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Prostaglandin E_1 potentiation of the spontaneous phasic contraction of rat isolated portal vein by a cyclopiazonic acid-sensitive mechanism

Takaaki Miwa, [1]Masayuki Endou, & Fukuichiro Okumura

Department of Anesthesiology, Yokohama City University School of Medicine, Yokohama 236, Japan

1 The effect of prostaglandin E_1 (PGE_1) on the spontaneous phasic contraction of the rat isolated portal vein was studied.

2 The isolated portal vein exhibited spontaneous phasic contractions. Removal of Ca^{2+} from Krebs-Ringer solution or application of nifedipine abolished the spontaneous contraction, indicating that the contraction depends exclusively on Ca^{2+} influx through L-type Ca^{2+} channels. On the other hand, cyclopiazonic acid (CPA), a specific inhibitor of Ca^{2+}-ATPase of sarcoplasmic reticulum (SR) increased the amplitude of the contractions, suggesting that the SR regulates the spontaneous contractions negatively by sequestration of Ca^{2+} entering through L-type Ca^{2+} channels and buffering the rise in cytosolic Ca^{2+}.

3 PGE_1 increased the amplitude of the spontaneous contraction in a concentration-dependent manner without affecting the resting tension. The effect was completely abolished by nifedipine. Bay K 8644 and phenylephrine (PE) also increased the amplitude of the contraction in a concentration-dependent manner. PGE_1 at a concentration of 1 μM, Bay K 8644 at 100 nM and PE at 30 nM doubled the amplitude, respectively.

4 Pretreatment with 1 μM CPA abolished the effect of PGE_1, but the effects of Bay K 8644 and PE were not inhibited by pretreatment with CPA. In contrast, 10 μM ryanodine attenuated the effect of PE without affecting the contractile effect of PGE_1.

5 When the SR was depleted of Ca^{2+} by repeated applications of caffeine in a nominally Ca^{2+}-free Krebs-Ringer solution, it took about 120 s to restore the spontaneous contraction after addition of Ca^{2+} into the solution. In CPA-treated veins, the time taken to restore the contraction was shortened significantly. Pretreatment with 1 μM PGE_1 shortened the time to the same extent as pretreatment with CPA did.

6 These results suggest that PGE_1 increases the amplitude of the spontaneous phasic contraction by a different mechanism from those by which PE and Bay K 8644 increase it. Inhibition of Ca^{2+}-ATPase of the SR might be involved in the vasoactive effect of PGE_1.

Keywords: Prostaglandin E_1; portal vein; sarcoplasmic reticulum; Ca^{2+}-ATPase; cyclopiazonic acid; ryanodine

Introduction

Prostaglandin E_1 (PGE_1) is quite an important therapeutic drug for keeping the arterial duct open in patients with congenital heart disease whose pulmonary blood flow depends on patency of the arterial duct (Silove, 1986). PGE_1 is also administered to patients with severe peripheral vascular disease (Clifford *et al*., 1980), and used to induce hypotension for reduction in blood loss during surgery (Goto *et al*., 1982). The vasodilator effect of PGE_1 is considered to be mediated by activating adenylate cyclase and a subsequent increase in adenosine 3′:5′-cyclic monophosphate (cyclic AMP) (Wilson & Warren, 1993). Thus PGE_1 seems to be one of vasodilator agents, such as isoprenaline and calcitonin gene-related peptide, which relax vascular smooth muscle by increasing the cyclic AMP content (Wood & Owen, 1992).

Although PGE_1/E_2 are considered clinically to be vasodilator agents, vasoconstriction induced by these agents is observed in some isolated vessels including human pulmonary artery (Pipkin & Chinnery, 1985; Qian *et al*., 1994). In general, the effects of PGE_1/E_2 are mediated via at least four subtypes of the receptor, termed EP_1-EP_4 (for review, see Coleman *et al*., 1994). These receptor subtypes are coupled to different signal transduction pathways; EP_1 is coupled to an increase in intracellular Ca^{2+}, EP_2 and EP_4 are coupled to stimulation of adenylate cyclase, and EP_3 is coupled to inhibition of adenylate cyclase and acceleration of hydrolysis of phosphoinositide. The complexity of the vasoactive effects of PGE_1/E_2 could result from species- and tissue-differences in the distribution of the EP receptor subtypes.

The rat portal vein possesses spontaneous contractile activity which is accompanied by burst of action potentials (Axelsson *et al*., 1967). This character is thought to be similar to that of the arteriolar resistance vessel with myogenic tone (Johansson, 1989). Thus the rat portal vein could provide a useful experimental model when we attempt to investigate the pharmacological actions of agents on systemic vascular resistance.

The present study was undertaken to evaluate the effect of PGE_1 on the spontaneous phasic contraction of the rat portal vein. Among several vasodilators, we showed that PGE_1 uniquely enhanced the amplitude of the portal vein contraction. We also suggested that the effect might be mediated by inhibition of the sarcoplasmic reticulum (SR) Ca^{2+}-ATPase of the portal vein, and subsequent impairment of the Ca^{2+} buffering function of the SR.

Methods

All procedures with animals in this study were in accordance with the guidelines of the Animal care Committee of Yokohama City University, School of Medicine.

[1] Author for correspondence.

Male Wistar rats weighing 200–250 g were anaesthetized with diethyl ether. The portal vein was rapidly excised and placed in a dissection dish filled with oxygenated Krebs-Ringer solution of the following composition (mM): NaCl 119.0; KCl 4.5; $CaCl_2$ 2.5; $MgCl_2$ 0.5; NaH_2PO_4 1.2; $NaHCO_3$ 25 and glucose 11. The vessels were carefully cleaned of adherent connective tissues and cut into rings (3–4 mm long). The rings were suspended longitudinally in a 10 ml double-walled glass chamber filled with Krebs-Ringer solution, gassed continuously with 95% O_2 and 5% CO_2 (pH 7.4) at 37°C. The bulk of the smooth muscle of the portal vein is oriented longitudinally, and longitudinal strips or whole veins mounted longitudinally are thought to be the usual preparations (Sutter, 1965). The preparations were connected to a force transducer (Minebea UL-10GR, Nagano, Japan) for isometric tension recordings with a pen recorder (Sanei RECTI-HORIZ-8K, Tokyo, Japan) through a preamplifier (Sanei AS2103). After a resting tension of 1 g was applied, the preparations contacted spontaneously at a frequency of 0.03–0.06 Hz and the amplitude of the contraction was 250–500 mg. We confirmed that the frequency and the amplitude of the phasic contraction were maintained in a steady level of 90 min after the stabilization. The concentration-response curve for the effects of agents used on the amplitude of the spontaneous phasic contraction were determined in a cumulative manner. The amplitude of the spontaneous phasic contraction just before the addition of the agents was taken as control (100%). When the contractile effects of PGE_1, phenylephrine (PE) and Bay K 8644 in the presence of cyclopiazonic acid (CPA) or ryanodine were examined, the amplitude of the contraction in the presence of CPA or ryanodine was taken as control. The nominally Ca^{2+}-free solution was prepared by omitting $CaCl_2$ from the Krebs-Ringer solution.

Drugs

The following drugs were used: nifedipine, indomethacin, carbachol chloride, (−)-phenylephrine hydrochloride, cyclopiazonic acid, caffeine, L-NMMA (N^G-monomethyl-L-arginine, Sigma Chemical Co., St. Louis, MO), ryanodine (Calbiochem., La Jolla, CA), Bay K 8644 (methyl-1,4-dihydro-2,6-dimethyl-3-nitro-4(2-trifluoromethyl-phenyl)pyridine-5-carboxylate, Research Biochemical International, Natick, MA), prostaglandin E_1 (kindly given by Ono Pharmaceutical Co., Tokyo, Japan), forskolin, sodium nitroprusside dihydrate (Wako Pharmaceutical Co., Ltd, Osaka, Japan), nicorandil (kindly given by Chugai Pharmaceutical Co., Ltd, Tokyo, Japan).

Nifedipine, nicorandil and indomethacin were dissolved in dimethyl sulphoxide (DMSO) and Bay K 8644 was dissolved in ethanol. Further dilutions were made with distilled water; at the concentrations used, DMSO and ethanol had no effect on any preparation in this study. All other chemicals were dissolved in distilled water.

Statistics

All values are presented in terms of mean ± s.e.mean. Comparisons of variables obtained during the concentration-response curves and comparisons of time needed to restore the spontaneous contraction by application of Ca^{2+} in the Ca^{2+}-free solution were made by one-way analysis of variance followed by Scheffe's t test or Bonferroni's t test. Analysis by Student's t test was performed for unpaired comparisons. A value of $P<0.05$ was considered statistically significant.

Results

Characteristics of spontaneous phasic contractions of the rat portal vein

As shown in Figure 1, the portal vein possessed a spontaneous phasic contractile activity at a frequency of 0.03–0.06 Hz, and the amplitude of the contraction was 250–500 mg, which is consistent with previous findings (Funaki & Bohr, 1964). This contraction disappeared within a minute, when Ca^{2+}-free Krebs-Ringer solution was applied into the organ bath (Figure

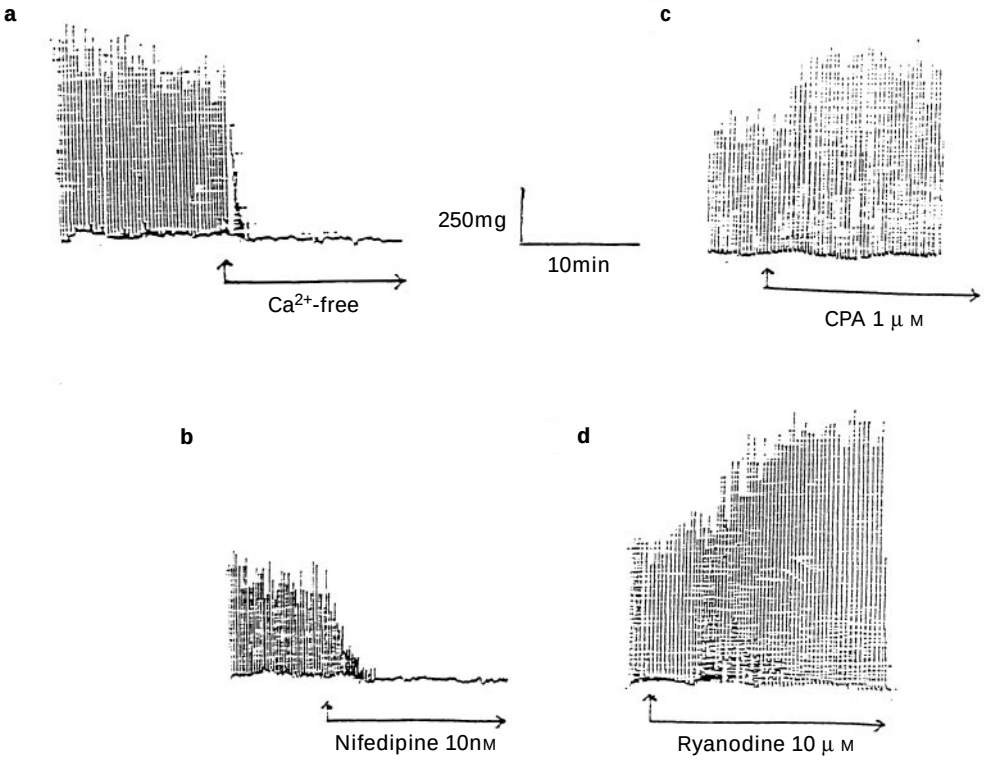

Figure 1 Representative effects of nominally Ca^{2+}-free Krebs-Ringer solution (a), nifedipine (b), cyclopiazonic acid (CPA) (c) and ryanodine (d) on the spontaneous phasic contraction of the rat portal vein.

1a). Nifedipine at a concentration of 10 nM also abolished the contraction (Figure 1b). These results indicate that the spontaneous contraction depends exclusively on Ca^{2+} influx through L-type Ca^{2+} channels.

We examined a role of the SR in the intracellular Ca^{2+} handling of the portal vein by means of using CPA and ryanodine. Both CPA and ryanodine increased the amplitude of the phasic contraction with little effect on the resting tone and the frequency of the spontaneous contraction (Figure 1c, d). CPA 1 μM and ryanodine 10 μM increased the amplitude by $84.4 \pm 10.1\%$ ($n=6$) and $68.3 \pm 8.7\%$ ($n=6$), respectively. There is no statistically significant difference between these effects of CPA and ryanodine.

Effects of PGE_1 and other vasoactive agents on the phasic contraction

A representative tracing depicting the effect of PGE_1 on the spontaneous phasic contraction is shown in Figure 2a. PGE_1 1 μM increased the amplitude by 102% without any effect on the resting tension and the frequency of the contraction. Figure 2c illustrates the concentration-response curve for the contractile effect of PGE_1. PGE_1 increased the amplitude in a concentration-dependent manner. PGE_1 1 μM increased it by $113.8 \pm 18.3\%$ ($n=6$). Pretreatment with either L-NMMA (100 μM) or indomethacin (1 μM) did not influence the effect of PGE_1 on the spontaneous contraction (data not shown). Since it has been postulated that PGE_1 dilates vascular smooth muscle by elevating cyclic AMP levels, we examined the effect of forskolin, an adenylate cyclase activator, on the portal vein contraction. In contrast to PGE_1, forskolin diminished the spontaneous contraction (Figure 2b) in a concentration-dependent manner (Figure 2d. The effect of PGE_1 was completely abolished by nifedipine (data not shown), which implies that Ca^{2+} influx through an L-type Ca^{2+} channel is mandatory for the effect of PGE_1. The clinically used vasodilators sodium nitroprusside and nicorandil at concentrations of 1 μM also reduced the amplitude of the contraction by $64.5 \pm 3.5\%$ ($n=8$) and $53.2 \pm 9.9\%$ ($n=6$), respectively.

Figure 3a and 3b demonstrates the typical effects of the Ca^{2+} channel activator Bay K 8644 and phenylephrine (PE). Bay K 8644 100 nM increased the amplitude of the contraction by 127% without affecting the resting tension (Figure 3a). PE 30 nM also enhanced the contraction by 105% with little affect on the resting tension (Figure 3b). PE did not change the frequency, while Bay K 8644 increased it by about 40%. Concentration-dependent effects of PE and Bay K 8644 on the amplitude of the contraction are shown in Figure 4a and Figure 5c, respectively. PE 30 nM increased the amplitude by $127.1 \pm 16.8\%$ ($n=6$), and Bay K 8644 100 nM increased it by $121.1 \pm 25.9\%$ ($n=6$). PE at higher concentrations caused an increase in the basal tone (tonic contraction). There was no significant difference between the increasing effects of 1 μM PGE_1, 100 nM Bay K 8644 and 30 nM PE on the amplitude of the spontaneous contraction.

Modulation of the contractile effects of PGE_1, phenylephrine and Bay K 8644 by ryanodine and CPA

Figure 4 shows the inhibitory effect of ryanodine on phenylephrine-induced increase in the amplitude of the contraction, but not on PGE_1-induced increase in the amplitude. The concentration-response curve for the contractile effect of PE was shifted downward by pretreatment with 10 μM ryanodine (Figure 4a). PE at a concentration of 30 nM in the absence and presence of ryanodine increased the amplitude by $127.1 \pm 16.8\%$ ($n=6$) and $44.4 \pm 3.7\%$ ($n=6$), respectively ($P<0.05$). On the other hand, the contractile effect of PGE_1 was not attenuated by pretreatment with 10 μM ryanodine

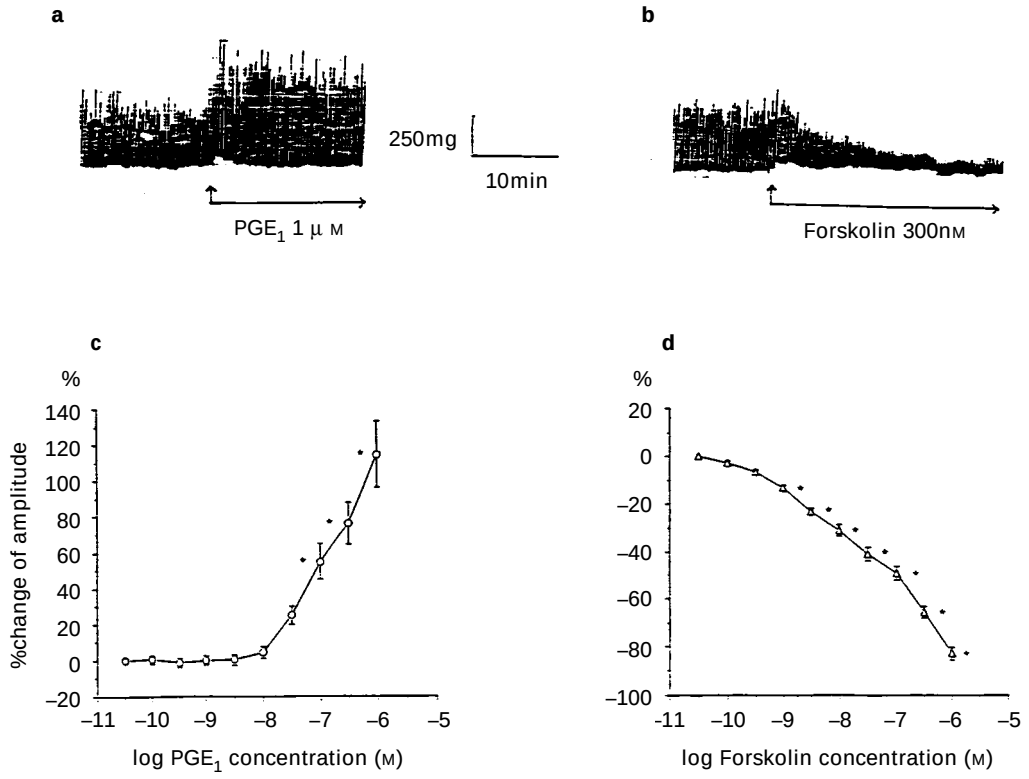

Figure 2 Effects of prostaglandin E_1 (PGE_1) and forskolin on the spontaneous phasic contraction of the rat portal vein. (a) Representative effect of PGE_1; (b) representative effect of forskolin. (c) Concentration-response curve for the PGE_1-induced increase in the amplitude of the spontaneous phasic contraction ($n=6$). (d) Concentration-response curve for forskolin-induced decrease in the amplitude ($n=6$). (c and d) Points are means of % change in the amplitude of the contraction; vertical lines show s.e.mean. Predrug control amplitude was taken as 100%. *Significantly different from control value ($P<0.05$) by one-way analysis of variance followed by Scheffe's t test.

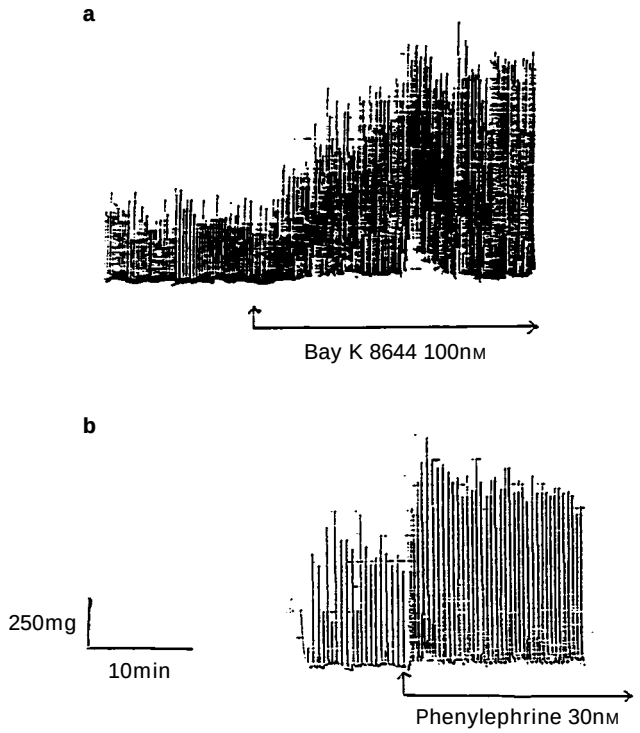

Figure 3 Representative effects of Bay K 8644 (a) and phenylephr-

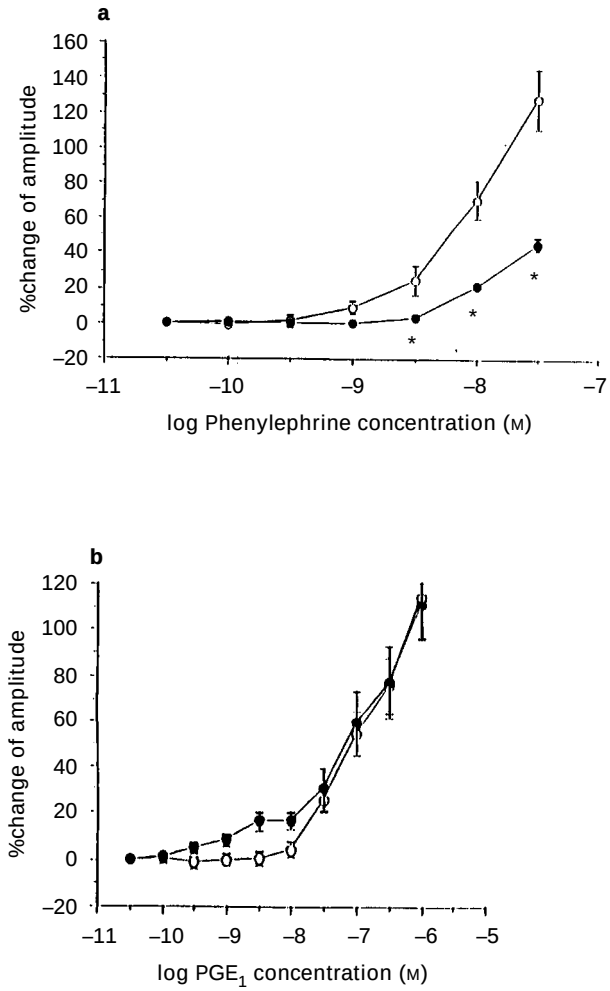

Figure 4 Concentration-response curves for the effects of phenylephrine (a), and prostaglandin E_1 (b) on the amplitude of the spontaneous phasic contraction of the rat portal vein in the absence (○) and presence (●) of ryanodine 10 μM. Points are means (vertical lines show s.e.mean; $n=6$ for each group) of % change in the amplitude of the contraction. Predrug control amplitudes in the absence and presence of ryanodine were taken as 100%, respectively. *Significantly different from corresponding control ($P<0.05$) by analysis of variance followed by Bonferroni's t test.

(Figure 4b). PGE_1 1 μM in the absence and presence of ryanodine increased the amplitude by $113.8\pm18.3\%$ ($n=6$) and $109\pm13.7\%$ ($n=6$), respectively.

In contrast, pretreatment with CPA 1 μM abolished the contractile effect of PGE_1 (Figure 5a), while the concentration-response curve for PE-induced increase in the amplitude was hardly affected by CPA (Figure 5b). PE 30 nM in the absence and presence of CPA increased the amplitude by $127.1\pm16.8\%$ ($n=6$) and $99.2\pm15.8\%$ ($n=6$), respectively ($P>0.05$). Similarly, the increase in the amplitude by Bay K 8644 was not affected by pretreatment with CPA (Figure 5c). Bay K 8644 100 nM in the absence and presence of CPA increased the amplitude by $121.1\pm25.9\%$ ($n=6$) and $115.0\pm19.2\%$ ($n=6$), respectively ($P>0.05$).

Restoration of the spontaneous phasic contraction by addition of Ca^{2+} in the Ca^{2+} depleted portal veins

The SR was depleted of Ca^{2+} by exposure to 25 mM caffeine three times at an interval of 20 min in nominally Ca^{2+}-free Krebs-Ringer solution. The first application of caffeine produced a contraction the amplitude of which was about 20% of that of the spontaneous phasic contraction in the normal Krebs-Ringer solution. The preparation no longer responded to the second and third application of caffeine. Then, addition of 1 mM Ca^{2+} to the organ bath restored the spontaneous phasic contraction in 120 s (Figure 6a). Treatment of the preparation with 1 μM CPA 15 min before the addition of Ca^{2+} accelerated the appearance of the spontaneous contraction (Figure 6b). As well as CPA, treatment with 1 μM PGE_1 shortened the time elapsing until the apperarance of the spontaneous contraction to the same extent (Figure 6c). Pretreatment with 30 nM PE (Figure 6d), 10 μM ryanodine (Figure 6e) and 100 nM Bay K 8644 (Figure 6f) also shortened it between Ca^{2+} application and the restoration of the contraction. However, the extent of the shortening effects seemed to vary between the agents. As summarized in Figure 7, the time taken to restore the spontaneous phasic contraction after the addition of Ca^{2+} was 120.3 ± 1.9 s ($n=4$). Although treatment with CPA, PGE_1, PE, ryanodine and Bay K 8644 all shortened the time statistically significantly, these five agents could be classified into three groups based on the extent of the shortening effects. As a slight-shortening group, PE and ryanodine shortened the time to 97.3 ± 4.3 s ($n=4$) and 98.1 ± 6.3 s ($n=4$), respectively. As a moderate-shortening group, CPA and PGE_1 shortened the time to 49.7 ± 2.8 s ($n=4$) and 49.8 ± 2.0 s ($n=4$), respectively. Bay K 8644 drastically shortened it to 25.5 ± 3.1 s ($n=4$).

Discussion

We have found that PGE_1 enhanced the amplitude of the spontaneous phasic contraction of the rat isolated portal vein without affecting its basal tone. Other vasodilators used in this study, such as nifedipine, sodium nitroprusside, nicorandil and forskolin, suppressed the spontaneous contraction, indicating the unique property of PGE_1 among the vasodilators. The Ca^{2+} channel activator, Bay K 8644, and the α-adrenoceptor agonist, PE, also increased the amplitude of the spontaneous contraction. The SR Ca^{2+}-ATPase inhibitor, CPA abolished the vasoactive effect of PGE_1 without affecting those of Bay K 8644 and PE. On the other hand, low concentrations of ryanodine, which fix caffeine-sensitive SR Ca^{2+} release channels in an open state, attenuated the vasoactive effect of PE without affecting that of

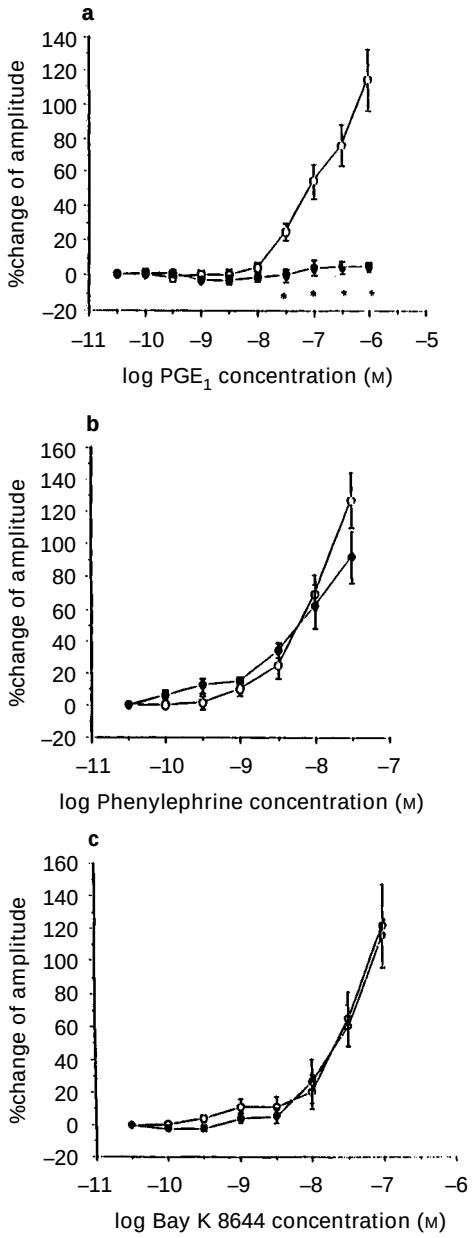

Figure 5 Concentration-response curves for the effects of prostaglandin E_1 (a), phenylephrine (b) and Bay K 8644 (c) on the amplitude of the spontaneous phasic contraction of the rat portal vein in the absence (○) and presence (●) of cyclopiazonic acid 1 μM. Points are means (vertical lines show s.e.mean; $n=6$ for each group) of % change in the amplitude of the contraction. Predrug control amplitudes in the absence and presene of cyclopiazonic acid were taken as 100%, respectively. *Significantly different from corresponding control ($P<0.05$) by analysis of variance followed by Bonferroni's t test.

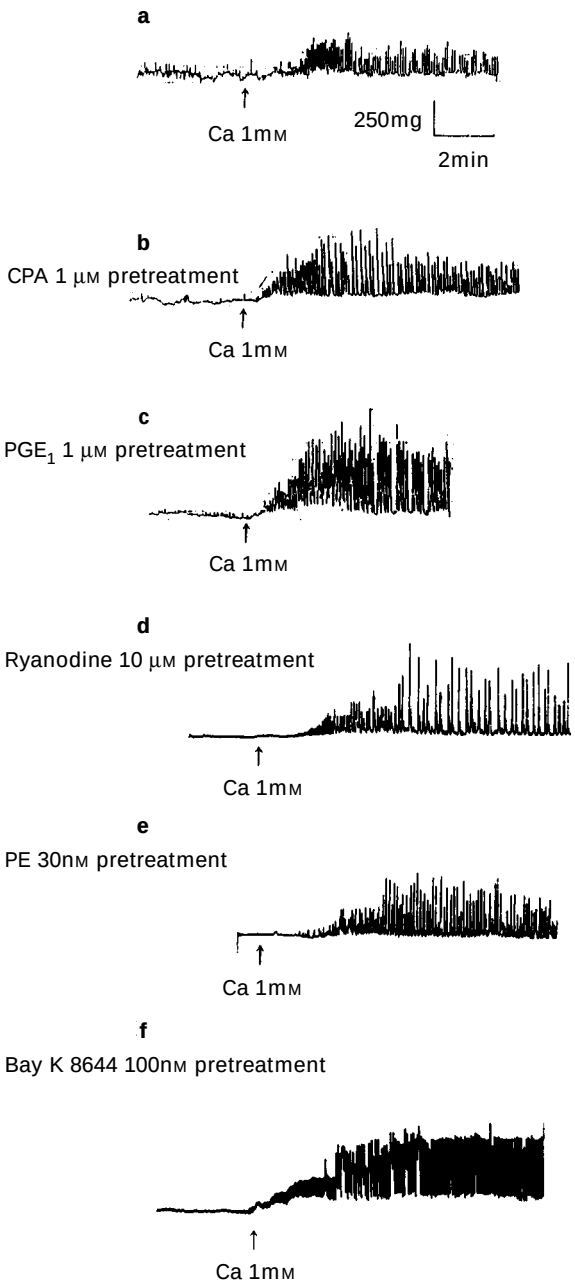

Figure 6 Restoration of the spontaneous phasic contraction by addition of Ca^{2+} in Ca^{2+}-depleted portal vein. After the preparations were stabilized in a Krebs-Ringer solution, 25 mM caffeine was applied three times in a nominally Ca^{2+}-free Krebs-Ringer solution at an interval of 20 min. Subsequent application of 1 mM Ca^{2+} restored the spontaneous phasic contraction in 120 s (a). Typical effects of pretreatment with cyclopiazonic acid (CPA), PGE_1, ryanodine, phenylephrine (PE) and Bay K 8644 on the restoration of the contraction are shown in (b), (c), (d), (e) and (f), respectively. Cyclopiazonic acid, PGE_1, ryanodine, phenylephrine and Bay K 8644 were applied 15 min before the addition of Ca^{2+}.

PGE_1. These results suggest strongly that the underlying mechanism of the PGE_1-induced increase in the amplitude of the contraction is different from those of Bay K 8644 and PE.

The spontaneous phasic contraction of the rat isolated portal vein is thought to be caused by the spontaneous occurrence of bursts of action potentials (Axelsson *et al.*, 1967). Removal of extracellular Ca^{2+} or application of nifedipine abolished the spontaneous contraction, indicating that Ca^{2+} influx via L-type Ca^{2+} channels during burst of the action potential is indispensable for the generation of the spontaneous contraction (Figure 1a, b; Mikkelsen, 1985). In general, the contractility of vascular smooth muscle is controlled by $[Ca^{2+}]_i$ and the Ca^{2+}-sensitivity of the contractile apparatus (van Breemen *et al.*, 1986). Although transsarcolemmal Ca^{2+} influx and efflux are the most important determinants of $[Ca^{2+}]_i$, SR also participates in determining $[Ca^{2+}]_i$ and regulating the contractility of the vascular smooth muscle (for review, see van Breemen & Saida, 1989; van Breemen *et al.*, 1995). Thus, we investigated the role of the SR in the spontaneous contraction of the portal vein by using two specific agents, CPA and ryanodine, which modulate the function of the SR. CPA, an indole tetramic acid metabolite of *Aspergillus* and *Penicillium*, is a selective inhibitor of SR/ER Ca^{2+}-ATPase of skeletal muscle (Seidler *et al.*, 1989), smooth muscle (Deng & Kwang, 1991) and endothelial cell (Zhang *et al.*, 1994). On the other hand, the

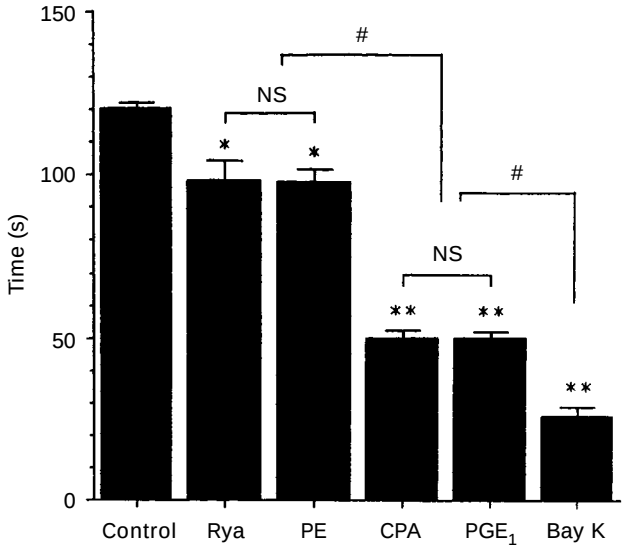

Figure 7 The effects of cyclopiazonic acid (CPA), PGE_1, ryanodine (Rya), phenylephrine (PE) and Bay K 8644 on the restoration of the spontaneous phasic contraction by addition of Ca^{2+} in Ca^{2+}-depleted portal vein. Each column represented the time needed to restore the spontaneous phasic contraction after the application of Ca^{2+} (means ± s.e.mean; $n=4$ for each group.) Significantly different from control, $*P<0.05$, $**P<0.01$. Significantly different from each other, $\#P<0.01$, by analysis of variance followed by Scheffe's t test.

plant alkaloid ryanodine, at concentrations up to 10 μM used in the present study, fixes caffeine-sensitive SR Ca^{2+} release channels in an open state (for review, see Coronado *et al*., 1994).

CPA increased the amplitude of the spontaneous contraction (Figure 1c). If we interpret this phenomenon simply, it implies that the SR takes up and sequesters some of the Ca^{2+} entering through L-type Ca^{2+} channels and buffers the rise in $[Ca^{2+}]_i$. This is in good agreement with the theory proposed by van Breemen and colleagues as the superficial buffer barrier hypothesis (for review, see van Breeman & Saida, 1989; van Breemen *et al*., 1995). Sequestered Ca^{2+} is thought to be released from the SR preferentially into the subsarcolemmal space to be extruded from the cell via Na^+-Ca^+ exchange (Stehno-Bittel & Sturek, 1992; Juhaszova *et al*., 1994). CPA inhibited Ca^{2+}-uptake into the SR, resulting in a large increase in $[Ca^{2+}]_i$ and amplitude of the spontaneous contraction. Ryanodine also increased the amplitude of the spontaneous contraction (Figure 1d). Inasmuch as ryanodine makes the SR leaky by means of fixing the caffeine-sensitive SR Ca^{2+}-release channel in an open state, we think that ryanodine impairs the ability of the SR to trap Ca^{2+}, resulting in an increase in $[Ca^{2+}]_i$. The 'capacitative Ca^{2+} entry hypothesis' advocated originally by Putney (1986) proposes that depletion of Ca^{2+} in an intracellular Ca^{2+} store promotes Ca^{2+}-influx from the extracellular space (for review, see Fasolato *et al*., 1994). Therefore, it is possible that CPA and ryanodine increase $[Ca^{2+}]_i$ by the same mechanism because both agents can deplete the SR of Ca^{2+} (Shima & Blaustein, 1992). However, we do not think that this is the case in the rat portal vein, because the results depicted in Figures 6 and 7 suggest strongly that CPA and ryanodine modulate the intracellular Ca^{2+} homeostasis by a different mechanism. After the intracellular Ca^{2+} stores had been depleted by caffeine in the nominally Ca^{2+}-free solution, it took about 120 s to restore the spontaneous contraction by addition of Ca^{2+} into the solution. CPA accelerated very much the restoration of the spontaneous contraction, suggesting that a large amount of Ca^{2+} was taken up into the depleted SR to refill it during the first few minutes. Luckhoff and Busse (1990) obtained similar results on preincubation of porcine aortic endothelial cells with another Ca^{2+}-ATPase inhibitor, 2,5-di(tert-butyl)-1,4-benzohydroquinone (BuBHQ); this induced a large increase in $[Ca^{2+}]_i$ during the refilling phase, while only a small increase was observed during the refilling phase in the absence of BuBHQ. On the other hand, ryanodine accelerated the restoration of the spontaneous contraction slightly, although 10 μM ryanodine and 1 μM CPA increased the amplitude of the spontaneous contraction to the same extent (Figure 1). This suggests that ryanodine could make Ca^{2+} leak out of the SR after a considerable part of the store had been refilled with Ca^{2+}. Thus, we think that CPA could increase the amplitude of the spontaneous contraction by means of inhibiting Ca^{2+}-uptake into the SR, while ryanodine could increase it by means of impairing the ability of the SR to trap Ca^{2+}.

Bay K 8644, which potentiates Ca^{2+} influx via voltage-dependent Ca^{2+} channels (Schramm *et al*., 1983), increased the amplitude and the frequency of the spontaneous contraction (Figure 3a) as shown by Mikkelsen (1985). It has been found that depolarization-evoked Ca^{2+} entry through voltage-dependent Ca^{2+} channels can induce the release of Ca^{2+} from the intracellular Ca^{2+} stores in the rat portal vein smooth muscle (Gregoire *et al*., 1993). Thus the Ca^{2+}-induced Ca^{2+} release mechanism might be also involved in the contractile effect of Bay K 8644. PE at concentrations up to 30 nM increased the amplitude of the phasic contraction without changing the resting tone (Figure 3b), which is consistent with the results obtained by Schwietert *et al*. (1991). Dacquet *et al*. (1987) demonstrated that the contractile effect of PE on the rat isolated portal vein was abolished by nifedipine, indicating that Ca^{2+} influx through L-type Ca^{2+} channels is also indispensable for the vasoactive effect of PE. Recent studies have demonstrated, in the rat portal vein smooth muscle, that Cl^- channels which are activated by Ca^{2+} released from the SR via an inositol (1,4,5) triphosphate (IP_3)-induced Ca^{2+}-release mechanism play a key role in depolarization of the plasma membrane and subsequent activation of L-type Ca^{2+} channels (Pacaud *et al*., 1991; 1995). Thus, for both Bay K 8644 and PE, the buffering function of the SR for Ca^{2+} entry would be inhibited by Ca^{2+}-induced Ca^{2+}-release or an IP_3-induced Ca^{2+}-release mechanism.

Similar to Bay K 8644 and PE, PGE_1 also increased the amplitude of the spontaneous phasic contraction without affecting the basal tone (Figure 2a). In addition, the contractile effect of PGE_1 was abolished by nifedipine, suggesting that PGE_1 might augment Ca^{2+} influx via voltage-dependent Ca^{2+} channels as Bay K 8644 and PE do. However, pretreatment with CPA and ryanodine revealed a difference between the underlying mechanisms of PGE_1 and the two agents. As shown in Figure 5a, CPA abolished the contractile effect of PGE_1. In sharp contrast, CPA did not modify the contractile effects of either PE or Bay K 8644 (Figure 5b, c). On the other hand, ryanodine attenuated significantly the contractile effect of PE without affecting the effect of PGE_1 (Figure 4a, b). As discussed above, Bay K 8644 and PE would make sequestered Ca^{2+} in the SR available for generation of the contraction by Ca^{2+}-induced Ca^{2+}-release and Ins (1,4,5)P_3-induced Ca^{2+}-release mechanisms. If so, CPA might change the pathway of Ca^{2+} via the SR to a new pathway which leads straightforwardly to the deep cytoplasm. We think that this is the reason why CPA did not inhibit the contractile effects of Bay K 8644 and PE. On the other hand, PE could not fully exhibit its ability to release Ca^{2+} sequestered in the SR in the presence of ryanodine, because ryanodine had already impaired the sequestration of Ca^{2+} in the SR. Thus, ryanodine attenuated the contractile effect of PE.

Other mechanisms which are thought to be independent of the underlying mechanisms for the Bay K 8644- and PE-induced increase in amplitude of the spontaneous contraction are (1) inhibition of basal release of a vasodilating substance released from the endothelial cells, if any, (2) an increase in Ca^{2+} sensitivity of the contractile proteins, (3) inhibition of SR Ca^{2+}-ATPase. Taking into consideration that L-NMMA and indomethacin did not affect either the basal amplitude of the contraction or the contractile effect of

PGE_1, and that CPA itself increased the amplitude and pretreatment of the vessel while it abolished the contractile effect of PGE_1, it seems likely that PGE_1 increases the amplitude of the spontaneous contraction by the same mechanism as CPA. This idea is supported by the observation that PGE_1 as well as CPA accelerate Ca^{2+}-induced restoration of the spontaneous contraction in the portal vein of which SR had been depleted of Ca^{2+} in nominally Ca^{2+}-free solution. When the store is depleted, Ca^{2+} entering through the plasmalemma may be taken up into the SR by Ca^{2+}-ATPase initially to refill the Ca^{2+} store, subsequently Ca^{2+} influx may increase $[Ca^{2+}]_i$, resulting in the restoration of the spontaneous contraction. Thus, we expected CPA to shorten the onset time of the spontaneous contraction by short-circuiting the SR, resutling in Ca^{2+} entry into the cytosol directly. We found, as illustrated in Figure 6b, exactly what we expected. Similar results have been obtained in the dog mesenteric artery with thapsigargin, another putative selective inhibitor of the SR Ca^{2+}-ATPase; pretreatment with thapsigargin changed the slow onset of K^+-induced contraction to a fast one in the Ca^{2+}-depleted artery (Low *et al*., 1991). PGE_1 induced the same effect on the Ca^{2+}-depleted portal vein as CPA did (Figures 6c and 7), suggesting strongly that PGE_1 also inhibits Ca^{2+}-ATPase of the SR. We also examined the effects of ryanodine, PE and Bay K 8644 on the Ca^{2+}-induced restoration of the spontaneous contraction. Interestingly, Bay K 8644 accelerated the restoration potently. In contrast, ryanodine and PE accelerated it slightly compared to CPA and PGE_1, as summarized in Figure 7. These results further suggest the similarity between PGE_1 and CPA.

The electrical and contractile properties of the portal vein are thought to resemble those of the small arterioles which have myogenic tone and play a pivotal role in determining the peripheral resistance and blood flow to the tissues (Johansson, 1989). Therefore, the isolated portal vein preparation is a much better model than large conduit arteries for *in vitro* assessment of effects of vasoactive agents on peripheral vascular resistance. In the present study we demonstrated that PGE_1 potentiated the spontaneous contraction of the rat portal vein, implying that PGE_1 could constrict small arterioles with myogenic tone, if the arterioles possess a PGE_1 receptor subtype which mediates the contractile effect of PGE_1.

In conclusion, PGE_1 increases the amplitude of the spontaneous contraction of the rat isolated portal vein. The underlying mechanism of PGE_1 is different from those of Bay K 8644 and PE, and seems to be related uniquely to inhibition of SR Ca^{2+}-ATPase of the portal vein smooth muscle. Further experiments are needed to determine the receptor subtype and intracellular signalling transduction pathway.

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