JCMM
Available online at
www.jcmm.ro
www.jcmm.org

*J. Cell. Mol. Med. Vol 10, No 2, 2006 pp. 407-422*

# Vascular physiology of a $Ca^{2+}$ mobilizing second messenger - cyclic ADP - ribose

**Andrew Y. Zhang, Pin - Lan Li ***

*Department of Pharmacology & Toxicology, Medical College of Virginia, Virginia Commonwealth University, VA, USA*

*Received: March 10, 2006; Accepted: April 3, 2006*



## Abstract

Cyclic ADP-ribose (cADPR) is a novel $Ca^{2+}$ mobilizing second messenger, which is capable of inducing $Ca^{2+}$ release from the sarcoplasmic reticulum (SR) *via* activation of ryanodine receptors (RyR) in vascular cells. This signaling nucleotide has also been reported to participate in generation or modulation of intracellular $Ca^{2+}$ sparks, $Ca^{2+}$ waves or oscillations, $Ca^{2+}$-induced $Ca^{2+}$ release (CICR) and spontaneous transient outward currents (STOCs) in vascular smooth muscle cells (VSMCs). With respect to the role of cADPR-mediated signaling in mediation of vascular responses to different stimuli, there is accumulating evidence showing that cADPR is importantly involved in the $Ca^{2+}$ response of vascular endothelial cells (ECs) and VSMCs to various chemical factors such as vasoactive agonists acetylcholine, oxotremorine, endothelin, and physical stimuli such as stretch, electrical depolarization and sheer stress. This cADPR-RyR-mediated $Ca^{2+}$ signaling is now recognized as a fundamental mechanism regulating vascular function. Here we reviewed the literature regarding this cADPR signaling pathway in vascular cells with a major focus on the production of cADPR and its physiological roles in the control of vascular tone and vasomotor response. We also summarized some publish results that unveil the underlying mechanisms mediating the actions of cADPR in vascular cells. Given the importance of $Ca^{2+}$ in the regulation of vascular function, the results summarized in this brief review will provide new insights into vascular physiology and circulatory regulation.

**Keywords**: calcium mobilization • signal transduction • arterial myocytes • circulation • second messenger

* Correspondence to: Pin-Lan LI, M.D, Ph.D.
Department of Pharmacology and Toxicology,
Medical College of Virginia, Virginia Commonwealth University, 410 N 12th, Richmond, VA 23298, USA.
Tel.: (804) 828-4793; Fax: (804) 828-2117
E-mail: pli@vcu.edu

Reprinted from:
**Journal of Cellular and Molecular Medicine**

Published by:
the CMM Foundation

## Introduction

Numerous studies have indicated that cytosolic free calcium plays an essential role in mediating or modulating functions of vascular cells including vascular endothelial cells (ECs) and smooth muscle cells (VSMCs). The $Ca^{2+}$-associated signaling is now well known as one of the important determinants of the peripheral vascular resistance and vascular tone, thereby critically participating in the control of arterial blood pressure [1–4]. Over the last 20 years, substantial evidence has accumulated that intracellular [$Ca^{2+}$] ([$Ca^{2+}]_i$) in vascular cells, particularly in VSMCs, is primarily controlled by the influx of extracellular $Ca^{2+}$ and the mobilization of $Ca^{2+}$ from intracellular stores. It is generally accepted that the influx of extracellular $Ca^{2+}$ may initiate vasoconstriction or stimulate $Ca^{2+}$ release from the sarcoplasmic reticulum (SR) through $Ca^{2+}$-induced $Ca^{2+}$ release (CICR) and thereby lead to vasoconstriction. It has also been well documented that the $Ca^{2+}$ mobilization importantly mediates the vasomotor response to many vasoactive substances such as hormones, autocrine or paracrine, and other stimuli. This intracellular $Ca^{2+}$ release and consequent vasomotor responses play a critical role in the homeostasis of blood circulation.

Since the 1980s, inositol 1,4,5-tris-phosphate ($IP_3$) has been recognized as the first intracellular second messenger mediating the vasomotor effects of various agonists such as norepinephrine (NE), angiotensin II (AngII), vasopressin (ADH), endothelin (ET), thromboxane $A_2$ and others. In this process, the agonists activate phospholipase C, which converts phosphatidylinositol-4,5,-biphosphate ($PIP_2$) into $IP_3$ and diacylglycerol (DAG). $IP_3$ induces $Ca^{2+}$ release through $IP_3$ receptors ($IP_3R$) on the SR and thereby leads to the rise of $[Ca^{2+}]_i$, producing vasoconstriction [3–6]. Although $IP_3$ as a second messenger mediates the effects of a number of endogenous or exogenous vasoactive substances, importantly participates in the control of vascular tone and mediates vasomotor responses, there are many other agonists or stimuli that induce intracellular $Ca^{2+}$ release independently of the $IP_3$ pathway. For example, caffeine, $Ca^{2+}$, 5-hydroxytryptamine (5-HT), acetylcholine (Ach), endothelin-1, and prostaglandin $F_{2\alpha}$ produce $Ca^{2+}$ mobilization from the SR through activation of ryanodine receptors (RyR) on the SR, which is not related to the action of $IP_3$. cADPR as a novel $Ca^{2+}$ mobilizing second messenger has now been demonstrated to be another mediator for the action of different agonists or stimuli independent of $IP_3$-mediated $Ca^{2+}$ activation. The major focus of this review is to present evidence to reveal the production of cADPR in vascular cells and to address the physiological roles of cADPR-mediated $Ca^{2+}$ signaling in the control of vascular tone and vasomotor responses.

## Production and metabolism of cADPR in vascular cells

cADPR was first found in sea urchin eggs [7] and then detected in a variety of mammalian tissues or cells such as heart, liver, spleen, brain, red blood cells, lymphocytes, pituitary cells and cultured renal epithelial cells [8–12]. Basal concentrations of cADPR in cardiac muscle, liver and brain are estimated as 100-200 nM [13]. Homogenates prepared from dissected small bovine coronary arteries, cultured arterial ECs and VSMCs produced cADPR and its metabolite ADPR, when incubated with NAD [14–17]. Tissue cADPR concentration in the coronary smooth muscle is about 150 nM [14]. The cADPR extracted from the reaction mixture of NAD with cultured VSMCs or arterial homogenates is capable of stimulating $Ca^{2+}$ release *in vitro* using single cell $Ca^{2+}$ fluorospectrometry. Recently, we also detected cADPR in coronary arterial ECs, which is also in nM range [18].

cADPR can be synthesized from NAD *via* the action of ADP-ribosylcyclase. Once formed, cADPR can be further hydrolyzed by cADPR hydrolase to ADPR. Therefore, the cellular cADPR level is determined by the expression and activities of these enzymes. Both ADP-ribosylcyclase and cADPR hydrolase are membrane-bound enzymes found in a wide range of mammalian tissues [19, 20]. Our HPLC analyses revealed that the production of cADPR and ADPR is greater in the microsomal fraction than in the cytosolic fraction from bovine coronary arteries [15]. Therefore, the enzymes responsible for both production and metabolism of cADPR are primarily present on the cell membrane. In regard to the identity of ADP-ribosylcyclase in mammalian cells, the human lymphocyte differentiation antigens, CD38 and CD157,

have been reported to have high homology with *Aplysia* ADP-ribosylcyclase, and they possess multiple activities including NAD glycohydrolase, ADP-ribosylcyclase and cADPR hydrolase activities [19–21]. These CD proteins are considered as a molecular switch to regulate the cellular cADPR level by balancing its synthetic and hydrolytic activities. In response to stimuli, this multi-functional enzyme can be aggregated and internalized in the cytoplasm and thereby more efficiently produce or metabolize cADPR. By Western blot analysis and RT-PCR, we demonstrated that CD38 was detectable in coronary arterial smooth muscle. In these experiments, two immunoreactive bands with molecular sizes of 42 and 90 kDa were recognized by a monoclonal antibody against CD38 in coronary arterial homogenates and microsomes [15]. Removal of CD38 by immunoprecipitation significantly decreased the production and catabolism of cADPR in these arterial homogenates. In $CD38^{-/-}$ mice, very low cADPR levels and no detectable ADP-ribosylcyclase activity were observed in airway tissue dissected from these mice [22]. Our HPLC analysis also showed that ADP-ribosylcyclase activity in isolated small coronary septal arteries was reduced by 90–95% in $CD38^{-/-}$mice than in wild type mice [23]. These results led us conclude that CD38 is an important enzyme responsible for the production and metabolism of cADPR in vascular cells. In other studies, we found that ADP-ribosylcyclase activity can be detected in various arteries or arterioles, even in the smallest renal arteriole with diameters less than 10 μm such as afferent arterioles and vasa recta [16]. Interestingly, this CD38-associated enzyme in coronary VSMCs not only produces cADPR, but also metabolizes cADPR into ADPR by its bifunctional domain. Therefore, intracellular cADPR levels could be dynamically maintained by switching the function of different CD38 domains [14]. It is now well accepted that an enzymatic pathway responsible for the formation and metabolism of cADPR is present in vascular cells. cADPR is formed from $NAD^+$ by ADP-ribosylcyclase and may be metabolized into ADPR by the hydrolase activity of the same enzyme, which could be CD38 associated multifunctional activity [14, 15, 24, 25]. In addition to detection of the basal level of cADPR and approaches to its production and metabolism, over the last 10 years many investigators have been particularly interested in the response of this $Ca^{2+}$ signaling nucleotide to different agonists or stimuli, as this response may reveal its possible physiological relevance as a novel intracellular second messenger. Below we present some important evidence to provide an overview of this aspect.

## Production of cADPR in response to different agonists

Many agonists have been demonstrated to stimulate ADP-ribosylcyclase activity and increase production of cADPR in vascular cells. These agonists can be categorized based on their action on the ADP-ribosylcyclase as receptor-dependent or receptor–independent. The neurotransmitter acetylcholine (ACh) contracts the coronary arteries without the endothelium [26]. There are four pharmacologically defined subtypes of mAChRs, namely $M_1$, $M_2$, $M_3$ and $M_4$ [27]. In NG108-15 neuronal cells, activation of $M_1$ mAChRs increases the production of cADPR [28]. In our experiments, incubation of coronary VSMCs with oxotremorine, a specific $M_1$ mAChR agonist, produced a time- and concentration-dependent activation of ADP-ribosylcyclase, which was blocked by its inhibitor, nicotinamide, and a specific M1 mAChR antagonist, pirenzepine (PIR). The activation of ADP-ribosylcyclase occurred rapidly even in the first minute of incubation of coronary VSMCs with oxotremorine [29]. In porcine airway smooth muscle, activation of ADP-ribosylcyclase seems to be mediated *via* $M_2$–receptor since Ach-induced cADPR response was attenuated by a selective $M_2$ mAChR antagonist, methoctramine [30].

Another vasoconstrictor agonist that stimulates cADPR production is endothelin-1. This endothelium-derived peptide possesses potent vasoconstrictor action in a variety of vascular beds. Its vasoconstrictor responses are mediated by receptors $ET_A$–R or $ET_B$-R. Using nicotinamide or $Zn^{2+}$ as an inhibitor of ADP-ribosylcyclase, several reports have suggested that endothelin-1 increased cADPR production via activation of this enzyme. It has been shown that inhibition of ADP-ribosylcyclase significantly attenuated endothelin-1-induced $Ca^{2+}$ release in rat mesenteric small arteries [31], porcine airway smooth muscle [31], peritubular smooth muscle [32] and shark anterior mesenteric arteries

[33]. Most of these studies have suggested that $ET_B$-R is exclusively coupled to cADPR signaling, whereas $ET_A$-R activation may be linked to both $IP_3$ and cADPR signaling pathways. However, recent studies have demonstrated that although this vasoactive peptide can activate ADP-ribosylcyclase, it may stimulate the production of another signaling nucleotide, namely NAADP from $NADP^+$. NAADP is another $Ca^{2+}$ signaling messenger related to this cADPR pathway, but the mechanism of its actions may be different from cADPR. Readers are directed to read two recent reviews to know more details about NAADP-mediated $Ca^{2+}$ signaling [34, 35].

In the field of vascular biology, AngII is a well known vasoactive factor, which plays an important role in the regulation of arterial blood pressure. The vasoconstrictor effect of AngII is primarily associated with activation of its type I receptor (AT1) on VSMCs. Although many studies have indicated that activation of AT1 receptors may be linked to IP3-mediated $Ca^{2+}$ signaling, recent studies reported that ADP-ribosylcyclase can be activated by AngII in the cardiovascular tissues. More recently, AngII has been demonstrated to activate ADP-ribosylcyclase in neonatal rat cardiac myocytes [36], and inhibition of cADPR production attenuated the vasoconstrictor response of renal afferent arterioles to AngII [37].

In addition to vasoconstrictor agonists, there is evidence that some vasodilators may also alter the production of cADPR in VSMCs and thereby decreases release of intracellular $Ca^{2+}$, producing vasodilation. In this regard, NO represents one of the potent endogenous inhibitors of ADP-ribosylcyclase. It has been documented that an NO donor, sodium nitroprusside (SNP), decreased ADP-ribosylcyclase activity, inhibiting the production of cADPR in coronary VSMCs [38] or from airway smooth muscle [39]. These results have suggested that cADPR-mediated signaling may serve as a target for the action of various endogenous or exogenous vasodilator factors (also see details below). However, the view that NO inhibits cADPR-mediated $Ca^{2+}$ release is not consistent with previous findings in nonvascular cells which have reported that NO increases the cADPR production and consequently increases $Ca^{2+}$ release from the endoplasmic reticulum (ER) in nonvascular cells such as pancreatic-β-cells [40], rat parotid acinar cells [41] and sea urchin eggs [42]. The mechanism by which NO decreases $[Ca^{2+}]_i$ in VSMCs and airway smooth muscle cells but increases $[Ca^{2+}]_i$ in some other cells remains unknown. It has been assumed that ADP-ribosylcyclase or CD38 associated enzyme may have different redox status in different cell types, whereby NO may alter the redox status to either stimulate the production of cADPR or enhance its degradation. This redox-related change in enzyme activity is well studied in the coupling or uncoupling of NO synthase (NOS). For example, a reduced NOS is able to produce NO, but an oxidized or uncoupled NOS only produces superoxide ($O_2^{-\cdot}$) [43, 44]. This redox-related mechanism may also determine the activity of CD38 functional domains.

Another vasodilator factor that may exert its action through cADPR signaling pathway is bradykinin, an endothelium-dependent vasodilator. There is evidence that bradykinin increases $[Ca^{2+}]_i$, resulting in NO production and vasodilation [45]. Recently, we have reported that bradykinin activates ADP-ribosylcyclase activity in bovine coronary arterial ECs [18]. This bradykinin-induced activation of ADP-ribosylcyclase is a rapid (within 1 minute) and concentration-dependent response, which is blocked by its inhibitor, nicotinamide, rather than by phospholipase C inhibitor, U-73122. These results are consistent with the findings from airway smooth muscle and NG108-15 neuronal cells, in which bradykinin significantly increased the activity of ADP-ribosylcyclase [46, 47]. Evidence revealed that the coupling of membrane-bound ADP-ribosylcyclase could be attributed to $B_2$ receptors of bradykinin [46, 47].

## Production of cADPR in response to other vasoactive stimuli

As mentioned above, redox status of cells seems to be an important factor determining the activity of ADP-ribosylcyclase. In recent studies, we demonstrated that incubation of VSMCs from coronary arteries with a $O_2^{-\cdot}$ generating system, xanthine/xanthine oxidase (X/XO), produced activation of ADP–ribosylcyclase [48]. In the myocardium, $O_2^{-\cdot}$ generated by X/XO was also found to stimulate the synthesis of cADPR from $NAD^+$ [49]. However, $H_2O_2$ did not significantly alter ADP–ribosylcyclase activity even with the highest concentration (100 μM) studied [48], sug-

gesting that $O_2^{-\cdot}$ is the oxidant stimulating the production of cADPR. These results further support the view that $O_2^{-\cdot}$-induced activation of ADP-ribosylcyclase may be an important regulatory mechanism of cADPR production.

There is evidence that cell membrane depolarization activates ADP-ribosylcyclase in VSMCs [50, 51]. Incubation of arteries with KCl depolarizes the membrane of VSMCs and produces potent vasoconstriction. In the presence of inhibitor of ADP-ribosylcyclase, nicotinamide, KCl-induced $Ca^{2+}$ mobilization in these VSMCs was significantly attenuated, suggesting that cell membrane depolarization may activate the production of cADPR. Similarly, in adjacent longitudinal muscle membrane depolarization by $Cl^-$ channel activation also resulted in opening of voltage-sensitive $Ca^{2+}$ channels and activation of ADP-ribosylcyclase [52]. It seems that activation of ADP-ribosylcyclase is importantly involved in cell membrane depolarization-induced $Ca^{2+}$ signaling in muscle cells. The mechanism for membrane depolarization-induced ADP-ribosylcyclase activation may be attributed to $Ca^{2+}$ influx. Initial $Ca^{2+}$ influx by membrane depolarization induces $Ca^{2+}$ release by activating SR $Ca^{2+}$ channels. The increase in $[Ca^{2+}]_i$ activates membrane-bound ADP-ribosylcyclase, resulting in the formation of cADPR and consequent enhancement of CICR.

## Intracellular regulators of cADPR production

The production and hydrolysis of cADPR may be regulated by a number of intracellular factors such as ATP, ADP, pH, cGMP, polyamines, $Ca^{2+}$ and redox status [25, 53–59]. Three of the more important regulatory mechanisms are probably involved in the production of cADPR in vascular cells.

### cGMP/cAMP

It has been reported that increases in cGMP levels enhance the production of cADPR in nonvascular tissues [58]. Since cGMP-producing enzyme, guanylate cyclase may be an endogenous receptor for NO, this cGMP-induced enhancement of cADPR synthesis possibly contributes to the action of NO to increase $[Ca^{2+}]_i$ in these non-smooth muscle tissues. However, NO inhibits ADP-ribosylcyclase activity in coronary and airway VSMCs in cGMP-independent manner [38, 39]. There is also evidence that both 8-Br-cGMP and guanylate cyclase inhibitor, ODQ are without effect on ADP-ribosylcyclase activity in VSMCs. However, the role of cAMP in the regulation of ADP-ribosylcyclase is largely unknown. A recent study reported that β-adrenergic receptor-mediated regulation of $[Ca^{2+}]_i$ in rat cardiomyocytes is primed by activation of ADP-ribosylcyclase *via* cAMP/PKA signaling. Interestingly, cAMP was found to increase NAADP synthesis in sea urchin egg homogenates [60]. It seems that in vascular cells both cGMP and cAMP may not mediate or modulate the production of cADPR.

### G-proteins

G-proteins play an important role in linking cell surface receptors to intracellular second messengers. G proteins may also participate in the signaling between surface receptors and the production of cADPR. In this context, G-proteins link acetylcholine (Ach) receptors to ADP-ribosylcyclase and therefore Ach stimulates the production of cADPR through G protein activation. This view has been supported by several reports from vascular and nonvascular tissues or cells [30, 61, 62]. However, the conclusion cannot be drawn until further studies confirm a direct structural or functional connection between G proteins and any enzyme determining intracellular cADPR levels.

### Redox molecules

As discussed above, ADP-ribosylcyclase activity is regulated by redox status in many tissues or cells. cADPR production is enhanced when intracellular oxidants are increasingly produced. It has been proposed that ADP-ribosylcyclase serves as a link between intracellular oxidants and $[Ca^{2+}]_i$. Oxidants activate ADP-ribosylcyclase and result in the conversion of $NAD^+$ into cADPR, thereby increasing $[Ca^{2+}]_i$ [54, 58]. There is substantial evidence that $[Ca^{2+}]_i$ is increased by oxidative stress in the cells [63]. Increase in cADPR may be importantly involved in this oxidant-induced $Ca^{2+}$ mobilization. This oxidant-induced activation of ADP-ribosylcyclase may also affect the actions of NO on the production of cADPR in VSMCs, since a rapid interaction of NO with $O_2^{-\cdot}$ may decrease intracellular oxidants, resulting in reduction of cADPR production. Although it remains unknown what mech-

anism mediates oxidant-induced activation of ADP-ribosylcyclase of CD38 in vascular cells, a dimerization of ADP-ribosylcyclase or CD38 in response to increases in intracellular oxidants is proposed as one possible mechanism. This dimerization of ADP-ribosylcyclase has been reported to enhance the catalytic activity of ADP-ribosylcyclase [64, 65]. Studies have demonstrated that cysteine residues in the CD38 or ADP-ribosylcyclase determine the ability of this enzyme to function as ADP-ribosylcyclase or cADPR hydrolase [66]. The oxidation of cysteine molecules may lead to the formation of one or several disulfide bonds, which induces the dimerization of the enzyme protein. In some studies, the activity of ADP-ribosylcyclase was found to be inhibited by disulfide bond reducing reagents [54, 58]. Taken together, these results imply that intracellular oxidants, particularly $O_2^{-\cdot}$, are importantly involved in the regulation of ADP-ribosylcyclase activity, which is associated with the dimerization of this enzyme.

## cADPR-mediated $Ca^{2+}$ signaling in vascular cells

### Direct action on $Ca^{2+}$ release *via* RyR activation

In VSMCs, cADPR was demonstrated to stimulate $Ca^{2+}$ release from intracellular $Ca^{2+}$ stores when given into cells. Kannan *et al.* reported that cADPR induces the SR $Ca^{2+}$ release in β-escin-permeabilized VSMCs freshly isolated from porcine coronary arteries. In α-toxin permeabilized cells, we found that cADPR produces $Ca^{2+}$ release from SR in both cultured and freshly dissociated cow coronary and rat renal VSMCs [16, 67]. This cADPR-induced $Ca^{2+}$ release from the SR can be completely blocked by cADPR antagonist, 8-Br-cADPR, but not by $IP_3R$ blockers [16, 67]. It is concluded that cADPR mobilizes intracellular $Ca^{2+}$ through a mechanism independent of $IP_3$ in VSMCs. Recently, we also determined whether bradykinin-induced vasodilator response is directly linked to cADPR-mediated $Ca^{2+}$ release from the ER in bovine coronary arterial ECs [18]. Using a newly developed fluorescence imaging system to simultaneously measure $Ca^{2+}$ transient and NO production in the intact arterial endothelium, we showed that bradykinin produced a rapid and transient increase in $[Ca^{2+}]_i$, which was accompanied by enhanced NO production [18]. However, bradykinin-induced $Ca^{2+}$ release and NO production were significantly attenuated by pretreatment of the arteries with cADPR-RyRs signaling inhibitors such as nicotinamide, 8-Br-cADPR or ryanodine. This supports the view that bradykinin-induced $Ca^{2+}$ increase in arterial ECs is through cADPR-mediated $Ca^{2+}$ release from the ER and *via* RyR activation.

With respect to the action of cADPR on RyR activity, there is considerable electrophysiological evidence showing that the RyR/$Ca^{2+}$ release channels reconstituted into a planar lipid bilayer are activated by cADPR. In coronary arterial smooth muscle, a calcium channel with 245 pS conductance is present on the SR membrane [68]. cADPR increases the NPo of these RyR/$Ca^{2+}$ release channels in a concentration-dependent manner [68]. In the presence of high concentrations of ryanodine (50 μM), cADPR-induced activation of these channels is completely abolished. These results provide direct evidence that cADPR activates RyR and therefore may serve as an endogenous activator or modulator of the RyR in these VSMCs. However, there is evidence indicating that cADPR releases $Ca^{2+}$ independently of RyR in neurons and cells from the myocardium and other smooth muscle [69–71]. The reason for these discrepancies remains unknown. It is possible that there is a tissue specific effect of cADPR on the RyR that may be associated with the intermediate proteins or accessory proteins that regulate RyR activity.

### CICR and $Ca^{2+}$ waves

It is generally accepted that cADPR mobilizes intracellular $Ca^{2+}$ by activating RyRs, producing $Ca^{2+}$ release from the SR or ER in different tissues or cells which is completely independent of $IP_3$. An important property of RyRs is that they can be opened by $Ca^{2+}$ and can mediate CICR. In VSMCs, a large $Ca^{2+}$ influx and rise of $Ca^{2+}$ around the deep SR leads to CICR, thereby giving rise to a global $Ca^{2+}$ signal throughout the cytoplasm and nucleus and leading to vasoconstriction [2]. CICR is not only an important mechanism increasing global $[Ca^{2+}]_i$, but also participates in the formation of $Ca^{2+}$ waves or oscillations in these cells [72, 73].

Repeating cycles of $Ca^{2+}$ uptake and release in the SR constitute intracellular $Ca^{2+}$ waves or oscillations. By $Ca^{2+}$ waves, $Ca^{2+}$ is guided from the plasma membrane to myofilaments, instead of direct diffusion from the plasma membrane through the cytoplasm. Therefore, the $Ca^{2+}$ oscillations may importantly contribute to the tension development in VSM in response to different agonists such as phenylephrine [72, 73].

In cerebral circulation, RyR-mediated CICR largely contributes to the vasoconstrictor response of cerebral resistance arteries to stimuli [74]. In pulmonary vascular bed, CICR is crucial for the development of basal vascular tone and production of hypoxic vasoconstriction [75, 76]. More recently, we have reported that cADPR participates in KCl, $CaCl_2$, Bay K 8644 ($Ca^{2+}$ channel activator) and caffeine-induced $Ca^{2+}$ release response in coronary and renal VSM cells, strongly suggesting that cADPR contributes to CICR [77]. In these smooth muscle cells, we also found that high extracellular $Ca^{2+}$ (5 mM $CaCl_2$) and agonist Ach produced 1–1.5 Hz oscillations, which are blocked by CICR inhibitor, tetracaine and cADPR antagonist 8-Br-cADPR [16]. Kannan *et al.* have also reported that cADPR increases Ach-induced $Ca^{2+}$ oscillations and cADPR blocker, 8-amino-cADPR diminished Ach-induced $[Ca^{2+}]_i$ oscillations in porcine tracheal smooth muscle cells [78]. Taken together, these results demonstrate that cADPR is necessary for CICR and intracellular $Ca^{2+}$ oscillation and RyR are the mechanistic link between cADPR and CICR or $Ca^{2+}$ oscillations.

Based on these results, two mechanistic models have been proposed to elucidate the role of endogenous cADPR in mediating vascular $Ca^{2+}$ mobilization [77]. The first model proposed that cADPR acts as a mediator. According to this model, various agonists or stimuli activate ADP-ribosylcyclase to produce cADPR, leading to activation of $Ca^{2+}$ release from the SR through the RyR. This activation of ADP-ribosylcyclase may also occur when intracellular $Ca^{2+}$ levels increases even slightly, thereby resulting in CICR. Another model considers cADPR as a modulator of CICR or RyR activity. In this way, cytosolic cADPR sensitizes the RyR, enhancing CICR activated by agonists or $Ca^{2+}$ influx. The relative contribution of these two mechanisms to the vascular reactivity may vary depending upon the concentrations of intracellular cADPR, $[Ca^{2+}]$, and calmodulin and the functional status of RyRs in different cells.

## $Ca^{2+}$ sparks and STOCs

$Ca^{2+}$ sparks are local and spontaneous transient $Ca^{2+}$release through the RyRs on the SR. $Ca^{2+}$ sparks occur in the relation to $Ca^{2+}$-dependent K ($K_{Ca}$) channel activation and muscle relaxation in VSM. A spontaneous $Ca^{2+}$ spark in a superficial area close to the cell membrane activates 10–100 $K_{Ca}$ channels (namely, spontaneous transient outward currents (STOCs)) and induces membrane hyperpolarization, which reduces $Ca^{2+}$ channel activity, resulting in decrease in vascular tone [79, 80]. $Ca^{2+}$ sparks occur by spontaneous opening of RyR/$Ca^{2+}$ release channels in smooth muscle cells. Therefore, the sensitivity of RyRs may largely determine the occurrence of $Ca^{2+}$ sparks. Since cADPR is also reported to sensitize the RyR on the SR, it is not surprising that cADPR participates in the $Ca^{2+}$ sparks. Recent studies have reported that exogenous cADPR increases $Ca^{2+}$ sparks in cardiac cells [81]. However, in VSM cells, ryanodine and cADPR transiently enhances $Ca^{2+}$ sparks and then produces a sustained inhibition of $Ca^{2+}$ sparks [80, 82, 83]. This sustained inhibition of $Ca^{2+}$ sparks may be due to the depletion of $Ca^{2+}$ stores [80, 82, 83]. In addition to its effect to increase global $[Ca^{2+}]$, cADPR-induced $Ca^{2+}$ depletion of the superficial SR, inhibition of $Ca^{2+}$ sparks and consequent membrane depolarization may be importantly involved in the control of vascular tone and vasomotor response [68].

## FKBP 12.6 protein and action of cADPR in VSMCs

The mechanism by which cADPR activates RyR in vascular cells is poorly understood. Increasing evidence indicate that FKBP12.6 plays an important role in the activation of the RyR [74, 84, 85]. FKBP12.6 is a ubiquitous 12.6-kDa cytosolic protein, which can bind to one RyR monomer and its activity is inhibited by the immunosuppressant drugs, FK506 and rapamycin. In nonvascular cells, $Ca^{2+}$ release from the SR is inhibited when FKBP12.6 is bound to the RyR, and dissociation of

FKBP 12.6 from the RyR releases $Ca^{2+}$. This 12.6 kDa protein is also expressed in coronary arterial smooth muscle [86]. Blockade, dissociation or removal of FKBP12.6 protein from the RyR substantially abolish cADPR-induced activation of RyR/$Ca^{2+}$ release channels on lipid bilayer membrane. Ligand binding experiments have demonstrated that cADPR can direct bind to FKBP12.6 in islet microsomes [87]. Recently, using confocal fluorescence imaging, we have demonstrated that FKBP12.6 colocalizes with RyRs in renal arterial myocytes [77]. $Ca^{2+}$ influx by $CaCl_2$ significantly decreased this colocalization and 8-Br-cADPR reversed $CaCl_2$ effects suggesting that cADPR is involved in the dissociation of FKBP 12.6 protein from RyRs under this condition. Based on these results, it can be proposed that cADPR exerts a modulator action to enhance the sensitivity of RyRs by dissociating FKBP12.6, thereby resulting in $Ca^{2+}$ release from the SR in VSMCs.

Interestingly, methylation of FKBP12.6 by arginine N-methyltranserase (PRMT1) activates RyR/$Ca^{2+}$ channels in the SR of coronary VSM [88]. Therefore, cADPR may also regulate methylation of FKBP12.6 resulting in an indirect activation of RyRs. Taken together, it is clear that FKBP12.6 plays a critical role in mediating cADPR-induced activation of RyR/$Ca^{2+}$ release channels in the SR of VSM.

# Role of cADPR in the regulation of vascular function

## Role of cADPR in the control of vascular tone

It is well known that vascular smooth muscle (VSM) usually operates in a contracted state, which is referred to vascular tone. Intracellular $Ca^{2+}$ importantly contributes to the production of this "resting" vascular tone. Under resting conditions, $[Ca^{2+}]_i$ in VSM is dependent upon the $Ca^{2+}$ influx, spontaneous brief releasing bursts of $Ca^{2+}$ from the SR into the cytoplasm and CICR [1–4]. cADPR participates in the control of the resting $Ca^{2+}$ levels in these smooth muscle cells through RyRs and CICR [68, 89, 90]. Therefore, cADPR may play an important role in the development of basic vascular tone. There is conceivable evidence supporting this view. In the isolated, perfused and pressurized small coronary arteries, basic vascular tone or spontaneous tension can be developed during a 1.5-hour equilibration period. Under this condition, the SR $Ca^{2+}$-ATPase inhibitor, thapsigargin, decreases the arterial diameter, and CICR blocker, tetracaine and cADPR antagonist, 8-Br-cADPR slightly dilates these arteries, suggesting that $[Ca^{2+}]_i$ associated with cADPR-RyR signaling pathway and CICR is one of the determinants of the basic vascular tone [51].

## Role of cADPR in vasomotor responses

### Vasoconstrictor response to agonists

Numerous studies have shown that coronary arteries or other blood vessels constrict in $Ca^{2+}$ free medium in response to a variety of agonists such as acetylcholine, endothelin, $PGF_{2\alpha}$ and histamine. cADPR mediates the effects of M-type mAchR in adrenal chromaffin cells, estrogen receptors in uterus, retinoic acid in renal tubular and arterial smooth muscle [91–93]. In coronary VSM, Ach has been selected as a prototype agonist to study the contribution of cADPR-mediated signaling pathway to agonist-induced vasoconstriction [29]. As discussed above, $M_1$ mAchR agonist, oxotremorine, markedly enhances the activity of ADP-ribosylcyclase and increases the production of cADPR in cultured coronary VSMCs, which is blockable by $M_1$ mAChR blocker, pirenzepine (PIR) and by ADP-ribosylcyclase inhibitor, nicotinamide [29]. It seems that ADP-ribosylcyclase is directly coupled to $M_1$ mAChRs through G-proteins. In isolated, perfused and pressurized small coronary arteries, vasoconstriction induced by Ach or oxotremorine is also attenuated by the inhibition of ADP-ribosylcyclase or blockade of cADPR action. These results confirm that cADPR is linked to M1 mAChRs and mediates the vasoconstrictor action of this subtype of mAChRs in VSM cells [29]. The oxotremorine-induced vasoconstriction is also directly associated with cADPR-mediated $Ca^{2+}$ release from the SR in VSMCs. oxotremorine produces a rapid $Ca^{2+}$ release in single VSMCs bathed with $Ca^{2+}$-free solution, which was significantly attenuated by inhibition of cADPR production by nicotinamide, blockade of cADPR action by 8-Br-cADPR [29]. In isolated and perfused small

coronary septal arteries from CD38$^{-/-}$ mice, oxotremorine produced much smaller vasoconstrictor response than in the same arteries from wild type mice [23]. Further, oxotremorine-induced intracellular $Ca^{2+}$ increase was significantly lowered in freshly isolated septal arterial VSMCs from CD38$^{-/-}$ mice than in VSMCs isolated from wild mice [23]. Taken together, these results provide direct evidence that endogenous cADPR contributes to oxotremorine-induced $Ca^{2+}$ mobilization in VSMCs and that cADPR serves as a second messenger to mediate vasoconstrictor response to M1 receptor activation [94].

Endothelin-1 is a potent endothelium-derived vasoconstrictor peptide, which increases intracellular $Ca^{2+}$ *via* activation of $ET_A$ and/or $ET_B$ receptors in different vascular beds. Recent studies have shown that endothelin-1-induced $Ca^{2+}$ response is associated with cADPR/RyR signaling. In rat mesenteric arteries, Giulumian *et al.* reported that endothelin-1-induced $Ca^{2+}$ increase and vasoconstriction were significantly attenuated by ADP-ribosylcyclase inhibitor nicotinamide and RyR $Ca^{2+}$ release channel inhibitor, dantrolene [31]. In isolated pulmonary arteries, Dipp *et al.* demonstrated that a membrane-permeant cADPR antagonist, 8-Br-cADPR, blocked sustained hypoxic pulmonary vasoconstriction [95]. In rat peritubular VSMCs, Barone *et al.* observed that both $ET_A$- and $ET_B$-mediated $Ca^{2+}$ signaling were completely abolished by cADPR antagonist, 8-$NH_2$-cADPR [32]. In porcine airway smooth muscle, White *et al.* showed that endothelin-1-induced $Ca^{2+}$ response was inhibited by 8-Br-cADPR [30]. In airway VSMCs isolated from CD38$^{-/-}$ mice, Deshpande *et al.* found that the intracellular $Ca^{2+}$ response to endothelin-1 was significantly lower than that in control animals [22]. Although there was direct detection of cADPR production in response to endothelin-1 in some vascular beds studied in these studies, these functional data have suggested that cADPR/RyR $Ca^{2+}$ signaling may importantly participate in endothelin-1-indcued vasoconstrictor response in these vascular beds.

**Vasoconstriction induced by $Ca^{2+}$ entry associated with cell membrane depolarization**

In various arteries, cell membrane depolarization and activation of L-type $Ca^{2+}$ channels were found to produce the influx of extracellular $Ca^{2+}$ and thereby increases $[Ca^{2+}]_i$, leading to vasoconstriction. In this process, the entry of $Ca^{2+}$ is further amplified by CICR, thereby producing an increase in global $[Ca^{2+}]$ throughout the cytoplasm and nucleus and consequent vasoconstriction [1, 2]. In spite of the essential effect of $Ca^{2+}$ influx, the amplification of $Ca^{2+}$ signal or $Ca^{2+}$ propagation within VSMCs also plays a critical role in the vasoconstriction in response to membrane depolarization and activation of membrane $Ca^{2+}$ channels. The SR $Ca^{2+}$-ATPase inhibitor, thapsigargin, significantly blocks KCl depolarization-induced vasoconstriction in coronary arteries, suggesting that $Ca^{2+}$ refilling and release in the SR mediated by this enzyme are critical for KCl-induced $Ca^{2+}$ release and vasoconstriction [51]. Similarly, CICR blocker tetracaine and cADPR antagonist 8-Br-cADPR also significantly attenuate KCl-induced decrease in arterial diameters. However, a cell permeable $IP_3$ blocker, xestospongin C at a concentration that attenuated U46619-induced contraction, has no effect on KCl-induced vasoconstriction in the same small resistant coronary arteries [51]. Using a $Ca^{2+}$ channel activator, Bay K8644 and $CaCl_2$ to stimulate $Ca^{2+}$ influx, the vasoconstrictor effects are also attenuated by thapsigargin, tetracaine or 8-Br-cADPR [51]. This role of CICR in vasoconstrictor response to $Ca^{2+}$ influx has also been documented in other vascular beds such as aorta, cerebral, renal and pulmonary arteries [12, 51, 74–76]. It is concluded that cADPR-mediated $Ca^{2+}$ signaling is of importance in amplification of intracellular $Ca^{2+}$ signal and vasoconstriction through CICR associated with cell membrane depolarization.

**Oxidative stress-induced vasoconstriction**

Recent studies have highlighted that the contribution of redox status to the control of vascular tone or vasomotor response in different vascular beds [96–98]. It has been demonstrated that reactive oxygen species (ROS) serve as a vascular $O_2$-sensing factor to activate the vascular reactivity in response to tissue metabolic activity [99]. An important mechanism by which ROS regulate the vascular tone may be due to the changes in the intracellular $Ca^{2+}$ homeostasis. In previous studies, ROS have been shown to cause $Ca^{2+}$ mobilization in cardiac, skeletal, and smooth muscles [98, 100–102]. This ROS-induced change in $Ca^{2+}$ homeostasis in muscles could be mediated *via* a variety of mechanisms such as inhibition of $Ca^{2+}$-ATPase of SR, modifica-

tion in gating activities of SR $Ca^{2+}$ release channels, or alteration of $Ca^{2+}$ release from either $IP_3$-sensitive or ryanodine-sensitive $Ca^{2+}$ stores [98, 100–102]. However, the underlying mechanisms by which ROS exert their actions on $Ca^{2+}$ homeostasis in VSM are still poorly understood.

In coronary VSMCs, cADPR antagonism, RyR and CICR blockade, and inhibition of cADPR production significantly attenuated $O_2^{-\cdot}$-induced $Ca^{2+}$ release [48]. Consistently, these inhibitors or blockers of cADPR/RyR signaling pathway could attenuate $O_2^{-\cdot}$-induced vasoconstriction in isolated and perfused small coronary arteries [48]. These results provide direct evidence that the cADPR signaling pathway participates in the vasoconstrictor response to ROS in the coronary circulation, supporting the view that oxidative stress, especially increased $O_2^{-\cdot}$ production results in vasoconstriction in part through cADPR/RyR activation pathway in coronary VSMCs. These findings that ROS exert their effects on $Ca^{2+}$ signaling in VSMCs through cADPR/RyR activation further extend our understanding that ROS serve as important mediators in regulating vascular reactivity in response to oxidative stress under physiological and pathological conditions.

**Nicotinamide-induced vasodilation**

Nicotinamide, an amide derivative of vitamin $B_3$, is a potent vasodilator. Nicotinamide increases the perfusion and oxygenation of tumors, thereby increasing the sensitivity of the tumors to radio- or chemical therapies [50]. However, the mechanism by which nicotinamide produces vasodilation and increases tissue perfusion is poorly understood. Nicotinamide inhibits the activity of purified ADP-ribosylcyclase, decreases cADPR production and blocks the $Ca^{2+}$-mobilizing action of β-$NAD^+$, cGMP and NO in nonvascular cells [103]. In this regard, the effect of nicotinamide on ADP-ribosylcyclase activity may produce vasodilation in VSM. Therefore, cADPR-mediated $Ca^{2+}$ mobilization may be a target for the vasodilator effect of nicotinamide. Consistent with this hypothesis, we have shown that this ADP-ribosylcyclase inhibitor, nicotinamide produces a concentration-dependent relaxation in U46619-precontracted coronary arteries [50]. This relaxation can be markedly blocked by pretreatment of coronary arteries with either 8-Br-cADPR or ryanodine. It has been proposed that cADPR-mediated $Ca^{2+}$ signaling may be a therapeutic target for the action of vasodilator compounds like nicotinamide.

**NO-induced vasodilation**

NO, an endothelium-derived relaxing factor, plays an important role in the control of vascular tone [17]. In nonvascular tissues, NO activates guanylyl cyclase and increases cGMP levels, which promotes cADPR production and consequently increases $Ca^{2+}$ release from the SR [42, 58, 103]. Obviously, this NO-induced rise in $[Ca^{2+}]_i$ could not contribute to its vasodilator effect, since NO decreases $[Ca^{2+}]_i$ in VSMCs [38, 39]. Endogenous cADPR-induced $Ca^{2+}$ release in the resting state probably contributes to the control of basal $[Ca^{2+}]_i$ in VSMCs, and NO decreases intracellular cADPR concentrations and thereby lowers $[Ca^{2+}]_i$. This cADPR-dependent changes in $[Ca^{2+}]_i$ may be one of the mechanisms mediating NO-induced vasodilation. This view is supported by the fact that NO donors such as SNP and deta NONOate-induced vasodilator response is not completely blocked by a guanylyl cyclase inhibitor, ODQ, unless a cADPR antagonist is added in combination [17, 38].

By determining $Ca^{2+}$ release response in single VSMC, NO has no direct effect on either cADPR- or $IP_3$-induced $Ca^{2+}$ release response in permeabilized cells, but inhibits KCl- and caffeine-induced $Ca^{2+}$ release in intact cells [17, 38, 100]. It appears that NO decreases $[Ca^{2+}]_i$ by inhibiting endogenous cADPR-mediated $Ca^{2+}$ release, but not by direct effect on $Ca^{2+}$ release machinery on the SR of VSMCs. This inhibitory effect of NO on cADPR-induced $Ca^{2+}$ release has also been confirmed in porcine tracheal smooth muscle cells [69, 104]. The mechanism by which NO decreases $[Ca^{2+}]_i$ in coronary and porcine tracheal VSMCs, but increases $[Ca^{2+}]_i$ in some other cells remains unknown. NO may have different effects on the enzyme activities responsible for the production or degradation of cADPR in different cells. NO decreases the cADPR production in a concentration-dependent manner, but it has no effect on cADPR hydrolase [38]. Based on these results, it is concluded that inhibitory effect of NO on the production of endogenous cADPR plays an important role in mediating NO-induced vasodilation.

**EETs-induced vasodilation**

Epoxyeicosatrienoic acids (EETs), endothelium-derived hyperpolarizing factors (EDHF), also produce vasodilation in a variety of vascular beds and thus play an important role in the regulation of vascular tone [105]. EETs induce vasodilation through the activation of $K_{Ca}$ channels in the VSMCs. ADPR, a metabolite of cADPR, directly stimulates $K_{Ca}$ channels in coronary VSMCs [24]. Interestingly, EETs can activate both NAD glycohydrolase and cADPR hydrolase, increasing ADPR levels in VSMCs [24]. Increased intracellular ADPR may serve as an intracellular signaling molecule mediating the effect of EETs on $K_{Ca}$ channels [24]. Patch clamp studies have shown that selective inhibition of NAD glycohydrolase by 3GA or inhibition of cADPR hydrolase by novobiocin completely abolished the activation of the $K_{Ca}$ channels induced by 11,12-EET in coronary VSMCs [24]. It appears that EETs-induced activation of KCa channels is associated with the production of ADPR. Consistent with these findings, 11,12-EET-induced vasodilation can be attenuated by 3GA or novobiocin in epicardial coronary arteries and small resistance coronary arteries [24, 106]. These results indicate that the NAD or cADPR metabolite, ADPR, may serve as a signaling molecule mediating the vasodilator effects of EDHF. This role of ADPR may represent a new signaling pathway regulating the vasomotor response.

**Role of cADPR in mediating production of endothelium-derived relaxing factors.**

$Ca^{2+}$ activation of ECs is an important mechanism mediating the production of endothelium-derived relaxing factors (EDRFs) such as NO, EETs and prostacyclins [45, 48, 107]. These EDRFs may mediate endothelium-dependent vasodilation (EDVD). Despite extensive studies, it is still controversial which $Ca^{2+}$ signaling pathway is responsible for the elevation of intracellular $Ca^{2+}$ in ECs in the process of EDVD. In regard to the regulation of $Ca^{2+}$ level in the vascular endothelium, it has been demonstrated that upon stimulation with inflammatory agonists such as thrombin, histamine, bradykinin and oxidants, the $[Ca^{2+}]_i$ could increase 5–10 times compared with the basal level. This agonist-induced increase in $[Ca^{2+}]_i$ occurs in two distinct phases, a transient rise due to intracellular $Ca^{2+}$ store depletion, which involves generation of $IP_3$ and $IP_3$-induced $Ca^{2+}$ release from the ER and a sustained phase due to $Ca^{2+}$ entry into the cell from extracellular medium ($Ca^{2+}$ influx). Increased $Ca^{2+}$ stimulate the enzymatic binding of $Ca^{2+}$/CaM to endothelial NO synthase (eNOS), resulting in rapid conversion of L-arginine into citrulline, producing NO [108-110]. However, there is considerable evidence that blockade of $IP_3$ signaling could only partially attenuate agonist-induced $Ca^{2+}$ release as well as store-operated calcium influx and partially decreased NO production in ECs [111]. Other studies have reported that $IP_3$ is not involved in the regulation of bradykinin-induced $Ca^{2+}$ increase in ECs [112] and that cyclopiazonic acid-enhanced $Ca^{2+}$ increase and EDRF release in bovine pulmonary arterial ECs is not $IP_3$-dependent [113]. In addition, the activation of protein tyrosine kinases and phosphatases is also found to participate in the process of endothelial NO generation independently of the phospholipase C signaling pathway [114]. Interestingly, ADP-ribosylcyclase gives rise to bradykinin signal transduction from receptors to its effector enzymes [46, 47, 115]. These results suggest that cADPR/RyR signaling may be present in ECs and modulate endothelial function by regulating of EDRF production.

Recently, we reported that inhibition of cADPR production or antagonism of its action significantly attenuated bradykinin-induced concentration-dependant coronary arterial vasodilation [18]. Since blockade of cADPR/RyR signaling causes relaxation of VSM, this inhibition of bradykinin-induced dilation is attributed to the interference of endothelial function (*i.e.* the ability to produce EDRFs) [29, 51]. Given the biochemical results that stimulation of coronary arterieal ECs with bradykinin caused a significant increase in endothelial ADP-ribosylcyclase activity and intracellular cADPR concentrations, these results tell us that cADPR mediates endothelial NO formation independent of $IP_3$ pathway and that activation of ADP-ribosylcyclase in ECs plays an important role in mediating NO production and EDVD.

By direct measurement of intracellular $Ca^{2+}$ release in the intact coronary arterial endothelium, we found that bradykinin evokes a $Ca^{2+}$ release from RyRs-sensitive stores, which was accompanied by an increase in NO production [18]. Blockade of $IP_3$ pathway by 2-APB only partially inhibited bradykinin-induced intracellular $Ca^{2+}$

mobilization and NO production, while 8-Br-cADPR together with 2-APB more substantially blocked these $Ca^{2+}$ release responses than using 8-Br-cADPR alone. It is concluded that this bradykinin-induced intracellular $Ca^{2+}$ increase and NO response is not mainly associated with $IP_3$ signaling but with cADPR levels in these cells. Other studies also reported that the maximal inhibition effect of carboxyamidotriazole on vascular endothelial growth factor A-induced NO production in human umbilical vein ECs was only about 50% depending on $IP_3$ pathway [111]. It is possible that cADPR primarily influences bradykinin-induced coronary vasodilation *via* its $Ca^{2+}$ release and consequent NO production. Taken together, we conclude that cADPR serves as an intracellular second messenger in mediating bradykinin-induced $Ca^{2+}$ mobilization in arterial ECs and thereby stimulates the production of NO and participates in the EDVD.

## Conclusion

Recent studies have demonstrated that a membrane-bound enzyme system is present in vascular ECs and VSMCs, which can synthesize and metabolize cADPR. In ECs, cADPR mediates agonist (such as bradykinin)-induced $Ca^{2+}$ mobilization from the ER, resulting in production of NO or other EDRFs, participating in the EDVD response. In VSMCs, cADPR serves a second messenger to stimulate $Ca^{2+}$ release from the SR *via* RyRs and involves in the regulation of CICR and consequent $Ca^{2+}$ waves, producing global $Ca^{2+}$ increase within these cells. cADPR activates RyRs by binding to FKBP12.6 and results in dissociation of this accessory protein from RyRs, whereby $Ca^{2+}$ from the SR was enhanced. There is considerable evidence showing that this cADPR-mediated $Ca^{2+}$ signaling plays a critical role in $Ca^{2+}$ release and vasoconstrictor response to different agonist, oxidative stress, cell membrane depolarization, and $Ca^{2+}$ influx. Moreover, decrease in this cADPR-mediated $Ca^{2+}$ mobilization may importantly contribute to the mediation or modulation of vasodilator response through its action on the production of EDRFs in ECs and on the activation of RyRs in VSMCs.

## Acknowledgment

Most of works from our laboratory cited in this report were supported by NIH Grants HL057244, HL075316 and DK054927.

## References

1. **Berridge MJ.** The biology and medicine of calcium signalling. *Mol Cell Endocrinol.* 1994; 98: 119–24.
2. **Berridge MJ.** Elementary and global aspects of calcium signalling. *J Physiol.* 1997; 499: 291–306.
3. **Himpens B, Missiaen L, Casteels R.** $Ca^{2+}$ homeostasis in vascular smooth muscle. *J Vasc Res.* 1995; 32: 207–19.
4. **Nelson MT, Patlak JB, Worley JF, Standen NB.** Calcium channels, potassium channels, and voltage dependence of arterial smooth muscle tone. *Am J Physiol* 1990; 259: C3–18.
5. **Fasolato C, Innocenti B, Pozzan T.** Receptor-activated $Ca^{2+}$ influx: how many mechanisms for how many channels? *Trends Pharmacol Sci.* 1994; 15: 77–83.
6. **Petersen OW, Hoyer PE, van Deurs B.** Effect of oxygen on the tetrazolium reaction for glucose 6-phosphate dehydrogenase in cryosections of human breast carcinoma, fibrocystic disease and normal breast tissue. *Virchows Arch B Cell Pathol Incl Mol Pathol.* 1985; 50: 13–25.
7. **Lee HC, Walseth TF, Bratt GT, Hayes RN, Clapper DL.** Structural determination of a cyclic metabolite of NAD+ with intracellular $Ca^{2+}$-mobilizing activity. *J Biol Chem.* 1989; 264: 1608–15.
8. **Takasawa S, Nata K, Yonekura H, Okamoto H.** Cyclic ADP-ribose in insulin secretion from pancreatic beta cells. *Science* 1993; 259: 370–3.
9. **Lee HC, Aarhus R:** Wide distribution of an enzyme that catalyzes the hydrolysis of 34 cyclic ADP-ribose. *Biochim Biophys Acta* 1993; 1164: 68–74.
10. **Galione A, Lee HC, Busa WB.** Ca(2+)-induced $Ca^{2+}$ release in sea urchin egg homogenates: modulation by cyclic ADP-ribose. *Science* 1991; 253: 1143–6.
11. **Koshiyama H, Lee HC, Tashjian AH Jr.** Novel mechanism of intracellular calcium release in pituitary cells. *J Biol Chem* 1991; 266: 16985–8.
12. **Beers KW, Chini EN, Lee HC, Dousa TP.** Metabolism of cyclic ADP-ribose in opossum kidney renal epithelial cells. *Am J Physiol* 1995; 268: C741–6.
13. **Lee HC.** A signaling pathway involving cyclic ADP-ribose, cGMP, and nitric oxide. *News Physiol Sci.* 1994; 9: 134–7.
14. **Li PL, Zou AP, Campbell WB.** Regulation of KCa-channel activity by cyclic ADP-ribose and ADP-ribose in coronary arterial smooth muscle. *Am J Physiol.* 1998; 275: H1002–10.
15. **Li P, Zou AP, Campbell WB.** Metabolism and actions of ADP-riboses in coronary arterial smooth muscle. *Adv Exp Med Biol.* 1997; 419: 437–41.

16. **Li N, Teggatz EG, Li PL, Allaire R, Zou AP.** Formation and actions of cyclic ADP-ribose in renal microvessels. *Microvasc Res.* 2000; 60: 149–59.
17. **Li N, Zou AP, Ge ZD, Campbell WB, Li PL.** Effect of nitric oxide on calcium-induced calcium release in coronary arterial smooth muscle. *Gen Pharmacol.* 2000; 35: 37–45.
18. **Zhang G, Teggatz EG, Zhang AY, Koeberl MJ, Yi F, Chen L, Li PL.** Cyclic ADP ribose-mediated $Ca^{2+}$ signaling in mediating endothelial nitric oxide production in bovine coronary arteries. *Am J Physiol Heart Circ Physiol.* 2006; 290: H1172–81.
19. **Franco L, Zocchi E, Calder L, Guida L, Benatti U, De Flora A.** Self-aggregation of the transmembrane glycoprotein CD38 purified from human erythrocytes. *Biochem Biophys Res Commun.* 1994; 202: 1710–5.
20. **Zocchi E, Franco L, Guida L, Benatti U, Bargellesi A, Malavasi F, Lee HC, De Flora A:** A single protein immunologically identified as CD38 displays $NAD^+$ glycohydrolase, ADP-ribosyl cyclase and cyclic ADP-ribose hydrolase activities at the outer surface of human erythrocytes. *Biochem Biophys Res Commun.* 1993; 196: 1459–65.
21. **Adebanjo OA, Koval A, Moonga BS, Wu XB, Yao S, Bevis PJ, Kumegawa M, Zaidi M, Sun L.** Molecular cloning, expression, and functional characterization of a novel member of the CD38 family of ADP-ribosyl cyclases. *Biochem Biophys Res Commun.* 2000; 273: 884–9.
22. **Deshpande DA, White TA, Guedes AG, Milla C, Walseth TF, Lund FE, Kannan MS.** Altered airway responsiveness in CD38-deficient mice. *Am J Respir Cell Mol Biol.* 2005; 32: 149–56.
23. **Teggatz EG, Zhang G, Yi F, Zou AP, Li PL.** Vasoconstrictor responses of coronary arteries in CD38 gene knockout mice: role of cyclic ADP-ribose. *FASEB J.* 2005; 19: 1088.
24. **Li PL, Zhang DX, Ge ZD, Campbell WB.** Role of ADP-ribose in 11,12-EET-induced activation of K(Ca) channels in coronary arterial smooth muscle cells. *Am J Physiol Heart Circ Physiol.* 2002; 282: H1229–36.
25. **Zhang DX, Zou AP, Li PL.** Adenosine diphosphate ribose dilates bovine coronary 36 small arteries through apyrase- and 5'-nucleotidase-mediated metabolism. *J Vasc Res.* 2001; 38: 64–72.
26. **Furchgott RF, Zawadzki JV.** The obligatory role of endothelial cells in the relaxation of arterial smooth muscle by acetylcholine. *Nature* 1980; 288: 373–6.
27. **van Zwieten PA, Doods HN.** Muscarinic receptors and drugs in cardiovascular medicine. *Cardiovasc Drugs Ther* 1995; 9: 159–67.
28. **Higashida H, Yokoyama S, Hashii M, Taketo M, Higashida M, Takayasu T, Ohshima T, Takasawa S, Okamoto H, Noda M.** Muscarinic receptor-mediated dual regulation of ADP-ribosyl cyclase in NG108-15 neuronal cell membranes. *J Biol Chem.* 1997; 272: 31272–7.
29. **Ge ZD, Zhang DX, Chen YF, Yi FX, Zou AP, Campbell WB, Li PL.** Cyclic ADP-ribose contributes to contraction and $Ca^{2+}$ release by M1 muscarinic receptor activation in coronary arterial smooth muscle. *J Vasc Res.* 2003; 40: 28–36.
30. **White TA, Kannan MS, Walseth TF.** Intracellular calcium signaling through the cADPR pathway is agonist specific in porcine airway smooth muscle. *FASEB J.* 2003; 17: 482–4.
31. **Giulumian AD, Meszaros LG, Fuchs LC.** Endothelin-1-induced contraction of mesenteric small arteries is mediated by ryanodine receptor $Ca^{2+}$ channels and cyclic ADP-ribose. J Cardiovasc Pharmacol 2000; 36: 758–63.
32. **Barone F, Genazzani AA, Conti A, Churchill GC, Palombi F, Ziparo E, Sorrentino V, Galione A, Filippini A.** A pivotal role for cADPR-mediated $Ca^{2+}$ signaling: regulation of endothelin-induced contraction in peritubular smooth muscle cells. *FASEB J.* 2002; 16: 697–705.
33. **Fellner SK, Parker LA.** Endothelin B receptor $Ca^{2+}$ signaling in shark vascular smooth muscle: participation of inositol trisphosphate and ryanodine receptors. *J Exp Biol.* 2004; 207: 3411–7.
34. **Lee HC.** Nicotinic acid adenine dinucleotide phosphate (NAADP)-mediated calcium signaling. *J Biol Chem.* 2005; 280: 33693–6.
35. **Yamasaki M, Churchill GC, Galione A.** Calcium signalling by nicotinic acid adenine dinucleotide phosphate (NAADP). *FEBS J* 2005; 272: 4598–606.
36. **Higashida H, Zhang J, Hashii M, Shintaku M, Higashida C, Takeda Y.** Angiotensin II stimulates cyclic ADP-ribose formation in neonatal rat cardiac myocytes. *Biochem J.* 2000; 352 Pt 1: 197–202.
37. **Fellner SK, Arendshorst WJ.** Angiotensin II $Ca^{2+}$ signaling in rat afferent arterioles: stimulation of cyclic ADP ribose and IP3 pathways. *Am J Physiol Renal Physiol.* 2005; 288: F785–91.
38. **Yu JZ, Zhang DX, Zou AP, Campbell WB, Li PL.** Nitric oxide inhibits Ca(2+) mobilization through cADP-ribose signaling in coronary arterial smooth muscle cells. *Am J Physiol Heart Circ Physiol.* 2000; 279: H873–81.
39. **White TA, Walseth TF, Kannan MS.** Nitric oxide inhibits ADP-ribosyl cyclase through a cGMP-independent pathway in airway smooth muscle. *Am J Physiol Lung Cell Mol Physiol.* 2002; 283: L1065–71.
40. **Willmott NJ, Galione A, Smith PA.** A cADP-ribose antagonist does not inhibit secretagogue-, caffeine- and nitric oxide-induced $Ca^{2+}$ responses in rat pancreatic beta-cells. *Cell Calcium.* 1995; 18: 411–9.
41. **Looms DK, Tritsaris K, Nauntofte B, Dissing S.** Nitric oxide and cGMP activate $Ca^{2+}$-release processes in rat parotid acinar cells. *Biochem J.* 2001; 355: 87–95.
42. **Willmott N, Sethi JK, Walseth TF, Lee HC, White AM, Galione A.** Nitric oxide-induced mobilization of intracellular calcium *via* the cyclic ADP-ribose signaling pathway. *J Biol Chem.* 1996; 271: 3699–705.
43. **Katusic ZS.** Vascular endothelial dysfunction: does tetrahydrobiopterin play a role? *Am J Physiol Heart Circ Physiol.* 2001; 281: H981–6.
44. **Vasquez-Vivar J, Kalyanaraman B, Martasek P.** The role of tetrahydrobiopterin in superoxide generation

from eNOS: enzymology and physiological implications. *Free Radic Res.* 2003; 37: 121–7.
45. **Yi FX, Zhang AY, Campbell WB, Zou AP, Van Breemen C, Li PL.** Simultaneous *in situ* monitoring of intracellular $Ca^{2+}$ and NO in endothelium of coronary arteries. *Am J Physiol Heart Circ Physiol.* 2002; 283: H2725–32.
46. **Deshpande DA, Walseth TF, Panettieri RA, Kannan MS.** CD38/cyclic ADP-ribose-mediated $Ca^{2+}$ signaling contributes to airway smooth muscle hyper-responsiveness. *FASEB J.* 2003; 17: 452–4.
47. **Higashida H, Egorova A, Hoshi N, Noda M.** Streptozotocin, an inducer of NAD+ decrease, attenuates M-potassium current inhibition by ATP, bradykinin, angiotensin II, endothelin 1 and acetylcholine in NG108-15 cells. *FEBS Lett.* 1996; 379: 236–8.
48. **Zhang AY, Yi F, Teggatz EG, Zou AP, Li PL.** Enhanced production and action of cyclic ADP-ribose during oxidative stress in small bovine coronary arterial smooth muscle. *Microvasc Res.* 2004; 67: 159–67.
49. **Kumasaka S, Shoji H, Okabe E.** Novel mechanisms involved in superoxide anion radical-triggered $Ca^{2+}$ release from cardiac sarcoplasmic reticulum linked to cyclic ADP-ribose stimulation. *Antioxid Redox Signal.* 1999; 1: 55–69.
50. **Geiger J, Zou AP, Campbell WB, Li PL.** Inhibition of cADP-ribose formation produces vasodilation in bovine coronary arteries. *Hypertension* 2000; 35: 397–402.
51. **Zhang DX, Harrison MD, Li PL.** Calcium-induced calcium release and cyclic ADP-ribose-mediated signaling in the myocytes from small coronary arteries. *Microvasc Res.* 2002; 64: 339–48.
52. **Kuemmerle JF, Murthy KS, Makhlouf GM.** Longitudinal smooth muscle of the mammalian intestine. A model for $Ca^{2+}$ signaling by cADPR. *Cell Biochem Biophys.* 1998; 28: 31–44.
53. **Lee HC.** A unified mechanism of enzymatic synthesis of two calcium messengers: cyclic ADP-ribose and NAADP. *Biol Chem.* 1999; 380: 785–93.
54. **Berruet L, Muller-Steffner H, Schuber F.** Occurrence of bovine spleen CD38/$NAD^+$ glycohydrolase disulfide-linked dimers. *Biochem Mol Biol Int.* 1998; 46: 847–55.
55. **Vu CQ, Coyle DL, Tai HH, Jacobson EL, Jacobson MK.** Intramolecular ADP-ribose transfer reactions and calcium signalling. Potential role of 2'-phospho-cyclic ADP-ribose in oxidative stress. *Adv Exp Med Biol.* 1997; 419: 381–8.
56. **Tohgo A, Munakata H, Takasawa S, Nata K, Akiyama T, Hayashi N, Okamoto H:** Lysine 129 of CD38 (ADP-ribosyl cyclase/cyclic ADP-ribose hydrolase) participates in the binding of ATP to inhibit the cyclic ADP-ribose hydrolase. *J Biol Chem.* 1997; 272: 3879–82.
57. **Takasawa S, Tohgo A, Noguchi N, Koguma T, Nata K, Sugimoto T, Yonekura H, Okamoto H.** Synthesis and hydrolysis of cyclic ADP-ribose by human leukocyte antigen CD38 and inhibition of the hydrolysis by ATP. *J Biol Chem.* 1993; 268: 26052–4.
58. **Galione A, White A, Willmott N, Turner M, Potter BV, Watson SP.** cGMP mobilizes intracellular $Ca^{2+}$ in sea urchin eggs by stimulating cyclic ADP-ribose synthesis. *Nature* 1993; 365: 456–9.
59. **Chini EN, Beers KW, Chini CC, Dousa TP.** Specific modulation of cyclic ADP-ribose-induced $Ca^{2+}$ release by polyamines. *Am J Physiol.* 1995; 269: C1042–7.
60. **Wilson HL, Galione A.** Differential regulation of nicotinic acid-adenine dinucleotide phosphate and cADP-ribose production by cAMP and cGMP. *Biochem J* 1998; 331: 837–43.
61. **Higashida H, Egorova A, Higashida C, Zhong ZG, Yokoyama S, Noda M, Zhang JS.** Sympathetic potentiation of cyclic ADP-ribose formation in rat cardiac myocytes. *J Biol Chem.* 1999; 274: 33348–54.
62. **Wu Y, Kuzma J, Marechal E, Graeff R, Lee HC, Foster R, Chua NH.** Abscisic acid signaling through cyclic ADP-ribose in plants. *Science* 1997; 278: 2126–30.
63. **Morad M, Suzuki YJ.** Redox regulation of cardiac muscle calcium signaling. *Antioxid Redox Signal* 2000; 2: 65–71.
64. **Chidambaram N, Wong ET, Chang CF.** Differential oligomerization of membrane-bound CD38/ADP-ribosyl cyclase in porcine heart microsomes. *Biochem Mol Biol Int.* 1998; 44: 1225–33.
65. **Guida L, Franco L, Zocchi E, De Flora A.** Structural role of disulfide bridges in the cyclic ADP-ribose related bifunctional ectoenzyme CD38. *FEBS Lett.* 1995; 368: 481–4.
66. **Tohgo A, Takasawa S, Noguchi N, Koguma T, Nata K, Sugimoto T, Furuya Y, Yonekura H, Okamoto H.** Essential cysteine residues for cyclic ADP-ribose synthesis and hydrolysis by CD38. *J Biol Chem.* 1994; 269: 28555–7.
67. **Yu J, Chait BT, Toll L, Kreek MJ.** Nociceptin *in vitro* biotransformation in human blood. Peptides 1996; 17: 873-6.
68. **Li PL, Tang WX, Valdivia HH, Zou AP, Campbell WB.** cADP-ribose activates reconstituted ryanodine receptors from coronary arterial smooth muscle. *Am J Physiol Heart Circ Physiol.* 2001; 280: H208–15.
69. **Kannan MS, Fenton AM, Prakash YS, Sieck GC.** Cyclic ADP-ribose stimulates sarcoplasmic reticulum calcium release in porcine coronary artery smooth muscle. *Am J Physiol.* 1996; 270: H801–6.
70. **Lahouratate P, Guibert J, Faivre JF.** cADP-ribose releases $Ca^{2+}$ from cardiac sarcoplasmic reticulum independently of ryanodine receptor. *Am J Physiol.* 1997; 273: H1082–9.
71. **Sitsapesan R, McGarry SJ, Williams AJ.** Cyclic ADP-ribose competes with ATP for the adenine nucleotide binding site on the cardiac ryanodine receptor Ca(2+)-release channel. *Circ Res.* 1994; 75: 596–600.
72. **Bolton TB, Gordienko DV.** Confocal imaging of calcium release events in single smooth muscle cells. *Acta Physiol Scand.* 1998; 164: 567–75.
73. **Ruehlmann DO, Lee CH, Poburko D, van Breemen C.** Asynchronous Ca(2+) waves in intact venous smooth muscle. *Circ Res.* 2000; 86: E72–9.
74. **Kamishima T, McCarron JG.** Regulation of the cytosolic $Ca^{2+}$ concentration by $Ca^{2+}$ stores in single

smooth muscle cells from rat cerebral arteries. J Physiol 1997; 501: 497–508.

75. **Vandier C, Delpech M, Rebocho M, Bonnet P.** Hypoxia enhances agonist-induced pulmonary arterial contraction by increasing calcium sequestration. *Am J Physiol.* 1997; 273: H1075–81.
76. **Jabr RI, Toland H, Gelband CH, Wang XX, Hume JR.** Prominent role of intracellular $Ca^{2+}$ release in hypoxic vasoconstriction of canine pulmonary artery. *Br J Pharmacol.* 1997; 122: 21–30.
77. **Teggatz EG, Zhang G, Zhang AY, Yi F, Li N, Zou AP, Li PL.** Role of cyclic ADP-ribose in Ca2+-induced Ca2+ release and vasoconstriction in small renal arteries. *Microvasc Res.* 2005; 70: 65–75.
78. **Kannan MS, Prakash YS, Brenner T, Mickelson JR, Sieck GC.** Role of ryanodine receptor channels in $Ca^{2+}$ oscillations of porcine tracheal smooth muscle. *Am J Physiol.* 1997; 272: L659–64.
79. **Nelson MT, Cheng H, Rubart M, Santana LF, Bonev AD, Knot HJ, Lederer WJ.** Relaxation of arterial smooth muscle by calcium sparks. *Science* 1995; 270: 633–7.
80. **Jaggar JH, Porter VA, Lederer WJ, Nelson MT:** Calcium sparks in smooth muscle. *Am J Physiol Cell Physiol.* 2000; 278: C235–56.
81. **Lukyanenko V, Gyorke S.** $Ca^{2+}$ sparks and $Ca^{2+}$ waves in saponin-permeabilized rat ventricular myocytes. *J Physiol.* 1999; 521: 575–85.
82. **Bychkov R, Gollasch M, Ried C, Luft FC, Haller H:** Regulation of spontaneous transient outward potassium currents in human coronary arteries. *Circulation* 1997; 95: 503–10.
83. **Cui Y, Galione A, Terrar DA.** Effects of photoreleased cADP-ribose on calcium transients and calcium sparks in myocytes isolated from guinea-pig and rat ventricle. *Biochem J.* 1999; 342: 269–73.
84. **Franzini-Armstrong C, Protasi F.** Ryanodine receptors of striated muscles: a complex channel capable of multiple interactions. *Physiol Rev.* 1997; 77: 699–729.
85. **Wang YX, Zheng YM, Mei QB, Wang QS, Collier ML, Fleischer S, Xin HB, Kotlikoff MI.** FKBP12.6 and cADPR regulation of $Ca^{2+}$ release in smooth muscle cells. *Am J Physiol Cell Physiol.* 2004; 286: C538–46.
86. **Tang WX, Chen YF, Zou AP, Campbell WB, Li PL.** Role of FKBP12.6 in cADPR-induced activation of reconstituted ryanodine receptors from arterial smooth muscle. *Am J Physiol Heart Circ Physiol.* 2002; 282: H1304–10.
87. **Noguchi N, Takasawa S, Nata K, Tohgo A, Kato I, Ikehata F, Yonekura H, Okamoto H.** Cyclic ADP-ribose binds to FK506-binding protein 12.6 to release Ca2+ from islet microsomes. *J Biol Chem.* 1997; 272: 3133–6.
88. **Chen YF, Zhang AY, Zou AP, Campbell WB, Li PL:** Protein methylation activates reconstituted ryanodine receptor-ca release channels from coronary artery myocytes. *J Vasc Res.* 2004; 41: 229–40.
89. **Evans AM, Wyatt CN, Kinnear NP, Clark JH, Blanco EA:** Pyridine nucleotides and calcium signalling in arterial smooth muscle: from cell physiology to pharmacology. *Pharmacol Ther.* 2005; 107: 286–313.
90. **Deshpande DA, White TA, Dogan S, Walseth TF, Panettieri RA, Kannan MS.** CD38/cyclic ADP-ribose signaling: role in the regulation of calcium homeostasis in airway smooth muscle. *Am J Physiol Lung Cell Mol Physiol.* 2005; 288: L773–88.
91. **Chini EN, de Toledo FG, Thompson MA, Dousa TP.** Effect of estrogen upon cyclic ADP ribose metabolism: beta-estradiol stimulates ADP ribosyl cyclase in rat uterus. *Proc Natl Acad Sci USA.* 1997; 94: 5872–6.
92. **de Toledo FG, Cheng J, Dousa TP.** Retinoic acid and triiodothyronine stimulate ADP-ribosyl cyclase activity in rat vascular smooth muscle cells. *Biochem Biophys Res Commun.* 1997; 238: 847–50.
93. **Morita K, Kitayama S, Dohi T.** Stimulation of cyclic ADP-ribose synthesis by acetylcholine and its role in catecholamine release in bovine adrenal chromaffin cells. *J Biol Chem.* 1997; 272: 21002–9.
94. **Prakash YS, Kannan MS, Walseth TF, Sieck GC.** Role of cyclic ADP-ribose in the regulation of $[Ca^{2+}]_i$ in porcine tracheal smooth muscle. *Am J Physiol.* 1998; 274: C1653–60.
95. **Dipp M, Evans AM.** Cyclic ADP-ribose is the primary trigger for hypoxic pulmonary vasoconstriction in the rat lung *in situ*. *Circ Res.* 2001; 89: 77–83.
96. **Schnackenberg CG.** Oxygen radicals in cardiovascular-renal disease. *Curr Opin Pharmacol.* 2002; 2: 121–5.
97. **Rathaus M, Bernheim J.** Oxygen species in the microvascular environment: regulation of vascular tone and the development of hypertension. *Nephrol Dial Transplant.* 2002; 17: 216–21.
98. **Chakraborti T, Ghosh SK, Michael JR, Batabyal SK, Chakraborti S.** Targets of oxidative stress in cardiovascular system. *Mol Cell Biochem.* 1998; 187: 1–10.
99. **Wolin MS.** Interactions of oxidants with vascular signaling systems. *Arterioscler Thromb Vasc Biol.* 2000; 20: 1430–42.
100. **Kawakami M, Okabe E.** Superoxide anion radical-triggered $Ca^{2+}$ release from cardiac sarcoplasmic reticulum through ryanodine receptor $Ca^{2+}$ channel. *Mol Pharmacol.* 1998; 53: 497–503.
101. **Kourie JI.** Interaction of reactive oxygen species with ion transport mechanisms. *Am J Physiol.* 1998; 275: C1–24.
102. **Suzuki YJ, Ford GD.** Redox regulation of signal transduction in cardiac and smooth muscle. *J Mol Cell Cardiol* 1999; 31: 345–53.
103. **Graeff RM, Franco L, De Flora A, Lee HC.** Cyclic GMP-dependent and -independent effects on the synthesis of the calcium messengers cyclic ADP-ribose and nicotinic acid adenine dinucleotide phosphate. *J Biol Chem.* 1998; 273: 118–25.
104. **Kannan MS, Prakash YS, Johnson DE, Sieck GC.** Nitric oxide inhibits calcium release from sarcoplasmic reticulum of porcine tracheal smooth muscle cells. *Am J Physiol* 1997; 272: L1–7.
105. **Falck JR, Krishna UM, Reddy YK, Kumar PS, Reddy KM, Hittner SB, Deeter C, Sharma KK, Gauthier KM, Campbell WB:** Comparison of

vasodilatory properties of 14,15-EET analogs: structural requirements for dilation. *Am J Physiol Heart Circ Physiol.* 2003; 284: H337–49.

106. **Archer SL, Gragasin FS, Wu X, Wang S, McMurtry S, Kim DH, Platonov M, Koshal A, Hashimoto K, Campbell WB, Falck JR, Michelakis ED:** Endothelium-derived hyperpolarizing factor in human internal mammary artery is 11,12-epoxyeicosatrienoic acid and causes relaxation by activating smooth muscle BK(Ca) channels. *Circulation* 2003; 107: 769–76.
107. **Zhang AY, Teggatz EG, Zou AP, Campbell WB, Li PL.** Endostatin uncouples NO and $Ca^{2+}$ response to bradykinin through enhanced $O2^{-\cdot}$ production in the intact coronary endothelium. *Am J Physiol Heart Circ Physiol* 2005; 288: H686–94.
108. **Tiruppathi C, Minshall RD, Paria BC, Vogel SM, Malik AB.** Role of $Ca^{2+}$ signaling in the regulation of endothelial permeability. *Vascul Pharmacol.* 2002; 39: 173–85.
109. **Putney JW Jr.** TRP, inositol 1,4,5-trisphosphate receptors, and capacitative calcium entry. *Proc Natl Acad Sci USA.* 1999; 96: 14669–71.
110. **Freichel M, Suh SH, Pfeifer A, Schweig U, Trost C, Weissgerber P, Biel M, Philipp S, Freise D, Droogmans G, Hofmann F, Flockerzi V, Nilius B:** Lack of an endothelial store-operated $Ca^{2+}$ current impairs agonist-dependent vasorelaxation in TRP4-/- mice. *Nat Cell Biol.* 2001; 3: 121–7.
111. **Faehling M, Kroll J, Fohr KJ, Fellbrich G, Mayr U, Trischler G, Waltenberger J:** Essential role of calcium in vascular endothelial growth factor A-induced signaling: mechanism of the antiangiogenic effect of carboxyamidotriazole. *FASEB J.* 2002; 16: 1805–7.
112. **Graier WF, Schmidt K, Kukovetz WR.** Bradykinin-induced Ca(2+)-influx into cultured aortic endothelial cells is not regulated by inositol 1,4,5-trisphosphate or inositol 1,3,4,5-tetrakisphosphate. *Second Messengers Phosphoproteins* 1991; 13: 187–97.
113. **Pasyk E, Inazu M, Daniel EE.** CPA enhances $Ca^{2+}$ entry in cultured bovine pulmonary arterial endothelial cells in an $IP_3$-independent manner. *Am J Physiol.* 1995; 268: H138–46.
114. **Fleming I, Busse R.** Tyrosine phosphorylation and bradykinin-induced signaling in endothelial cells. *Am J Cardiol.* 1997; 80: 102A–09A.
115. **Higashida H, Yokoyama S, Hoshi N, Hashii M, Egorova A, Zhong ZG, Noda M, Shahidullah M, Taketo M, Knijnik R, Kimura Y, Takahashi H, Chen XL, Shin Y, Zhang JS.** Signal transduction from bradykinin, angiotensin, adrenergic and muscarinic receptors to effector enzymes, including ADP-ribosyl cyclase. *Biol Chem.* 2001; 382: 23–30.