British Journal of Pharmacology (2002) 137, 545–553

www.nature.com/bjp

In the presence of L-NAME SERCA blockade induces endothelium-dependent contraction of mouse aorta through activation of smooth muscle prostaglandin H2/thromboxane A2 receptors

*,[1]Elena B. Okon, [1]Ali Golbabaie & [1]Cornelis van Breemen

[1]iCAPTUR[4]E Center, University of British Columbia and St. Paul's Hospital, Vancouver, British Columbia, Canada

1 The mechanism of transient contractions induced by the sarcoplasmic–endoplasmic reticulum calcium ATPase (SERCA) blocker cyclopiazonic acid (CPA) in the presence of L-NAME was investigated in mouse aorta.

2 The contractions elicited by 10 μM CPA required an intact endothelium, were dependent upon external Ca^{2+} and were prevented by 10 μM indomethacin, the inhibitor of prostaglandin synthesis, or 1 μM SQ29548, the specific prostaglandin H2/thromboxane A2 (PGH2/TXA2) receptor blocker.

3 A blocker of receptor/store operated Ca^{2+} channels and voltage gated calcium channels (VGCC), SK&F 96365 (10 μM), completely abolished the contractions, while a specific blocker of VGCC nifedipine (1 μM) inhibited them by one third.

4 Dichlorobenzamyl hydrochloride, a blocker of Na^+/Ca^{2+} exchange effectively prevented return of tension to baseline value.

5 At higher concentrations (30–100 μM) CPA induced indomethacin-resistant tonic contractions of mouse aorta. The CPA dose response curve for tonic contractions is shifted to the right compared to the transient contractions suggesting that smooth muscle is less sensitive to CPA than endothelium.

6 PGH2/TXA2 receptors in mouse aorta are highly sensitive to the thromboxane analogue U46619 (EC_{50}: 1.93 nM). This compound stimulates contractions even in the absence of external Ca^{2+}, which are abolished by the Rho-kinase inhibitor HA-1077.

7 The results suggest that 10 μM CPA induced capacitive Ca^{2+} entry in endothelial cells stimulating the release of PGH2/TXA2, which subsequently caused smooth muscle contraction dependent on Ca^{2+} influx and myofilament sensitization by Rho-kinase. Higher concentrations of CPA (30–100 μM) directly induced contraction of mouse aortic smooth muscle.

British Journal of Pharmacology (2002) **137,** 545–553. doi:10.1038/sj.bjp.0704884

Keywords: SERCA; cyclopiazonic acid; mouse aorta; prostaglandin H2/thromboxane A2 receptor

Abbreviations: 2APB, diphenylboric acid 2-aminoethyl ester; AA, arachidonic acid; Ach, acetylcholine; ATP, adenosine 5′-triphosphate; COX, cyclo-oxygenase; CPA, cyclopiazonic acid; DCB, Dichlorobenzamyl hydrochloride; EC(s), endothelial cell(s); EC_{50}, 50% effective concentration; ER, endoplasmic reticulum; IP_3, inositol 1,4,5-trisphosphate; IP_3R, inositol 1,4,5-trisphosphate receptor; L-NAME, N^{ω}-nitro-L-arginine methylester; NCX, Na^+/Ca^{2+} exchanger; NO, nitric oxide; PG(s), prostaglandin(s); PLA2, phospholipase A2; ROC, receptor-operated channel; SERCA, sarcoplasmic-endoplasmic reticulum calcium ATPase; SMC(s), smooth muscle cell(s); SOC, store-operated channel; SQ29548, ([1S-[1a,2a(Z),3a,4a]]-7-[3-[[2-(Phenylamino)carbonyl]hidrazino]-methyl]-7-oxabicyclo[2.2.1]hept-2-yl]-5-heptenoic acid); SR, sarcoplasmic reticulum; TXA2, thromboxane A2; TXR, PGH2/TXA2 receptor; U46619, 9,11-dideoxy-11α,9α-epoxymethano-prostaglandin F(2α); VGCC, voltage gated calcium channel.

Introduction

The pioneering research of Vane (Moncada & Vane, 1979a; de Nucci *et al*., 1988) and Furchgott (Furchgott & Zawadzki, 1980; Furchgott & Vanhoutte, 1989) has shown that endothelial secretions play an important role in modulating vascular tone. Release of both relaxing and contracting factors from the endothelium is regulated by elevation of $[Ca^{2+}]_i$. For this reason, it is essential to separate the actions of pharmacological agents, which target Ca^{2+} transport molecules on endothelial cells (ECs) from those on the underlying smooth muscle. An agent for which this is particularly relevant is the SERCA blocker CPA, which in most intact vascular preparations induces relaxation by elevating endothelial $[Ca^{2+}]_i$ in ECs (Zhang *et al*., 1994) and releasing nitric oxide (NO) (Zheng *et al*., 1993; Moritoki *et al*., 1994; Rahimian *et al*., 1997). On the other hand, when

*Author for correspondence at: iCAPTUR[4]E Center, McDonald Research Laboratories, University of British Columbia, room 292, St. Paul's Hospital, 1081 Burrard Street, V6Z 1Y6, Vancouver, BC, Canada; E-mail: eokon@mrl.ubc.ca

SERCA blockade induces contraction, it is often interpreted to indicate depletion of smooth muscle sarcoplasmic reticulum (SR) Ca^{2+} and activation of capacitative Ca^{2+} entry (Tosun *et al*., 1998a; Tanaka *et al*., 2000; Ng & Gurney, 2001). Alterations in endothelial control of vascular tone in pathological states necessarily involve Ca^{2+} fluxes in both types of cells.

Considering the importance of the mouse in genetic and molecular biological research, especially in the creation of animal models of cardiovascular diseases, we have begun to characterize Ca^{2+} regulation in the mouse aorta. In the course of these studies we found that in the mouse aorta, under conditions where NO synthesis is blocked by L-NAME, CPA elicited a large transient contraction, which was due to release of prostaglandin H2 and/or thromboxane A2 from the endothelium.

Methods

Tissue preparations

Male C57BL/6J mice from the Jackson Laboratory (U.S.A.), and the male CD1 mice from Animal Care Center of the University of British Columbia (Canada), were maintained under standard animal room conditions (12 h light – 12 h dark, 26°C, three per a cage, Purina chow-diet) for one week after arrival from the supplier. Mice were sacrificed at the weight of 20.6 ± 0.25 g for C57BL/6J strain and 28 ± 1.5 g for CD1 strain by halothane anaesthesia and exsanguination. Aortae were isolated and cleaned of connective tissue, fat, and blood with special care to preserve the endothelium.

Organ bath studies

Aortic rings were mounted isometrically in Small Vessel Myograph (Danish MyoTechnology A/S) and equilibrated for 1 h in oxygenated (5% CO_2, 95% O_2) Krebs' solution, which was changed at least three times. During equilibration the resting tension gradually increased to 3 mN per mm of ring width and was kept at this level for 30 min. The tissues were contracted two times with 80 mM K^+ buffer with an interval of 15 min between the contractions and treated further according to the specific protocols. All experiments were performed in the presence of 200 μM of N^{ω}-nitro-L-arginine methylester (L-NAME) to eliminate the effect of NO-induced relaxation.

In the experiments with denuded vessels, the endothelium was completely removed mechanically by gentle rotation of the vessels around a thin polished wooden stick. Denudation was documented by the absence of acetylcholine-induced relaxation after precontraction with 1 μM phenylephrine.

Pretreatment with L-NAME, indomethacin, SQ29548, nifedipine, SK&F 96365, DCB and 2APB lasted 30 min before CPA or U46619 addition. Effects of the compounds, dissolved in dimethylsulfoxide (indomethacin, SQ29548, nifedipine, DCB, 2APB, ryanodine) were controlled with the vehicle.

In the Ca^{2+}-replenishment protocol, the solution was replaced with Krebs' buffer, containing all the additions, needed according to the protocol, but without Ca^{2+}; tissues were incubated in Ca^{2+}-free buffer for 10 – 12 min, where after Ca^{2+}-containing Krebs' solution with all the additions required was returned.

Data analysis

Tension was recalculated per mm of ring width. Data are expressed as mean ± s.e.mean of n animals. The significance is presented according to Student's t-test (two-tailed). For dose response curves for phenylephrine and U46619 the compounds were added in cumulative manner. EC_{50} values for those compounds were determine for each curve using 3-parameter logistic fitting in SigmaPlot, thereafter mean Log EC_{50} were calculated as average for the group of animals. Responses to CPA ± indomethacin were measured for each CPA concentration separately per each ring. EC_{50} values and s.e.mean for CPA were determined from 3-parameter logistic fitting in SigmaPlot for the entire group of data for the amplitude of the transient peak and for the indomethacin resistant contraction.

Chemicals

Krebs' solution contained: (mM) NaCl 119; KCl 4.7; KH_2PO_4 1.18; $MgSO_4$ 1.17; $NaHCO_3$ 24.9; $CaCl_2$ 2.5; D-glucose 11; pH 7.4. 80 mM K^+ buffer contained: (mM) NaCl 44.9; KCl 78.82; KH_2PO_4 1.18; $MgSO_4$ 1.17; $NaHCO_3$ 24.9; $CaCl_2$ 2.5; D-glucose 11; pH 7.4.

Drugs were obtained from the following sources: acetylcholine, ATP, bradykinin, cyclopiazonic acid (CPA), diphenylboric acid 2-aminoethyl ester (2APB), indomethacin, N^{ω}-nitro-L-arginine methylester (L-NAME), nifedipine, phelylephrine, 9,11-dideoxy-11α,9α-epoxymethano-prostaglandin F(2α) (U46619), from Sigma; SQ29548 ([1S-[1a,2a(Z),3a, 4a]]-7-[3-[[2-(Phenylamino)carbonyl]hidrazino]methyl]-7-oxabicyclo[2.2.1]hept-2-yl]-5-heptenoic acid) and HA-1077 (1-(5-isoquinolinesulfonyl)-homopiperazine), from Sigma-RBI; dichlorobenzamyl hydrochloride (DCB) from Molecular Probes and SK&F 96365 hydrochloride ({1-[β-3-(4-methoxyphenyl)-propoxy]-4-methoxyphenethyl]-1H-imidazole, HCl}) from CALBIOCHEM.

Results

The principal results of this study are shown in Figure 1. In aortae of both the C57BL/6J and CD1 mice 10 μM CPA induced a large transient contraction, which was characterized by an initial 'foot' or a latent time period (Figure 1a). The amplitudes of the contractions induced by 10 μM CPA in the presence of 200 μM of L-NAME were 57 ± 11% of the maximal amplitude that could be developed by a maximally effective concentration of phenylephrine in the presence of L-NAME. Occasionally after the transient contractions, tonic contractions with much lower amplitude were observed. In Ca^{2+}-free buffer 10 μM CPA did not induce a contraction; force developed immediately after re-admission of the Ca^{2+} (Figure 1b). The transient contraction in the Ca^{2+} replenishment protocol had a lower amplitude and was never followed by a tonic contraction. Indomethacin, an inhibitor of cyclooxygenase (COX), completely abolished the transient contractions induced by 10 μM CPA. The average amplitudes of the peaks are presented in Figure 1c.

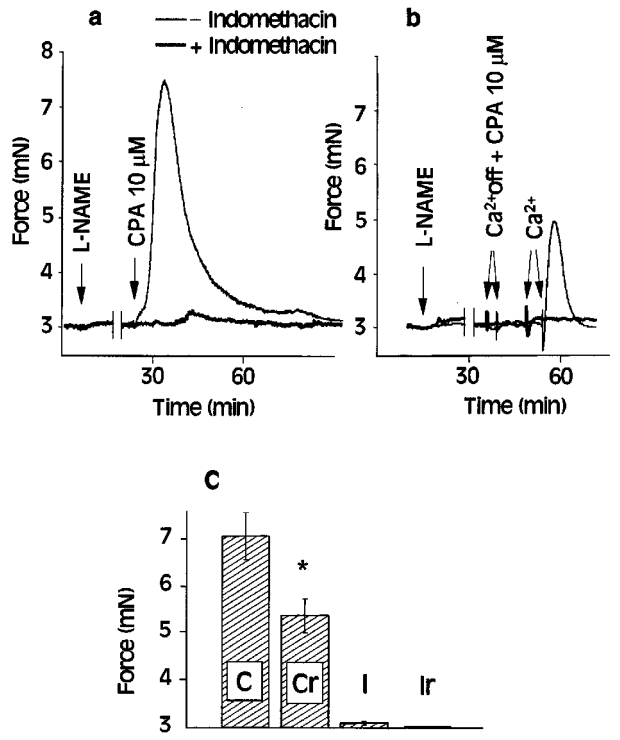

Figure 1 The effect of indomethacin (10 μM) on contraction, induced by 10 μM CPA, in C57BL/6J mouse aortae. (a) Individual traces of force development in Ca^{2+}-containing Krebs' buffer. (b) 10 μM CPA-induced contraction initiated by the readmission of Ca^{2+} after preincubation in Ca^{2+}-free solution with CPA. (c) Average amplitudes ($n=6$) of CPA-induced transient contractions and maximal tensions in the presence of indomethacin in the Ca^{2+}-contaning buffer and in the Ca^{2+} replenishment protocol. C, CPA-induced transient contractions; Cr, CPA-induced transient contractions after the readmission of Ca^{2+}; I, CPA-induced maximal tensions in the presence of indomethacin; Ir, CPA-induced maximal tensions in the presence of indomethacin after the readmission of Ca^{2+}. *Significantly different from the amplitude in the Ca^{2+}-containing buffer with $P<0.05$.

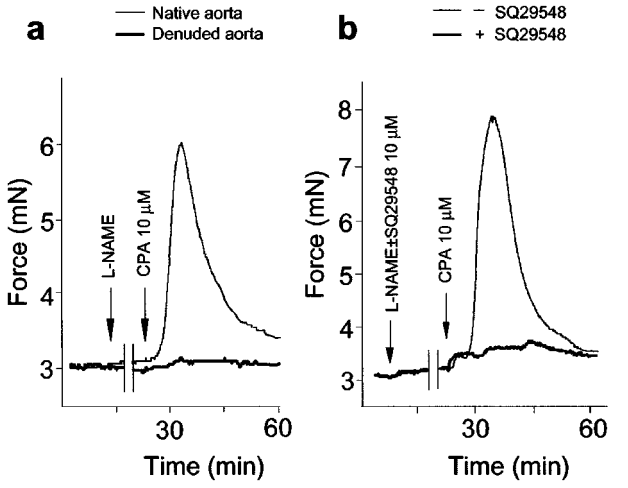

Figure 2 (a), 10 μM CPA-induced contraction in native and denuded aortae of C57BL/6J mice (representative of $n=6$). (b) Effect of SQ29548 (1 μM) on the 10 μM CPA-induced contraction of C57BL/6J mouse aorta (representative of $n=6$).

Table 1 Inhibition of the amplitude of the 10 μM CPA-induced transient contraction in C57BL/6J mice

Effector	*% of inhibition*	n	P
SQ29548 (1 μM)	96.07±0.62	5	<0.001
Nifedipine (1 μM)	33.77±5.72	5	<0.05
SK&F 96365 (10 μM)	99.23±0.76	5	<0.001
2APB (75 μM)	99.41±0.52	5	<0.001

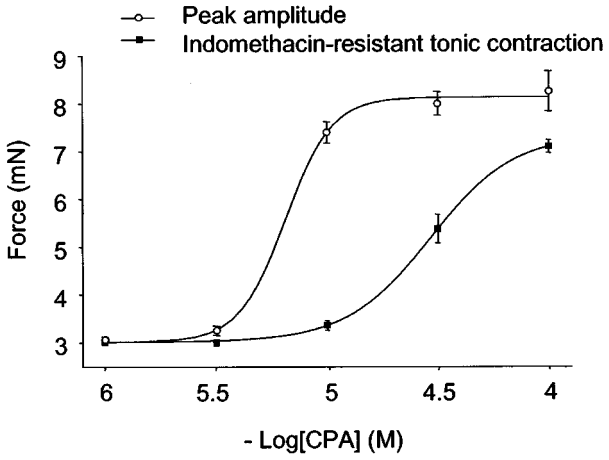

Figure 3 CPA concentration response curves for the peak amplitudes of transient contractions and the indomethacin-resistant tonic contractions of mouse aortae (CD1mice, 5–8 animals per point). The points were fitted with 3-parameter logistic curves.

These results suggest that CPA stimulates the production and release of prostaglandins (PGs), which initiate force development. The pertinent question is, which cells release which PGs? Figure 2a answers the first part of the question, in that denudation of the endothelium results in the loss of the CPA-induced transient, indicating a key role for ECs in prostaglandin synthesis. Figure 2b answers the second part of this question by showing that the PGH2/TXA2 receptor (TXR) antagonist SQ29548 abolishes the transient CPA-induced contraction (see also Table 1).

We thus conclude that in the mouse aorta blockade of SERCA in ECs stimulates the synthesis and release of PGH2 and/or TXA2, which leads to marked, but transient vasoconstriction. Higher concentrations of CPA induced PG-independent tonic contractions (in the presence of 10 μM indomethacin) due to direct activation of smooth muscle. The dose response curve for the CPA-activated smooth muscle tone lies well to the right of the dose response curve for the endothelium-dependent transient CPA response (Figure 3). Non-linear fitting for the amplitude of the transient contraction and for the maximum of the indomethacin-resistant contraction gives the estimations of the CPA EC_{50} values, 6.58±0.82 μM and 27.65±0.39 μM, respectively.

In the absence of L-NAME, the contractile response to blockade of endothelial SERCA by 10 μM CPA in mouse aorta is mostly balanced by the relaxing effect of NO production, so that the integral contractile peak is several times less than that seen in the presence of L-NAME. In the absence of L-NAME, lower concentrations of CPA (0.3 μM–

1.0 μM) induced pronounced relaxation developing over several minutes when added to the peak of a 1 μM PE-induced contraction in the mouse aorta. To the contrary, however, CPA induced contraction at higher concentrations (10 μM or more. Data not shown).

The initial steps in the chain of events leading to the transient contraction in response to 10 μM CPA are thought to be: the increase in EC $[Ca^{2+}]_i$ as a consequence of capacitative Ca^{2+} entry, resulted from SERCA blockade; the activation of phospholipase A2 (PLA2) and production of arachidonic acid (AA); transformation of AA into PGH2 and/or TXA2, and activation of TXR of smooth muscle cells (SMCs). In the subsequent part of this study we explored the sources of Ca^{2+}, which support transient CPA-induced contraction by blocking candidate Ca^{2+} channels. However in order to distinguish between effects of various inhibitors on endothelium and smooth muscle we also investigated contractions initiated by direct activation of the TXR. The synthetic TXA2 analogue U46619 was very potent in causing maintained contraction of the mouse aorta. Its EC_{50} is 40 times less than EC_{50} for the α-adrenergic agonist phenylephrine ($-$Log $EC_{50} = 8.777 \pm 0.156$ *vs* 7.176 ± 0.091, respectively; $EC_{50} = 1.93$ nM). The amplitude of maximal contraction achieved with 1 μM U46619 was 163.2% of that with 10 μM phenylephrine (8.860 ± 0.638 mN *vs* 5.430 ± 0.416 mN).

Blocking voltage gated Ca^{2+} channels (VGCC) with 1 μM nifedipine inhibited the transient 10 μM CPA-induced contraction by one-third (Table 1), while 10 μM SK&F 96365, which blocks both VGCC and receptor/store-operated channels (ROC/SOC), abolished practically all responses to CPA. On the other hand 10 nM U46619 still induced contractions in the presence of 10 μM SK&F 96365 as well as in Ca^{2+}-free solution, although their amplitudes were decreased by $59.84 \pm 4.20\%$ and $53.90 \pm 6.63\%$, respectively (Figure 4, curve 2, and Figure 5). One μM nifedipine caused approximately 30% inhibition of the U46619-induced contraction (data not shown). Figure 5 also shows that the Rho-kinase inhibitor HA-1077 (50 μM) blocks the Ca^{2+} insensitive portion of the U46619-induced contraction. These results suggest that Ca^{2+} entry through endothelial SOC is required for the release of PGH2/TXA2, while activation of the smooth muscle TXR induces contraction by Ca^{2+} influx through VGCC and ROC/SOC as well as by enhancement of myofilament Ca^{2+} sensitivity in a Rho-kinase dependent fashion.

The involvement of intracellular Ca^{2+} stores was investigated by blocking the SR Ca^{2+}-release channels. The inositol 1,4,5-trisphosphate receptor (IP_3R) blocker 2APB (75 μM) completely eliminated the 10 μM CPA-induced transient contraction (Table 1) and abolished the maintained phase of the contraction stimulated by 30 μM CPA (Figure 6). In contrast 2APB had only a minor effect on contractions in response to 10 nM of U46619 (Figure 4, curves 1 and curve 3 in comparison with curve 2). These results suggest that 2APB inhibits the opening of SOC of both endothelial and smooth muscle cells, but that the activation of IP_3R does not play a significant role in the response to direct activation of the TXR in smooth muscle.

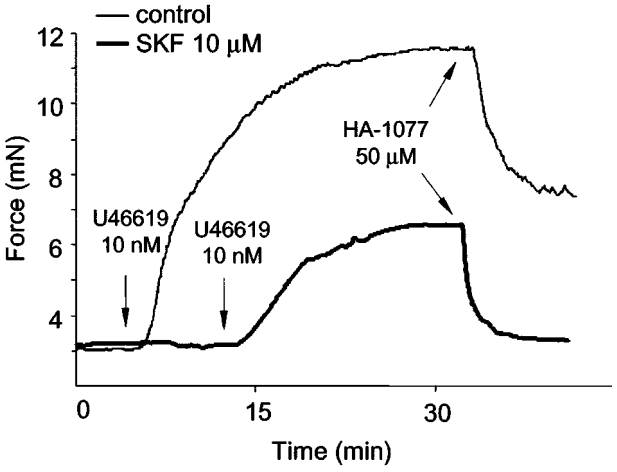

Figure 5 Effect of SKF (10 μM) and HA-1077 (50 μM) on 10 nM U46619-induced contractions of CD1 mouse aorta (representative of $n = 4$).

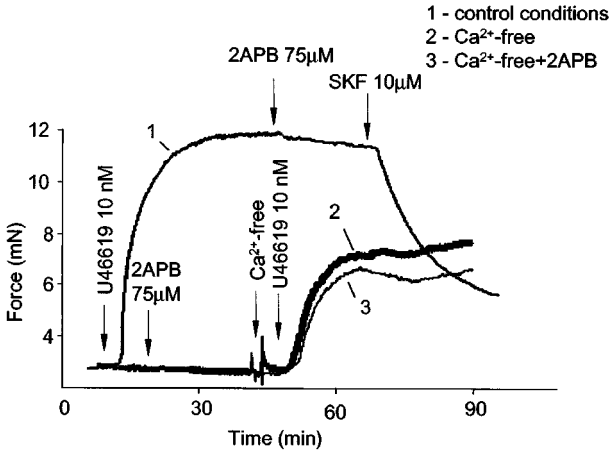

Figure 4 10 nM U46619-induced contractions of CD1 mouse aorta. Curve 1, Ca^{2+}-containing buffer; Curve 2, Ca^{2+}-free buffer; Curve 3, after preincubation with 75 M 2APB in Ca^{2+}-free buffer (representative of $n = 5$).

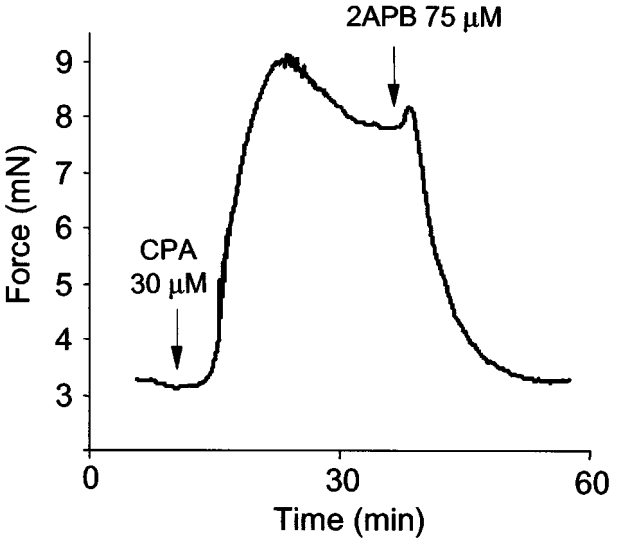

Figure 6 Effect of 75 μM 2APB on the force developed by CD1 mouse aorta in the presence of 30 μM CPA (representative of $n = 6$).

The results illustrated in Figure 7 demonstrate that ryanodine receptors do not play a role in the transient CPA-induced contraction. Pretreatment with 100 μM ryanodine, which releases Ca^{2+} from smooth muscle SR, did not affect the peak force development in response to 10 μM CPA.

Since the Na^+/Ca^{2+}-exchanger (NCX) has under different conditions been shown to contribute to smooth muscle contraction as well as relaxation (Blaustein & Lederer, 1999), we applied dichlorobenzamyl hydrochloride (DCB), a NCX blocker to test the contribution of the NCX in the transient CPA-induced contraction. NCX inhibition induced a gradual increase in resting tension and supported a high level of the residual tension and an indomethacin-resistant component after 10 μM CPA, but did not significantly affect peak tension (Figure 8). The residual tension and the indomethacin-resistant component in the presence of DCB were also abolished by 10 μM SK&F 96365.

Endothelial agonists, although less effective than CPA, also caused contractions of the mouse aorta dependent on the PG synthesis (Figure 9). Indomethacin-sensitive contractions induced by adenosine 5′-triphosphate (ATP) were approximately one-third of those induced by CPA. Bradykinin and acetylcholine (Ach) were considerably less effective than ATP and CPA in inducing contraction of the mouse aorta. In the absence of L-NAME ATP and Ach, but not bradykinin, induced relaxation of the aorta pre-contracted with phenylephrine (data not shown). Concentrations of ATP and Ach, which induced contractions, were an order of magnitude higher than those used to induce relaxation.

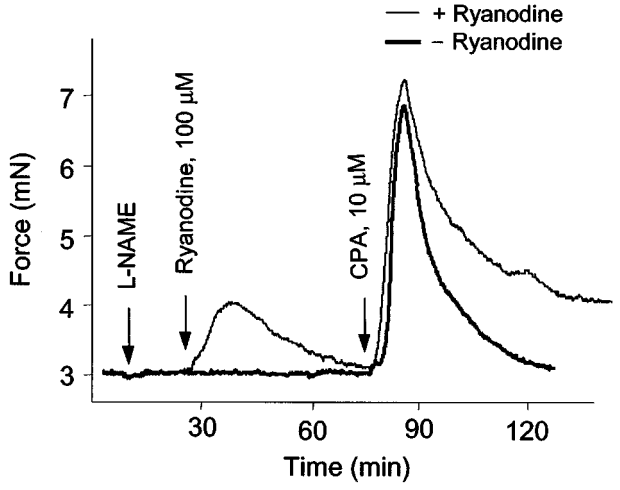

Figure 7 Effect of ryanodine (100 μM) on 10 μM CPA-induced contraction of C57BL/6J mouse aortae (representative of $n=3$).

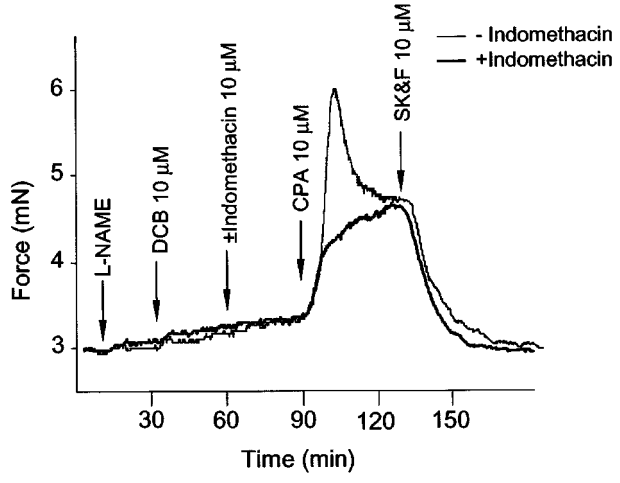

Figure 8 Effect of DCB (10 μM) on 10 μM CPA-induced contraction of C57BL/6J mouse aortae in the presence of indomethacin (10 μM) (representative of $n=6$).

Discussion

This study reports the novel finding that SERCA inhibition in the intact mouse aorta stimulates contraction due to release of an endothelium derived constricting agent. Although vasodilation initiated by SERCA inhibition is accepted as mediated by the endothelium (Zheng *et al*., 1993; Moritoki *et al*., 1994; Fukao *et al*., 1995; Rahimian *et al*., 1997), contraction is commonly interpreted to result from Ca^{2+} release from smooth muscle SR and activation of SOC (Tosun *et al*., 1998a; Tanaka *et al*., 2000; Ng & Gurney, 2001). In the present study, however, blockade of the transient CPA-induced contraction by either endothelial denudation, application of indomethacin or SQ29548, a specific blocker of TXR, suggested an indirect contractile effect due to endothelial synthesis and release of PGH2/TXA2. In addition the abolition of the transient CPA contraction, but not the U46619-induced contraction by Ca^{2+} removal, showed that capacitative Ca^{2+} entry in endothelial cells was required for release of the contracting factor(s). This conclusion is further supported by the finding, that blocking the IP_3R with 2APB abolished the CPA contraction, but had a little effect on force development in response to direct activation of the TXR.

The deduced signalling mechanisms leading to the CPA-induced transient contraction are illustrated in Figure 10. Ten μM CPA inhibits the endothelial SERCA, which has been reported to be of the SERCA3 type (Ozog *et al*., 1998; Paul, 1998; Khan *et al*., 2000; Mountian *et al*., 2001), and depletes

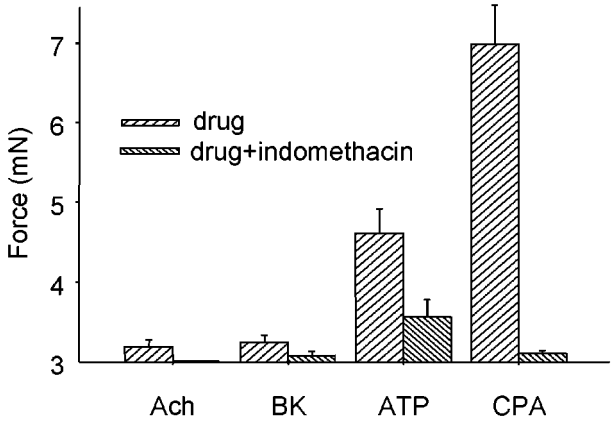

Figure 9 Endothelial agonist-induced contraction of C57BL/6J mouse aorta in comparison with 10 μM CPA-induced contraction in the presence and the absence of 10 μM indomethacin. Acetylcholine (Ach) 1 μM ($n=4$); bradykinin (BK) 100 nM ($n=5$); adenosine 5′-triphosphate (ATP) 300 μM ($n=6$).

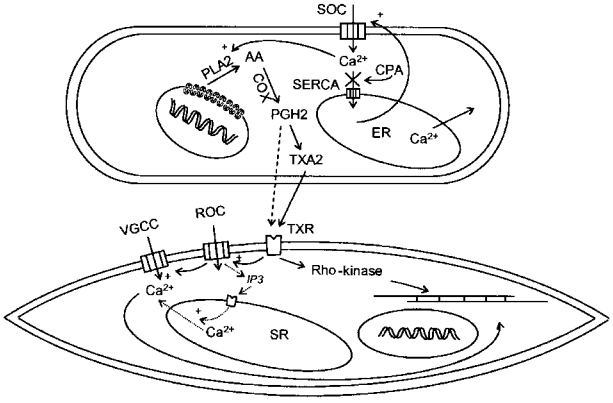

Figure 10 Paracrine effect of arachidonic acid (AA) metabolites in mouse aorta. Sequence of events: (1) CPA binds to SERCA in endothelial cells (ECs) and holds it open depleting the endoplasmic reticulum (ER); (2) depletion of ER opens SOC and induces Ca^{2+} entry into ECs; (3) $[Ca^{2+}]_i$ activates phospholipase A2 (PLA2) leading to AA production; (4) AA is transformed by cyclo-oxygenase (COX) into prostaglandin H2, possibly followed by its transformation into thromboxane A2; (5) PGH2/TXA2 activate the PGH2/TXA2 receptors (TXR) of smooth muscle cells thereby initiating signal transduction cascades; (6) TXR activation induces Ca^{2+} entry into the smooth muscle cells through ROC and VGCC with minor contribution of IP3 pathway; (7) elevated $[Ca^{2+}]_i$ induces contraction of SMCs; (8) TXR activation induces myofilament sensitization to Ca^{2+}.

the endoplasmic reticulum (ER). The depleted ER signals, in a yet to be defined way, the opening of SOC in the endothelial plasma membrane (Oike *et al*., 1994; Zhang *et al*., 1994; Pasyk *et al*., 1995) and the resulting Ca^{2+} influx activates PLA2 (Millanvoye-Van Brussel *et al*., 1999; Sa *et al*., 1995; Kan *et al*., 1996; Channon & Leslie, 1990). Arachidonic acid is converted by COX to PGH2 and an undetermined fraction of this is converted by TXA2-synthase to TXA2 (Fitzpatrick & Soberman, 2001; Hamberg *et al*., 1975; Mullane *et al*., 1979). PGH2 and/or TXA2 activate the TXR on the smooth muscle (Auch-Schwelk *et al*., 1990; Taddei & Vanhoutte, 1993; Shimizu *et al*., 1993; Ge *et al*., 1995) to initiate contraction *via* stimulation of three pathways: VGCC, ROC and Rho-kinase mediated sensitization of the myofilaments.

CPA also inhibits SERCA in the smooth muscle SR, which belongs predominantly to the type 2b isoform (Wu *et al*., 2001), but the contractile consequences of this action appear only at higher concentrations. We have recently shown that the concentration of CPA required to maximally block refilling of vascular smooth muscle SR is 30 μM (Szado *et al*., 2001). At this concentration CPA-induced tonic contractions are dependent on Ca^{2+} entry through SOC, which is supported by the fact that this contraction is inhibited by SKF 96365 and 2APB (Figure 6). We thus conclude that at the intermediate concentration of 10 μM CPA endothelial ER depletion was sufficient to activate the SOC (Sasajima *et al*., 1997), while SR of the smooth muscle was not depleted sufficiently to activate SOC in these cells. The higher concentration of CPA needed to block SERCA of SMCs than SERCA of ECs could result from different isoforms of the enzyme in these two types of cells. SERCA3 isoform is shown to be predominant in endothelium of murine arteries (Ozog *et al*., 1998; Paul, 1998; Khan *et al*., 2000; Mountian *et al*., 2001), while SERCA2b is expressed in SMCs (Magnier *et al*., 1992; Papp *et al*., 1993; Khan *et al*., 2000; Wu *et al*., 2001). Of considerable additional interest is the observation that SOC were blocked by 2APB in both tissues even when depletion was not mediated *via* opening of IP_3R. This could be due to prevention of the interaction of IP_3R with SOC, which is thought to be essential for SOC activation (van Rossum *et al*., 2000; Ma *et al*., 2000) or to direct blockade of SOC by 2APB as was observed in hepatocytes (Gregory *et al*., 2001). Our observation that the tonic contraction induced by U46619 was resistant to 2APB, suggests that TXR activation opens a ROC rather than the SOC. However, both types of channels were blocked by SK&F 96365. Ca^{2+}-influx during the endothelium dependent, 10 μM CPA-induced contraction is mediated partly through VGCC (1/3 of the amplitude of 10 μM CPA-induced contraction was blocked by nifedipine) as well as by ROC. The remaining 40–50% of the contraction was due to myofilament sensitization to Ca^{2+} by Rho-kinase phosphorylation of myosin light chain phosphatase. This is in agreement with a previous observation (Tosun *et al*., 1998b), where the contractions, induced by U46619 in rat aorta were shown to be supported by calcium entering through L-type and non-L-type Ca^{2+} channels combined with an increase in Ca^{2+} sensitivity. The involvement of Rho-kinase in U46619-induced vasoconstriction has recently been reported (Batchelor *et al*., 2001; Nobe & Paul, 2001; Sakurada *et al*., 2001).

Dependence of PG synthesis solely on extracellular Ca^{2+} is in agreement with the work of Millanvoye-Van Brussel *et al*. (1999) who showed that in the absence of Ca^{2+} influx endothelial arachidonic acid release was suppressed despite the marked elevation of $[Ca^{2+}]_i$ in both histamine- and thapsigargin-stimulated cells. The dependence of the PG synthesis solely on Ca^{2+} influx also can explain the several minutes' delay in the development of the contraction in Ca^{2+}-containing buffer in our experiment. This time may be needed for depletion of ER and opening of SOC as it was shown for rabbit aortic endothelial cells (Sasajima *et al*., 1997). In the Ca^{2+}-replenishment protocol, the delay after Ca^{2+} readmission is absent (Figure 1B) because depletion was completed during incubation of the tissue in Ca^{2+}-free buffer. Immediate elevation of $[Ca^{2+}]_i$ has been shown previously in thapsigargin-treated human umbilical vein endothelial cells after reapplication of extracellular Ca^{2+} (Oike *et al*., 1994).

The transient nature of the 10 μM CPA-induced contraction could be due to self-inactivation of TXA2 synthase (Fitzpatrick & Soberman, 2001; Shram *et al*., 1999) and PGH2-1 and -2 synthase (Barnett *et al*., 1994; Shi *et al*., 2000; Wu *et al*., 1999). Alternatively, the Ca^{2+} peak itself could be transient, as this was shown for another SERCA blocker thapsigargin in the Chinese hamster ovary (Lund *et al*., 2000).

Ryanodine receptors did not appear to participate in the capacitative Ca^{2+} entry in ECs, as the 10 μM CPA-induced transient contraction was not affected by a concentration of ryanodine, which either locks the release channel in a subconductance state or blocks it (Figure 7). This is in agreement with an earlier observation that partial depletion of the EC Ca^{2+} store in the presence of ryanodine does not activate SOC in ECs, while a similar degree of depletion in the presence of CPA does activate SOC (Sasajima *et al*., 1997).

In several smooth muscle preparations it has been shown that the NCX contributes to agonist-initiated Ca^{2+} entry especially where SOC mediate Na^{+} influx (Blaustein & Lederer, 1999; Lee *et al*., 2001). We investigated this possibility by blocking NCX with DCB. In contrast with the rabbit vena cava smooth muscle, where DCB inhibits the phenylephrine-induced contraction (Lee *et al*., 2001), it had no effect on the transient component, but promoted a slow increase in force development and increased the indomethacin-resistant component of 10 μM CPA concentrations (Figure 8). From these data we conclude that in the mouse aorta ROC is responsible for Ca^{2+} influx, while the NCX functions in the forward mode to remove Ca^{2+} from the cytoplasm.

Under physiological conditions the equilibrium between contractile and relaxing responses to EC receptor activation favours relaxation. In the presence of lower concentrations of CPA (<10 μM) capacitative Ca^{2+} entry in mouse aorta results in a dilating effect, while higher concentrations of CPA ($\geqslant 10$ μM) result in a contractile response to the capacitative Ca^{2+} entry. This difference may be due to Ca^{2+} penetrating deeper into the cytoplasm in the absence of SERCA-mediated Ca^{2+} sequestration resulting in increased activation of PLA2 associated with internal membranes (Kan *et al*., 1996).

Receptor activation of endothelial cells also caused contraction of mouse aorta (Figure 9), which was dependent on PG synthesis. Higher concentrations of agonists were required to induce contractions than relaxation. The differences observed between the effects of agonists and CPA may be related to different patterns of Ca^{2+} release, entry and distribution.

The clinical relevance of this study may lie in the general observation that in vascular disease the balance of endothelial modulation of the underlying smooth muscle shifts from relaxation towards contraction. SERCA activity could be depressed in anoxia, which causes endothelium- and PG synthesis-dependent contractions in a variety of isolated arteries and veins (Vanhoutte, 1987; Lin *et al*., 1991; Hoshino *et al*., 1994). Atherosclerosis with its transformed endothelium is characterized by decreased NO bioavailability (Kurowska, 2002) and increased superoxide formation (Hathaway *et al*., 2002). Increased production of TXA2 itself down-regulates NO accumulation in vascular smooth muscle (Shiokoshi *et al*., 2002). Diabetes is another disease with impaired NO secretion favouring the production of PGs, especially PGH2/TXA2 (Laight *et al*., 1999; Napoli & Franco, 1998; Hishinuma *et al*., 1999; Lasserre *et al*., 2000; Tesfamariam *et al*., 1989). In the diabetic conditions, when NO is quenched by superoxide anion (Pieper, 1998) and Ca^{2+} sequestration by ER might be impaired (Kim *et al*., 2001), the contractile influence of the endothelium could participate in hypertension and accelerated development of atherosclerosis. Endothelium dependent smooth muscle activation through PGH2/TXA2 receptors could also contribute to vasospasm in the coronary (Zou & Bachschmid, 1999) and cerebral arteries (Tanaka *et al*., 2001; Takeuchi *et al*., 1999) as well as that seen in arterial bypass graft complications (He & Yang, 1999; Houppe *et al*., 1983). In support, a protective role of TXR antagonist was shown in myocardial infarction (Gurbel *et al*., 1999; Singh *et al*., 1997; Takase *et al*., 1996), which is known for its decreased NO bioavailability (Wiemer *et al*., 2001). Besides inducing vascular contraction, thromboxane, a classic platelet agonist, is secreted by the endothelium in the lumen where it participates in blood coagulation and development of atherosclerosis.

This work was supported by Vancouver Vascular Biology Research Center and grant 20R91189 from the Canadian Institute of Health Research.

References

AUCH-SCHWELK, W., KATUSIC, Z.C. & VANHOUTTE, P.M. (1990). Thromboxane A2 receptor antagonists inhibit endothelium-dependent contractions. *Hypertension,* **15,** 699 – 703.

BARNETT, J., CHOW, J., IVES, D., CHIOU, M., MACKENZIE, R., OSEN, E., NGUYEN, B., TSING, S., BACH, C., FREIRE, J. *et al.* (1994). Purification, characterization and selective inhibition of human prostaglandin G/H synthase 1 and 2 expressed in the baculovirus system. *Biochim. Biophys. Acta,* **1209,** 130 – 139.

BATCHELOR, T.J., SADABA, J.R., ISHOLA, A., PACAUD, P., MUNSCH, C.M. & BEECH, D.J. (2001). Rho-kinase inhibitors prevent agonist-induced vasospasm in human internal mammary artery. *Br. J. Pharmacol.,* **132,** 302 – 308.

BLAUSTEIN, M.P. & LEDERER, W.J. (1999). Sodium/calcium exchange: its phisiological implication. *Physiological reviews,* **79,** 763 – 854.

CHANNON, J.Y. & LESLIE, C.C. (1990). A calcium-dependent mechanism for associating a soluble arachidonoyl-hydrolyzing phospholipase A2 with membrane in the macrophage cell line RAW 264.7. *J. Biol. Chem.,* **265,** 5409 – 5413.

DE NUCCI, G., GRYGLEWSKI, R.J., WARNER, T.D. & VANE, J.R. (1988). Receptor-mediated release of endothelium-derived relaxing factor and prostacyclin from bovine aortic endothelial cells is coupled. *Proc. Natl. Acad. Sci., U.S.A.,* **85,** 2334 – 2338.

FITZPATRICK, F.A. & SOBERMAN, R. (2001). Regulated formation of eicosanoids. *J. Clin. Invest.,* **107,** 1347 – 1351.

FUKAO, M., HATTORI, Y., KANNO, M., SAKUMA, I. & KITABATAKE, A. (1995). Thapsigargin- and cyclopiazonic acid-induced endothelium-dependent hyperpolarization in rat mesenteric artery. *Br. J. Pharmacol.,* **115,** 987 – 992.

FURCHGOTT, R.F. & VANHOUTTE, P.M. (1989). Endothelium-derived relaxing and contracting factors. *FASEB J.,* **3,** 2007 – 2018.

FURCHGOTT, R.F. & ZAWADZKI, J.V. (1980). The obligatory role of endothelial cells in the relaxation of arterial smooth muscle by acetylcholine. *Nature,* **288,** 373 – 376.

GE, T., HUDHES, H., JUNQUERO, D.C., WU, K.K., VANHOUTTE, P.M. & BOULANGER, C.M. (1995). Endothelium-dependent contractions are associated with both augmented expression of prostaglandin H syntase-1 and hypersensitivity to prostaglandin H2 in the SHR aorta. *Circ. Res.,* **76,** 1003 – 1010.

GREGORY, R.B., RYCHKOV, G. & BARRITT, G.J. (2001). Evidence that 2-aminoethyl diphenylborate is a novel inhibitor of store-operated Ca^{2+} channels in liver cells, and acts through a mechanism which does not involve inositol triphosphate receptors. *Biochem. J.,* **354,** 285 – 290.

GURBEL, P.A., MURUGESAN, S.R., LOWRY, D.R. & SEREBRUANY, V.L. (1999). Plasma thromboxane and prostacyclin are linear related and increased in patients presenting with acute myocardial infarction. *Prostaglandins Leukot. Essent. Fatty Acids,* **61,** 7 – 11.

HAMBERG, M., SVENSSON, J. & SAMUELSSON, B. (1975). Thromboxanes: a new group of biologically active compounds derived from prostaglandin endoperoxides. *Proc. Natl. Acad. Sci. U.S.A.,* **72,** 2994 – 2998.

HATHAWAY, C.A., HEISTAD, D.D., PIEGORS, D.J. & MILLER, F.J. (2002). Regression of atherosclerosis in monkeys reduces vascular superoxide levels. *Circ. Res.,* **90,** 277 – 283.

HE, G.W. & YANG, C.Q. (1999). Inhibition of vasoconstriction by the thromboxane A2 antagonist GR32191B in the human radial artery. *Br. J .Clin. Pharmacol.,* **48,** 207 – 215.

HISHINUMA, T., KOSEKI, Y., MURAI, Y., YAMAZAKI, T., SUZUKI, K. & MIZUGAKI, M. (1999). Urinary thromboxane A2/prostacyclin balance reflects the pathological state of a diabetics. *Prostaglandins Other Lipid Mediat.,* **58,** 263 – 271.

HOSHINO, Y., MORRISON, K.J. & VANHOUTTE, P.M. (1994). Mechanisms of hypoxic vasoconstriction in the canine isolated pulmonary artery: role of endothelium and sodium pump. *Am. J. Physiol.,* **267,** L120 – L127.

HOUPPE, J.P., VILLEMOT, J.P., AMREIN, D., LABOUREL, L., CLAVEY, M. & MATHIEU, P. (1983). Early coronary spasm after myocardial revascularization surgery. *Presse Med.,* **12,** 2667 – 2670.

KAN, H., RUAN, Y. & MALIK, K.U. (1996). Involvement of mitogen-activated protein kinase and translocation of cytosolic phospholipase A2 to the nuclear envelope in acetylcholine-induced prostacyclin synthesis in rabbit coronary endothelial cells. *Mol. Pharmacol.,* **50,** 1139 – 1147.

KHAN, I., SANDHU, V., MISQUITTA, C.M. & GROVER, A.K. (2000). SERCA pump isoform expression in endothelium of vein and arteries: every endothelium is not the same. *Mol. Cell. Biochem.,* **203,** 11 – 15.

KIM, H.W., CH, Y.S., LEE, H.R., PARK, S.Y. & KIM, Y.H. (2001). Diabetic alterations in cardiac sarcoplasmic reticulum Ca^{2+}-ATPase and phospholamban protein expression. *Life Sci.,* **70,** 367 – 379.

KUROWSKA, E.M. (2002). Nitric oxide therapies in vascular diseases. *Curr. Pharm. Des.,* **8,** 155 – 166.

LAIGHT, D.W., CARRIER, M.J. & ÅNGGÅRD, E.E. (1999). Endothelial cell dysfunction and pathogenesis of diabetic macroangiopathy. *Diabet. Metab. Res. Rev.,* **15,** 274 – 282.

LASSERRE, B., NAVARRO-DELMASURE, C., PHAM HUU CHANH, A., CATALA, J. & HOLLANDE, E. (2000). Modifications in the TXA(2) and PGI(2) plasma levels and some other biochemical parameters during the initiation and development of non-insulin-dependent diabetes mellitus (NIDDM) syndrome in the rabbit. *Prostaglandins Leukot. Essent. Fatty Acids,* **62,** 285 – 291.

LEE, C.-H., POBURKO, D., SAHOTA, P., SANDHU, J., RUEHLMANN, D. & VAN BREEMEN, C. (2001). The mechanism of phenylephrine-mediated $[Ca^{2+}]_i$ oscillations underlying tonic contraction in the rabbit inferior vena cava. *J. Physiol.,* **534,** 641 – 650.

LIN, P.J., PEARSON, P.J. & SCHAFF, H.V. (1991). Endothelium-dependent contraction and relaxation of human and canine internal mammary artery: Study on bypass graft vasospasm. *Surgery,* **110,** 127 – 135.

LUND, P.-E., SHARIATMADARI, R., UUSTARE, A., DETHEUX, M., PARMENTIER, M., KUKKONEN, J.P. & ÅKERMAN, K.E.O. (2000). The Orexin OX1 Receptor Activates a Novel Ca^{2+} Influx Pathway Necessary for Coupling to Phospholipase C. *J. Biol. Chem.,* **275,** 30806 – 30812.

MA, H.-T., PATTERSON, R.L., VAN ROSSUM, D.B., BIRNBAUMER, L., MIKOSHIBA, K. & GILL, D.L. (2000). Requirement of the inositol triphosphate receptor for activation of store-operated Ca^{2+} channels. *Science,* **287,** 1647 – 1651.

MAGNIER, C., PAPP, B., CORVAZIER, E., BREDOUX, R., WUYTACK, F., EGGERMONT, J., MASLOUF, J. & ENOUF, J. (1992). Regulation of sarco-endoplasmic reticulum Ca^{2+}-ATPases during platelet-derived growth factor-induced smooth muscle cell proliferation. *J. Biol. Chem.,* **267,** 15808 – 15815.

MILLANVOYE-VAN BRUSSEL, E., DAVID-DUFILHO, M., PHAM, T.D., IOUZALEN, L. & DEVYNCK, M.A. (1999). Regulation of arachidonic acid release by calcium influx in human endothelial cells. *J. Vasc. Res.,* **36,** 235 – 244.

MONCADA, S. & VANE, J.R. (1979). The role of prostacyclin in vascular tissue. *Fed. Proc.,* **38,** 66 – 71.

MORITOKI, H., HISAYAMA, T., TAKEUCHI, S., KONDOH, W. & IMAGAWA, M. (1994). Relaxation of rat thoracic aorta induced by the Ca^{2+}-ATPase inhibitor, cyclopiazonic acid, possibly through nitric oxide formation. *Br. J. Pharmacol.,* **111,** 655 – 642.

MOUNTIAN, I.I., BABA-AISSA, F., JONAS, J.C., DE SMEDT, H., WUYTACK, F. & PARYS, J.B. (2001). Expression of Ca^{2+} Transport Genes in Platelets and Endothelial Cells in Hypertension. *Hypertension,* **37,** 135 – 141.

MULLANE, K.M., DUSTING, G.J., SALMON, J.A., MONCADA, S. & VANE, J.R. (1979). Biotransformation and cardiovascular effects of arachidonic acid in the dog. *Eur. J. Pharmacol.,* **54,** 217 – 228.

NAPOLI, E. & FRANCO, N. (1998). Thromboxane A2 production in the pathogenesis of diabetic complications. *Minerva Endocrinol.,* **23,** I – 5.

NG, L.C. & GURNEY, A.M. (2001). Store-operated channels mediate Ca^{2+} influx and contraction in rat pulmonary artery. *Circ. Res.,* **89,** 923 – 929.

NOBE, K. & PAUL, R.J. (2001). Distinct pathways of Ca^{2+} sensitization in porcine coronary artery: effects of Rho-related kinase and protein kinase C inhibition on force and intracellular Ca^{2+}. Circ. Res., **88,** 1283 – 1290.

OIKE, M., GERICKE, M., DROOGMANS, G. & NILIUS, B. (1994). Calcium entry activated by store depletion in human umbilical vein endothelial cells. *Cell Calcium,* **16,** 367 – 376.

OZOG, A., POUZET, B., BOBE, R. & LOMPRE, A.M. (1998). Characterization of the 3′ end of the mouse SERCA 3 gene and tissue distribution of mRNA spliced variants. *FEBS Lett.,* **427,** 349 – 352.

PAPP, B., CORVAZIER, E., MAGNIER, C., KOVACS, T., BOURDEAU, N., LEVY-TOLEDANO, S., BREDOUX, R., LEVY, B., POITEVIN, P., LOMPRE, A.M. *et al.* (1993). Spontaneously hypertensive rats and platelet Ca^{2+}-ATPases: specific up-regulation of the 97 kDa isoform. *Biochem. J.,* **295,** 685 – 690.

PASYK, E., INAZU, M. & DANIEL, EE. (1995). CPA enhances Ca^{2+} entry in cultured bovine pulmonary arterial endothelial cells in an IP_3-independent manner. *Am. J. Physiol.,* **268,** H138 – H146.

PAUL, R.J. (1998). The role of phospholamban and SERCA3 in regulation of smooth muscle-endothelial cell signalling mechanisms: evidence from gene-ablated mice. *Acta Physiol. Scand.,* **164,** 589 – 597.

PIEPER, G. (1998). Review of alteration in endothelial nitric oxide production in diabetes. *Hypertension,* **31,** 1047 – 1060.

RAHIMIAN, R., VAN BREEMEN, C., KARKAN, D., DUBE, G. & LAHER, I. (1997). Estrogen augments cyclopiazonic acid-mediated, endothelium-dependent vasodilation. *Eur. J. Pharmacol.,* **327,** 143 – 149.

SA, G., MURUGESAN, G., JAYE, M., IVASHENKO, Y. & FOX, P.L. (1995). Activation of cytosolic phospholipase A2 by basic fibroblast growth factor via a p42 mitogen-activated protein kinase-dependent phosphorylation pathway in endothelial cells. *J. Biol. Chem.,* **270,** 2360 – 2366.

SAKURADA, S., OKAMOTO, H., TAKUWA, N., SUGIMOTO, N. & TAKUWA, Y. (2001). Rho activation in excitatory agonist-stimulated vascular smooth muscle. *Am. J. Physiol. Cell Physiol.,* **281,** C571 – C578.

SASAJIMA, H., WANG, X. & VAN BREEMEN, C. (1997). Fractional Ca2+ release from the endoplasmic reticulum activates Ca^{2+} entry in freshly isolated rabbit aortic endothelial cells. *Biochem. Biophys. Res. Commun.,* **241,** 471 – 475.

SHI, W., HOGANSON, C.W., ESPE, M., BENDER, C.J., BABCOCK, G.T., PALMER, G., KULMACZ, R.J. & TSAI, A. (2000). Electron paramagnetic resonance and electron nuclear double resonance spectroscopic identification and characterization of the tyrosyl radicals in prostaglandin H synthase 1. *Biochemistry,* **39,** 4112 – 4121.

SHIMIZU, K., MURAMATSU, M., KAKEGAWA, Y., ASANO, H., TOKI, Y., MIYAZAKI, Y., OKUMURA, K., HASHIMOTO, H. & ITO, T. (1993). Role of prostaglandin H2 as an endothelium-derived contracting factor in diabetic state. *Diabetes,* **42,** 1246 – 1252.

SHIOKOSHI, T., OHSAKI, Y., KAWABE, J., FUJINO, T. & KIKUCHI, K. (2002). Downregulation of nitric oxide accumulation by cyclooxygenase-2 induction and thromboxane A2 production in interleukin-1beta-stimulated rat aortic smooth muscle cells. *J. Hypertens.,* **20,** 455 – 461.

SHRAM, S.I., LAZURKINA, T.YU., MYASOEDOV, N.F. & VARFOLOMEEV, S.D. (1999). Enzyme self-activation is a main limitation of the preparation of eicosanoids. Enzymatic synthesis of thromboxane and 12(S)-hydroxytetraenoic acid. *Appl. Biochem. Biotechnol.,* **82,** 37–56.

SINGH, J., SETH, S.D., MANCHANDA, S.C. & SETH, S. (1997). Protective actions of a thromboxane receptor antagonist, SQ 29548 on the ischemic myocardium: morphologic and hemodynamic effects. *Prostaglandins Leukot. Essent. Fatty Acids,* **56,** 105–110.

SZADO, T., MCLARNON, M., WANG, X. & VAN BREEMEN, C. (2001). Role of sarcoplasmic reticulum in regulation of tonic contraction of rabbit basilar artery. *Am. J. Physiol. Heart Circ. Physiol.,* **281,** H1481–1489.

TADDEI, S. & VANHOUTTE, P.M. (1993). Endothelium-dependent contractions to endothelin in the rat aorta are mediated by thromboxane A2. *J. Cardiovasc. Pharmacol.,* **22,** S328–S331.

TAKASE, B., MARUYAMA, T., KURITA, A., UEHATA, A., NISHIOKA, T., MIZUNO, K., NAKAMURA, H., KATSURA, K. & KANDA, Y. (1996). Arachidonic acid metabolites in acute myocardial infarction. *Angiology,* **47,** 649–661.

TAKEUCHI, H., TANABE, M., OKAMOTO, H. & YAMAZAKI, M. (1999). Effects of thromboxane synthetase inhibitor (RS-5186) on experimentally-induced cerebral vasospasm. *Neurol. Res.,* **21,** 513–516.

TANAKA, Y., IMAI, T., IGARASHI, T., TAKAYANAGI, K., OTSUKA, K., YAMAKI, F., TANAKA, H. & SHIGENOBU, K. (2000). Comparison of the Ca^{2+} entry channels responsible for mechanical responses of guinea-pig aorta to noradrenaline and thapsigargin using SK&F 96365 and LOE 908. *Naunyn Schmiedebergs Arch. Pharmacol.,* **362,** 160–168.

TANAKA, Y., SAITO, M. & YAMADA, T. (2001). Inhibitory effects of verapamil and nitroglycerin on contraction and cytosolic Ca^{2+} levels in cerebrovascular smooth muscle during chronic cerebral vasospasm. *Neurol. Med. Chir. (Tokyo),* **41,** 238–244.

TESFAMARIAM, B., JAKUBOWSKI, J.A. & COHEN, R.A. (1989). Contraction of diabetic rabbit aorta caused by endothelium-derived PGH2-TXA2. *Am. J. Physiol.,* **257,** HI327–H1333.

TOSUN, M., PAUL, R.J. & RAPOPORT, R.M. (1998a). Coupling of store-operated Ca^{2+} entry to contraction in rat aorta. *J. Pharmacol. Exp. Ther.,* **285,** 759–766.

TOSUN, M., PAUL, R.J. & RAPOPORT, R.M. (1998b). Role of extracellular Ca^{2+} influx via L-type and non-L-type Ca++ channels in thromboxane A2 receptor-mediated contraction in rat aorta. *J. Pharmacol. Exp. Ther.,* **284,** 921–928.

VANHOUTTE, P.M. (1987). Endothelium-dependent contractions in arteries and veins. *Blood Vessels,* **24,** 141–144.

VAN ROSSUM, D.B., PATTERSON, R.L., MA, H.T. & GILL, D.L. (2000). Ca^{2+} entry mediated by store depletion, S-nitrosylation, and TRP3 channels. Comparison of coupling and function. *J. Biol. Chem.,* **275,** 28562–28568.

WIEMER, G., ITTER, G., MALINSKI, T., & LINZ, W. (2001). Decreased nitric oxide availability in normotensive and hypertensive rats with failing hearts after myocardial infarction. *Hypertension,* **38,** 1367–1371.

WU, K.-D., BUNGARD, D. & LYTTON, J. (2001). Regulation of SERCA Ca^{2+} pump expression by cytoplasmic $[Ca^{2+}]_i$ in vascular smooth muscle cells. *Am. J. Physiol. Cell Physiol.,* **280,** C843–C851.

WU, G., WEI, C., KULMACZ, R.J., OSAWA, Y. & TSAI, A.L. (1999). A mechanistic study of self-inactivation of the peroxidase activity in prostaglandin H synthase-1. *J. Biol. Chem.,* **274,** 9231–9237.

ZHANG, H., INAZU, M., WEIR, B., BUCHANAN, M. & DANIEL, E. (1994). Cyclopiazonic acid stimulates Ca^{2+} influx through non-specific cation channels in endothelial cells. *Eur. J. Pharmacol.,* **251,** 119–125.

ZHENG, X.F., GUAN, Y.Y. & KWAN, C.Y. (1993). Cyclopiazonic acid causes endothelium-dependent relaxation in rat aorta. *Zhongguo Yao Li Xue Bao,* **14,** 21–26.

ZOU, M.H. & BACHSCHMID, M. (1999). Hypoxia-reoxygenation triggers coronary vasospasm in isolated bovine coronary arteries via tyrosine nitration of prostacyclin synthase. *J. Exp. Med.,* **190,** 135–139.

(Received June 13, 2002
Accepted July 22, 2002)