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Possible mechanism underlying the potent vasoconstrictor actions of cyclopiazonic acid on dog cerebral arteries

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Abstract

A sustained Ca^{2+} influx via L-type Ca^{2+} channels has been shown in the resting state of dog cerebral arteries. Sarcoplasmic reticulum is now recognized to serve as a buffer barrier to Ca^{2+} entry in vascular smooth muscle cells. To clarify whether sarcoplasmic reticulum of the cerebral arteries can buffer the sustained Ca^{2+} influx, effects of cyclopiazonic acid (CPA), an inhibitor of sarcoplasmic reticulum Ca^{2+}-ATPase, were determined in endothelium-denuded strips of the cerebral (basilar, posterior communicating, middle cerebral), mesenteric and coronary arteries of the dog. The addition of CPA (0.1–10 μM) during the resting state of the strips caused a concentration-dependent contraction in the three cerebral arteries. The CPA-induced contraction was extremely small in the mesenteric or coronary artery. The CPA-induced contractions in the cerebral arteries were inhibited concentration-dependently by nifedipine (1–100 nM). Nifedipine itself induced relaxation from the resting state of cerebral arteries, suggesting a maintenance of basal tone. The CPA-induced potent contraction seen in the cerebral arteries could be mimicked in the mesenteric artery by elevating the extracellular K^+ concentration (14.9 mM) or adding Bay k 8644 [methyl-1,4-dihydro-2,6-dimethyl-3-nitro-4-(2-trifluoromethylphenyl)-pyridine-5-carboxylate] (100 nM) to produce an increase in Ca^{2+} influx via L-type Ca^{2+} channels. We conclude that, in the resting state of dog cerebral arteries, (1) the greater part of the sustained Ca^{2+} influx is buffered by Ca^{2+} uptake into the sarcoplasmic reticulum, (2) therefore, the inhibition of sarcoplasmic reticulum Ca^{2+}-ATPase by CPA causes a potent contraction, and (3) the maintenance of basal tone suggests that some Ca^{2+} that entered via L-type Ca^{2+} channels always reaches the myofilaments in the resting state.

Keywords: Cyclopiazonic acid; Sarcoplasmic reticulum; Ca^{2+} buffering function; Arterial basal tone; Ca^{2+} channel, L-type; Cerebral artery, dog

1. Introduction

Cerebral arteries differ electrically and pharmacologically from arteries in other areas. Even during a resting state of smooth muscle strips of cerebral arteries, a sustained Ca^{2+} influx and a high cytosolic Ca^{2+} level ($[Ca^{2+}]_i$) have been shown when compared with those in peripheral arteries (Asano et al., 1987, 1993, 1996b; Tanoi et al., 1991). The high resting $[Ca^{2+}]_i$ in the cerebral arteries has been shown to maintain a spontaneous active tone and this tone is abolished by removal of extracellular Ca^{2+} and by blockers of L-type Ca^{2+} channels (Asano et al., 1987, 1993, 1996b; Tanoi et al., 1991). It is thus possible that L-type Ca^{2+} channels in the resting state of cerebral arteries are in different states of activation from those in peripheral arteries.

The high resting $[Ca^{2+}]_i$ also immediately triggers a number of compensatory mechanisms, aimed overall at reducing $[Ca^{2+}]_i$ back to the resting level. We have already shown that Ca^{2+}-activated K^+ (K_{Ca}) channels are highly activated in the resting state of cerebral arteries (Asano et al., 1993). Thus, the activation of K_{Ca} channels is acting as a negative feedback mechanism (via closure of L-type Ca^{2+} channels) to regulate the level of resting tone in the cerebral arteries. Moreover, the high resting $[Ca^{2+}]_i$ could be extruded by Na^+–Ca^{2+} exchange and Ca^{2+} pumping across the plasmalemma, and sequestered by Ca^{2+} uptake into the sarcoplasmic reticulum. According to the 'superficial buffer barrier' hypothesis proposed by Van Breemen and colleagues (Van Breemen and Saida, 1989; Chen et al., 1992; Van Breemen et al., 1995; Laporte and Laher, 1997), the superficial sarcoplasmic reticulum serves

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as a buffer barrier to Ca^{2+} entry in vascular smooth muscle cells. To eliminate the Ca^{2+} buffering function of the sarcoplasmic reticulum, ryanodine and cyclopiazonic acid (CPA) have been used. We have previously examined the effects of ryanodine on tension and cellular Ca^{2+} level during the resting state of cerebral arteries, and found that the contribution of sarcoplasmic reticulum to buffer the entered Ca^{2+} is relatively high in these arteries (Asano et al., 1996b). The mechanism of action of ryanodine is considered to be binding of this alkaloid to Ca^{2+}-induced Ca^{2+} release channels that are in an open state, and then locking open of the channels.

The present study was designed to confirm the conclusion drawn from the ryanodine action, by using CPA, another tool to eliminate the Ca^{2+} buffering function of the sarcoplasmic reticulum. CPA has been introduced as an inhibitor of Ca^{2+}-ATPase of sarcoplasmic/endoplasmic reticulum (Seidler et al., 1989), and shown to inhibit Ca^{2+} uptake into sarcoplasmic reticulum in smooth muscles (Deng and Kwan, 1991; Uyama et al., 1992; Nomura et al., 1996). Thus, CPA can also be used as a valuable tool to eliminate the Ca^{2+} buffering function of sarcoplasmic reticulum in arterial smooth muscle. Therefore, the effects of CPA on isometric tension were determined in the cerebral arteries, and these data were compared with the data from the mesenteric or coronary artery. The prediction is that if sarcoplasmic reticulum of the cerebral arteries is a buffer barrier to the sustained Ca^{2+} influx, the functional elimination of sarcoplasmic reticulum by CPA will cause a large elevation of $[Ca^{2+}]_i$ and a concomitant contraction. Here, we show that the addition of CPA during the resting state of cerebral arteries caused a potent contraction, confirming that the contribution of a Ca^{2+} buffering function of sarcoplasmic reticulum is relatively high in these arteries. A preliminary account of these findings was presented to the 71st Annual Meeting of the Japanese Pharmacological Society (Asano and Nomura, 1998).

2. Materials and methods

2.1. Preparation of arterial strips

Mongrel dogs of either sex weighing 8 to 12 kg were anesthetized with sodium pentobarbital (30 mg/kg i.v.) and then exsanguinated. The brain, heart and mesenteric artery (in situ o.d. of 0.6–0.8 mm) were excised and placed in a Krebs solution of the following composition (in mM): NaCl 115.0, KCl 4.7, $CaCl_2$ 2.5, $MgCl_2$ 1.2, $NaHCO_3$ 25.0, KH_2PO_4 1.2 and dextrose 10.0. Basilar (0.7–0.9 mm o.d.), posterior communicating (PC, 0.6–0.8 mm o.d.) and middle cerebral (MC, 0.6–0.8 mm o.d.) arteries were isolated from the brain, and the left anterior descending coronary artery (0.6–0.9 mm o.d.) was isolated from the heart. The arteries were cut into helical strips (0.8 mm in width) as described previously (Asano et al., 1987, 1988, 1993, 1996b). To avoid possible effects of the endothelium-derived factors (e.g., relaxing, hyperpolarizing and contracting factors), the endothelium of the strip was removed by gentle rubbing of the endothelial surface with a cotton swab.

2.2. Measurement of isometric tension

Arterial strips (0.8×7 mm) were mounted vertically in water-jacketed muscle baths containing 10 ml Krebs solution. Krebs solutions were maintained at 37°C and aerated with 95% O_2 and 5% CO_2. Isometric tension was recorded with a force-displacement transducer (TB-612T, Nihon Kohden Kogyo, Tokyo, Japan). Strips were stretched passively to their optimal length by imposing a resting tension

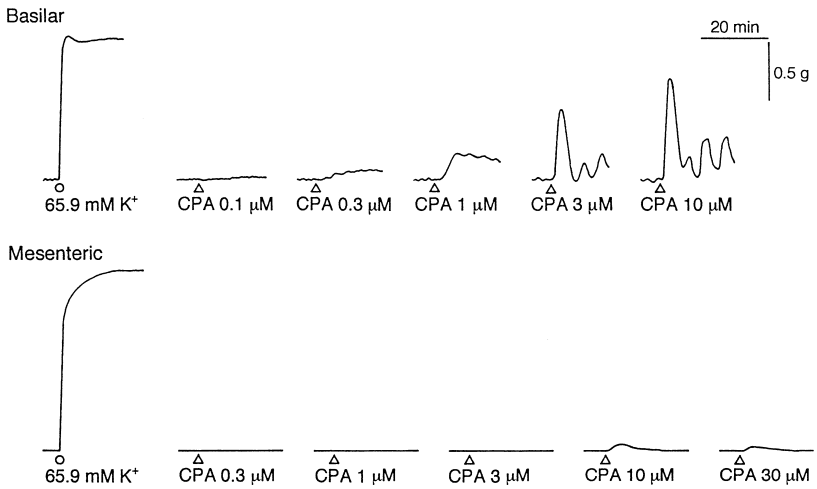

Fig. 1. Typical recordings of the contractions induced by cyclopiazonic acid (CPA) in strips of basilar and mesenteric arteries of the dog. After determination of the maximum contraction induced by 65.9 mM KCl (K^+), CPA was added in a non-cumulative fashion with a washout time of 45 min. The response of the basilar artery to 3 or 10 μM CPA was relatively transient, but a small rhythmic contraction developed when the application of CPA was prolonged (3 μM CPA for 15–20 min, 10 μM CPA for 20–25 min).

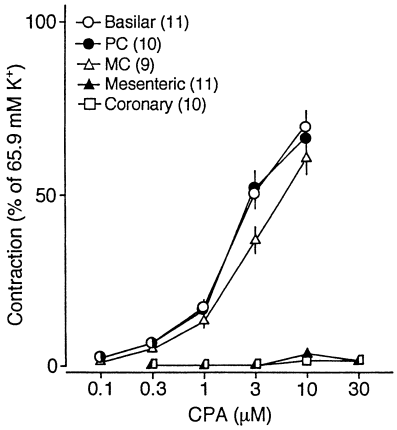

Fig. 2. Concentration–response curves for the CPA-induced contractions in strips of basilar (○), posterior communicating (PC, ●), middle cerebral (MC, △), mesenteric (▲) and coronary (□) arteries of the dog. Experimental conditions were the same as in Fig. 1. Peak contractions induced by each concentration of CPA are expressed as % of the 65.9 mM K^+-induced maximum contraction. Data points are means for the number of preparations indicated in parentheses and S.E.M. are shown by vertical bars.

(basilar, 0.8 g; posterior communicating, 0.6 g; middle cerebral, 0.7 g; coronary, 0.8 g; mesenteric, 0.8 g) and having a 90-min equilibration period precede each experiment. The optimal resting tension was determined in a length-passive tension study (Asano et al., 1987, 1993, 1996b).

After the equilibration, contractile responses of the strips to the Krebs solution containing 65.9 mM KCl (K^+) (equimolar replacement of Na^+ with K^+) were repeated two or three times until the responses were reproducible. After washout of the strips with Krebs solution, contractile responses to CPA were determined. To characterize the CPA-induced contractions, effects of nifedipine (a blocker of L-type Ca^{2+} channels) on these contractions were determined.

2.3. Statistical analysis

The results are expressed as means ± S.E.M. (n = number of preparations, with one preparation from each dog). Student's t-test for unpaired data or variance analysis was used to determine the significance of differences between means, and a P value of < 0.05 was taken as statistically significant.

2.4. Drugs

The drugs used were CPA (Sigma Chemical, St. Louis, USA), nifedipine (a kind gift from Bayer Yakuhin, Osaka, Japan) and Bay K 8644 [methyl-1,4-dihydro-2,6-dimethyl-3-nitro-4-(2-trifluoromethylphenyl)-pyridine-5-carboxylate] (a kind gift from Bayer, Wuppertal, Germany).

CPA (3 mM) was dissolved in 100% dimethyl sulfoxide. Nifedipine (1 mM) and Bay K 8644 (1 mM) were dissolved in 100% ethanol, with further dilution in distilled water before use. Concentrations of drugs are expressed as final molar concentrations.

3. Results

3.1. CPA-induced contractions

After the determination of the maximum contraction induced by 65.9 mM K^+ in the strips, the addition of CPA (0.1–10 μM) caused a concentration-dependent contraction in the basilar artery (Fig. 1). At relatively high concentrations (3 and 10 μM), CPA induced a transient peak contraction followed by a small rhythmic contraction in the basilar artery (Fig. 1). No further contraction was observed when 30 μM CPA was added (data not shown). The CPA-induced contraction was extremely small in the mesenteric artery (Fig. 1). Even at high concentrations (10 and 30 μM), CPA induced a transient contraction in the mesenteric artery.

Similar experiments were then performed with posterior communicating, middle cerebral and coronary arteries, and the concentration–response curves for the CPA-induced

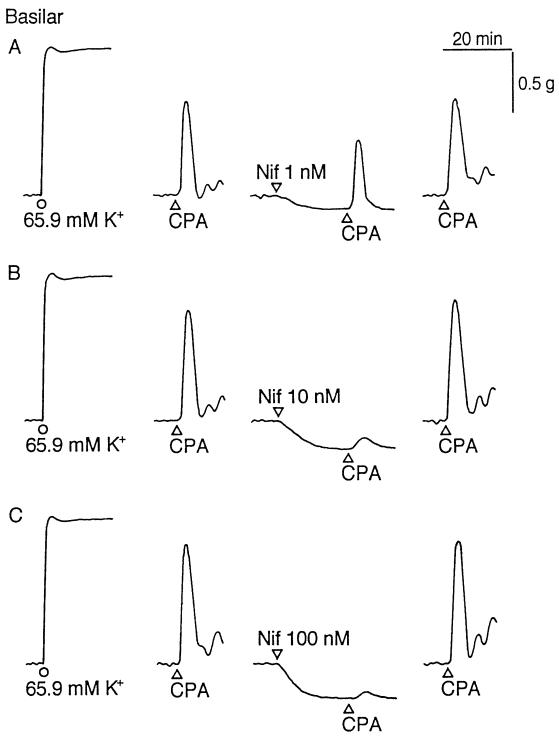

Fig. 3. Typical recordings of the effects of nifedipine (Nif) on the CPA-induced contraction in strips of dog basilar artery. After determination of the maximum contraction induced by 65.9 mM KCl (K^+) (first panels), the control contraction induced by 10 μM CPA was determined (second panels). After the relaxation induced by nifedipine (A, 1 nM; B, 10 nM; C, 100 nM) had reached a plateau (20 min), CPA was added (third panels). The CPA-induced contraction recovered after washout for 60 min (fourth panels).

Table 1
Effects of nifedipine on cyclopiazonic acid (CPA)-induced contractions in strips of basilar and middle cerebral arteries of the dog

Condition	CPA-induced contraction (% of 65.9 mM K^+)	
	Basilar	Middle cerebral
CPA 10 μM (Control)	62.8 ± 5.4 (4)	61.9 ± 5.3 (5)
+ Nifedipine 1 nM	48.1 ± 4.7 (4)	44.9 ± 4.6[a] (5)
CPA 10 μM (Control)	77.8 ± 3.0 (8)	61.9 ± 6.8 (5)
+ Nifedipine 10 nM	8.5 ± 0.7[a] (8)	11.6 ± 1.0[a] (5)
CPA 10 μM (Control)	81.7 ± 5.2 (4)	64.0 ± 2.4 (3)
+ Nifedipine 100 nM	5.2 ± 0.6[a] (4)	5.4 ± 1.1[a] (3)

Experimental conditions were the same as for Fig. 3.
Contractions induced by 10 μM CPA are expressed as % of the 65.9 mM K^+-induced maximum contraction.
Nifedipine was added 20 min before the addition of CPA.
Data are expressed as means ± S.E.M., and numbers in parentheses indicate the number of preparations used.
[a]Significantly different from the respective 'Control' ($P < 0.05$).

contractions are shown in Fig. 2. CPA induced a potent contraction in the posterior communicating and middle cerebral arteries also, but almost failed to induce a contraction in the coronary artery. Therefore, the CPA-induced contractions in the three cerebral arteries were significantly greater than those in mesenteric and coronary arteries (Fig. 2).

3.2. Effects of nifedipine on the CPA-induced contractions

Preincubation of the strips with nifedipine greatly inhibited the CPA-induced contractions in the cerebral arteries. The contraction induced by 10 μM CPA in the basilar or middle cerebral artery was inhibited concentration-dependently by nifedipine (1–100 nM) (Fig. 3, Table 1). In the presence of nifedipine, CPA induced a transient contraction which was not followed by a rhythmic contraction (Fig. 3). The CPA-induced contraction had completely recovered after washout for 60 min (Fig. 3). The effects of nifedipine on the concentration–response curves for CPA were then determined with the basilar and middle cerebral arteries (Fig. 4). Nifedipine inhibited the CPA-induced contractions in a typical non-competitive fashion (Fig. 4). As shown in Fig. 3, nifedipine itself induced a relaxation from the resting state. The relaxation induced by 1, 10 and 100 nM nifedipine in the basilar artery was 9.1 ± 1.9% ($n = 9$), 18.6 ± 2.5% ($n = 15$) and 23.3 ± 3.6% ($n = 10$), respectively, of the 65.9 mM K^+-induced maximum contraction. When papaverine at 0.1 mM was added after the relaxation induced by 100 nM nifedipine had reached a plateau, no further relaxation was observed (data not shown).

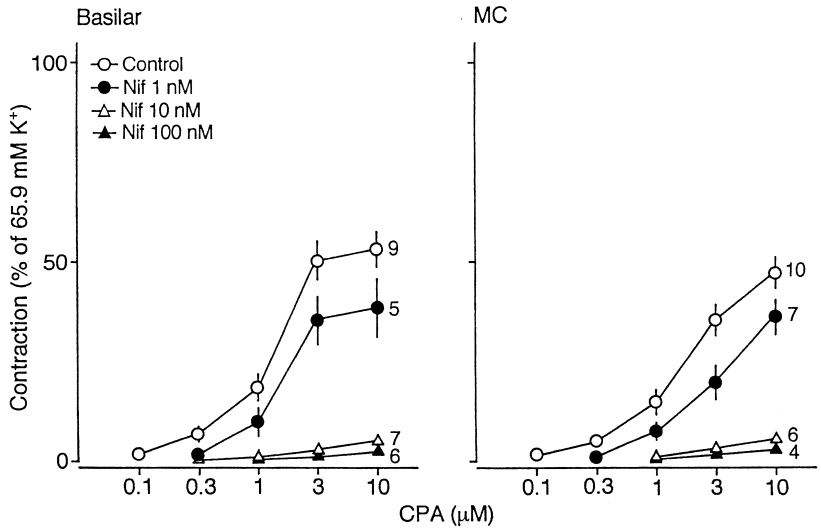

Fig. 4. Effects of nifedipine (Nif) on the concentration–response curve for CPA in strips of basilar and middle cerebral (MC) arteries of the dog. The concentration–response curve for the CPA-induced contractions was determined in a cumulative fashion (○). Nifedipine in a concentration of 1 nM (●), 10 nM (△) or 100 nM (▲) was added 20 min before the determination of the concentration–response curve for CPA. Peak contractions induced by each concentration of CPA are expressed as % of the 65.9 mM K^+-induced maximum contraction. Data points are means for the number of preparations indicated beside the concentration–response curve and S.E.M. are shown by vertical bars.

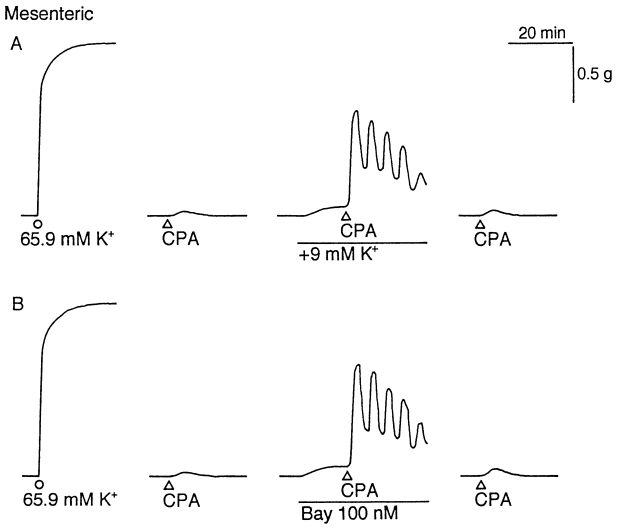

Fig. 5. Typical recordings of the effects of an increased extracellular K^+ concentration (A) and of Bay k 8644 (B) on the CPA-induced contraction in strips of dog mesenteric artery. After determination of the maximum contraction induced by 65.9 mM KCl (K^+) (first panels), the control contraction induced by 10 μM CPA was determined (second panels). After the small contraction induced by the addition of 9 mM K^+ (A) or 100 nM Bay K 8644 (B) to the bath had reached a plateau (12–17 min), CPA was added (third panels). The augmenting effects of K^+ and Bay K 8644 disappeared after washout for 60 min (fourth panels).

3.3. Augmentation by K^+ or Bay K 8644 of the CPA-induced contraction in the mesenteric artery

The CPA-induced potent contraction seen in the cerebral arteries can be mimicked in the mesenteric artery by elevating the extracellular K^+ concentration or adding Bay k 8644 to produce an increase in Ca^{2+} influx via L-type Ca^{2+} channels (Fig. 5). When the mesenteric artery was contracted by the addition of 9 mM K^+ to the bath (total K^+ concentration; 14.9 mM), the CPA-induced contraction was markedly augmented when compared with the contraction determined in the normal K^+ concentration (5.9 mM) (Fig. 5A vs. Fig. 1). An increase of extracellular K^+ to 14.9 mM caused a $5.1 \pm 0.9\%$ ($n = 5$) contraction in the mesenteric artery, and resulted in a $55.4 \pm 4.9\%$ ($n = 5$) contraction in response to 10 μM CPA (the values are expressed as % of the 65.9 mM K^+-induced maximum contraction). Similar results were obtained when 100 nM Bay k 8644 was added instead of increasing extracellular K^+ (Fig. 5B). After washout for 60 min, the augmenting effects of K^+ and of Bay K 8644 had disappeared (Fig. 5).

4. Discussion

Ryanodine and CPA have been used as valuable tools to eliminate the Ca^{2+} buffering function of sarcoplasmic reticulum, but the mechanism of action is quite different for the two agents. Ryanodine depletes Ca^{2+} stored in sarcoplasmic reticulum by locking open the Ca^{2+}-induced Ca^{2+} release channels in sarcoplasmic reticulum (for details, see Asano et al., 1996a,b). CPA inhibits Ca^{2+} uptake into the sarcoplasmic reticulum by inhibiting sarcoplasmic reticulum Ca^{2+}-ATPase (Seidler et al., 1989; Deng and Kwan, 1991; Uyama et al., 1992; Nomura et al., 1996). We have previously examined the effects of ryanodine on isometric tension and cellular Ca^{2+} level during the resting state of dog cerebral arteries (Asano et al., 1996b). Ryanodine itself induced a concentration-dependent contraction, which was significantly greater in the cerebral arteries than in the mesenteric or coronary artery. We concluded that (1) the basal Ca^{2+} influx via L-type Ca^{2+} channels was higher in the resting state of the cerebral arteries, (2) the greater part of the higher Ca^{2+} influx was buffered by Ca^{2+} uptake into the sarcoplasmic reticulum, and (3) therefore, the functional elimination of sarcoplasmic reticulum by ryanodine caused a potent contraction in these arteries (Asano et al., 1996b).

To confirm the conclusion drawn from the ryanodine action, CPA was used in the present study as another tool to eliminate the Ca^{2+} buffering function of sarcoplasmic reticulum. The prediction was that if sarcoplasmic reticulum of the cerebral arteries is a buffer barrier to the high resting $[Ca^{2+}]_i$, the functional elimination of sarcoplasmic reticulum by CPA will cause a large elevation of $[Ca^{2+}]_i$ and a contraction. Thus, the magnitude of contraction is a rough index of the Ca^{2+} buffering function of sarcoplasmic reticulum. When CPA was added during the resting state of the strip, a potent contraction occurred in the three cerebral arteries. Because the contraction was much greater in the cerebral arteries than in the mesenteric or coronary artery, it is likely that the contribution of the Ca^{2+} buffering function of sarcoplasmic reticulum is relatively high in the resting state of cerebral arteries.

As shown in Fig. 3, the basal tone and the CPA-induced contraction were almost abolished by 100 nM nifedipine, suggesting that these contractions were mainly due to Ca^{2+} influx via L-type Ca^{2+} channels. We consider that these contractions can be explained by the 'superficial buffer barrier' hypothesis proposed by Van Breemen et al. (Van Breemen and Saida, 1989; Chen et al., 1992; Van Breemen et al., 1995; Laporte and Laher, 1997). According to this hypothesis, sarcoplasmic reticulum is a buffer barrier to Ca^{2+} entry, based on mechanisms of Ca^{2+} uptake and unloading (extrusion) to the extracellular space. It has been reported that the magnitude of contraction in vascular smooth muscle depends on the rate, rather than on the net amount, of Ca^{2+} influx (Van Breemen, 1977; Van Breemen and Saida, 1989; Van Breemen et al., 1995). Thus, the effectiveness of sarcoplasmic reticulum Ca^{2+} buffering depends on the nature of the Ca^{2+} influx. For instance, if the Ca^{2+} influx is large but slow, such as the basal influx via a leak pathway, sarcoplasmic reticulum Ca^{2+} buffering would be effective to blunt the contraction. On the other hand, if the Ca^{2+} influx is slight but rapid,

such as the influx via L-type Ca^{2+} channels (e.g., cerebral arteries in the present study), then the Ca^{2+} uptake by the sarcoplasmic reticulum is less able to compete with the Ca^{2+} influx, with the result that delivery of Ca^{2+} to the myofilaments would be more effective to initiate contraction. Because the basal tone was abolished by nifedipine, some parts of the entered Ca^{2+} reached the myofilaments, resulting in the maintenance of basal tone in the basilar artery. The finding that nifedipine induced a relaxation that was 23% of the 65.9 mM K^+-induced contraction clearly indicates that this amount of Ca^{2+} always reaches the myofilaments in the resting state of the basilar artery.

However, as estimated from the CPA-induced contractions, it is possible that a large amount of Ca^{2+} enters the cell via L-type Ca^{2+} channels and is taken up into the sarcoplasmic reticulum in the resting state of cerebral arteries. The findings that CPA caused a contraction that is 60–70% of the 65.9 mM K^+-induced contraction and that the contractions were almost abolished by nifedipine, clearly indicate that this amount of Ca^{2+} is always taken up into the sarcoplasmic reticulum in the resting state of cerebral arteries. Therefore, it is possible that, during the inhibition of sarcoplasmic reticulum Ca^{2+}-ATPase by CPA, the distribution of cellular Ca^{2+} that entered the cell via L-type Ca^{2+} channels in the resting state is altered, and more Ca^{2+} bypassed the sarcoplasmic reticulum and reached the myofilaments than during the basal tone. This conclusion can also be supported by the results shown in Fig. 5, where the CPA-induced potent contraction seen in the cerebral arteries can be mimicked in the mesenteric artery by elevating the extracellular K^+ concentration or adding Bay K 8644 to produce an increase in Ca^{2+} influx via L-type Ca^{2+} channels. The finding that the Ca^{2+} influx via L-type Ca^{2+} channels is mainly involved in the CPA-induced contractions in the cerebral arteries is in good agreement with results obtained with aorta, carotid and femoral arteries isolated from spontaneously hypertensive rats (Low et al., 1993; Sekiguchi et al., 1996; Nomura et al., 1997).

We have reported a sustained Ca^{2+} influx in the resting state of cerebral arteries (Asano et al., 1987, 1993, 1996b; Tanoi et al., 1991). The precise mechanism responsible for the sustained Ca^{2+} influx is not clear, but appears to be that the cerebral arteries are more depolarized in the resting state than the mesenteric artery (for details, see Asano et al., 1993, 1996b). Since CPA at 10 μM has been shown to depolarize the smooth muscle cells, probably through inhibition of K_{Ca} channels and/or activation of Ca^{2+}-activated Cl^- channels or non-selective cation channels (Uyama et al., 1993; Maggi et al., 1995; Sekiguchi et al., 1996), this depolarization may also contribute to the CPA-induced contraction.

The present study clearly demonstrated that sarcoplasmic reticulum of the cerebral arteries can serve as a buffer barrier to Ca^{2+} entry by utilizing the Ca^{2+} uptake mechanism in the face of high resting $[Ca^{2+}]_i$. Thus, the presence of a potent Ca^{2+} buffering function of sarcoplasmic reticulum in the resting state of cerebral arteries was proposed from the previous study with ryanodine (Asano et al., 1996b) and was confirmed in the present study with CPA.

According to the 'superficial buffer barrier' hypothesis, Ca^{2+} taken up into the sarcoplasmic reticulum is continually extruded from the sarcoplasmic reticulum lumen to the extracellular space. This process is composed of two stages: vectorial release towards the inner surface of the plasmalemma followed by extrusion via the Na^+–Ca^{2+} exchanger and the Ca^{2+}-ATPase of the plasmalemma (Van Breemen and Saida, 1989; Chen et al., 1992; Van Breemen et al., 1995; Laporte and Laher, 1997). It is also possible that the K_{Ca} channels of the plasmalemma are activated during this process. We have already shown that K_{Ca} channels are highly activated in the resting state of cerebral arteries when compared with the mesenteric artery and this activation appears to be secondary to the increased Ca^{2+} influx via L-type Ca^{2+} channels (Asano et al., 1993). Activation of the K_{Ca} channels is acting as a negative feedback mechanism (via closure of L-type Ca^{2+} channels) to regulate the level of resting tone in the cerebral arteries.

The activation of K_{Ca} channels in the resting state of arteries is now widely accepted as the spontaneous transient outward currents (for review, see Bolton and Imaizumi, 1996). In many smooth muscle cells, spontaneous transient outward currents are often observed at the resting membrane potential and are seen more frequently as the membrane is depolarized. Buryi et al. (1994) showed that spontaneous transient outward currents are caused by vectorial release of Ca^{2+} from the superficial sarcoplasmic reticulum. Nelson et al. (1995) showed clearly that spontaneous transient outward currents in smooth muscle cells arise from Ca^{2+} sparks which represent the increase in $[Ca^{2+}]_i$ within a microvolume. Ca^{2+} sparks are now believed to arise due to vectorial release of Ca^{2+} from sarcoplasmic reticulum close to the plasmalemma. It is also suggested that a discharge of spontaneous transient outward currents probably reflects the Ca^{2+} overloading of sarcoplasmic reticulum. These findings make it possible that the Ca^{2+} stored in sarcoplasmic reticulum can activate K_{Ca} channels during the Ca^{2+} extrusion process (for review, see Bolton and Imaizumi, 1996; Laporte and Laher, 1997).

Together, our results suggest strongly that, in the resting state of dog cerebral arteries, (1) the greater part of the sustained Ca^{2+} influx via L-type Ca^{2+} channels is buffered by Ca^{2+} uptake into the sarcoplasmic reticulum, (2) therefore, the functional elimination of sarcoplasmic reticulum Ca^{2+} buffering by CPA causes a potent contraction, and (3) Ca^{2+} taken up into the sarcoplasmic reticulum appears to be extruded from the plasmalemma and also activates K_{Ca} channels. Furthermore, the maintenance of the basal tone in the cerebral arteries suggests that some Ca^{2+} that

entered via L-type Ca^{2+} channels always reaches the myofilaments in the resting state.

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