British Journal of Pharmacology (1996) 118, 1711-1716 © 1996 Stockton Press All rights reserved 0007-1188/96 $12.00

Effects of cyclopiazonic acid and ryanodine on cytosolic calcium and contraction in vascular smooth muscle

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1 In smooth muscle, both Ca^{2+} release from the sarcoplasmic reticulum (SR) and Ca^{2+} influx across the plasma membrane are responsible for the increase in the cytosolic Ca^{2+} level ($[Ca^{2+}]_i$). To understand further the role of SR on smooth muscle contraction, the effects of an inhibitor of the SR Ca^{2+} pump, cyclopiazonic acid (CPA 10 μM), an inhibitor of the Ca^{2+}-induced Ca^{2+} release, ryanodine, (10 μM), and an activator of the Ca^{2+}-induced Ca^{2+} release, caffeine (20 mM), on $[Ca^{2+}]_i$ and contractile force were examined in the ferret portal vein loaded with a photoprotein, aequorin.

2 CPA induced a small increase in the aequorin signal reaching a maximum at 7 min. Several minutes after the increase in the aequorin signal, muscle tension increased reaching a maximum at 21.5 min. In contrast, ryanodine changed neither the aequorin signal nor contraction. In the presence of ryanodine, caffeine induced a sustained increase in the aequorin signal and transient contraction. After washing ryanodine and caffeine, the aequorin signal and muscle tone returned to their respective control levels. After treatment with ryanodine and caffeine, the second addition of caffeine was almost ineffective whereas CPA still increased the aequorin signal and muscle tension.

3 In the presence of external Ca^{2+}, noradrenaline (NA, 10 μM) induced a transient increase followed by a sustained increase in the aequorin signal and sustained contraction. In contrast, KCl (70 mM) induced sustained increases in the aequorin signal and sustained contraction. In Ca^{2+}-free solution, NA induced a small transient increase in the aequorin signal and a small transient contraction. These changes were inhibited in the presence of CPA or on pretreatment of the muscle with ryanodine and caffeine. These results suggest that CPA or ryanodine and caffeine depleted Ca^{2+} in SR. High K^+ was ineffective in the absence of external Ca^{2+}.

4 In the presence of external Ca^{2+} and CPA, NA and high K^+ induced larger aequorin signals than in the absence of CPA, whereas the magnitude and shape of the contractions did not change. In contrast, pretreatment with ryanodine and caffeine did not have such an effect. In the muscle pretreated with ryanodine and caffeine, CPA changed the responses to high K^+ and NA in a similar manner to that in the muscle without the pretreatment with ryanodine and caffeine.

5 Dissociation of contraction from $[Ca^{2+}]_i$ as measured with aequorin suggests that NA and high K^+ increase Ca^{2+} in two compartments: a compartment containing contractile elements (contractile compartment) and another compartment unrelated to contractile elements (non-contractile compartment). Because CPA augmented the stimulant-induced increase in aequorin signal without changing contraction, the non-contractile compartment may be located near the SR and the CPA-sensitive SR Ca^{2+} pump may regulate the Ca^{2+} level in this compartment. However, because CPA changed neither the magnitude nor shape of the contractions in the presence of external Ca^{2+}, the SR Ca^{2+} pump may have little effect on regulation of Ca^{2+} level in the contractile compartment. Furthermore, the release of Ca^{2+} from SR seems to have little effect on the increase in the contractile Ca^{2+} because ryanodine and caffeine changed neither the aequorin signals nor contractions induced by NA and high K^+ in the presence of external Ca^{2+} in the ferret portal vein.

Keywords: Sarcoplasmic reticulum; cyclopiazonic acid; ryanodine; cytosolic Ca^{2+} level; Ca^{2+} localization; ferret portal vein

Introduction

Smooth muscle contraction is regulated by changes in cytosolic Ca^{2+} levels ($[Ca^{2+}]_i$) and Ca^{2+} sensitivity of contractile elements (Karaki, 1989). Measuring $[Ca^{2+}]_i$ simultaneously with contraction in the ferret portal vein loaded with a photoprotein, aequorin, we have reported that noradrenaline (NA 10 μM) or high K^+ increased the aequorin signal and elicted a sustained contraction. A second application of NA or high K^+ induced a reproducible contraction. In contrast, the aequorin signal resulting from a second application of NA or high K^+ was much smaller than the first signal. Following a 13 h but not a 3 h resting period, the aequorin signal recovered, while the contractile response to these interventions remained unchanged (Abe *et al.*, 1995b). From these results, we postulated that high K^+ and NA may increase Ca^{2+} in at least two cytosolic compartments: a compartment that is responsible for the contractile activation ('contractile' Ca^{2+} compartment; the major portion of cytoplasm containing contractile elements) and a compartment that is unrelated contractile activation ('non-contractile' Ca^{2+} compartment; a small sub-membrane area that does not contain contractile elements). On stimulation, the Ca^{2+} level in the 'contractile' compartment may increase to a level high enough to stimulate myosin light chain kinase but not so high as to consume aequorin rapidly. In contrast, the Ca^{2+} level in the 'non-contractile' compartment may increase so much that aequorin in this compartment is rapidly consumed. These two compartments may be separated by a diffusion barrier which restricts rapid diffusion of aequorin from the 'contractile' to the 'non-contractile' com-

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partment. Thus, a 13 h resting period was necessary to restore the full response of aequorin signals. The Ca^{2+}-dependent mechanisms in these two compartments may be regulated differentially, because the concentration of Ca^{2+} in these compartments changed independently.

In vascular smooth muscle cells, it has been suggested that the superficial sarcoplasmic reticulum (SR) accumulates a portion of the Ca^{2+} that enters cells and thus functions as a buffer barrier to Ca^{2+} entry into myoplasm. Such a superficial buffer barrier may generate a high Ca^{2+} compartment in restricted diffusion spaces between the plasma membrane and SR (for review see van Breemen *et al.*, 1995). This Ca^{2+} space may correspond to the non-contractile Ca^{2+} compartment that has been postulated by Abe *et al.* (1995b). To examine whether the non-contractile Ca^{2+} compartment is located adjacent to SR and whether the Ca^{2+} concentration in this compartment is regulated by SR, we studied the effects of an inhibitor of the SR Ca^{2+} pump, cyclopiazonic acid (Kurebayashi & Ogawa, 1991; Uyama *et al.*, 1992; Darby *et al.*, 1993), an inhibitor of the Ca^{2+}-induced Ca^{2} release from SR, ryanodine (Ito *et al.*, 1986; Hwang & van Breemen, 1987), and an activator of Ca^{2+}-induced Ca^{2+} release, caffeine (Endo, 1977), on $[Ca^{2+}]_i$ measured with aequorin and muscle tension in the ferret isolated portal vein.

Some of these results have been reported as an abstract (Abe *et al.*, 1995a).

Methods

Ferrets of either sex, weighing 700–1200 g, were killed by exsanguination under ether anaesthesia and the portal vein was isolated. To exclude the effects of endogenous adrenergic nerves, adventitia was removed (Abe & Endoh, 1990). The vein was cut into a helical strip of 2–3 mm width and the endothelium was removed by gently rubbing the intimal surface with a cotton swab moistened with physiological salt solution that contained (in mM): NaCl 116.4, KCl 5.4, $CaCl_2$ 1.8, $MgSO_4$ 1.0, $NaHCO_3$ 23.8, NaH_2PO_4 0.9, and glucose 5.5. High K^+ solution was made by substituting NaCl with equimolar KCl. Ca^{2+}-free solution was made by removing $CaCl_2$ and adding 0.5 mM EGTA. These solutions were saturated with 95% O_2 and 5% CO_2 mixture at 22°C to maintain the pH at 7.4.

Aequorin was loaded as reported previously (Abe *et al.*, 1995b). In brief, muscle strips were treated with Ca^{2+}-free physiological salt solution with 10 mM EGTA at 2°C for 15 min followed by an incubation with the medium containing 1 mM EGTA at 2°C for 20 min. The latter solution was similar to that developed for organ transplantation (Beltzer & Southard, 1988; Maurer *et al.*, 1990) with the following composition (in mM): potassium lactobionate 100.0, $MgSO_4$ 5.0, K_2HPO_4 25.0, raffinose 30.0, adenosine 5.0, glutathione 3.0, alloprinol 1.0, EGTA 1.0 and insulin 100 u l^{-1}. Strips were then treated with a similar solution containing 0.1 mM EGTA and 0.4 mg ml^{-1} aequorin (purchased from Dr J.R. Blinks, Washington University, Seattle, U.S.A.) for 18 to 20 h. Strips loaded with aequorin were treated with Ca^{2+}-free Eagle's MEM medium (Nissui Pharmaceutical, Tokyo) at 2°C for 30 min followed by a gradual increase in Ca^{2+} concentration to 0.5 mM and the temperature to 22°C. Strips were then treated with Trowell T-8 medium (Dainippon Pharmaceutical, Osaka) for 15 to 19 h at 22°C before starting the experiments. The aequorin signals were measured with a photomultiplier (R-268, Hamamatsu Photonics, Hamamatsu, Japan) at 22°C. Force of contraction was recorded isometrically under a resting force of 10 mN. Stimulants were added for 5 min followed by 55 min resting period. We have previously reported that repeated applications of stimulants, such as NA or high K^+, decrease progressively the aequorin signal until a steady state of response is achieved (Abe *et al.*, 1995b). In the present study, experiments were started when reproducible steady responses had been reached after several applications of NA or high K^+. NA or high K^+ was added for 5 min followed by a 55 min washout with normal solution. To inhibit the SR Ca^{2+} pump, CPA (10 μM) was added 50 min before addition of the stimulant. To deplete Ca^{2+} from the SR, 20 mM caffeine was added 10 min after the addition of 10 μM ryanodine. Ten minutes after the addition of caffeine, the muscle was washed out with normal solution for 50 min and then the stimulant was added. In some experiments, muscle strips were treated with Ca^{2+}-free solution for 10 min before the addition of the stimulant.

CPA, ryanodine, caffeine and NA were purchased from Wako Pure Chemicals, Osaka, Japan.

Results are expressed as mean ± s.e.mean. Student's t test was used for the statistical analysis of the results. $P<0.05$ was considered to be significant.

Results

Figure 1 shows the effect of CPA (10 μM) on contraction and the aequorin signal in the ferret portal vein. CPA induced a slow increase in the aequorin signal reaching a maximum 7.0 ± 0.6 min ($n=6$) after the application and then gradually decreased to a steady level that was slightly above the resting level. The maximum level of the aequorin signal was similar to the sustained level of the aequorin signal induced by 10 μM NA (79.1 ± 22.0%, $n=6$). Muscle tension also slowly increased. The onset of contraction was slower and the maximum was reached 21.5 ± 3.2 min ($n=6$) after the addition of CPA. The maximum contraction was 18.5 ± 6.3% ($n=6$) of the NA-induced contraction. The CPA-induced increases in aequorin signals and contraction were abolished in the absence of external Ca^{2+} (data not shown).

Figure 2 shows the effects of ryanodine (10 μM) and caffeine (20 mM). Ryanodine alone affected neither the aequorin signal nor muscle tension (Figure 2a). When caffeine was added 10 min after the addition of ryanodine, it induced a small transient increase followed by a sustained and fluctuating increase in aequorin signals. These changes were associated with a transient contraction followed by a long-lasting relaxation to a level below the resting tone (Figure 2a). The effects of caffeine in the absence of ryanodine were almost identical to those observed in the presence of ryanodine (data not shown). After washout of ryanodine and caffeine, the muscle tension transiently increased and then returned to resting level. Fifty minutes later, when caffeine was added again, it induced a sustained relaxation without affecting the aequorin signal (Figure 2b).

In the experiment shown in Figure 3, CPA was added after treating the muscle with ryanodine and caffeine. Addition of ryanodine (10 μM) and caffeine (20 mM) elicited similar effects to those shown in Figure 2. After washout of both agent until the aequorin signal and muscle tension returned to their respective resting levels (about 6.5 min), addition of 10 μM CPA increased both the aequorin signal and muscle tension in a manner similar to those observed in the absence of pretreatment with ryanodine and caffeine (see Figure 1).

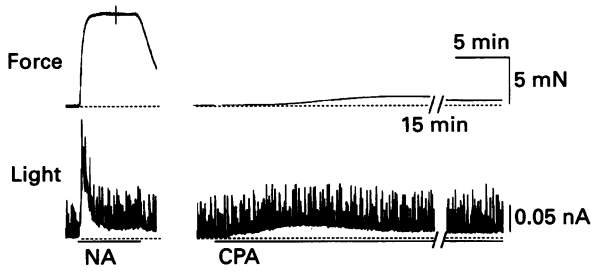

Figure 1 Effects of noradrenaline (NA, 10 μM) and cyclopiazonic acid (CPA, 10 μM) on the aequorin signal (as indicated by current) and contractile force in the ferret portal vein.

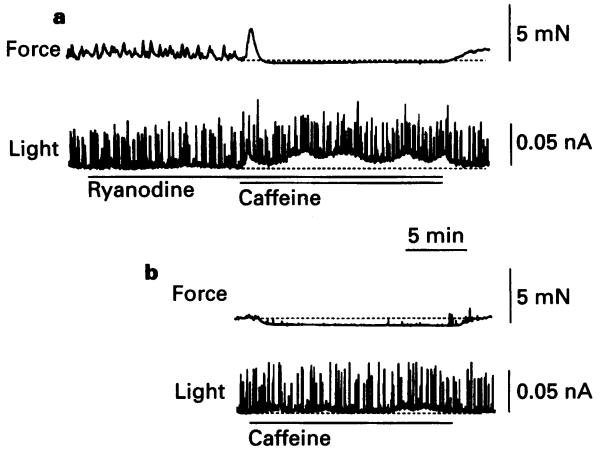

Figure 2 Effects of ryanodine (10 μM) and caffeine (20 mM) on the aequorin signal (as indicated by current) and contractile force in the ferret portal vein. The tracing in (b) was obtained 50 min after recording the tracing in (a) in the same muscle strip.

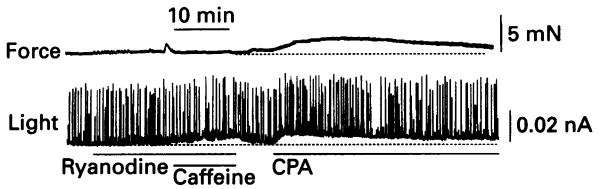

Figure 3 Effects of cyclopiazonic acid (CPA, 10 μM) on the aequorin signal (as indicated by current) and contractile force in the ferret portal vein pretreated with ryanodine (10 μM) and caffeine (20 mM).

Figure 4 shows the effects of CPA on the increase in the aequorin signal and muscle tension induced by NA or 70 mM KCl. In the absence of CPA, 10 μM NA induced a transient increase followed by a small sustained increase in aequorin signals in association with a sustained contraction. In contrast, 70 mM KCl induced a sustained increase in aequorin signals associated with a contraction. After washout of NA or KCl for 50 min, CPA (10 μM) was added to the muscle and, 50 min later, NA or KCl (70 mM) was added again. As shown in Figure 4, the increase in the peak aequorin signal induced by NA or KCl (70 mM) was augmented with little change in the maximum level and shape of the contractile response to either intervention. By washing out the agents applied, muscle tension returned to the resting level at a rate similar to that in the absence of CPA. The effects of CPA on the peak aequorin signal and the peak contraction in response to NA or KCl (70 mM) are summarized in Figure 5. In the presence of CPA, the increase in the aequorin signal induced by NA or KCl (70 mM) was augmented to 170% and 193%, respectively, whereas there was no significant change in the peak contraction induced by either stimulant. We also examined the influence of CPA (10 μM) on the effects of KCl (30 mM) that induced a smaller aequorin signal ($10.0 \pm 1.7\%$, $n=3$) and a smaller contraction ($37.7 \pm 5.4\%$, $n=3$) than those induced by KCl (70 mM). In the presence of CPA (10 μM), the response of the aequorin signal induced by KCl (30 mM) was augmented to $312.3 \pm 55.1\%$ ($n=4$) of that in the absence of CPA without changing the contractile response ($106.1 \pm 13.8\%$, $n=4$).

Figure 6 shows the effects of ryanodine and caffeine on the increase in the aequorin signal and muscle tension induced by NA (10 μM) or KCl (70 mM). The muscle was treated with ryanodine (10 μM) and caffeine (20 mM), as shown in Figure 2, and, 50 min later, NA or KCl (70 mM) was added. As shown in Figure 6, neither the aequorin signal nor the contraction induced by NA or KCl (70 mM) was altered by the treatment

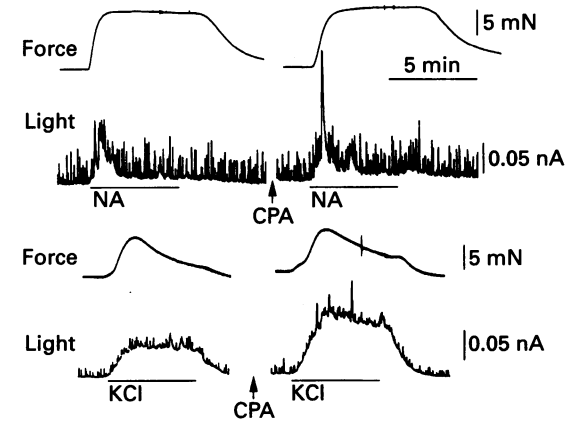

Figure 4 Effects of noradrenaline (NA, 10 μM) and KCl (70 mM) on the aequorin signal (as indicated by current) and contractile force in the ferret portal vein in the presence of cyclopiazonic acid (CPA, 10 μM). CPA was added 50 min before the addition of NA or KCl.

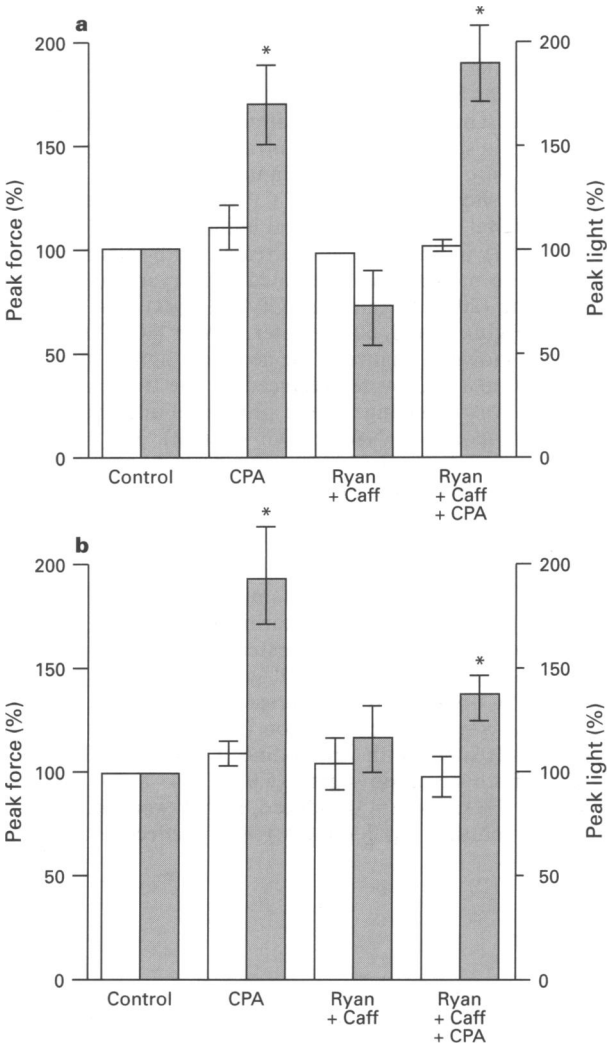

Figure 5 Effects of noradrenaline (NA, 10 μM) (a) or KCl (70 mM) (b) on the peak aequorin signal (solid column, right ordinate scale) and contractile force (open column; left ordinate scale) in the ferret portal vein pretreated with cyclopiazonic acid (CPA, 10 μM), ryanodine (Ryan, 10 μM) and caffeine (Caff, 20 mM) or a combination of CPA, Ryan and Caff. Experiments were done as shown in Figures 4, 5 and 6. Mean ± s.e.mean of 3–4 experiments are shown. *Significantly different from the response to the first addition of stimulants with $P<0.05$.

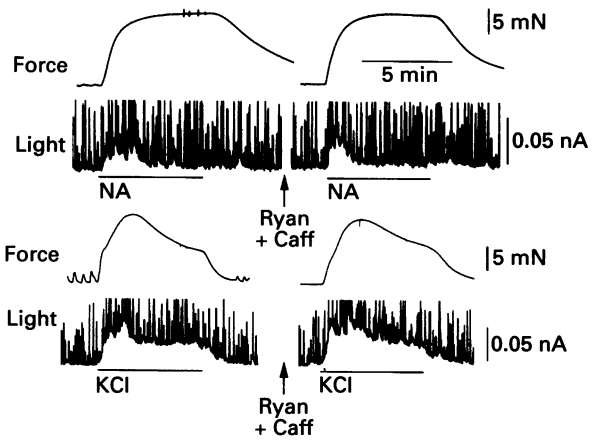

Figure 6 Effects of noradrenaline (NA, 10 μM) and KCl (70 mM) on the aequorin signal (as indicated by current) and contractile force in the ferret portal vein. The muscle was pretreated with ryanodine (Ryan, 10 μM) and caffeine (Caff, 20 mM) as shown in Figure 2 and 50 min later, NA or KCl was added.

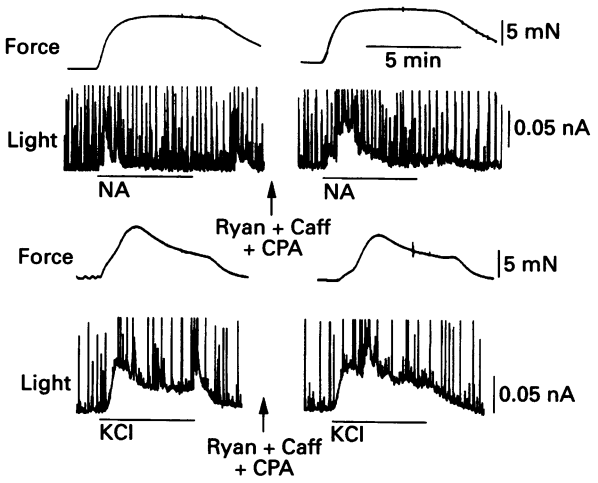

Figure 7 Effects of noradrenaline (NA, 10 μM) and KCl (70 mM) on the aequorin signal (as indicated by current) and contractile force in the ferret portal vein in the presence of cyclopiazonic acid (CPA, 10 μM). The muscle was pretreated with ryanodine (Ryan, 10 μM) and with caffeine (Caff, 20 mM) as shown in Figure 2 and 50 min later, NA or KCl was added.

with ryanodine. The rate of relaxation by washing out of the stimulants was also not changed by treatment with ryanodine and caffeine. The effects of pretreatment with ryanodine on the peak aequorin signal and peak contraction induced by NA and 70 mM KCl are summarized in Figure 5.

Figure 7 shows the effects of CPA on the increase in the aequorin signal and muscle tension induced by NA (10 μM) or KCl (70 mM) in the muscle pretreated with ryanodine and caffeine. The muscle was pretreated with ryanodine (10 μM) and caffeine (20 mM) and then CPA (10 μM) was added, as shown in Figure 3, and, 50 min later, NA or KCl (70 mM) was added. As shown in Figure 7, the aequorin signals induced by NA and 70 mM KCl were enhanced by the treatment. However, there was no change in the peak level and shape of the contractile reponse to NA or KCl (70 mM). The rate of relaxation induced by washing the stimulants was also not changed. The effects of CPA on the peak aequorin signal and peak contraction induced by NA and KCl (70 mM) in the muscle pretreated with ryanodine and caffeine are summarized in Figure 5.

In the Ca^{2+}-free solution containing EGTA (0.5 mM), NA induced a small increase in aequorin signal ($18.3 \pm 3\%$, $n=5$, of the signal in the presence of external Ca^{2+}) in association with a small contraction ($26.7 \pm 7\%$, $n=5$). In the presence of 10 μM CPA, NA scarcely increased the aequorin signal ($4.7 \pm 0.9\%$, $n=5$) and contraction ($2.9 \pm 2.3\%$, $n=5$). In the muscle pretreated with ryanodine (10 μM) and caffeine (20 mM), both aequorin signal ($5.0 \pm 1.8\%$, $n=3$) and contraction ($2.3 \pm 1.1\%$, $n=3$) induced by NA were inhibited. In Ca^{2+}-free solution, KCl (70 mM) was ineffective (data not shown).

Discussion

In the ferret isolated portal vein, both NA and KCl (70 mM) increased $[Ca^{2+}]_i$ as measured with aequorin and induced contraction. In Ca^{2+}-free solution, high K^+ was ineffective, whereas NA (10 μM) induced small transient increases in $[Ca^{2+}]_i$ and contraction. These results are consistent with those reported previously (Abe *et al.*, 1995b), indicating that the NA-induced increase in $[Ca^{2+}]_i$ is partially due to release of Ca^{2+} from SR. CPA (10 μM) inhibited the effects of NA in Ca^{2+}-free solution, suggesting that CPA inhibited the SR Ca^{2+} pump and depleted Ca^{2+} in SR.

In resting muscle, the inhibition of the SR Ca^{2+} pump induced by CPA produced a slowly developing elevation of the resting $[Ca^{2+}]_i$ level in association with a contraction. It is noteworthy that the development of contraction was delayed, following behind the elevation of $[Ca^{2+}]_i$ induced by CPA. Furthermore, although the peak $[Ca^{2+}]_i$ was comparable to the sustained response induced by NA (10 μM), the peak force was much lower than that induced by NA. The stimulatory effects of CPA were observed only in the presence of external Ca^{2+}. Since it has been suggested in previous studies that depletion of SR Ca^{2+} results in an increase in plasma membrane Ca^{2+} influx (for review see Irvine, 1992; Clapham, 1993), the increase in $[Ca^{2+}]_i$ induced by CPA may be due to such a mechanism reported previously.

When the CPA-sensitive Ca^{2+} pump is inhibited, NA and high K^+ induced a greater increase in $[Ca^{2+}]_i$. On the other hand, neither peak force nor shape of contractions induced by these stimulants was altered by CPA. As the reason for dissociation of contraction from the CPA-induced increase in $[Ca^{2+}]_i$, it is probably that NA (10 μM) or KCl (70 mM) alone induced the maximum contractions to cause saturation of the contractile system and the increase in $[Ca^{2+}]_i$, could not further increase the contractile force. To test this possibility, we studied the effects of a submaximal concentration of KCl (30 mM). Although, 10 μM CPA enhanced the aequorin response induced by KCl (30 mM), it did not augment the contractile response either, suggesting that this possibility is not likely. A second possibility is that CPA inhibited the Ca^{2+} sensitivity of contractile elements and thus suppressed the contractile response to the elevated $[Ca^{2+}]_i$. It has been shown, however, that CPA does not inhibit Ca^{2+}-induced contraction in permeabilized smooth muscle (Uyama *et al.*, 1992; Gonzalez de la Fuente *et al.*, 1995), indicating that CPA does not affect the Ca^{2+} sensitivity of contractile elements. A third possibility is that CPA increased $[Ca^{2+}]_i$ in the compartment which is only weakly coupled to the contractile response and changes in $[Ca^{2+}]_i$ in this compartment were detected by aequorin.

Previously, we have proposed that a part of the aequorin signal stimulated by NA or high K^+ may represent Ca^{2+} near the plasma membrane that is not coupled to contraction (non-contractile Ca^{2+}) (Abe *et al.*, 1995b). As shown in Figure 8, the non-contractile Ca^{2+} may be located near the CPA-sensitive SR Ca^{2+} pump and inhibition of this Ca^{2+} pump may increase the amount of Ca^{2+} in this restricted compartment. A part of Ca^{2+} in this compartment may slowly diffuse to the contractile compartment to induce a small contraction with a remarkable delay behind the elevation of aequorin signal.

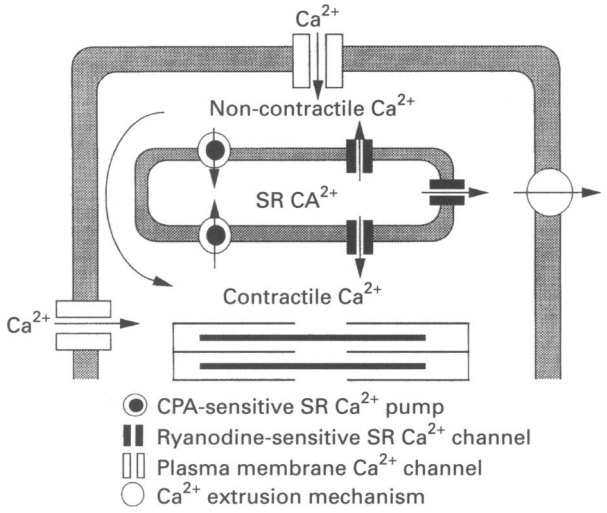

Figure 8 The proposed two Ca^{2+} compartments model in the ferret portal vein. The major Ca^{2+} compartment in the smooth muscle cell is the contractile Ca^{2+} compartment. In addition, there is a small Ca^{2+} compartment between plasma membrane and sarcoplasmic reticulum (SR) that does not contain contractile elements (the non-contractile compartment). The CPA-sensitive SR Ca^{2+} pump decreases Ca^{2+} in the non-contractile compartment with little effect on the contractile Ca^{2+} compartment. For further explanation, see text.

To examine further the relationship between SR and non-contractile Ca^{2+}, we tested the effects of ryanodine (10 μM) in combination with caffeine (20 mM). Caffeine (20 mM) induced a sustained increase in the aequorin signal and a transient contraction followed by a sustained relaxation. The increase in $[Ca^{2+}]_i$ in response to caffeine may be due to an initial release of Ca^{2+} followed by a sustained increase in Ca^{2+} influx whereas the dissociation of contraction from the increased $[Ca^{2+}]_i$ may be due to a decrease in Ca^{2+} sensitivity of contractile elements induced by accumulation of cyclic AMP and also by a direct inhibition of the contractile elements (Sato *et al.*, 1988). Ryanodine (10 μM), on the other hand, changed neither the resting $[Ca^{2+}]_i$ level nor the muscle tesion. Ryanodine also did not inhibit the changes in $[Ca^{2+}]_i$ and muscle tension induced by the first application of caffeine. By contrast, ryanodine inhibited the increase in $[Ca^{2+}]_i$ and muscle tension induced by the second application of caffeine without affecting the relaxant effect of caffeine. These results are consistent with previous findings obtained in the rat aorta (Sato *et al.*, 1988) and support the postulate that ryanodine inhibits the SR Ca^{2+} channel when it is in the open state (Jeffrey *et al.*, 1987).

When the SR Ca^{2+} channel activity was suppressed by treatment with ryanodine and caffeine, the NA-induced transient contraction observed in Ca^{2+}-free solution was abolished, indicating that the response is due to the NA-induced Ca^{2+} release from SR. Nevertheless, inactivation of the ryanodine-sensitive Ca^{2+} channel did not affect the resting $[Ca^{2+}]_i$; the resting muscle tension and the increase in $[Ca^{2+}]_i$ and contraction in response to NA or high K^+. Even in the muscle in which the SR Ca^{2+} channel had been inactivated, CPA increased the resting $[Ca^{2+}]_i$ and stimulated $[Ca^{2+}]_i$ as observed in the muscle in which the SR Ca^{2+} channel was not inhibited. These results support the view that the SR Ca^{2+} pump is operating to regulate $[Ca^{2+}]_i$ in the non-contractile Ca^{2+} compartment (Figure 8).

In rat isolated urinary bladder, Munro & Wendt (1994) reported that, in the presence of 10 μM CPA, electrical stimulation, carbachol and high K^+ induced greater increases in the $[Ca^{2+}]_i$ measured with a fluorescent Ca^{2+} indicator, fura-2, although the peak force levels in response to these different stimulants remained unaltered. These findings imply that the intracellular Ca^{2+} compartmentation exists not only in the ferret portal vein but also in other types of smooth muscle.

Inhibition of the SR Ca^{2+} pump and/or inactivation of SR Ca^{2+} channel inhibited the NA-induced release of Ca^{2+} from SR. However, neither the peak level nor the shape of contraction induced by high K^+ or NA was changed. Furthermore, the rate of relaxation induced by washout of the stimulants was also unaffected. These results indicate that Ca^{2+} uptake by SR does not play a crucial role in regulation of the contractile Ca^{2+}, while the initial small portion of the NA-induced contraction observed in the absence of external Ca^{2+} is due to the Ca^{2+} release from SR.

In conclusion, it is suggested that NA and high K^+ may increase $[Ca^{2+}]_i$ in two compartments: one compartment containing contractile elements (contractile compartment) and another compartment without contractile elements (non-contractile compartment). The non-contractile compartment may be located near SR and the CPA-sensitive SR Ca^{2+} pump may regulate the Ca^{2+} level in this compartment. However, this Ca^{2+} pump does not seem to play an important role in regulation of Ca^{2+} in the contractile compartment. The non-contractile Ca^{2+} may regulate the membrane Ca^{2+}-dependent mechanisms independently from the regulation of contractile elements. Furthermore, the release of Ca^{2+} from SR seems to have little influence on the increase in the contractile Ca^{2+} in the ferret portal vein.

This work was supported by a Grant-in-Aid for Scientific Research from the Ministry of Education, Science, Sports and Culture, Japan.

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(Received November 10, 1995
Revised April 15, 1996
Accepted April 23, 1996)