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Possible mechanism of the potent vasoconstrictor responses to ryanodine in dog cerebral arteries

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Abstract

Isolated cerebral (basilar, posterior communicating and middle cerebral) arteries exist in a partially contracted state. To determine the Ca^{2+}-buffering function of sarcoplasmic reticulum in the resting state of cerebral arteries, the effects of ryanodine that eliminates the function of sarcoplasmic reticulum, on tension and cellular Ca^{2+} level were compared in endothelium-denuded strips of the cerebral, coronary and mesenteric arteries of the dog. The addition of ryanodine to strips with basal tone caused a concentration-dependent contraction, which was significantly greater in the cerebral arteries than in the mesenteric or coronary artery. In the presence of 10^{-5} M ryanodine, the caffeine (20 mM)-induced contraction was greatly attenuated in these arteries. After washout, the basal tone was greatly elevated in the cerebral arteries. The elevated tone was abolished by 10^{-7} M nifedipine. The ryanodine-induced contractions were also abolished by 10^{-7} M nifedipine. Nifedipine itself caused a relaxation from the basal tone in the cerebral arteries, suggesting the maintenance of myogenic tone. The basal Ca^{2+} influx in arteries measured after a 5-min incubation with ^{45}Ca was significantly higher in the basilar artery than in the mesenteric artery. The basal Ca^{2+} influx was not increased by 10^{-5} M ryanodine in either artery. The basal Ca^{2+} influx was decreased by 10^{-7} M nifedipine in the basilar artery, but was unchanged in the mesenteric artery. These results suggest that: (1) the basal Ca^{2+} influx via L-type voltage-dependent 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. Furthermore, the maintenance of myogenic tone in the cerebral arteries suggests that more Ca^{2+} enters the smooth muscle cell than the buffering ability of sarcoplasmic reticulum can handle.

Keywords: Ryanodine; Sarcoplasmic reticulum; Ca^{2+}-buffering function; Ca^{2+} channel, voltage-dependent; Arterial myogenic tone; Cerebral artery; (Dog)

1. Introduction

Cerebral arteries differ electrically and pharmacologically from arteries in other areas. These arteries exist in a partially contracted state from which they can constrict further or dilate. Because the smooth muscle of cerebral arteries is more depolarized than that of peripheral arteries (Fujiwara et al., 1982; Kou et al., 1982), cerebral arteries are more sensitive than peripheral arteries to dihydropyridine Ca^{2+} antagonists (Allen and Banghart, 1979; Shimizu et al., 1980; Towart, 1981; Cauvin et al., 1983) and agonists (Uski and Andersson, 1985; Asano et al., 1987). It is thus possible that voltage-dependent Ca^{2+} channels (VDCCs) in cerebral arteries are in different states of activation from those in peripheral arteries.

Isolated cerebral arteries have been shown to maintain a myogenic tone even in the resting state (Asano et al., 1987, 1993a; Tanoi et al., 1991; Suzuki et al., 1992). It is likely that the myogenic tone in the cerebral arteries reflects the higher plasmalemmal Ca^{2+} influx via L-type VDCCs, since the tone is abolished by the removal of extracellular Ca^{2+} and by blockers of L-type VDCCs (Asano et al., 1987, 1993a; Tanoi et al., 1991; Suzuki et al., 1992). Because the cytosolic Ca^{2+} level ($[Ca^{2+}]_i$) plays a key role in arterial smooth muscle contraction, the elevation of $[Ca^{2+}]_i$ resulting from the higher Ca^{2+} influx may explain

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the maintenance of myogenic tone in the cerebral arteries. The elevated $[Ca^{2+}]_i$ also immediately triggers a number of compensatory mechanisms, which aim overall to reduce $[Ca^{2+}]_i$ back to the resting level. Thus, the elevated $[Ca^{2+}]_i$ 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 his colleagues (Van Breemen and Saida, 1989; Chen et al., 1992; Van Breemen et al., 1995), sarcoplasmic reticulum serves as an effective barrier to Ca^{2+} entry in vascular smooth muscle cells. Therefore, the maintenance of myogenic tone in the resting state of cerebral arteries may indicate two opposite possibilities: (1) the function of sarcoplasmic reticulum to buffer the entered Ca^{2+} is impaired; or (2) this function is increased but more Ca^{2+} enters the cell than the Ca^{2+}-buffering ability of sarcoplasmic reticulum can handle.

Ryanodine, a plant alkaloid, has been reported to accelerate Ca^{2+} release from sarcoplasmic reticulum having Ca^{2+}-induced Ca^{2+} release channels and then finally abolish the Ca^{2+} release (Fleischer et al., 1985; Rousseau et al., 1987; Hwang and Van Breemen, 1987). 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 (Fleischer et al., 1985; Rousseau et al., 1987; Iino et al., 1988). Thus, ryanodine can be used as a tool to eliminate the function of sarcoplasmic reticulum in vascular smooth muscle cells (Iino et al., 1988; Ito et al., 1991; Van Breemen et al., 1995). To determine the Ca^{2+}-buffering function of sarcoplasmic reticulum in the resting state of cerebral arteries, we examined the effects of ryanodine on tension and cellular Ca^{2+} level in the resting state of cerebral arteries, and these data were compared with the data from the mesenteric or coronary artery. The assumption is that if the sarcoplasmic reticulum of the cerebral arteries is an effective barrier to the increased Ca^{2+} influx, the functional elimination of sarcoplasmic reticulum by ryanodine will cause an elevation of $[Ca^{2+}]_i$ which results in a smooth muscle contraction. Here, we report that the addition of 10^{-5} M ryanodine during basal tone of the strips of cerebral arteries caused a potent contraction, suggesting that the buffering function of sarcoplasmic reticulum is increased in these arteries.

2. Materials and methods

2.1. Preparation of arterial strips

Mongrel dogs of either sex weighing 8–12 kg were anesthetized with sodium pentobarbital (30 mg/kg i.v.) and then exsanguinated. The brain, heart and mesenteric artery (in situ outside diameter 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 (0.6–0.8 mm o.d.) and middle cerebral (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). 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 (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, 1993a; Masuzawa et al., 1991). All experiments were conducted with phenoxybenzamine (2×10^{-6} M)-treated strips and 5×10^{-7} M timolol was added to the Krebs solution to eliminate possible α- and β-adrenoceptor responses to endogenously released noradrenaline (Asano et al., 1993a).

After the equilibration, contractile responses of the strips to the Krebs solution containing 65.9 mM KCl (K^+) (equimolar substitution 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 caffeine or ryanodine were determined. To characterize the ryanodine-induced contraction, the effects of nifedipine (a blocker of L-type VDCCs) and cromakalim (an opener of ATP-sensitive K^+ channels) on the contractile responses to ryanodine were determined. In some experiments, nifedipine was added to strips pretreated with ryanodine plus caffeine. This experiment supplied the data for the cumulative concentration-response curves for the relaxant effects of nifedipine (Asano et al., 1993b).

2.3. Measurement of basal Ca^{2+} influx

Basal ^{45}Ca influx was measured by using a substituted-La^{3+} solution at 0.5°C as described previously (Asano et al., 1993a). Briefly, isolated basilar and mesenteric arteries were opened longitudinally and equilibrated in a Tris-buffered solution of the following composition (in mM):

NaCl 154.0, KCl 5.4, $CaCl_2$ 2.5, dextrose 11.0 and Tris 6.0 (pH 7.4). Tris-buffered solutions were maintained at 37°C and aerated with 100% O_2. The arteries were then transferred to the Tris-buffered solution to which 1 μCi/ml ^{45}Ca had been added. After the 5-min incubation, arteries were placed in test tubes containing 80.8 mM La^{3+}-substituted solution (0.5°C) for 30 min to remove extracellular ^{45}Ca. The amount of ^{45}Ca taken up by the arteries during the 5-min incubation can be assumed to be primarily due to Ca^{2+} influx with some efflux components (Meisheri et al., 1981; Meisheri and Van Breemen, 1982; Asano et al., 1993a). The arteries were then dissolved in a glass scintillation vial containing 0.1 ml NCS tissue solubilizer (Amersham International, UK). The radioactivity in the solubilized tissues was counted in an Aloka liquid scintillation counter. The effects of ryanodine, 160 mM K^+ solution, or nifedipine on the basal ^{45}Ca influx were determined. The values for basal Ca^{2+} influx were then calculated and are expressed as nmol/g artery wet weight, as described previously (Asano et al., 1993a).

2.4. 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.

2.5. Drugs and isotope

The drugs used were ryanodine (lot 704RWP-1; S.B. Penick, Lyndhurst, NJ, USA), caffeine (Wako Pure Chemical Industries, Osaka, Japan), nifedipine (Bayer Yakuhin, Osaka, Japan), cromakalim (Beecham Pharmaceuticals Research Division, Harlow, Essex, UK), papaverine hydrochloride (Wako), phenoxybenzamine hydrochloride (Nacalai Tesque, Kyoto, Japan) and timolol maleate (Banyu Pharmaceuticals, Tokyo, Japan). $^{45}CaCl_2$ (specific activity initially 13.5–17.4 mCi/mg) was obtained from Amersham International (Buckinghamshire, UK).

Nifedipine (1 mM) and cromakalim (10 mM) were dissolved in 50 and 60% ethanol, respectively, with further dilution in distilled water before use. Phenoxybenzamine (2 mM) was dissolved in 99.5% ethanol. Caffeine (20 mM) was dissolved in Krebs solution. Aqueous stock solutions were prepared for other drugs. Concentrations of drugs are expressed as final molar concentrations.

3. Results

3.1. Ryanodine-induced contractions

After the determination of the maximum contraction induced by 65.9 mM K^+ in the strips, the addition of 20 mM caffeine caused a transient contraction followed by a sustained relaxation in the basilar artery and only a transient contraction in the mesenteric artery (Fig. 1). The caffeine-induced contraction was not significantly different in the two arteries (Table 1). Since 20 mM caffeine succeeded in depleting the stored Ca^{2+}, a transient contraction induced by caffeine could be a rough index of the amount of Ca^{2+} in the sarcoplasmic reticulum, as shown in other studies (Leijten and Van Breemen, 1986; Naganobu et al., 1994). After the repeated washout with Krebs solution, the addition of 10^{-5} M ryanodine caused a relatively sustained contraction, and this contraction was significantly greater in the basilar artery than in the mesenteric artery (Fig. 1, Table 1). In the presence of ryanodine, caffeine caused a small transient contraction in both arteries (Fig. 1). After washout with Krebs solution, the basal tone was significantly elevated, and the elevated tone was significantly greater in the basilar artery than in the mesenteric artery (Fig. 1). When caffeine was added after a 45-min washout, it caused only a relaxation in both arteries (Fig. 1).

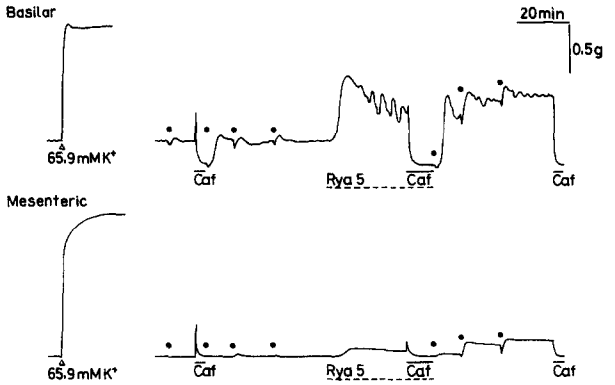

Fig. 1. Typical recordings of the contractions induced by caffeine and ryanodine in strips of basilar and mesenteric arteries of the dog. After the determination of the maximum contraction induced by 65.9 mM KCl (K^+), 20 mM caffeine (Caf) was added for 4 min. Following washout for 45 min, 10^{-5} M ryanodine (Rya 5) was added for 30 min. In the presence of ryanodine, Caf was added for 10 min. After washout for 45 min, Caf was added for 4 min. Dots denote the washing of the strips with Krebs solution.

Table 1
Contractions induced by caffeine and ryanodine in strips of basilar, posterior communicating, middle cerebral, coronary and mesenteric arteries of the dog

Artery	Contraction (% of 65.9 mM K^+)		
	n	Caffeine 20 mM	Ryanodine 10^{-5} M
Basilar	16	25.5±2.1	55.4±5.1 [a]
Posterior communicating	10	21.3±2.5	45.7±5.2 [a]
Middle cerebral	13	21.8±2.9	44.7±3.4 [a]
Coronary	14	21.2±1.1	15.7±2.8 [a]
Mesenteric	16	24.0±1.3	6.0±2.8

Experimental conditions were the same as in Fig. 1. Contractions are expressed as % of the 65.9 mM K^+-induced maximum contraction. Data are expressed as means±S.E.M. and n indicates the number of preparations used. [a] Significantly different from the mesenteric artery ($P < 0.05$).

Similar experiments were then performed with posterior communicating, middle cerebral and coronary arteries (Fig. 2, left). Ryanodine caused a potent contraction in the posterior communicating and middle cerebral arteries also. Therefore, the ryanodine-induced contractions in the three cerebral arteries were significantly greater than that in the mesenteric artery (Table 1). The ryanodine-induced contraction in the coronary artery was intermediate between these extremes (Fig. 2, left; Table 1).

Concentration-response curves for the contractile effects of ryanodine in the basilar and mesenteric arteries are shown in Fig. 3. Above the concentration of 1×10^{-7} M, ryanodine caused a greater contraction in the basilar artery than in the mesenteric artery (Fig. 3).

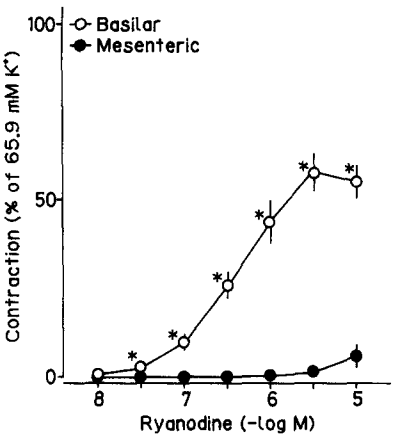

Fig. 3. Concentration-response curves for the ryanodine-induced contractions in strips of basilar (○) and mesenteric (●) arteries of the dog. The contraction induced by each concentration of ryanodine was determined in one strip, as shown in Fig. 1. Peak contractions induced by each concentration of ryanodine are expressed as % of the 65.9 mM K^+-induced maximum contraction. Data points are means for 5–16 preparations and S.E.M. are shown by vertical bars. * Significantly different from the mesenteric artery ($P < 0.05$).

3.2. Characteristics of the ryanodine-induced contraction

The effects of nifedipine were determined against the elevated basal tone that was observed after the treatment with ryanodine plus caffeine (Fig. 2). The addition of nifedipine to the elevated tone caused a concentration-dependent relaxation in the basilar, posterior communicating, middle cerebral and coronary arteries (Fig. 2, left). The nifedipine-induced relaxation was similar in the four arteries (Fig. 2, right).

Pretreatment of the strips with nifedipine also attenuated the ryanodine-induced contractions in the cerebral arteries (Fig. 4, Table 2). In the presence of 10^{-7} M nifedipine, the ryanodine-induced contractions were almost abolished (Table 2). Nifedipine itself caused a relaxation of the strips from the basal tone (Fig. 4). The relaxations induced by 10^{-8} and 10^{-7} M nifedipine in the basilar artery were 16.2 ± 3.9 ($n = 5$) and $20.1 \pm 3.7\%$ ($n = 5$) of the 65.9 mM K^+-induced maximum contraction. The addition of 10^{-5} M cromakalim also caused a relaxation from the basal tone and attenuated the ryanodine-induced contractions (Fig. 4, Table 2).

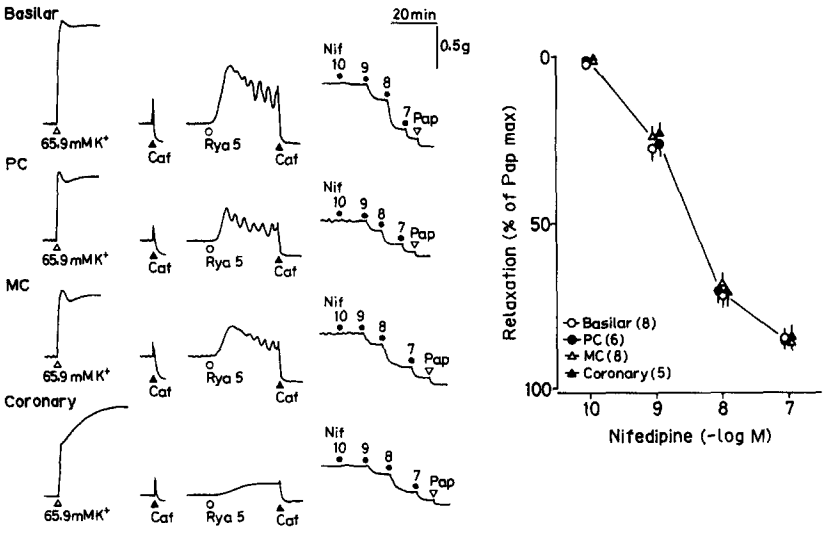

Fig. 2. Contractions induced by caffeine and ryanodine and the effects of nifedipine (Nif) on the elevated basal tone after the treatment with ryanodine plus caffeine in strips of basilar (○), posterior communicating (PC, ●), middle cerebral (MC, △) and coronary (▲) arteries of the dog. The left panel shows typical recordings. Strips were treated with 10^{-5} M ryanodine (Rya 5) plus 20 mM caffeine (Caf) and then were washed out with Krebs solution, as shown in Fig. 1. After a 45-min washout, Nif was added in a cumulative fashion. At the end of experiments, 10^{-4} M papaverine (Pap) was added to identify the position of the maximum relaxation. Concentrations of Nif are expressed as negative logs of the molar concentration. The elevated basal tone before the addition of Nif was $36.7 \pm 6.9\%$ (basilar, $n = 8$), $29.2 \pm 5.2\%$ (PC, $n = 6$), $32.8 \pm 5.9\%$ (MC, $n = 8$) and $32.1 \pm 4.2\%$ (coronary, $n = 5$), respectively, of the 65.9 mM K^+-induced maximum contraction. The right panel shows the concentration-response curves for the Nif-induced relaxations. Relaxations induced by each concentration of Nif are expressed as % of the Pap-induced maximum relaxation. Data points are means for the number of preparations indicated in parentheses and S.E.M. are shown by vertical bars.

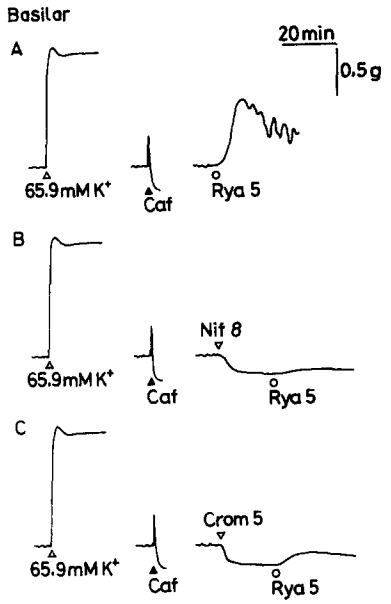

Fig. 4. Typical recordings of the effects of nifedipine (Nif) and cromakalim (Crom) on the ryanodine-induced contraction in strips of dog basilar artery. The control contraction induced by 10^{-5} M ryanodine (Rya 5) determined as in Fig. 1 is shown in A. Nif (10^{-8} M) or Crom (10^{-5} M) was added 20 min before the determination of the ryanodine-induced contraction (B,C). Concentrations of Nif and Crom are expressed as negative logs of the molar concentration.

The caffeine-induced contraction in the basilar artery was also attenuated by 10^{-7} M nifedipine, since the contractions were 23.1 ± 0.8 (control, $n = 10$) and 15.2 ± 0.7% (nifedipine, $n = 10$), respectively, of the 65.9 mM K^+-induced maximum contraction. In the mesenteric artery, however, the caffeine-induced contraction was not attenuated by 10^{-7} M nifedipine.

3.3. Effects of ryanodine on basal Ca^{2+} influx

The basal Ca^{2+} influx in the arteries measured after a 5-min incubation with ^{45}Ca was significantly higher in the basilar artery than in the mesenteric artery (Table 3). When the ^{45}Ca incubation was done in the presence of 10^{-5} M ryanodine, the basal Ca^{2+} influx was unchanged in the basilar artery but was significantly decreased in the mesenteric artery. Therefore, the Ca^{2+} influx in the presence of ryanodine was still significantly higher in the basilar artery. On the other hand, the Ca^{2+} influx was significantly increased by 160 mM K^+-substituted solution in both arteries (Table 3).

When the ^{45}Ca incubation was done in the presence of 10^{-7} M nifedipine, the basal Ca^{2+} influx was significantly decreased in the basilar artery but was unchanged in the mesenteric artery. The Ca^{2+} influx in the presence of nifedipine was still significantly higher in the basilar artery (Table 3).

3.4. Augmentation by K^+ of the ryanodine-induced contraction in the mesenteric artery

The ryanodine-induced potent contractions seen in the cerebral arteries could be mimicked in the mesenteric

Table 2
Effects of nifedipine and cromakalim on ryanodine-induced contractions in strips of basilar, posterior communicating (PC) and middle cerebral (MC) arteries of the dog

Condition	Ryanodine-induced contraction (% of 65.9 mM K^+)		
	Basilar	PC	MC
Ryanodine 10^{-5} M (control)	45.0 ± 5.0 (5)	41.8 ± 5.0 (4)	38.8 ± 3.9 (5)
+ Nifedipine 10^{-8} M	3.8 ± 1.9 [a] (5)	7.7 ± 3.4 [a] (4)	4.7 ± 1.9 [a] (5)
+ Nifedipine 10^{-7} M	3.0 ± 1.2 [a] (5)	3.3 ± 0.9 [a] (4)	2.3 ± 1.2 [a] (5)
+ Cromakalim 10^{-5} M	11.4 ± 6.6 [a] (5)	4.1 ± 0.6 [a] (4)	4.3 ± 1.3 [a] (5)

Experimental conditions were the same as in Fig. 4. Contractions induced by 10^{-5} M ryanodine are expressed as % of the 65.9 mM K^+-induced maximum contraction. Nifedipine or cromakalim was added 20 min before the application of ryanodine. 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' ($P < 0.05$).

Table 3
Effects of ryanodine, high-K^+ solution and nifedipine on basal Ca^{2+} influx in basilar and mesenteric arteries of the dog

^{45}Ca incubation condition	Ca^{2+} taken up by the artery (nmol/g wet artery)	
	Basilar	Mesenteric
5.4 mM K^+ (control)	90.3 ± 4.7 [a] (8)	70.4 ± 1.7 (8)
5.4 mM K^+ + Ryanodine 10^{-5} M	86.1 ± 6.7 [a] (4)	63.4 ± 2.3 [b] (4)
160 mM K^+	190.7 ± 12.7 [b] (6)	170.7 ± 8.2 [b] (8)
5.4 mM K^+ + Nifedipine 10^{-7} M	78.0 ± 2.3 [a,b] (8)	70.8 ± 1.3 (8)

Arteries were incubated for 5 min in each solution to which ^{45}Ca had been added. Ryanodine (10^{-5} M) was added only to the ^{45}Ca incubation solution. Nifedipine (10^{-7} M) was added 30 min before the application of ^{45}Ca and also during the ^{45}Ca incubation period. Data are expressed as means ± S.E.M. and numbers in parentheses indicate the number of measurements. [a] Significantly different from the mesenteric artery ($P < 0.05$). [b] Significantly different from the respective 'control' ($P < 0.05$).

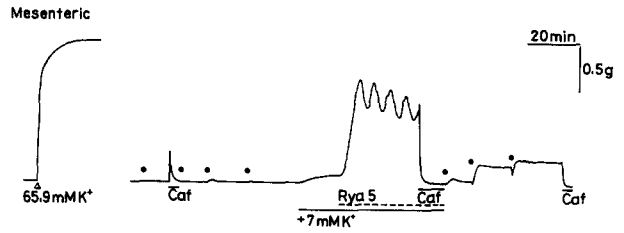

Fig. 5. Typical recordings of the effects of an increased extracellular K^+ concentration on the ryanodine-induced contraction in strips of dog mesenteric artery. After the small contraction induced by the addition of 7 mM K^+ to the bath had reached a plateau, 10^{-5} M ryanodine (Rya 5) and then 20 mM caffeine (Caf) were added as indicated. After washout for 45 min, Caf was added. Other experimental conditions were the same as in Fig. 1. Dots denote the washing of the strips with Krebs solution.

artery by elevating the extracellular K^+ concentration to produce an increase in Ca^{2+} influx via VDCCs (Fig. 5). When the mesenteric artery was contracted by the addition of 7 mM K^+ to the bath (total K^+ concentration; 12.9 mM), the ryanodine-induced contraction was greatly augmented when compared with the contraction determined in the normal K^+ concentration (5.9 mM) (Fig. 5 vs. Fig. 1). An increase of extracellular K^+ to 12.9 mM caused a $4.8 \pm 0.8\%$ ($n = 6$) contraction in the mesenteric artery, and resulted in a $69.0 \pm 2.4\%$ ($n = 6$) contraction in response to 10^{-5} M ryanodine (the values are expressed as % of the 65.9 mM K^+-induced maximum contraction).

After the treatment with ryanodine plus caffeine and washout with Krebs solution, the basal tone of the mesenteric artery was elevated (Fig. 5). When the elevated basal tone was compared with that shown in Fig. 1, this tone was similar to that of the mesenteric artery rather than of the basilar artery, because the extracellular K^+ concentration was returned to 5.9 mM by the washout.

4. Discussion

A major finding of present study was that the addition of ryanodine (10^{-7}–10^{-5} M) during the basal tone of arterial strips caused a concentration-dependent contraction, which was much greater in cerebral arteries than in the mesenteric or coronary artery. The greater ryanodine action in the cerebral arteries may have been due to their higher basal Ca^{2+} influx via L-type VDCCs, which would be expected to exert a greater effect if the Ca^{2+}-buffering function of sarcoplasmic reticulum was abolished. Since the myogenic tone was maintained in the resting state of cerebral arteries, this tone also appears to be due to the basal Ca^{2+} influx which exceeded the Ca^{2+}-buffering ability of sarcoplasmic reticulum in these arteries.

The mechanism of action of ryanodine is considered to be that this alkaloid binds to Ca^{2+}-induced Ca^{2+} release channels that are in an open state, and then locks them open (Fleischer et al., 1985; Rousseau et al., 1987; Iino et al., 1988). The initial action of ryanodine could thus be acceleration of Ca^{2+} release. After full development of the ryanodine action, the sarcoplasmic reticulum becomes empty and can no longer accumulate Ca^{2+}, so that the sarcoplasmic reticulum cannot buffer Ca^{2+} which enters cells from the extracellular space, nor release Ca^{2+} from there. Because ryanodine binds Ca^{2+}-induced Ca^{2+} release channels practically irreversibly, once the Ca^{2+}-induced Ca^{2+} release channels have been opened by ryanodine, they remain open for a long period even after the ryanodine has been washed out (Fleischer et al., 1985). In the present study, these actions of ryanodine on the arteries were confirmed by results of the experiments shown in Figs. 1 and 2. When Ca^{2+}-induced Ca^{2+} release channels in sarcoplasmic reticulum were locked open by ryanodine (plus caffeine), elevation of the basal tone occurred, and was accompanied by a considerable loss of caffeine-releasable Ca^{2+}. Therefore, it may be considered that the Ca^{2+} released from the sarcoplasmic reticulum is responsible for the ryanodine-induced potent contractions in the cerebral arteries. However, this is unlikely, because the ryanodine-induced contractions were totally dependent on the plasmalemmal Ca^{2+} influx via L-type VDCCs. The contribution of Ca^{2+} influx via L-type VDCCs to the ryanodine-induced contractions could suggest a direct action of ryanodine on L-type VDCCs to increase the Ca^{2+} influx. However, this is also unlikely, because the studies using a 5-min incubation with ^{45}Ca showed that ryanodine failed to increase the basal ^{45}Ca influx in the basilar artery.

Therefore, another explanation is necessary for the ryanodine-induced potent contractions. We have reported on a higher Ca^{2+} influx in the resting state of the cerebral arteries (Asano et al., 1987, 1993a; Tanoi et al., 1991; Suzuki et al., 1992). The present study also showed a higher basal ^{45}Ca influx in the basilar artery than in the mesenteric artery, as shown in Table 3. This change reflected mainly the increased opening of L-type VDCCs, because the change was reduced by nifedipine. The precise mechanism responsible for the higher basal Ca^{2+} influx via L-type VDCCs in the cerebral arteries is not clear, but appears to be that the cerebral arteries are more depolarized in the resting state than the mesenteric artery. The resting membrane potential of the smooth muscle of dog basilar or middle cerebral artery is approximately -50 mV (Fujiwara et al., 1982), while that of dog mesenteric artery measured in the same laboratory is approximately -68 mV (Kou et al., 1982). The level of the resting membrane potential in arterial smooth muscle largely determines the basal tone, and the sensitivity to vasoconstrictors that exert their action through the Ca^{2+} influx via VDCCs (Hirst and Edwards, 1989; Nelson et al., 1990). The present study showed clearly that nifedipine caused a relaxation from the basal tone, and concomitantly abolished the ryanodine-induced contraction in the cerebral arteries. Similarly, both the myogenic tone and the ryanodine-induced contraction in the cerebral arteries were attenuated by the hyperpolarization of the membrane

through the opening of K^+ channels by cromakalim. Therefore, we consider that the higher basal Ca^{2+} influx via L-type VDCCs is responsible for both the maintenance of the myogenic tone and the appearance of the ryanodine-induced contraction in the cerebral arteries.

Both the myogenic tone and the ryanodine-induced contraction may be explained by the 'superficial buffer barrier' hypothesis proposed by Van Breemen and his colleagues (Van Breemen and Saida, 1989; Chen et al., 1992; Van Breemen et al., 1995). According to this hypothesis, sarcoplasmic reticulum is an effective barrier to Ca^{2+} entry, based on mechanisms of Ca^{2+} uptake and unloading to the extracellular space. It is likely that, in the resting state of cerebral arteries, a relatively large amount of Ca^{2+} is taken up into the sarcoplasmic reticulum, as estimated by the contractile action of ryanodine. However, it is also possible that some Ca^{2+} can reach the myofilaments, since the myogenic tone was maintained in the cerebral arteries, as shown in Figs. 1, 2 and 4. Because the myogenic tone was abolished by nifedipine, some part of the basal Ca^{2+} influx via L-type VDCCs is involved in the maintenance of myogenic tone. Studies using a 5-min incubation with ^{45}Ca showed that the amount of Ca^{2+} influx via L-type VDCCs was relatively small when compared with the total basal Ca^{2+} influx in the basilar artery. It has been reported that the magnitude of contraction in vascular smooth muscle depends on the rate of Ca^{2+} influx 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 the sarcoplasmic reticulum 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 (e.g. mesenteric artery in the present study), sarcoplasmic reticulum buffering would be effective to blunt the contraction. On the other hand, if the Ca^{2+} influx is small but fast, such as the influx via L-type VDCCs (e.g. basilar artery), then the Ca^{2+} uptake by the sarcoplasmic reticulum is less able to compete with the Ca^{2+} influx, with the result that the delivery of Ca^{2+} to the myofilaments would be more effective to initiate contraction. Therefore, the myogenic tone in the cerebral arteries appears to be due to the basal Ca^{2+} influx via L-type VDCCs which exceeded the Ca^{2+}-buffering ability of sarcoplasmic reticulum in these arteries.

Because the ryanodine-induced contractions in the cerebral arteries were abolished by nifedipine, neither the Ca^{2+} release from the sarcoplasmic reticulum nor the resting Ca^{2+} influx through the leak pathway was involved in these contractions. Therefore, it is likely that the distribution of cellular Ca^{2+} which enters the cell via L-type VDCCs is altered during the addition of ryanodine, and more Ca^{2+} reached the myofilaments than during the myogenic tone. During the addition of ryanodine, the Ca^{2+} released from the sarcoplasmic reticulum appears to cycle through the sarcoplasmic reticulum; Ca^{2+} that leaked from the open-locked Ca^{2+}-induced Ca^{2+} release channels around the outer surface of the sarcoplasmic reticulum was again taken up into the sarcoplasmic reticulum by Ca^{2+}-ATPase. Under these conditions, it is likely that the sarcoplasmic reticulum cannot buffer the resting Ca^{2+} influx via L-type VDCCs, thus allowing a greater proportion of the entered Ca^{2+} to activate the myofilaments. This conclusion can also be supported by results of the experiment shown in Fig. 5, where the ryanodine-induced potent contraction can be mimicked in the mesenteric artery by elevating the extracellular K^+ concentration to produce an increase in Ca^{2+} influx via VDCCs.

Hwang and Van Breemen (1987) and Kanmura et al. (1988) have reported a ryanodine-induced increase in ^{45}Ca efflux from the plasmalemma of vascular smooth muscle. This action of ryanodine may be reflected by the observation that the basal ^{45}Ca influx in the mesenteric artery was decreased during the addition of ryanodine. Since the amount of ^{45}Ca taken up by the arteries during the 5-min incubation can be assumed to be primarily due to Ca^{2+} influx, with some efflux components (Meisheri et al., 1981; Meisheri and Van Breemen, 1982; Asano et al., 1993a), it is likely that the Ca^{2+} efflux component is increased by the functional elimination of sarcoplasmic reticulum from the mesenteric artery. Since the basal ^{45}Ca influx in the basilar artery was unchanged during the addition of ryanodine, the function of the plasmalemmal Ca^{2+} extrusion systems may be weak in the cerebral arteries. Such a weak function may also contribute to the ryanodine-induced potent contractions in the cerebral arteries.

As shown in Figs. 1 and 2, caffeine caused a relaxation after a transient contraction in the cerebral arteries. Moreover, after the development of the ryanodine action, caffeine caused only a relaxation without causing a contraction in all the arteries tested. The caffeine-induced relaxation has been demonstrated in vascular smooth muscle and the possible mechanism of this action was explained as follows: (1) caffeine increases the cAMP content by inhibiting cAMP phosphodiesterase; (2) caffeine inhibits the Ca^{2+} influx stimulated by K^+-depolarization and by noradrenaline; and (3) caffeine inhibits myosin light-chain kinase and actin-myosin interaction (Sato et al., 1988; Ozaki et al., 1990; Watanabe et al., 1992).

In conclusion, the present study clearly demonstrated the potent contraction in response to ryanodine in the dog cerebral arteries, indicating that the Ca^{2+}-buffering function of sarcoplasmic reticulum is increased in these arteries. The higher basal Ca^{2+} influx via L-type VDCCs is responsible for the myogenic tone and the ryanodine-induced contractions in these arteries. Since the sarcoplasmic reticulum of the cerebral arteries was functionally eliminated by ryanodine, the Ca^{2+} that entered the smooth muscle cells via L-type VDCCs bypassed the sarcoplasmic reticulum, and thus caused a potent contraction in these arteries.

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