Ca²⁺ Buffering Function of Sarcoplasmic Reticulum in Rat Tail Arteries: Comparison in Normotensive and Spontaneously Hypertensive Rats

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Received April 3, 2000 Accepted May 25, 2000

ABSTRACT—The superficial buffer barrier function of the sarcoplasmic reticulum (SR) during rest and that during stimulation with Bay k 8644, an agonist of L-type Ca²⁺ channels, were compared in endothelium-denuded strips of tail arteries from 13-week-old normotensive Wistar-Kyoto rats (WKY) and spontaneously hypertensive rats (SHR), by measuring the effects of cyclopiazonic acid (CPA) and thapsigargin that inhibit SR Ca²⁺-ATPase and the effect of ryanodine that depletes SR Ca²⁺. The addition of 10 μ M CPA induced a transient contraction that was not significantly different between WKY and SHR. The CPA-induced contraction was strongly inhibited by 100 nM nifedipine and was abolished by Ca²⁺-free solution in both strains. Thapsigargin (100 nM) or ryanodine (10 μ M) induced similar, small transient contractions in the two strains. The addition of Bay k 8644 (1–100 nM) almost failed to induce a contraction in both WKY and SHR. When the strips were preincubated with 10 μ M CPA, 100 nM thapsigargin or 10 μ M ryanodine, Bay k 8644 induced similar concentration-dependent contractions in the two strains. The amount of Ca²⁺ stored in the SR, as estimated from the 20 mM caffeine-induced contraction, was not significantly different between WKY and SHR. Our results suggest that the SR of rat tail arteries can buffer a large amount of Ca²⁺ that enters the cell during the rest and the Bay k 8644 stimulation, and these functions are not altered in SHR.

Keywords: Superficial buffer barrier function, Sarcoplasmic reticulum Ca²⁺-ATPase, Ca²⁺ influx via L-type Ca²⁺ channels, Tail artery, Spontaneously hypertensive rats (SHR)

The sarcoplasmic reticulum (SR) is an important regulator of cytosolic Ca²⁺ levels ([Ca²⁺]_i) in arterial smooth muscle cells by acting as a buffer barrier to Ca²⁺ influx via L-type Ca²⁺ channels. According to the “superficial buffer barrier” hypothesis proposed by van Breemen and his colleagues (1–3), Ca²⁺ that enters the peripheral cytoplasm through the plasmalemma is taken up into the SR by SR Ca²⁺-ATPase before it can diffuse to the deeper myoplasm. This hypothesis was derived from the observation that the magnitude of arterial smooth muscle contraction due to K⁺ depolarization depends on the rate, rather than on the net amount, of Ca²⁺ influx via L-type Ca²⁺ channels; and this was confirmed later by a number of studies using cyclopiazonic acid (CPA) and thapsigargin that inhibit SR Ca²⁺-ATPase or ryanodine that depletes SR Ca²⁺ (for details, see refs. 4–8).

Methyl-1,4-dihydro-2,6-dimethyl-3-nitro-4-(2-trifluoromethylphenyl)-pyridine-5-carboxylate (Bay k 8644) is an agonist of L-type Ca²⁺ channels that increases Ca²⁺ influx through an increase in the open probability of the channel.

This agonist induces a strong contraction in many arteries from spontaneously hypertensive rats (SHR) when compared with normotensive Wistar-Kyoto rats (WKY); e.g., femoral artery (9), carotid artery (10) and aorta (11). However, it also is shown that this agonist failed to induce a contraction in the tail artery, a cutaneous artery, from both SHR and WKY (9). The Ca²⁺ buffering function of the SR during stimulation of rabbit aortic smooth muscle with Bay k 8644 was proposed by Hwang and van Breemen (12), and it was confirmed in our previous study (8). In our previous study, the Ca²⁺ buffering function of the SR during the Bay k 8644 stimulation was examined in the WKY femoral artery. Bay k 8644 failed to induce a contraction under normal conditions, but induced a strong contraction when the SR Ca²⁺ buffering was inhibited by CPA, thapsigargin or ryanodine, suggesting that Ca²⁺ influx during the Bay k 8644 stimulation is completely buffered by the SR. Because the Ca²⁺ buffering functions of the SR during the rest and the Bay k 8644 stimulation have never been determined in the tail arteries, the present study was designed to determine these functions in the WKY tail artery through an interpretation of the contraction data by using the selec-

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tive inhibitors of the SR Ca^{2+} buffering, and the data were compared with those in SHR. Here, we show that rat tail arteries have strong Ca^{2+} buffering functions of the SR during the resting state and during Bay k 8644 stimulation, and these functions are not altered in SHR. A preliminary account of these findings was presented at the 73rd Annual Meeting of The Japanese Pharmacological Society (13).

MATERIALS AND METHODS

Preparation of arterial smooth muscle strips

Male SHR and WKY were inbred in our laboratory. They were studied at 13 weeks (12–14 weeks) of age. The systolic blood pressure at this age, measured by tail-cuff plethysmography (KN-210; Natsume Seisakusho, Tokyo), was significantly higher in SHR (200 ± 2.4 mmHg, $n=24$) than in WKY (138 ± 0.7 mmHg, $n=24$). The body weight was not significantly different between SHR (280 ± 4.4 g, $n=24$) and WKY (291 ± 3.5 g, $n=24$). After decapitation, the proximal parts of the tail arteries (0.6–0.7-mm outside diameter) were excised and placed in a Krebs solution of the following composition: 115.0 mM NaCl, 4.7 mM KCl, 2.5 mM CaCl_2 , 1.2 mM MgCl_2 , 25.0 mM NaHCO_3 , 1.2 mM KH_2PO_4 and 10.0 mM dextrose. Arteries were cut into helical strips (0.7–0.8 mm in width) as described previously (14). To avoid possible influences of endothelium-derived factors, the endothelium of the strip was removed by gently rubbing the endothelial surface with a cotton swab. Successful removal of the endothelium was confirmed later by the inability of acetylcholine (1 μM) to induce a relaxation in strips precontracted with prostaglandin $\text{F}_{2\alpha}$.

Arterial strips (7–8 mm in length) were mounted vertically in water-jacketed muscle baths containing 10 ml Krebs solution. Krebs solutions were aerated with 95% O_2 and 5% CO_2 and maintained at 37°C. The isometric tension was recorded with a force-displacement transducer (TB-612T; Nihon Kohden Kogyo, Tokyo). Strips were stretched passively to optimal length by imposing a resting tension of 0.5 g for WKY or 0.55 g for SHR, respectively, and a 60-min equilibration period preceded each experiment. The optimal resting tensions were determined by a length-passive tension study (14). All experiments were done under protection from light.

Experimental protocols

After the equilibration, the contractile responses of the strips to the Krebs solution containing 65.9 mM KCl (K^+) (equimolar substitution of Na^+ with K^+) were repeated (usually three times) until the responses were reproducible. After washout with a Krebs solution, the contractile responses to Bay k 8644 (1–100 nM) or elevated extracellular K^+ (3–30 mM addition) were determined in a cumulative fashion. At the end of the K^+ contraction, the bathing

solution was replaced with 65.9 mM K^+ to determine the maximum contraction of the strip. The contractile responses to K^+ were conducted in phenoxybenzamine (2 μM)-treated strips to eliminate possible α -adrenoceptor responses to endogenously released norepinephrine, and timolol (0.5 μM) was added to a Krebs solution to eliminate possible β -adrenoceptor responses (14). The sensitivity for Bay k 8644 or K^+ is expressed as the concentration inducing either 50% or 30% of the maximum contraction (EC_{50} or EC_{30} , respectively).

To examine the Ca^{2+} buffering function of the SR during stimulation with Bay k 8644 or K^+ , the effects of CPA, thapsigargin or ryanodine on the responses to Bay k 8644 or K^+ were determined. Because each inhibitor itself induced a relatively transient contraction that reflects the Ca^{2+} buffering function of the SR during rest, the possible mechanism underlying this contraction was also examined. In some experiments, a nominally Ca^{2+} -free solution was used. This solution was prepared by omitting Ca^{2+} from a Krebs solution and adding 0.1 mM EGTA.

Since higher concentrations of caffeine (>20 mM) have been shown to deplete Ca^{2+} stored in the SR, the transient contraction induced by 20 mM caffeine can be used as an index of the amount of Ca^{2+} stored in the SR (4–6, 15, 16).

Statistical analyses

Unless specified, the results are expressed as means $\pm$ S.E.M. (n = number of preparations, with one preparation from each rat). The Student's *t*-test for unpaired data was used to determine the significance of differences between means, and a *P* value of <0.05 was taken as significant.

Drugs

The drugs used were Bay k 8644 (Bayer, Wuppertal, Germany); CPA, thapsigargin, EGTA, timolol maleate and acetylcholine chloride (Sigma, St. Louis, MO, USA); ryanodine and caffeine (Wako, Osaka); nifedipine (Bayer Yakuhin Osaka); phenoxybenzamine hydrochloride (Nacalai Tesque, Kyoto); and prostaglandin $\text{F}_{2\alpha}$ (Ono Pharmaceutical, Osaka).

Bay k 8644 (1 mM) and nifedipine (1 mM) were dissolved in 99.5% ethanol with further dilution in distilled water before use. Phenoxybenzamine (2 mM) was dissolved in 99.5% ethanol. CPA (5 mM) and thapsigargin (100 μM) were dissolved in 100% dimethyl sulfoxide. Dimethyl sulfoxide at 0.2% (contained in the experiments with 10 μM CPA) neither induced a contraction nor augmented the responses to Bay k 8644 or K^+ . Caffeine (20 mM) was dissolved in the Krebs solution. Aqueous stock solutions were prepared for other drugs. Concentrations of drugs are expressed as final molar concentrations.

RESULTS

Contractions induced by CPA, thapsigargin and ryanodine

The addition of 65.9 mM K^+ induced a peak contraction followed by a plateau phase (6–10 min after the addition) in strips of tail arteries from WKY and SHR (Fig. 1). The peak contraction was significantly larger in SHR (938 ± 30 mg, $n=24$) than in WKY (698 ± 27 mg, $n=24$). For the relative comparison of the following contraction data between WKY and SHR, the magnitude of the contraction was always normalized by expressing the data as % of the 65.9 mM K^+ contraction obtained in the same strip. The addition of 20 mM caffeine induced a transient contraction in WKY and SHR (Fig. 1). This contraction was not significantly different between the two strains (Table 1A), suggesting that a similar amount of Ca^{2+} was stored in the SR.

Fig. 1. Typical recordings of the contractions induced by caffeine (Caf, 20 mM), cyclopiazonic acid (CPA, 10 μM) and thapsigargin (TG, 100 nM) in strips of tail arteries from WKY and SHR. After determination of the maximum contraction induced by 65.9 mM KCl (65.9 K^+), Caf was added for 3 min. Following washout for 35 min, CPA was added for 40 min (A) or TG was added for 50 min (B). In the presence of CPA or TG, Caf was added. Dots (•) denote the washout of the strips with Krebs solution.

The addition of 10 μM CPA induced a contraction that declined gradually with time in WKY and SHR (Fig. 1A). The peak contraction was not significantly different between the two strains (Table 1B). Also, the small contraction remaining at 35–45 min after the addition was not significantly different between WKY ($6.2 \pm 1.3\%$, $n=22$) and SHR ($3.6 \pm 1.2\%$, $n=22$). After the CPA contraction had declined, 20 mM caffeine induced a transient contraction that was not significantly different between WKY ($30.5 \pm 3.0\%$, $n=8$) and SHR ($31.6 \pm 3.1\%$, $n=8$), suggesting that a similar amount of Ca^{2+} leaked spontaneously from the SR during the addition of CPA.

The addition of 100 nM thapsigargin induced a small contraction in WKY and SHR (Fig. 1B). This contraction was not significantly different between the two strains (Table 1C). During the plateau phase of the thapsigargin contraction, 20 mM caffeine induced a transient contraction that was not significantly different between WKY ($14.2 \pm 2.4\%$, $n=7$) and SHR ($15.6 \pm 2.4\%$, $n=7$), again suggesting the similar leakage of SR Ca^{2+} .

The addition of 10 μM ryanodine also induced a small contraction in WKY and SHR (Fig. 2). This contraction was not significantly different between the two strains (Table 1D). During the plateau phase of the ryanodine contraction, 20 mM caffeine induced a small transient contraction in both strains. After washout, a small contraction appeared, and during this period, 20 mM caffeine induced a small transient contraction that was not significantly different between WKY ($8.1 \pm 2.1\%$, $n=7$) and SHR (6.9

Table 1. Contractile responses to caffeine (Caf, A), cyclopiazonic acid (CPA, B), thapsigargin (TG, C) and ryanodine (Rya, D) in strips of tail arteries from WKY and SHR and effects of nifedipine and Ca^{2+} -free solution (0-Ca) on these responses

Condition ^a	Contraction (% of 65.9 mM K^+) ^b	
	WKY	SHR
A. Caf, 20 mM (Control)	45.3 ± 1.6 (24)	45.8 ± 1.7 (24)
+ Nifedipine, 100 nM	46.7 ± 1.7 (6)	45.3 ± 2.4 (6)
in 0-Ca for 2 min	21.5 ± 1.7^c (5)	24.9 ± 0.9^c (6)
B. CPA, 10 μM (Control)	48.7 ± 3.1 (22)	49.6 ± 3.8 (22)
+ Nifedipine, 100 nM	9.5 ± 1.2^c (7)	12.5 ± 1.4^c (6)
in 0-Ca for 2 min	0.9 ± 0.6^c (4)	0.6 ± 0.4^c (4)
C. TG, 100 nM (Control)	9.2 ± 2.4 (15)	9.5 ± 2.5 (15)
+ Nifedipine, 100 nM	1.5 ± 0.8^c (7)	1.6 ± 0.8^c (6)
D. Rya, 10 μM (Control)	9.3 ± 1.9 (15)	9.6 ± 2.2 (15)
+ Nifedipine, 100 nM	0.8 ± 0.2^c (7)	0.9 ± 0.4^c (7)

^aControl contractions induced by Caf, CPA, TG and Rya were the total values of the present study. Experimental conditions for the effects of nifedipine and 0-Ca were the same as in Fig. 3. ^bThe peak contractions induced by Caf, CPA, TG and Rya are expressed as % of the 65.9 mM K^+ contraction. Data are expressed as means $\pm$ S.E.M.; numbers in parentheses indicate the number of preparations used. ^cSignificantly different from the respective Control value ($P < 0.05$). Each value in SHR was not significantly different from the respective WKY value.

Fig. 2. Typical recordings of the ryanodine (Rya, 10 μ M) treatment in strips of tail arteries from WKY and SHR. After determination of the maximum contraction induced by 65.9 mM KCl (65.9 K^+), caffeine (Caf, 20 mM) was added for 3 min. Following washout for 35 min, Rya was added for 30 min. In the presence of Rya, Caf was added for 10 min. After washout for 35 min, Caf was added. Dots (•) denote the washout of the strips with Krebs solution.

$\pm 1.8\%$, $n = 7$), suggesting the similar leakage of SR Ca^{2+} from Ca^{2+} -induced Ca^{2+} release (CICR) channels.

Effects of nifedipine and Ca^{2+} -free solution on the contractions induced by CPA, thapsigargin and ryanodine

The CPA-induced contraction was strongly inhibited by 100 nM nifedipine and was almost abolished by a 2-min exposure to a Ca^{2+} -free solution in WKY and SHR (Fig. 3, Table 1B). Each effect was not significantly different between the two strains (Table 1B). Nifedipine also inhibited the contractions induced by thapsigargin and ryanodine, and each effect was not significantly different between WKY and SHR (Table 1: C and D). On the other hand, the caffeine-induced contraction was not inhibited by nifedipine and was not abolished (but was reduced) by Ca^{2+} -free solution in WKY and SHR (Table 1A). The caffeine contraction in a Ca^{2+} -free solution was not significantly different between the two strains, suggesting the similar leakage of SR Ca^{2+} (Table 1A).

Effects of CPA, thapsigargin and ryanodine on the responses to Bay k 8644

The addition of Bay k 8644 (1 – 100 nM) almost failed to induce a contraction in WKY and SHR (Figs. 4A and 5, Table 2). To examine the Ca^{2+} buffering function of the SR during the Bay k 8644 stimulation, the effects of 10 μ M CPA on the Bay k 8644 response were determined (Figs. 4B and 5, Table 2). After the CPA contraction had declined, Bay k 8644 induced a concentration-dependent contraction in WKY and SHR. The maximum contraction and the EC_{50} value for Bay k 8644 were not significantly different between the two strains (Table 2). Similar augmented contractions to Bay k 8644 were observed when the effects of

Fig. 3. Typical recordings of the effects of nifedipine and Ca^{2+} -free solution on the cyclopiazonic acid (CPA, 10 μ M)-induced contraction in strips of the WKY tail artery. A: The control contraction induced by CPA was determined as in Fig. 1. B: After the addition of nifedipine (Nif, 100 nM) for 20 min, CPA was added. C: After the 2-min exposure of the strip to a Ca^{2+} -free solution (0-Ca), CPA was added.

100 nM thapsigargin were determined (Figs. 4C and 5, Table 2). The effects of ryanodine on the Bay k 8644 response were then determined (Figs. 4D and 5, Table 2). After the treatment of the strips with 10 μ M ryanodine plus 20 mM caffeine, the addition of Bay k 8644 also induced a concentration-dependent contraction in WKY and SHR.

The maximum contraction and the EC_{50} value for Bay k 8644 were not significantly different between the two strains (Table 2).

Under the inhibition of the SR Ca^{2+} buffering by CPA, thapsigargin and ryanodine, the maximum contraction and the EC_{50} value for Bay k 8644 were not significantly different among the three agents and also were not different between the two strains (Fig. 5, Table 2). Therefore, the inhibition of the SR Ca^{2+} buffering caused the Bay k 8644 contraction to be similar in the six experimental conditions (Fig. 5, Table 2).

Effects of CPA on the contractile responses to K^+

The addition of K^+ (3–30 mM) induced a concentration-dependent contraction at relatively high concentrations (above 14 mM) in WKY and SHR (Fig. 6). The EC_{30} value for K^+ was not significantly different between WKY

Fig. 4. Typical recordings of the effects of cyclopiazonic acid (CPA, 10 μM), thapsigargin (TG, 100 nM) and the ryanodine (Rya, 10 μM) treatment on the contractile responses to Bay k 8644 in strips of the WKY tail artery. After determination of the maximum contraction induced by 65.9 mM KCl (65.9 K^+), contractile responses to Bay k 8644 (Bay, 1–100 nM) were determined in a cumulative fashion. A: Control responses to Bay k 8644. Effects of CPA, TG and the Rya treatment on the Bay k 8644 response are shown in B, C and D, respectively. The preincubation of the strips with CPA or TG and the Rya treatment were done as in Figs. 1 and 2.

Fig. 5. Effects of cyclopiazonic acid (CPA, 10 μM) (Δ), thapsigargin (TG, 100 nM) ($\bullet$) and the ryanodine (Rya, 10 μM) ($\blacktriangle$) treatment ($\blacktriangle$) on the concentration-response curve for Bay k 8644 ($\circ$, Control) in strips of tail arteries from WKY and SHR. Experimental conditions were the same as in Fig. 4. At '0' on the abscissa, the CPA-, TG- or Rya-induced contractions remaining before the addition of Bay k 8644 are expressed as % of the 65.9 mM K^+ contraction. The peak contractions induced by each concentration of Bay k 8644 are then expressed as % of the 65.9 mM K^+ contraction. Data points are means of 8 preparations, and S.E.M.s are shown by vertical bars.

Table 2. Effects of cyclopiazonic acid (CPA), thapsigargin (TG) and the ryanodine (Rya) treatment on the contractile responses to Bay k 8644 in strips of tail arteries from WKY and SHR

Condition	n	Maximum Bay k 8644 contraction (% of 65.9 mM K ⁺) ^a		EC ₅₀ for Bay k 8644 (nM) ^a	
		WKY	SHR	WKY	SHR
Control	8	1.5 ± 0.6	0.5 ± 0.4	N.C. ^b	N.C. ^b
+ CPA, 10 μM	8	45.6 ± 6.0 ^c	46.4 ± 7.0 ^c	4.7 ± 1.5	5.2 ± 1.7
+ TG, 100 nM	8	43.7 ± 6.8 ^c	44.8 ± 7.0 ^c	3.3 ± 1.2	3.5 ± 1.6
+ Rya, 10 μM	8	41.8 ± 6.5 ^c	40.9 ± 4.6 ^c	3.6 ± 1.4	3.7 ± 1.6

^aThe maximum contractions and the EC₅₀ values for Bay k 8644 were calculated from the concentration-response curves shown in Fig. 5 and are expressed as % of the 65.9 mM K⁺ contraction and nM, respectively. Data are expressed as means ± S.E.M., n = number of preparations used. ^bNot calculated. ^cSignificantly different from the respective Control value ($P < 0.05$). Each value in SHR was not significantly different from the respective WKY value.

(23.7 ± 1.3 mM, n = 6) and SHR (24.9 ± 1.5 mM, n = 6). To examine the Ca²⁺ buffering function of the SR during the K⁺ stimulation, the effects of 10 μM CPA were determined (Fig. 6). The K⁺ contraction was highly augmented by CPA; after the CPA contraction had declined, low concentrations of K⁺ (3–14 mM) induced a strong contraction in WKY and SHR. The maximum contraction induced by 65.9 mM K⁺ was not affected by CPA in both strains. The EC₃₀ value for K⁺ in the augmented contraction was not significantly different between WKY (7.3 ± 0.8 mM, n = 6)

and SHR (6.5 ± 0.7 mM, n = 6), suggesting that the CPA-induced augmentation was the same in the two strains.

DISCUSSION

The present study compared the Ca²⁺ buffering function of the SR in tail arteries from WKY and SHR through an interpretation of the contraction data using the selective inhibitors of the SR Ca²⁺ buffering. The major findings are that Ca²⁺ influx during the resting state and during the

Fig. 6. Effects of cyclopiazonic acid (CPA, 10 μM) (Δ) on the concentration-response curve for K⁺ (○, Control) in strips of tail arteries from WKY and SHR. Experimental conditions were similar to those of Figs. 4 and 5. At '0' on the abscissa, the CPA-induced contractions remaining before the addition of K⁺ are expressed as % of the 65.9 mM K⁺ contraction. The peak contractions induced by each concentration of K⁺ are then expressed as % of the 65.9 mM K⁺ contraction. Data points are means of 6 preparations, and S.E.M.s are shown by vertical bars.

stimulation with Bay k 8644 or K^+ is completely buffered by Ca^{2+} uptake into the SR of the tail arteries, and these Ca^{2+} buffering functions are not altered in SHR. Thus, the SR of tail arteries acts as a strong buffer barrier to Ca^{2+} influx because it can take up Ca^{2+} through the action of SR Ca^{2+} -ATPase. To eliminate the Ca^{2+} buffering action of the SR, CPA, thapsigargin and ryanodine were used in the present study. CPA and thapsigargin inhibit Ca^{2+} uptake into the SR by inhibiting SR Ca^{2+} -ATPase (for details, see refs. 6–8). Therefore, under the inhibition of the Ca^{2+} uptake, Ca^{2+} that enters the cell across the plasmalemma bypasses the SR and can reach the myofilaments, resulting in an increased basal tone and a strong contraction in response to Bay k 8644 or K^+ . Ryanodine binds to CICR channels of the SR and then locks them open. The initial action of ryanodine could thus be acceleration of Ca^{2+} release. After full development of the ryanodine action, the SR becomes empty and cannot accumulate Ca^{2+} any longer, so that the SR cannot buffer Ca^{2+} that enters the cell, nor release Ca^{2+} from there (for details, see refs. 4, 5, 8). Therefore, after the ryanodine treatment, Ca^{2+} that enters the cell across the plasmalemma and is subsequently taken up into the SR, leaks from the SR and can reach the myofilaments.

The Ca^{2+} buffering function of the SR during rest was estimated from the increase in basal tone induced by CPA, thapsigargin and ryanodine. As shown in the present study, CPA, thapsigargin and ryanodine itself induced a contraction in the tail arteries. Because these contractions were strongly inhibited by nifedipine and were almost abolished by Ca^{2+} -free solution, the contractions are largely dependent on extracellular Ca^{2+} , especially Ca^{2+} influx via L-type Ca^{2+} channels. Thus, Ca^{2+} influx during rest was completely buffered by the SR of the tail arteries. Similar magnitude of the contraction induced by CPA, thapsigargin or ryanodine was reported in other arteries from WKY; e.g., aorta (17, 18), femoral artery (4, 7, 8, 19) and carotid artery (20). These contractions reflect the basal Ca^{2+} influx in the resting state of the artery, as already discussed in our previous studies (4–7). The finding that the contraction induced by CPA, thapsigargin or ryanodine was not different between the tail arteries from WKY and SHR suggests that the Ca^{2+} buffering function of the SR during rest is the same in WKY and SHR. Moreover, Ca^{2+} influx during rest is probably the same in the two strains. The contraction and the elevation of $[\text{Ca}^{2+}]_i$ induced by CPA, thapsigargin or ryanodine have been reported to be larger in many arteries from SHR when compared with WKY; e.g., aorta (17, 18), femoral artery (4, 7, 19) and carotid artery (20). This suggests that the basal Ca^{2+} influx is larger in these SHR arteries, and therefore the Ca^{2+} buffering function of the SR is secondarily increased. In fact, the increased basal Ca^{2+} influx has been reported in these SHR arteries

(21, 22). Thus, it is likely that such alterations do not occur in the SHR tail artery.

According to the “superficial buffer barrier” hypothesis (1–3), the effectiveness of the SR Ca^{2+} buffering depends on the state of Ca^{2+} loading of the SR; an empty SR will buffer Ca^{2+} influx and an overloaded SR cannot buffer the entering Ca^{2+} . The state of Ca^{2+} loading of the SR can be estimated from the contraction induced by 20 mM caffeine (see Materials and Methods). The finding that the caffeine contraction was not different between the tail arteries from WKY and SHR suggests that the state of Ca^{2+} loading of the SR is the same in WKY and SHR. This conclusion is also supported by the finding that the Ca^{2+} leakage from the SR during the CPA addition, the thapsigargin addition, the ryanodine treatment or the exposure to a Ca^{2+} -free solution was not different between WKY and SHR. Dohi et al. (23), however, have shown that the caffeine contraction was decreased in the tail artery from prehypertensive SHR (4 weeks of age) when compared with WKY. This finding was different from the present study. Since SHR at 13 weeks of age were used in the present study, the difference in the caffeine contraction may be due to the age of the rats, but further studies are needed to assess this difference. The caffeine contraction has been reported to be increased in many arteries from SHR when compared with WKY; e.g., mesenteric artery (24), aorta (18), femoral artery (4) and carotid artery (25). This suggests that these SHR arteries have an increased total capacity of the SR for Ca^{2+} storage as an attempt to compensate for the large Ca^{2+} influx. Thus, such a compensation was not observed in the tail artery from adult SHR.

The Ca^{2+} buffering function of the SR during the Bay k 8644 stimulation was estimated from the augmenting effects of CPA, thapsigargin or ryanodine on the responses to Bay k 8644. Bay k 8644 almost failed to induce a contraction in the tail arteries. Under the inhibition of the SR Ca^{2+} buffering by CPA, thapsigargin or ryanodine, Bay k 8644 induced a strong contraction, and this contraction was not different among the three agents. It is thus likely that during the Bay k 8644 stimulation, a large amount of Ca^{2+} enters the cell via L-type Ca^{2+} channels and is subsequently taken up into the SR before it can reach the myofilaments. Similar results were obtained in the WKY femoral artery of our previous study (8). Also, the K^+ contraction was highly augmented by CPA, as shown in Fig. 6. Thus, the SR of tail arteries can buffer a large amount of Ca^{2+} that enters the cell during stimulation with Bay k 8644 or K^+ .

The Ca^{2+} buffering function of the SR during stimulation with Bay k 8644 or K^+ was not altered in the SHR tail artery, as shown in the present study. Moreover, the Ca^{2+} influx induced by Bay k 8644 or K^+ was probably the same in WKY and SHR. Thus, the SHR tail artery also has

strong Ca^{2+} buffering functions of the SR during rest and during the stimulation with Bay k 8644 or K^+ . From the survey of reports that compared the augmenting effects of CPA, thapsigargin or ryanodine on the contractile responses to Bay k 8644 or K^+ between SHR and WKY, we found only one paper that compared the effects of CPA on the K^+ contraction in aortic strips (17). In this paper, CPA ($30\text{ }\mu\text{M}$) caused a sustained contraction and augmented the K^+ contraction in both strains. This augmentation was greater in SHR than in WKY. These augmentations, however, were extremely small when compared with the tail arteries of the present study or the WKY femoral artery of our previous study (8). Accordingly, the results of the present study suggest the strong Ca^{2+} buffering functions of the SR during stimulation with Bay k 8644 and K^+ in the SHR tail artery. As stated in the Introduction, a strong contraction in response to Bay k 8644 under normal conditions has been reported in many arteries from SHR when compared with WKY. Such an alteration was not observed in the SHR tail artery.

Although the reason for the unusual findings in the SHR tail artery is not assessed in the present study, a possible explanation is as follows: The size and length of the tail artery lends itself well to contraction studies, but this cutaneous artery represents a thermoregulatory vessel not implicated in blood pressure regulation. Hence, the Ca^{2+} buffering functions of the SR in the SHR tail artery may not resemble mechanisms in arteries primarily implicated in blood pressure regulation (e.g., mesenteric, skeletal muscle arteries). Thus, the findings in the tail arteries differ from those in resistance arteries potentially involved in the pathophysiology of genetic hypertension.

The difference in the tail arteries from WKY and SHR of the present study was noted in the absolute value (mg) of the contraction induced by 65.9 mM K^+ . The contraction was larger in SHR than in WKY. Since almost the same size of strip was used throughout the experiments, the large contraction in the SHR tail artery may be due to the increase in the number (hyperplasia) and/or size (hypertrophy) of the smooth muscle cells (26).

From the present study, we conclude that the SR of rat tail arteries can buffer a large amount of Ca^{2+} that enters the cell via L-type Ca^{2+} channels during rest and during the stimulation with Bay k 8644 or K^+ , and these functions are not altered in SHR.

Acknowledgments

This work was supported in part by a Grant-in-Aid for Scientific Research from the Ministry of Education, Science, Sports and Culture, Japan. Generous gifts of Bay k 8644 (Bayer) and nifedipine (Bayer Yakuhin) are gratefully acknowledged.

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