM EGTA caused a loss of Ca content in the tissue as shown in Fig. 6. More Ca was lost from the tissue in the Ca-free solution containing 4 mM EGTA than in the Ca-free solution without EGTA. Calcium content in the tissue was reduced to 30% of its original after 5 min, 12% after 30 min, 9% after 120 min in Ca-free solution containing 4 mM EGTA. A part of tissue Ca was lost faster within 30 min while another part was lost much more slowly in the Ca-free solution. There was no significance in tissue Ca loss in the Ca-

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![Figure 6](image)

**Fig. 6.** Loss of total Ca from taenia coli incubated in Ca-free solution.

![Figure 7](image)

**Fig. 7.** Loss of Ca content from taenia coli treated with 7 mM caffeine (A) and $1 \times 10^{-4}$ M carbachol (B) in Ca depleted medium.

\[ \text{Ca}_{o} = 2.5 \text{ mM} \]

- **Ca-free** - EGTA 5 mM
- **Ca-free** - EGTA 4 mM + Caffeine 7 mM or Carbachol $1 \times 10^{-4}$ M

In one series of experiments, 7 mM caffeine or $1 \times 10^{-4}$ M carbachol was added for 5 min immediately after the Ca depletion. In another series, different muscles were equilibrated for 30 min in Ca-free solution then muscles were treated with caffeine or carbachol for 15 min. Results of these two series are drawn together.
free solution containing 4 mM EGTA bubbled with 95% N₂ and 5% CO₂ gas compared with that bubbled with 95% O₂ and 5% CO₂ gas (Fig. 6).

Muscles treated with 7 mM caffeine for 5 min during exposure to Ca-free solution containing 4 mM EGTA had a lower Ca content in the tissue than the untreated muscle (Fig. 7-A). However, the loss of tissue Ca was rather inhibited by the application of $1 \times 10^{-6}$ M carbachol under the same experimental schedule as the application of caffeine (Fig. 7-B). After muscles had been exposed to the Ca-free solution containing 4 mM EGTA for 30 min, they were then treated with 7 mM caffeine or $1 \times 10^{-6}$ M carbachol for 15 min. The loss of tissue Ca remained unchanged (Fig. 7-A, B).

When caffeine was added at the same time the "Ca-free contraction" was relaxed, caffeine produced a transient contraction accompanied with loss of Ca content in the tissue which indicates a Ca release from the cellular site.

**DISCUSSION**

In the frog's skeletal muscle, a contraction induced by caffeine is sustained as long as caffeine remains in the bath and this drug effect is not mediated by changes in the resting potential (1). The smooth muscle produces, however, a contraction accompanied by slight depolarization by an application of caffeine (3). Thus caffeine has a different effect on the membrane excitability in both kinds of muscles.

On the other hand, caffeine increases resting Ca influx and efflux in the skeletal muscle and does not require the presence of Ca in the bathing medium in order to reveal its action and Ca efflux in Ca-free solution is also increased by caffeine (6), moreover, the store of Ca is depleted by successive caffeine contractures when the muscle is immersed in Ca-free solution (7). With an adequate dose of caffeine, Ca was released from frog isolated sarcoplasmic reticulum and this accounted for the force of the contracture which was inhibited by procaine (2). Purified phospholipase C which is known to destroy the ability of the sarcoplasmic reticulum to induce relaxation and to accumulate Ca ions, abolished caffeine-induced contraction of the skinned fibres (8). These results confirmed that the caffeine-induced contracture in skeletal muscle is a consequence of the release of Ca from the sarcoplasmic reticulum.

In smooth muscle of taenia coli, 7 mM caffeine caused an increase in Ca influx and efflux without the change of Ca content in the tissue during the contraction, thus demonstrating that, caffeine increased the Ca exchangeability in the muscle cell. The effect of caffeine on Ca movement in the present paper was demonstrated to be the same as the effect of carbachol in the early phase of the contractile response (5). However, another series of experiments was attempted to elucidate the source of Ca in the initiation of contractures induced by caffeine and carbachol. Seven mM caffeine-induced contraction was independent on external Ca above 2.5 mM, while muscle contraction induced by $1 \times 10^{-6}$ M carbachol was dependent. Moreover, 7 mM caffeine produced a transient contraction immediately after the "Ca-free contraction" while $1 \times 10^{-6}$ M carbachol produced no contraction. Under the same conditions, caffeine increased the loss of Ca
content in the tissue in Ca-free medium, while such was reduced with carbachol. On the other hand, the muscle exposed to Ca-free solution for 30 min did not exhibit contractile responses to caffeine or carbachol and neither of these drugs changed the rate of loss of Ca content in the tissue.

From these results, it is suggested that caffeine releases Ca for the contraction from the binding sites in the cell, after which extracellular Ca ions are supplied to the intracellular Ca sites, resulting in an increase in Ca exchangeability, and that sequestered Ca in the muscle cell which declines to 12% of its original Ca content in the tissue after the exposure for 30 min in Ca-free solution is not utilized for contraction by caffeine. Furthermore, it is proposed that the release of bound Ca in the cell is essential for the caffeine-induced contraction. On the other hand, the carbachol-induced contraction may be caused by an increase in a permeability of cell membrane to Ca ions.

Thus it is considered that the effect of caffeine on the Ca movement in taenia coli is fundamentally the same as it is on the Ca movement in intact skeletal muscle (Table 1). However, as the endoplasmic reticulum is not a well-developed system in the smooth muscle (9), it is still obscure whether or not sarcoplasmic reticulum of the smooth muscle is a source of intracellular Ca for caffeine-induced contraction.

**Table 1. Effect of caffeine on Ca movement in guinea pig taenia coli or frog sartorius muscle.**

| [Ca]o | Skeletal muscle (frog, sartorius m.) | Smooth muscle (guinea pig, taenia coli) |
|-------|-----------------------------------|----------------------------------------|
| rest  | Ca influx ↑                        | Ca influx ↑                            |
|       | Ca efflux ↑                       | Ca efflux ↑                            |
| K-depol.| Ca influx ↑                     | Ca influx ↑                            |
|       | Ca efflux ↑                       | Ca efflux ↑                            |
|       | (Bianchi, '61)                    | (Bianchi, '61)                         |
| [Ca]o excess | no observable effect on the contracture | no observable effect on the contraction |
|       | (Axelsson et al., '58)            | (Axelsson et al., '58)                 |
| [Ca]o 0 mM | contracture                       | transient contraction                   |
|       | Ca efflux ↑                       | tissue Ca ↓                            |
|       | (Bianchi, '61)                    | (Bianchi, '61)                         |

As spontaneous contraction can usually be elicited in smooth muscle, the state of smooth muscle in normal solution is termed as "normal".

It has been shown in a previous paper (5) that changes in Ca exchange by various stimulants in guinea pig taenia coli can be classified into three types, 1. increase in Ca exchange, 2. increase in Ca net influx and subsequent accumulation, 3. release of cellular Ca and subsequent accumulation. The effect of caffeine and cholinergic drugs in the early phase on Ca movement is classified under type 1. However, the initiation of contraction by caffeine or carbachol would necessarily be related to the different Ca movements, as mentioned above. Accordingly, it is reasonable that types of the Ca movement by carbachol and caffeine be identified as subtype a and b of the type 1, respectively.
REFERENCES
1) AXELSSON, J. AND THESLIF, S.: Acta Physiol. scand. 44, 55 (1958)
2) WEBER, A. AND HERZ, R.: J. gen. Physiol. 52, 750 (1968)
3) McFARLAND, S.A. AND PFAFFMAN, M.A.: Arch. int. Pharmacodyn. 198, 49 (1972)
4) NASU, T., KARAKI, H., IKEDA, M. AND URAKAWA, N.: Japan. J. Pharmacol. 21, 597 (1971)
5) NASU, T. AND URAKAWA, N.: Japan. J. Pharmacol. 23, 553 (1973)
6) BIANCHI, C.P.: J. gen. Physiol. 44, 845 (1961)
7) FRANK, G.B.: J. Physiol. 163, 254 (1962)
8) ENDO, M., TANAKA, M. AND OGAWA, Y.: Nature 228, 34 (1970)
9) DERVINE, C.E., SOMLYO, A.V. AND SOMLYO, A.P.: J. cell. Biol. 52, 690 (1972)