Caffeine-induced contraction of rat portal vein and effects of K-depolarization, Na-removal and low temperature

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Abstract

Contribution of Ca influx from extracellular pool and Ca-release from store sites in caffeine-induced contraction of rat portal vein longitudinal muscle were examined. At 37°C caffeine induced a phasic contraction and the contraction was inhibited by verapamil or in the absence of Ca. Under low temperature, it was not decreased remarkably by verapamil or by the removal of extracellular Ca. Na-removal potentiated caffeine-induced contraction in the absence of Ca. Caffeine-induced contraction was also potentiated by high K-depolarization. These contractions were at both temperature inhibited greatly by ryanodine. Caffeine induced the burst of the action potential at 37°C but it was not remarkable at 17°C. These results indicate that both extracellular Ca influx and release of stored Ca are involved in the caffeine-induced contraction. However, dependence of the contraction on Ca sources are influenced by temperature, extracellular Na and membrane potential.

Caffeine is known to release stored Ca and, thereby, induces contraction in the absence of extracellular Ca (Bond et al., 1984). Caffeine has also been shown to induce membrane excitation increasing spike discharges in the presence of extracellular Ca (Nanjo, 1984) in guinea-pig portal vein. These results indicated that Ca influx due to an increase of electrical activity is also responsible for the caffeine-induced contraction in guinea-pig portal vein. In rat portal vein, on the other hand, it has been reported that caffeine induces only phasic contraction both in the presence and absence of Ca (Dacquet et al., 1987), or the contraction can hardly be induced in Ca-free solution (Feletou et al., 1986). Thus, contribution of Ca release to the caffeine-induced contraction is still controversial in rat portal vein. Several mechanisms can be considered to contribute to the weakness or the abolition of the caffeine-induced contraction in the absence of Ca in rat portal vein. In the present study, contribution of Ca-influx from extracellular pool and Ca release from store sites in caffeine-induced contraction and influences of possible mechanisms which may affect caffeine-induced contraction in rat portal vein were studied.

Material and methods

Male Wistar rats of 16 weeks old were anesthetized with ether and sacrificed by bleeding. Portal veins were extracted immediately and attached tissues were removed under a binocular
microscope. Adventitia of the vein was also removed and longitudinal muscle preparations of 200 µm wide, 5 mm long were made. The preparations were suspended in organ baths containing 10 ml incubating medium. One end of the preparation was fixed to a force-displacement transducer (Shinkoh U gage 2.0) with tungsten wire (30 µm diameter) by stretching to 1.5 times length of relaxed state which provided maximum contraction of the tissue in previous study (Shimamura et al., 1989). They were allowed to stand for 2 hr before experiments and then mechanical activities of preparations were recorded isometrically. The composition of the normal incubating physiological salt solution was; NaCl 137, KCl 5.4, NaH2PO4 • 2H2O 0.4, NaHCO3 11.9, MgCl2 1.0, CaCl2 2.0 and glucose 5.6 in mM. The solution was aerated with gas mixture of 95% O2 and 5% CO2 maintaining pH at 7.3. Ca-free solution was made by omission of CaCl2 from the Tyrode’s solution. High K (K 80 mM) solution was made by equimolar replacement of Na with K. Na-free solution was made by equimolar replacement of NaCl, NaHCO3 and NaH2PO4 respectively with Tris-HCl, KHCO3 and KH2PO4. The temperature of the incubating medium was controlled by changing temperature of outer chamber of organ bath with circulating water of desired temperature. Temperature of incubating medium was monitored with thermistor probe. When the temperature was changed, tissues were allowed to stand at least 30 min at new temperature for further experiment.

Membrane electrical activities of smooth muscle cells in the longitudinal layer were recorded with microelectrode technique as reported in previous paper (Shimamura and Sunano, 1988). Briefly, each vein was cut longitudinally and adventitia was removed to expose longitudinal muscle. Preparation was mounted on a silicon rubber in a chamber of 0.5 ml volume and was superfused continuously with physiological solution at a rate of 3 ml/min. Glass capillary microelectrodes filled with 3M KCl (tip resistance 30–50 megohm) were inserted to the longitudinal muscle layer of the tissue. In the most of the experiments, mechanical activities of preparation were recorded simultaneously connecting one end of preparation to a force-displacement transducer.

Caffeine was dissolved in incubation mediums described above. Ryanodine was dissolved in ethylalcohol and diluted with Tyrode’s solution. Final concentration of ethylalcohol in the bath solution containing ryanodine 10 µM was 0.01% which was demonstrated to induce no effect in preliminary experiment. Ryanodine treatment was performed by incubating the tissues in Tyrode’s solution containing ryanodine 10 µM for 1 h.

Prior to each caffeine contraction, preparations were subjected to K 80 mM induced-contracture for 10 minutes. Caffeine was applied by exchanging half of incubating solution with a solution containing caffeine of double of expected concentration to avoid mechanical disturbance. After each caffeine application, preparation was washed twice with Tyrode’s solution at 10 min interval. These procedures were applied at intervals of over 1 hr.

Heights of caffeine-induced contraction were expressed as percentages of high (80 mM) K-induced phasic contraction at 37°C. When there is a sustained tension increase such as in high K or in the absence of Na, height of caffeine-induced contraction was measured from the elevated level to the peak. Duration of phasic contraction in control medium was measured at the level of 25% of peak height.

Values are expressed as mean ± SE with number of preparation except in data of resting
membrane potential where number of cells are indicated. Student's t-test was used for statistical evaluation.

Following drugs were used in the present experiments: Ryanodine (Wako pure chemical, Japan), caffeine (Sigma, U.S.A.), verapamil (gift from Eisai, Japan), prazosin (gift from Taito-Pfeizer, Japan), phentolamine (gift from Ciba-Geigy, Japan), procaine (Sigma, U.S.A.).

Results

Mechanical recording at 37°C

The preparations exhibited periodic phasic contractions which appeared about 30 min after incubation. The force of the spontaneous contractions was $0.69 \pm 0.15 \text{ mN (n=12)}$, being $60.9 \pm 4.8\% (n=12)$ of K 80 mM-induced phasic contraction ($1.18 \pm 0.18 \text{ mN n=12}$), and the frequency of them was 2–4 per min. In the present experiment, caffeine was applied in the quiescent period between phasic contractions.

Caffeine of concentrations 0.1 to 0.5 mM caused slight inhibition of subsequent spontaneous contraction, but no stimulating action was observed. Caffeine of concentration higher than 1 mM, however, induced a phasic contraction which was followed by an inhibition of spontaneous contraction (Fig. 1). The height of the caffeine-induced phasic contraction increased concen-

![Fig. 1. Effect of caffeine on portal vein at 37°C and 17°C. Caffeine of different concentration (indicated in left column) was applied at arrows. Each application was preceded by incubation in K 80 mM solution for 10 min and following incubation in normal Tyrode for 30 min. Recordings are obtained at 37°C (left) and at 17°C (right).](image)

![Fig. 2. Concentration–response curve of caffeine at 37°C. Contractions in control solution (○) and in the presence of verapamil $10^{-5} \text{ M (●)}$ were expressed as % of K phasic contraction at 37°C.](image)
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Fig. 3. Effects of verapamil and Ca removal on caffeine-induced contraction. Caffeine (10 mM) was applied at arrows. Contractions were obtained 10 min after application of verapamil (10^{-6} M) or 1 min after Ca removal.

Fig. 4. Concentration-response curve of caffeine-induced contraction in K 80 mM-induced tonic contraction. Caffeine (10 mM)-induced contraction in high-K tonic contraction was completely inhibited in the presence of ryanodine (left). Concentration-response curve in high-K depolarized preparation shows lower threshold for contraction than polarized preparation (right) from control.

Caffeine concentration dependently in the range between 1 mM and 10 mM (Fig. 2). The amplitude at 1 mM and 10 mM was 45.2±2.5% (n=8) and 71.7±7.7% (n=20) of high-K induced phasic contraction, respectively. Thus, the dose–response curve rose abruptly between 0.5 to 1 mM. Duration of 10 mM caffeine-induced contraction measured at the level of 25% of peak height was 5.00±0.18 sec (n=15). Caffeine of concentrations higher than 10 mM did not cause further increase in the contraction amplitude but blocked the spontaneous contraction completely. The spontaneous contraction which was abolished by caffeine was restored by washing out of the drug. These results were not affected by treatment with phentolamine (10^{-6} M) or prazosin (10^{-6} M).

High-K–induced contraction was completely abolished within 30 sec after removal of Ca or immediately after the application of verapamil of same concentration. Caffeine–induced
contraction was also reduced by the removal of extracellular Ca. At 1 min after the removal of Ca, caffeine (10 mM)-induced contraction was 4.4±0.49% (n=4) of 80 mM K-induced phasic contraction (Fig. 3), and decreased further with the time after Ca-removal. Although the caffeine (10 mM) contraction was also reduced remarkably by the treatment with verapamil 10^{-5} M, the contraction of the amplitude of 6.62±0.49% (n=4) of high-K-induced contraction was still remained. The caffeine-induced contraction in the presence of verapamil had threshold of 1-3 mM and increased its amplitude concentration dependently (Fig. 2).

Caffeine contraction in high-K-depolarized preparation
When caffeine was applied during the course of K(80 mM)-induced tonic contraction, it induced a phasic contraction which was followed by relaxation. In this high K-depolarized preparation, threshold concentration of caffeine for contraction was 0.05 mM which was lower than that in polarized preparation. The height of contraction was dose dependent and reached its maximum at 10 mM, being 77.5±4.6% (n=8) of the amplitude of high-K-induced contraction (Fig. 4). The height of contraction in the depolarized preparation was higher than the polarized preparation especially in contractions induced by lower concentration of caffeine. Duration of the contraction induced by 10 mM caffeine in K 80 mM solution was 4.36±0.21 sec (n=14). Removal of extracellular Ca reduced the caffeine-induced contraction also in K-depolarized preparation. The amplitude of 10 mM caffeine-induced contraction 1 min after Ca removal was 16.1±1.39% (n=4) and that in the presence of verapamil was 19.1±1.45% (n=4) of that of high-K-induced phasic contraction. These values were significantly greater than those observed in polarized preparations (p<0.01, respectively).

Effects of Na removal
Removal of Na also exerted effects on caffeine-induced contraction. The removal of Na itself caused the elevation of the basal tension by 8.1±1.4% (n=4) of amplitude of high-K-induced contraction. Caffeine-(10 mM) induced contraction in Na-free solution was not

![Fig. 5. Effect of Na removal on caffeine contraction.](image)
left: Caffeine contraction in the presence and absence of Na was induced in the presence and absence of extracellular Ca (1 min after Ca removal).
right: Comparison of caffeine contraction height. Asterisk indicates significant difference (p<0.01) from control.
significantly different from that in normal Tyrode's solution when Ca concentration was normal. It was 69.4±9.6% (n=4) in Na–free solution and 60.9±4.8% (n=12) in normal Tyrode’s solution (Fig. 5). However, the amplitude observed 1 min after Ca removal was 33.3±6.0% (n=4) of high-K-induced contraction being significantly higher than that in the presence of Na (p<0.01) and the reduction of the contraction after Ca removal was less prominent.

Caffeine-induced contraction at low temperature

Caffeine-induced contraction was affected by change of the temperature of the organ baths. Although the amplitude of the contraction by higher concentration of caffeine was not markedly altered, the contraction by lower concentration of the drug increased significantly, so that caffeine of the concentration lower than 1 mM could induce contraction. The duration of the contraction induced by caffeine (10 mM) was increased remarkably by lowering the temperature and was 20.4±2.3 sec (n=11) at 17°C which was significantly longer than that at 37°C (5.00±0.18 sec, n=14, p<0.01). Height of caffeine (10 mM)–induced contraction was 67.0±4.1% (n=4) in the presence of verapamil (10⁻⁵ M), 42.0±4.5% (n=4) in the absence of

![Fig. 6. Concentration-response curve of caffeine at 17°C and effect of verapamil (10⁻⁵ M).](image)

![Fig. 7. Effect of verapamil, Ca-free and ryanodine on caffeine-induced contraction at 17°C. Caffeine was applied as same condition as in Fig. 3.](image)

![Fig. 8. Effect of caffeine on mechanical (upper trace) and electrical (bottom trace) activities recorded simultaneously. Dotted line in bottom trace indicates resting membrane potential of control.](image)
Ca, respectively of high-K-induced contraction (Fig. 6, Fig. 7). Contraction induced by caffeine during the course of the high-K-induced tonic contraction was $86.2 \pm 3.1\%$ ($n=4$) of that of control high-K-induced phasic contraction. All of these values were significantly greater than those at $37^\circ$C.

**Effect of ryanodine and procaine on contractions**

At $37^\circ$C, treatment with ryanodine ($10 \mu$M) depressed the caffeine-induced contraction markedly from $71.7 \pm 7.7\%$ ($n=20$) to $7.3 \pm 3.1\%$ ($n=6$) of that of high-K-induced phasic contraction (Fig. 3). Caffeine-induced contraction in the presence of verapamil was also abolished in ryanodine pretreated preparation. Ryanodine did not affect high-K-induced contracture, although it inhibited caffeine-induced contraction of high-K-depolarized preparation completely (Fig. 4).

Potentiated caffeine-induced contraction of both polarized and high-K-depolarized preparation at $17^\circ$C were also completely blocked by the treatment with ryanodine of the same concentration (Fig. 7). Similar blocking effect was observed by treating the preparation with procaine under all conditions described above.

**Effect of caffeine on electrical activity**

Resting membrane potential of the smooth muscle cells of the portal vein was $-43.8 \pm 0.61$ mV ($n=6$) at $37^\circ$C. Periodic bursts of the action potential and associated contractions were observed in all preparation. Caffeine exerted its effect on membrane electrical activities depending on the rate of the application. At flow rate of 3 ml/min, caffeine of 10 mM or 20 mM initiated action potential burst with membrane depolarization to $-41.9 \pm 1.97$ mV ($n=9$) and $-32.0 \pm 1.77$ mV ($n=16$), respectively. The action potential was abolished within a few minutes but depolarization persisted as long as caffeine was present (Fig. 8). When the flow rate of the incubation medium containing caffeine was slow (1 ml/min), on the other hand, no excitatory effect on the action potentials but abolition of the spike activity was observed by caffeine of the same range of concentration despite the membrane depolarization. The membrane depolarization recovered gradually by washing out of the drug.

At $17^\circ$C, no spontaneous electrical activity was observed and caffeine seldom induced action potential burst although caffeine-induced depolarization was observed.

**Discussion**

Caffeine has been known to release Ca from sarcoplasmic reticulum in skeletal muscle and, therefore, initiates contraction without membrane potential changes (See Sandow, 1965; Endo, 1977). In smooth muscle, on the other hand, caffeine shows stimulating action on cell membrane in addition to Ca-releasing action. Therefore, at least, part of the caffeine-induced contraction is thought to be initiated through membrane excitation (Ito and Kuriyama, 1971; Ito et al., 1973; Sunano and Miyazaki, 1973), and the contraction mediated with membrane excitation is thought to be initiated by Ca-influx through voltage-dependent Ca channel (Bolton, 1979).

In the present experiment with rat portal vein, it was shown that caffeine initiated the
action potential burst and induced a phasic contraction. Since the contraction was markedly reduced by removal of extracellular Ca or by the application of Ca antagonist, it can be thought that the caffeine-contraction under normal condition is induced mainly by Ca from extracellular space through voltage-dependent Ca channel.

Although the depolarization of smooth muscle membrane is thought to be the cause of the increase in the burst of the spike potentials, the mechanisms of the depolarizing action of caffeine remained unsolved in the present experiment. These effects would not be mediated by the action on the intrinsic excitatory nerves, since the contraction was not blocked by adrenergic blocking agents or tetrodotoxin.

The remaining part of caffeine-induced contraction in the absence of extracellular Ca or in the presence of Ca antagonist is thought to be initiated by the released Ca from intracellular binding sites. The release of intracellular stored Ca by caffeine has been presented in other vascular smooth muscles (Leijten and Van Breemen, 1984; Sumimoto and Kuriyama, 1986; Kobayashi et al., 1986; Sato et al., 1988). This is supported by the result that the contraction by caffeine was depressed by ryanodine which has been known to reduce stored Ca in sarcoplasmic reticulum which is released by caffeine (Ito et al., 1986; Ueno et al., 1987; Hwang and Van Breemen, 1987; Sato et al., 1988; Ahn and Karaki, 1988).

Caffeine-induced contraction was phasic and the preparations relaxed quickly. The spontaneous action potentials of the portal vein also disappeared after caffeine-induced contraction, although the depolarization of the muscle membrane by caffeine persisted unaltered. Therefore, it can be considered that some spike blocking action of caffeine is a cause of quick relaxation to form only a phasic contraction, although mechanisms of the block of the action potential remain unsolved in the present studies.

Caffeine induced a phasic contraction also in depolarized preparation. This result indicates that caffeine could induce contraction also without changing membrane potential. Two possibilities can be thought as cause of Ca-dependent contraction induced by caffeine; increase in Ca permeability of cell membrane and Ca-release from intracellular binding sites such as sarcoplasmic reticulum. The contraction was completely blocked by the treatment with ryanodine, although ryanodine had no effect on high-K-induced contraction. Similar results have been presented in other types of vascular smooth muscle (Ito et al., 1986; Ashida et al., 1988; Julou and Freslon, 1988). Thus, it is indicated that ryanodine does not affect Ca-influx through voltage-dependent Ca channel and that caffeine-induced contraction in K-depolarized preparation is not mediated by an increase in Ca permeability through voltage-dependent channel. As caffeine contraction was blocked by the treatment with ryanodine, it may be concluded that caffeine-induced contraction of K-depolarized preparation is brought about solely by the release of Ca from sarcoplasmic reticulum even in the presence of extracellular Ca. The potentiation in caffeine-induced contraction in K-depolarized preparation, then, may be explained by an increased release of Ca from sarcoplasmic reticulum. The increase in Ca release would be brought about by increased Ca store which is caused by an increased Ca influx through voltage-dependent channel due to high-K-induced depolarization (Leijten and Van Breemen, 1984: see also Jones et al., 1987; Novailhetas et al., 1988).

Caffeine-induced contraction was transient both in polarized and K-depolarized prepara-
tions. In addition, relaxation of the tonic component of high-K-induced contraction was brought about after the phasic contraction. Similar results have been observed in rabbit aorta (Leijten and Van Breemen, 1984) and rat aorta (Sato et al., 1988). The effects has been explained by the inhibition of Ca influx (Leijten and Van Breemen, 1984). Sato et al. (1988) have suggested the direct inhibitory action of caffeine on contractile elements in addition to decrease in intracellular free Ca concentration. These actions of caffeine can be considered as cause of the relaxation of K-depolarized portal vein. Caffeine-induced contraction mediated by released Ca was transient suggesting that the increase in intracellular Ca is also transient. This has been proved in the experiment with intracellular Ca indicator (Sato et al., 1988; Kobayashi et al., 1986; Sunano et al. 1992). The sequestration of increased intracellular free Ca are thought to be brought about by Ca extrusion through cell membrane and Ca reuptake by sarcoplasmic reticulum (Van Breemen et al., 1986; Verbist et al., 1986; see Jones et al., 1987). In addition, Na–Ca exchange mechanism may also be involved (Smith et al., 1989; Ashida and Blaustein, 1987; Nabel et al., 1988; see Verbist et al., 1986; Blaustein et al., 1986). Then, treatments which may inhibit these mechanisms would potentiate caffeine-induced contraction. Lowering the temperature performed in the present experiment which has been reported to depress Ca–pump activities of cell membrane and sarcoplasmic reticulum (Hurwitz et al., 1975; Hurwitz et al., 1977; Deth, 1978; Droogmans and Casteels, 1981) for example, potentiated caffeine-induced contraction markedly. Since it has been shown that the lowering of the temperature in the range performed in the present experiment dose not increase the degree of the contraction of smooth muscle of rat portal vein (Peiper et al., 1975), it may be concluded that the potentiation of caffeine–induced contraction at lower temperature is brought about by the inhibition of Ca–sequestration. Then, caffeine would cause, somehow the stimulation of Ca–sequestration in addition to its releasing action. Similar assumption has been presented in rabbit aorta (Ahn et al., 1988). It is also shown in the present experiment that at 17°C verapamil or Ca-removal depressed the contraction only partially. This indicates that the contraction under low temperature is initiated mostly by released Ca.

In the present experiment, it was shown that the reduction of extracellular Na concentration also potentiated caffeine–induced contraction. Similar result has been reported in rat aorta (Ashida and Blaustein, 1987). The reduction of extracellular Na has been known to depress the Ca efflux in arterial smooth muscle (Reuter et al., 1973; Van Breemen et al. 1979), and therefore, the sequestration of intracellular free Ca through this mechanism would also be depressed. The result, then, suggest that Na–Ca exchange mechanism would be involved, at least in part, in the relaxing phase of caffeine–induced contraction in rat portal vein. Decreased Ca efflux and Ca sequestration in the absence of Na has been proved with intracellular Ca indicator although Ca release was initiated by angiotensin II (Smith and Smith, 1987). Thus, it may be considered that the reduction of extracellular Na concentration caused the potentiation of caffeine contraction by inhibiting Na–Ca exchange through cell membrane (Reuter et al., 1973: Van Breemen et al., 1979), however, whether caffeine stimulates Na–Ca exchange mechanism has not still been known.

In conclusion, caffeine induces phasic contractions in rat portal vein acting directly on the smooth muscle cells. A part of the contraction is mediated by Ca influx through membrane
excitation and the rest of the contraction is induced by the released Ca from sarcoplasmic reticulum. When Ca sequestration mechanisms are inhibited by low temperature or Na removal, released Ca is sufficient to induce contraction. However, under normal condition, the contraction induced by Ca release is suppressed because of strong Ca sequestering action of smooth muscle cells through Ca-pump activity and Na-Ca exchange mechanism.

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