Handling of Cytoplasmic Ca^{2+} by the Sarcoplasmic Reticulum during α_1 -Adrenoceptor-Mediated Contraction of Rat Mesenteric Resistance Arteries

Kiyokazu Naganobu and Katsuaki Ito*

Department of Veterinary Pharmacology, Faculty of Agriculture, Miyazaki University, Miyazaki 889–21, Japan

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ABSTRACT—We investigated the role of the sarcoplasmic reticulum (SR) in the regulation of cytoplasmic Ca^{2+} ($[\text{Ca}^{2+}]_i$) during α_1 -adrenoceptor-mediated contraction in rat mesenteric resistance arteries. Phenylephrine (PE) at 1 μM elevated the tension and $[\text{Ca}^{2+}]_i$ measured with fura-2 in Ca^{2+} -containing PSS, but did not do so in Ca^{2+} -free PSS, suggesting that the contraction elicited by this concentration depends on the Ca^{2+} influx. Caffeine (100 mM) was shown to discharge Ca^{2+} in the SR, and cyclopiazonic acid (CPA, 10 μM) was shown to inhibit the Ca^{2+} uptake into the SR in these arteries. In resting arteries, both CPA and ryanodine (10 μM) sustainedly elevated $[\text{Ca}^{2+}]_i$ without affecting the tension. In PE-stimulated arteries, both agents caused transient increase in $[\text{Ca}^{2+}]_i$, which was larger than that in resting arteries, and augmented the contraction. In the presence of PE, the caffeine-evoked $[\text{Ca}^{2+}]_i$ transient was more greatly decreased after the application of ryanodine than after CPA. The CPA-induced rise in $[\text{Ca}^{2+}]_i$ could be ascribed to inhibition of Ca^{2+} buffering by the SR, and the ryanodine-induced one can be attributed to the acceleration of Ca^{2+} release. It is suggested that both Ca^{2+} release from and Ca^{2+} uptake into the SR are enhanced during PE-induced contraction, which depends on the transmembrane Ca^{2+} influx.

Keywords: α_1 -Adrenoceptor, Sarcoplasmic reticulum, Ryanodine, Cyclopiazonic acid, Resistance vessel

As a general understanding, α_1 -adrenoceptor-coupled Ca^{2+} mobilization in vascular smooth muscles involves Ca^{2+} release from the sarcoplasmic reticulum (SR) and Ca^{2+} influx from the extracellular fluid. The former is believed to be mediated by inositol 1,4,5-trisphosphate (IP_3), a product of membrane inositol phospholipid by phospholipase C following α_1 -adrenoceptor activation. In this regard, the significance of the SR as a source of activator Ca^{2+} in α_1 -adrenoceptor mediated contraction is well established. However, it is still to be clarified whether a role of the SR in the contraction is limited to IP_3 -mediated Ca^{2+} release. For example, it is uncertain whether the SR increases cytoplasmic Ca^{2+} ($[\text{Ca}^{2+}]_i$) as a result of Ca^{2+} release triggered by Ca^{2+} influx (namely Ca^{2+} -induced Ca^{2+} release, CICR) or decreases it by buffering Ca^{2+} provided by Ca^{2+} entry.

Ryanodine has been reported to accelerate Ca^{2+} efflux from the SR (1, 2), and then finally abolish Ca^{2+} release from the SR having CICR channels, due to its substantial-

ly irreversible action (3, 4). The action of caffeine resembles that of ryanodine because it also accelerates Ca^{2+} release through CICR channels (5–7). The advantages of caffeine over ryanodine as a tool are that a high concentration of caffeine immediately discharges Ca^{2+} in the SR and that the effect is reversible, while as its disadvantages, caffeine interferes with Ca^{2+} influx and changes the Ca^{2+} -sensitivity of the contractile elements (8, 9). On the other hand, cyclopiazonic acid (CPA) has been shown to selectively inhibit the Ca^{2+} pump of the SR (10–12). If we use these agents properly, we may be able to know the functional state of the SR under various conditions. In this paper, we analyzed the role of the SR in α_1 -adrenoceptor mediated contraction of rat mesenteric resistance vessels through the actions of ryanodine, CPA and caffeine on the fura-2 Ca^{2+} fluorescence and the tension.

MATERIALS AND METHODS

Tension measurement

Male Wistar rats (10–14 weeks, 300–360 g) were

*To whom all correspondence should be addressed.

anesthetized with pentobarbital sodium (45 mg/kg, i.p.). Cylindrical segments (1.5–2 mm in length) of artery were cut from the first branch of the mesenteric arcade (13) and then cleaned of fat and connective tissues. The internal diameter of the arterial segment was 70–150 μm in a slack condition. Endothelium was removed by gently passing a stainless steel wire (30 or 40 μm in diameter) bearing a plastic ball (70–160 μm in diameter) through the lumen of the segment. Then two stainless steel wires (30 μm in diameter) were placed through the lumen. One wire was fixed horizontally at the bottom of an organ bath (fluid volume of 0.2 ml), and the other wire was connected to a strain-gage transducer (UL-10 GR; Minebea, Nagano) to measure the isometric tension. The segment was stretched to 1.5 times the original diameter and stabilized for 1 hr. Preparations were steadily superfused with physiological saline solution (PSS) warmed at 37°C at a rate of 3.7 ml/min. After reproducible contractile responses to isotonic 80 mM K^+ were obtained, the experiments were started. Normal PSS had the following composition: 129.5 mM NaCl, 4.7 mM KCl, 1.2 mM MgSO_4 , 15.5 mM NaHCO_3 , 1.2 mM KH_2PO_4 , 2.4 mM CaCl_2 and 11.0 mM glucose, gassed with 95% O_2 and 5% CO_2 (pH 7.4).

Cytoplasmic free Ca^{2+} measurement

$[\text{Ca}^{2+}]_i$ was measured in a separate experiment, because the preparation was so small that it was difficult to measure $[\text{Ca}^{2+}]_i$ and tension simultaneously. The segments of the mesenteric artery (ca. 3 mm in length) prepared as described above were treated with 10 μM fura-2/AM and 0.03% cremophor EL for 6–12 hr at room temperature. Both ends of the segment were pinned and fixed in an organ bath (fluid volume of 0.5 ml) constructed in a fluorimeter (CAF-100; JASCO, Tokyo). The segment was superfused with PSS warmed at 37°C at a rate of 3.7 ml/min. The ratio of the fluorescence due to excitation at 340 nm to that at 380 nm (F_{340}/F_{380}) was calculated from successive illumination periods. The emitted light from the segment was collected to a photomultiplier through a 500 nm filter.

A number of investigators have calculated the absolute $[\text{Ca}^{2+}]_i$ in vascular smooth muscles based on the equation by Grynkiewicz et al. (14), using ionomycin and EGTA to get R_{max} and R_{min} , respectively. However, we did not determine the absolute $[\text{Ca}^{2+}]_i$ in the tissue used, because $[\text{Ca}^{2+}]_i$ -responses to stimulants decreased time-dependently within the experimental period, probably due to leak of fura-2, and ionomycin markedly promoted the leakage (15). Instead, we evaluated the change in $[\text{Ca}^{2+}]_i$ due to interventions as a percentage of the F_{340}/F_{380} ratio in response to 100 mM caffeine that was applied at the beginning of the experiments.

Drugs

The following drugs were used: fura-2/AM (Dojindo Laboratories, Kumamoto), cremophor EL (Nacalai Tesque, Kyoto), *l*-phenylephrine hydrochloride (PE) and caffeine (Wako Pure Chemicals, Osaka), CPA (Sigma, St. Louis, MO, USA) and ryanodine (Penick, Lyndhurst, NJ, USA). CPA was dissolved in dimethylsulfoxide in a concentration of 10 mM as a stock solution. The final concentration of dimethylsulfoxide did not exceed 0.1%.

Statistics

Data are expressed as the mean $\pm$ S.E. Significance was calculated by Student's *t*-test, and differences were considered to be significant at the level of $P < 0.05$.

RESULTS

Effects of PE on tension and cytoplasmic Ca^{2+} level in rat mesenteric resistance arteries

Figure 1A shows the contraction induced by 1 and 100 μM PE, an α_1 -adrenoceptor agonist, in Ca^{2+} -containing PSS or Ca^{2+} -free PSS. In Ca^{2+} -containing PSS, either 1 or 100 μM PE induced a sustained contraction, the magnitude of which depended on the concentration. In Ca^{2+} -free PSS, 1 μM PE did not cause a contraction, whereas 100 μM PE produced a transient contraction, the peak of which was $52 \pm 4\%$ ($n=5$) of the contraction induced in normal PSS, followed by a small, sustained contraction. Figure 1B shows changes in $[\text{Ca}^{2+}]_i$ (F_{340}/F_{380}) measured in separate experiments. PE at 1 μM caused a monotonic, sustained increase of $[\text{Ca}^{2+}]_i$ in normal PSS, whereas it did not cause any change in Ca^{2+} -free PSS. On the other hand, 100 μM PE produced an initial rapid increase in $[\text{Ca}^{2+}]_i$ followed by a little lower, sustained level of $[\text{Ca}^{2+}]_i$. In Ca^{2+} -free PSS, 100 μM PE produced only a transient increase in $[\text{Ca}^{2+}]_i$. Thus, the contraction induced by 1 μM PE seemed to depend on transmembrane Ca^{2+} influx. In the following experiments, we analyzed how the SR regulated $[\text{Ca}^{2+}]_i$ during the contraction induced by 1 μM PE.

Effects of CPA on caffeine-induced contraction

In normal PSS, caffeine (100 mM) caused a transient contraction, which was followed by a relaxation to the baseline. The response was reproducible if a 10-min Ca^{2+} -loading period intervened. When CPA (10 μM) was added to muscles equilibrated in normal PSS, it decreased the subsequent caffeine-contraction elicited 5 min later to $68 \pm 4\%$ ($n=6$) of the control response (Fig. 2A). We tested how quickly CPA affected the caffeine-contraction (Fig. 2B). To obtain the control caffeine-contraction, caffeine (100 mM) was applied simultaneously with switching the medium to Ca^{2+} -free PSS. This concentration of caffeine

Fig. 1. Changes in the tension (A) and the ratio of fura-2 fluorescence (B) in response to 1 or 100 μM phenylephrine (PE) in normal PSS or Ca^{2+} -free PSS (with 2 mM EGTA) of rat mesenteric resistance arteries. When tested in Ca^{2+} -free PSS, the arteries were incubated for 3 min in Ca^{2+} -free PSS until PE was applied. In B, the calibration represents the F_{340}/F_{380} ratio.

depleted the SR Ca^{2+} , since the second application of caffeine during the continued exposure to Ca^{2+} -free PSS did not induce any contraction (data not shown). Three minutes after washing out caffeine, the muscle was exposed to normal PSS to refill the SR with Ca^{2+} . At 2 min in normal PSS, caffeine was added in order to measure the state of refilling in the SR. The caffeine-contraction after 2 min Ca^{2+} refilling was $42 \pm 8\%$ ($n=5$) of the control contraction. If CPA (10 μM) was present during the refilling period, the caffeine-contraction was significantly decreased to $22 \pm 5\%$ ($n=5$) of the control caffeine-con-

traction. These results indicate that the inhibitory effect of CPA on the Ca^{2+} uptake appears earlier than 2 min.

Effects of CPA and ryanodine on 1 μM PE-induced contraction

Neither CPA (10 μM) nor ryanodine (10 μM) affected the muscle tension in normal PSS. When applied during the sustained phase of 1 μM PE-induced contraction, however, CPA caused a transient potentiation of the contraction (Fig. 3A). The peak of potentiation was $226 \pm 46\%$ ($n=6$), when expressed as a percent of the 1 μM PE-induced contraction. Ryanodine (10 μM) also transiently potentiated the 1 μM PE-induced contraction by $200 \pm 37\%$ ($n=6$) at the maximum effect (Fig. 3B). The potentiation due to CPA subsided within 5 min, while it took more than 10 min for ryanodine-induced potentiation to subside.

Changes in cytosolic Ca^{2+} caused by CPA and ryanodine

Next, we measured the change in $[\text{Ca}^{2+}]_i$ due to CPA and ryanodine in the absence or presence of PE. Furthermore, we also measured the transient rise in $[\text{Ca}^{2+}]_i$ induced by 100 mM caffeine to determine how the Ca^{2+} content in the SR changed after the above interventions. Since a single dose of 100 mM caffeine depleted the SR Ca^{2+} as mentioned above, we took the initial $[\text{Ca}^{2+}]_i$ response to caffeine as the index of Ca^{2+} content in the SR. One problem concerning the measurement of fura-2 fluorescence is a time-dependent decay of the fluorescence response to stimulants, probably due to leakage of fura-2 from the cells (15). For example, when isotonic 80 mM K^+ was applied two times with an interval of 35 min, the change of the ratio (F_{340}/F_{380}) caused by a second application of K^+ decreased to $86 \pm 5\%$ ($n=5$) of the first one (data not shown). We observed the response to 100 mM caffeine twice with an interval of 15 min and took the response to the second caffeine application in the absence of any intervention as the time-matched control (Fig. 4A, left panel). In the following section, we express the fluorescence response to various stimulants as a percentage of the response to the first caffeine application (CAF 1 in Fig. 4). Caffeine, applied in normal PSS for 2 min, caused a rapid increase in $[\text{Ca}^{2+}]_i$ followed by a decrease to a level slightly higher than the resting level. After termination of caffeine superfusion, $[\text{Ca}^{2+}]_i$ showed a small and transient increase. In the control muscles, the F_{340}/F_{380} ratio in response to the second caffeine application decreased to $91 \pm 5\%$ ($n=7$) of the response to the first caffeine application (Fig. 4A). This decay of the response is considered to be due to the leak of fura-2, but not due to the decrease of the Ca^{2+} releasing action, since the tension response to caffeine was the same between the two trials as aforementioned.

Fig. 2. Effects of cyclopiazonic acid (CPA) on caffeine (CAF)-induced contractions of mesenteric arteries. A, the muscle equilibrated in normal PSS was treated with 10 μ M CPA, and then 100 mM caffeine was added twice, at 5 min and 15 min after the addition of CPA. Caffeine was present for 2 min for each addition. B, first caffeine (100 mM) was applied to discharge the SR Ca^{2+} when the external medium was changed to Ca^{2+} -free PSS containing 2 mM EGTA. This response is referred as 100%. After the muscle was soaked in Ca^{2+} -free PSS for 5 min, it was incubated in normal PSS for 2 min to refill the SR with Ca^{2+} , and then 100 mM caffeine was added. Left: control response to caffeine. Right: the caffeine-response when CPA was present during the refilling period.

Fig. 3. Potentiation by cyclopiazonic acid (CPA) and ryanodine (RY) of phenylephrine (PE)-induced contraction. CPA (10 μ M, A) or RY (10 μ M, B) was added in normal PSS (left) or 10 min after the induction of 1 μ M PE-contraction (right). Each trace was obtained from different preparations.

In resting arteries CPA (10 μ M) or ryanodine (10 μ M) slightly but sustainedly increased $[\text{Ca}^{2+}]_i$ (Fig. 4, B and C). The rises in $[\text{Ca}^{2+}]_i$ due to both agents were comparable (Table 1). Caffeine (100 mM), which was added 5 min after the application of CPA or ryanodine, induced a transient rise in $[\text{Ca}^{2+}]_i$ with an amplitude of $64 \pm 4\%$ ($n=6$) or $60 \pm 2\%$ ($n=7$), respectively (Fig. 5). In Ca^{2+} -free PSS, neither CPA nor ryanodine caused a change in $[\text{Ca}^{2+}]_i$ (data not shown).

PE (1 μ M) increased the F_{340}/F_{380} ratio by $23 \pm 7\%$ ($n=8$). If caffeine was added 10 min after application of PE, it caused a transient elevation of $[\text{Ca}^{2+}]_i$. The peak of the transient was $71 \pm 7\%$ ($n=7$, Fig. 4A). When CPA was added 5 min after PE, it increased $[\text{Ca}^{2+}]_i$ more greatly than when it was applied to resting arteries (Fig. 4B, Table 1). The rise in $[\text{Ca}^{2+}]_i$ due to CPA in PE-treated arteries was transient, since $[\text{Ca}^{2+}]_i$ gradually decreased after reaching the peak at 1.6 ± 0.1 min ($n=7$). Similarly, ryanodine, applied during the PE-induced contraction, further elevated $[\text{Ca}^{2+}]_i$, the amplitude being larger than that observed in resting arteries. The ryanodine-induced rise in $[\text{Ca}^{2+}]_i$ in PE-treated arteries was also transient since it began to decline 3–5 min later, but more slowly than the case of CPA. When caffeine was added 5 min

Fig. 4. Effects of cyclopiazonic acid (CPA) and ryanodine (RY) on $[Ca^{2+}]_i$ in resting or phenylephrine (PE)-stimulated mesenteric resistance arteries. In all panels, responses to caffeine (100 mM) were observed twice with an interval of 15 min. The response to the first caffeine (CAF 1) application is referred as 100%, as shown in the calibration bar. In right panels of A-C, PE (1 μ M) was introduced 5 min after washing out the first caffeine. B, CPA (10 μ M) was added 10 min after the first caffeine application. C, ryanodine (10 μ M) was added 10 min after the first caffeine application.

after CPA or ryanodine in PE-treated arteries, it induced a smaller increase in $[Ca^{2+}]_i$ than the increase in the absence of CPA and ryanodine. The Ca^{2+} transient caused by caffeine was more greatly depressed after application of ryanodine ($13 \pm 4\%$, $n=7$) than after application of CPA ($39 \pm 6\%$, $n=7$, Fig. 5). In arteries that had been exposed to a combination of ryanodine and caffeine, the

Table 1. Increases in the F_{340}/F_{380} ratio by cyclopiazonic acid (CPA) and ryanodine in resting or 1 μ M phenylephrine-stimulated rat mesenteric resistance arteries

	Resting	Phenylephrine-stimulated
CPA (10 μ M)	22 ± 2 (6)	$55 \pm 7^*$ (7)
Ryanodine (10 μ M)	19 ± 1 (7)	$48 \pm 6^*$ (7)

Data are expressed as a percent of the response to the first caffeine. Number in parentheses represents the number of experiments. *Significantly different from the response in resting arteries ($P < 0.05$).

Fig. 5. Modification of caffeine-induced Ca^{2+} transient by cyclopiazonic acid (CPA) and ryanodine (RY) in the absence (left two columns) or presence (right two columns) of phenylephrine (PE, 1 μ M). The experimental protocol is shown in Fig. 4. The response to the first caffeine application in each preparation is referred as 100%. Hatched column: arteries treated with CPA (10 μ M). Open column: arteries treated with ryanodine (10 μ M). Data are means $\pm$ S.E. of 6 or 7 experiments.

subsequent application of caffeine did not induce a rise in $[Ca^{2+}]_i$ even after washing out of both drugs (data not shown).

DISCUSSION

As shown in Fig. 1, in rat mesenteric resistance arteries, the 1 μ M PE-induced contraction seemed to mainly depend on the transmembrane Ca^{2+} influx, whereas the 100 μ M PE-induced contraction clearly involved both Ca^{2+} release from the SR and Ca^{2+} influx. We analyzed how the SR regulated $[Ca^{2+}]_i$ in the contraction where the Ca^{2+} influx predominated.

Activation of the CICR during α_1 -adrenoceptor stimulation

Ryanodine increased $[Ca^{2+}]_i$ in resting arteries and more greatly did so in PE-stimulated arteries, whereas it elevated the tension in only PE-contracted arteries. Since ryanodine has been reported to accelerate the Ca^{2+} efflux from the SR through CICR channels (2, 16), the transient increase in $[Ca^{2+}]_i$ and tension due to ryanodine could be explained by this action.

The increase in $[Ca^{2+}]_i$ following application of ryanodine was larger during the PE-stimulation than during the resting condition. This means that ryanodine released more Ca^{2+} during the PE-stimulation than during the resting state. Ryanodine binds to CICR channels that are in an open state, and then locks them open (1, 2, 4). Consistent with this, our previous report (17) revealed that the manifestation of ryanodine-action depended on the active state of CICR channels. From the comparison of responses to ryanodine, it can be said that more CICR channels were in an active state during the PE-induced contraction than during the resting state.

Because the CICR depends on the cytoplasmic Ca^{2+} (18, 19), acceleration of the CICR in the presence of PE could be due to increased Ca^{2+} in the cytoplasm which entered from the extracellular space. The similar acceleration of the CICR has been shown in the situation where depolarization-induced Ca^{2+} influx occurred in guinea pig aortas (20). Although production of IP_3 by 1 μ M PE is guessed to be very scarce from the data shown in Fig. 1, the role of IP_3 may not be neglected, because IP_3 -induced Ca^{2+} release could be enhanced depending on the concentration of cytoplasmic Ca^{2+} through a positive feedback mechanism (21). IP_3 produced by 1 μ M PE may be able to release Ca^{2+} in Ca^{2+} -containing medium, even though it does not release Ca^{2+} in Ca^{2+} -free medium, and thereby secondarily accelerate the CICR.

The role of SR Ca^{2+} pump during α_1 -adrenoceptor activation

As one of the functions of the SR in smooth muscles, the Ca^{2+} pump promotes the uptake of Ca^{2+} influxed from the extracellular space; thereby, the SR works to reduce $[Ca^{2+}]_i$ (22, 23). In such a situation, an intervention to inhibit the SR Ca^{2+} pump would increase $[Ca^{2+}]_i$ (24). The action of CPA to inhibit Ca^{2+} uptake into the SR was demonstrated in the experiment shown in Fig. 2. On the assumption that CPA is a selective inhibitor of Ca^{2+} -ATPase (10), an increase of $[Ca^{2+}]_i$ by CPA in resting or PE-stimulated arteries (Fig. 4) could be explained by the inhibition of Ca^{2+} uptake into the SR. However, an alternative explanation is also possible that CPA accelerated the Ca^{2+} leak from the SR, since Baró and Eisner (25) reported that thapsigargin, another SR Ca^{2+} pump inhibitor, released Ca^{2+} from the SR in rat mesenteric artery cells, in either Ca^{2+} -containing or Ca^{2+} -free medium. Unlike their case, however, CPA did not increase $[Ca^{2+}]_i$ in Ca^{2+} -free PSS. Furthermore, the $[Ca^{2+}]_i$ -response to caffeine after CPA was larger than that after ryanodine in PE-stimulated arteries, indicating that the SR retained more Ca^{2+} after CPA than after ryanodine. This suggests that the acceleration of Ca^{2+} leakage, if any, was negligible in the effect of CPA observed here. Therefore, the CPA-induced rise in $[Ca^{2+}]_i$ could be attributed to the inhibition of Ca^{2+} buffering of the SR. Since, as discussed in the previous section, Ca^{2+} release from the SR was involved in the rise in $[Ca^{2+}]_i$ due to PE, the inhibition of Ca^{2+} uptake by CPA would secondarily decrease the release-dependent component in $[Ca^{2+}]_i$ elevation. This may be a cause for the transient nature of the CPA-induced rise in $[Ca^{2+}]_i$.

After PE increased $[Ca^{2+}]_i$ and the tension, CPA further increased both parameters, whereas CPA produced a smaller increase in $[Ca^{2+}]_i$ and no tension development in resting arteries. These results suggest that the Ca^{2+} uptake into the SR was enhanced during the α_1 -adrenoceptor activation. It was shown that in microsomes from vascular smooth muscles, the Ca^{2+} -uptake depended on the free Ca^{2+} concentration outside the vesicles (26). Sturek et al. (23) showed that when $[Ca^{2+}]_i$ was higher, the subsequent caffeine-induced $[Ca^{2+}]_i$ transient was proportionally larger, indicating a greater Ca^{2+} uptake at higher cytoplasmic Ca^{2+} . Therefore, an elevation of $[Ca^{2+}]_i$ due to increased Ca^{2+} influx by PE may be associated with the enhancement of Ca^{2+} uptake into the SR in the presence of PE.

SR Ca^{2+} cycling in resting arteries

When CPA was applied for 5 min to arteries equilibrated in normal PSS, it decreased the subsequent caffeine-induced contraction to $68 \pm 4\%$ (Fig. 2). This suggests that

the SR is exchanging Ca^{2+} by taking up Ca^{2+} when the SR resides in an equilibrated condition. The data that CPA increased the F_{340}/F_{380} ratio by $23 \pm 7\%$ in resting arteries and decreased the $[\text{Ca}^{2+}]_i$ response to subsequently added caffeine from $91 \pm 5\%$ to $64 \pm 4\%$ are consistent with the idea that Ca^{2+} uptake occurs in resting arteries. Therefore, it can be said that 20–30% of the Ca^{2+} in the SR is exchanged for 5 min by the cytoplasmic Ca^{2+} under an equilibrium condition. According to 'superficial buffer barrier' hypothesis (22, 24), Ca^{2+} provided by the SR under the resting condition is extruded from cells rather than utilized for activating contraction. The sustained increase in $[\text{Ca}^{2+}]_i$ following inhibition of Ca^{2+} uptake by CPA suggests that the SR works to lower the Ca^{2+} concentration in the bulk of the cytoplasm by taking up Ca^{2+} under a resting condition, favoring the hypothesis.

The significant increase in $[\text{Ca}^{2+}]_i$ following treatment with ryanodine in resting arteries implies that the CICR operated under such a condition. Thus, the CICR may be involved in the exchange of SR Ca^{2+} . A part of the released Ca^{2+} would be extruded from the cells, and the remainder would be again taken up by the SR. The lack of effect of CPA and ryanodine on $[\text{Ca}^{2+}]_i$ in Ca^{2+} -free PSS suggests that the transmembrane Ca^{2+} influx is important for the CICR and the replenishment of the SR Ca^{2+} during the resting condition.

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