

## Vascular physiology of a Ca<sup>2+</sup> mobilizing second messenger - cyclic ADP - ribose

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### Abstract

Cyclic ADP-ribose (cADPR) is a novel Ca<sup>2+</sup> mobilizing second messenger, which is capable of inducing Ca<sup>2+</sup> 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<sup>2+</sup> sparks, Ca<sup>2+</sup> waves or oscillations, Ca<sup>2+</sup>-induced Ca<sup>2+</sup> 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<sup>2+</sup> 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<sup>2+</sup> 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<sup>2+</sup> 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

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## 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  $\text{Ca}^{2+}$ -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  $[\text{Ca}^{2+}]_i$  in vascular cells, particularly in VSMCs, is primarily controlled by the influx of extracellular  $\text{Ca}^{2+}$  and the mobilization of  $\text{Ca}^{2+}$  from intracellular stores. It is generally accepted that the influx of extracellular  $\text{Ca}^{2+}$  may initiate vasoconstriction or stimulate  $\text{Ca}^{2+}$  release from the sarcoplasmic reticulum (SR) through  $\text{Ca}^{2+}$ -induced  $\text{Ca}^{2+}$  release (CICR) and thereby lead to vasoconstriction. It has also been well documented that the  $\text{Ca}^{2+}$  mobilization importantly mediates the vasomotor response to many vasoactive substances such as hormones, autocrine or paracrine, and other stimuli. This intracellular  $\text{Ca}^{2+}$  release and consequent vasomotor responses play a critical role in the homeostasis of blood circulation.

Since the 1980s, inositol 1,4,5-tris-phosphate ( $\text{IP}_3$ ) 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  $\text{A}_2$  and others. In this process, the agonists activate phospholipase C, which converts phosphatidylinositol-4,5-bisphosphate ( $\text{PIP}_2$ ) into  $\text{IP}_3$  and diacylglycerol (DAG).  $\text{IP}_3$  induces  $\text{Ca}^{2+}$  release through  $\text{IP}_3$  receptors ( $\text{IP}_3\text{R}$ ) on the SR and thereby leads to the rise of  $[\text{Ca}^{2+}]_i$ , producing vasoconstriction [3–6]. Although  $\text{IP}_3$  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  $\text{Ca}^{2+}$  release independently of the  $\text{IP}_3$  pathway. For example, caffeine,  $\text{Ca}^{2+}$ , 5-hydroxytryptamine (5-HT), acetylcholine (ACh), endothelin-1, and prostaglandin  $\text{F}_{2\alpha}$  produce  $\text{Ca}^{2+}$  mobilization from the SR through activation of ryanodine receptors (RyR) on the SR, which is not related to the action

of  $\text{IP}_3$ . cADPR as a novel  $\text{Ca}^{2+}$  mobilizing second messenger has now been demonstrated to be another mediator for the action of different agonists or stimuli independent of  $\text{IP}_3$ -mediated  $\text{Ca}^{2+}$  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  $\text{Ca}^{2+}$  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  $\text{Ca}^{2+}$  release *in vitro* using single cell  $\text{Ca}^{2+}$  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<sup>-/-</sup> 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<sup>-/-</sup> 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  $\mu\text{m}$  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<sup>+</sup> by ADP-ribosylcyclase and may be metabolized into ADPR by the hydrolase activity of the same enzyme, which could be CD38 associated multi-functional 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 par-

ticularly interested in the response of this Ca<sup>2+</sup> 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<sub>1</sub>, M<sub>2</sub>, M<sub>3</sub> and M<sub>4</sub> [27]. In NG108-15 neuronal cells, activation of M<sub>1</sub> mAChRs increases the production of cADPR [28]. In our experiments, incubation of coronary VSMCs with oxotremorine, a specific M<sub>1</sub> mAChR agonist, produced a time- and concentration-dependent activation of ADP-ribosylcyclase, which was blocked by its inhibitor, nicotinamide, and a specific M<sub>1</sub> 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<sub>2</sub>-receptor since ACh-induced cADPR response was attenuated by a selective M<sub>2</sub> 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<sub>A</sub>-R or ET<sub>B</sub>-R. Using nicotinamide or Zn<sup>2+</sup> 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<sup>2+</sup> 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  $IP_3$ -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- $\beta$ -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  $\mu M$ ) 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  $\beta$ -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 non-vascular 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  $O_2^-$ , 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  $\beta$ -escin-permeabilized VSMCs freshly isolated from porcine coronary arteries. In  $\alpha$ -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  $IP_3$ R 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  $\mu$ M), 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  $\text{Ca}^{2+}$  uptake and release in the SR constitute intracellular  $\text{Ca}^{2+}$  waves or oscillations. By  $\text{Ca}^{2+}$  waves,  $\text{Ca}^{2+}$  is guided from the plasma membrane to myofilaments, instead of direct diffusion from the plasma membrane through the cytoplasm. Therefore, the  $\text{Ca}^{2+}$  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,  $\text{CaCl}_2$ , Bay K 8644 ( $\text{Ca}^{2+}$  channel activator) and caffeine-induced  $\text{Ca}^{2+}$  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  $\text{Ca}^{2+}$  (5 mM  $\text{CaCl}_2$ ) 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  $\text{Ca}^{2+}$  oscillations and cADPR blocker, 8-amino-cADPR diminished Ach-induced  $[\text{Ca}^{2+}]_i$  oscillations in porcine tracheal smooth muscle cells [78]. Taken together, these results demonstrate that cADPR is necessary for CICR and intracellular  $\text{Ca}^{2+}$  oscillation and RyR are the mechanistic link between cADPR and CICR or  $\text{Ca}^{2+}$  oscillations.

Based on these results, two mechanistic models have been proposed to elucidate the role of endogenous cADPR in mediating vascular  $\text{Ca}^{2+}$  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  $\text{Ca}^{2+}$  release from the SR through the RyR. This activation of ADP-ribosylcyclase may also occur when intracellular  $\text{Ca}^{2+}$  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  $\text{Ca}^{2+}$  influx. The relative contribution of these two mechanisms to the vascular reactivity may vary depending upon the concentrations of intracellular

cADPR,  $[\text{Ca}^{2+}]_i$ , and calmodulin and the functional status of RyRs in different cells.

### **$\text{Ca}^{2+}$ sparks and STOCs**

$\text{Ca}^{2+}$  sparks are local and spontaneous transient  $\text{Ca}^{2+}$  release through the RyRs on the SR.  $\text{Ca}^{2+}$  sparks occur in the relation to  $\text{Ca}^{2+}$ -dependent  $\text{K}^+$  ( $\text{K}_{\text{Ca}}$ ) channel activation and muscle relaxation in VSM. A spontaneous  $\text{Ca}^{2+}$  spark in a superficial area close to the cell membrane activates 10–100  $\text{K}_{\text{Ca}}$  channels (namely, spontaneous transient outward currents (STOCs)) and induces membrane hyperpolarization, which reduces  $\text{Ca}^{2+}$  channel activity, resulting in decrease in vascular tone [79, 80].  $\text{Ca}^{2+}$  sparks occur by spontaneous opening of RyR/ $\text{Ca}^{2+}$  release channels in smooth muscle cells. Therefore, the sensitivity of RyRs may largely determine the occurrence of  $\text{Ca}^{2+}$  sparks. Since cADPR is also reported to sensitize the RyR on the SR, it is not surprising that cADPR participates in the  $\text{Ca}^{2+}$  sparks. Recent studies have reported that exogenous cADPR increases  $\text{Ca}^{2+}$  sparks in cardiac cells [81]. However, in VSM cells, ryanodine and cADPR transiently enhances  $\text{Ca}^{2+}$  sparks and then produces a sustained inhibition of  $\text{Ca}^{2+}$  sparks [80, 82, 83]. This sustained inhibition of  $\text{Ca}^{2+}$  sparks may be due to the depletion of  $\text{Ca}^{2+}$  stores [80, 82, 83]. In addition to its effect to increase global  $[\text{Ca}^{2+}]_i$ , cADPR-induced  $\text{Ca}^{2+}$  depletion of the superficial SR, inhibition of  $\text{Ca}^{2+}$  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,  $\text{Ca}^{2+}$  release from the SR is inhibited when FKBP12.6 is bound to the RyR, and dissociation of

FKBP 12.6 from the RyR releases  $\text{Ca}^{2+}$ . 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/ $\text{Ca}^{2+}$  release channels on lipid bilayer membrane. Ligand binding experiments have demonstrated that cADPR can directly 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].  $\text{Ca}^{2+}$  influx by  $\text{CaCl}_2$  significantly decreased this colocalization and 8-Br-cADPR reversed  $\text{CaCl}_2$  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  $\text{Ca}^{2+}$  release from the SR in VSMCs.

Interestingly, methylation of FKBP12.6 by arginine N-methyltransferase (PRMT1) activates RyR/ $\text{Ca}^{2+}$  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/ $\text{Ca}^{2+}$  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 as vascular tone. Intracellular  $\text{Ca}^{2+}$  importantly contributes to the production of this “resting” vascular tone. Under resting conditions,  $[\text{Ca}^{2+}]_i$  in VSM is dependent upon the  $\text{Ca}^{2+}$  influx, spontaneous brief releasing bursts of  $\text{Ca}^{2+}$  from the SR into the cytoplasm and CICR [1–4]. cADPR participates in the control of the resting  $\text{Ca}^{2+}$  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 con-

ceivable 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  $\text{Ca}^{2+}$ -ATPase inhibitor, thapsigargin, decreases the arterial diameter, and CICR blocker, tetracaine and cADPR antagonist, 8-Br-cADPR slightly dilates these arteries, suggesting that  $[\text{Ca}^{2+}]_i$  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  $\text{Ca}^{2+}$  free medium in response to a variety of agonists such as acetylcholine, endothelin,  $\text{PGF}_{2\alpha}$  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  $M_1$  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  $\text{Ca}^{2+}$  release from the SR in VSMCs. Oxotremorine produces a rapid  $\text{Ca}^{2+}$  release in single VSMCs bathed with  $\text{Ca}^{2+}$ -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<sup>-/-</sup> mice, oxotremorine produced much smaller vasoconstrictor response than in the same arteries from wild type mice [23]. Further, oxotremorine-induced intracellular Ca<sup>2+</sup> increase was significantly lowered in freshly isolated septal arterial VSMCs from CD38<sup>-/-</sup> mice than in VSMCs isolated from wild mice [23]. Taken together, these results provide direct evidence that endogenous cADPR contributes to oxotremorine-induced Ca<sup>2+</sup> 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<sup>2+</sup> *via* activation of ET<sub>A</sub> and/or ET<sub>B</sub> receptors in different vascular beds. Recent studies have shown that endothelin-1-induced Ca<sup>2+</sup> response is associated with cADPR/RyR signaling. In rat mesenteric arteries, Giulumian *et al.* reported that endothelin-1-induced Ca<sup>2+</sup> increase and vasoconstriction were significantly attenuated by ADP-ribosylcyclase inhibitor nicotinamide and RyR Ca<sup>2+</sup> 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<sub>A</sub>- and ET<sub>B</sub>-mediated Ca<sup>2+</sup> signaling were completely abolished by cADPR antagonist, 8-NH<sub>2</sub>-cADPR [32]. In porcine airway smooth muscle, White *et al.* showed that endothelin-1-induced Ca<sup>2+</sup> response was inhibited by 8-Br-cADPR [30]. In airway VSMCs isolated from CD38<sup>-/-</sup> mice, Deshpande *et al.* found that the intracellular Ca<sup>2+</sup> 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<sup>2+</sup> signaling may importantly participate in endothelin-1-induced vasoconstrictor response in these vascular beds.

#### **Vasoconstriction induced by Ca<sup>2+</sup> entry associated with cell membrane depolarization**

In various arteries, cell membrane depolarization and activation of L-type Ca<sup>2+</sup> channels were found to produce the influx of extracellular Ca<sup>2+</sup> and thereby increases [Ca<sup>2+</sup>]<sub>i</sub>, leading to vasoconstriction.

In this process, the entry of Ca<sup>2+</sup> is further amplified by CICR, thereby producing an increase in global [Ca<sup>2+</sup>] throughout the cytoplasm and nucleus and consequent vasoconstriction [1, 2]. In spite of the essential effect of Ca<sup>2+</sup> influx, the amplification of Ca<sup>2+</sup> signal or Ca<sup>2+</sup> propagation within VSMCs also plays a critical role in the vasoconstriction in response to membrane depolarization and activation of membrane Ca<sup>2+</sup> channels. The SR Ca<sup>2+</sup>-ATPase inhibitor, thapsigargin, significantly blocks KCl depolarization-induced vasoconstriction in coronary arteries, suggesting that Ca<sup>2+</sup> refilling and release in the SR mediated by this enzyme are critical for KCl-induced Ca<sup>2+</sup> 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<sub>3</sub> blocker, xestospongine 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<sup>2+</sup> channel activator, Bay K8644 and CaCl<sub>2</sub> to stimulate Ca<sup>2+</sup> influx, the vasoconstrictor effects are also attenuated by thapsigargin, tetracaine or 8-Br-cADPR [51]. This role of CICR in vasoconstrictor response to Ca<sup>2+</sup> 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<sup>2+</sup> signaling is of importance in amplification of intracellular Ca<sup>2+</sup> 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<sub>2</sub>-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<sup>2+</sup> homeostasis. In previous studies, ROS have been shown to cause Ca<sup>2+</sup> mobilization in cardiac, skeletal, and smooth muscles [98, 100–102]. This ROS-induced change in Ca<sup>2+</sup> homeostasis in muscles could be mediated *via* a variety of mechanisms such as inhibition of Ca<sup>2+</sup>-ATPase of SR, modifica-

tion in gating activities of SR  $\text{Ca}^{2+}$  release channels, or alteration of  $\text{Ca}^{2+}$  release from either  $\text{IP}_3$ -sensitive or ryanodine-sensitive  $\text{Ca}^{2+}$  stores [98, 100–102]. However, the underlying mechanisms by which ROS exert their actions on  $\text{Ca}^{2+}$  homeostasis in VSM are still poorly understood.

In coronary VSMCs, cADPR antagonism, RyR and CICR blockade, and inhibition of cADPR production significantly attenuated  $\text{O}_2^{\cdot-}$ -induced  $\text{Ca}^{2+}$  release [48]. Consistently, these inhibitors or blockers of cADPR/RyR signaling pathway could attenuate  $\text{O}_2^{\cdot-}$ -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  $\text{O}_2^{\cdot-}$  production results in vasoconstriction in part through cADPR/RyR activation pathway in coronary VSMCs. These findings that ROS exert their effects on  $\text{Ca}^{2+}$  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  $\text{B}_3$ , 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  $\text{Ca}^{2+}$ -mobilizing action of  $\beta\text{-NAD}^+$ , 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  $\text{Ca}^{2+}$  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  $\text{Ca}^{2+}$  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  $\text{Ca}^{2+}$  release from the SR [42, 58, 103]. Obviously, this NO-induced rise in  $[\text{Ca}^{2+}]_i$  could not contribute to its vasodilator effect, since NO decreases  $[\text{Ca}^{2+}]_i$  in VSMCs [38, 39]. Endogenous cADPR-induced  $\text{Ca}^{2+}$  release in the resting state probably contributes to the control of basal  $[\text{Ca}^{2+}]_i$  in VSMCs, and NO decreases intracellular cADPR concentrations and thereby lowers  $[\text{Ca}^{2+}]_i$ . This cADPR-dependent changes in  $[\text{Ca}^{2+}]_i$  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  $\text{Ca}^{2+}$  release response in single VSMC, NO has no direct effect on either cADPR- or  $\text{IP}_3$ -induced  $\text{Ca}^{2+}$  release response in permeabilized cells, but inhibits KCl- and caffeine-induced  $\text{Ca}^{2+}$  release in intact cells [17, 38, 100]. It appears that NO decreases  $[\text{Ca}^{2+}]_i$  by inhibiting endogenous cADPR-mediated  $\text{Ca}^{2+}$  release, but not by direct effect on  $\text{Ca}^{2+}$  release machinery on the SR of VSMCs. This inhibitory effect of NO on cADPR-induced  $\text{Ca}^{2+}$  release has also been confirmed in porcine tracheal smooth muscle cells [69, 104]. The mechanism by which NO decreases  $[\text{Ca}^{2+}]_i$  in coronary and porcine tracheal VSMCs, but increases  $[\text{Ca}^{2+}]_i$  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 arterial 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<sup>2+</sup> release responses than using 8-Br-cADPR alone. It is concluded that this bradykinin-induced intracellular Ca<sup>2+</sup> increase and NO response is not mainly associated with IP<sub>3</sub> 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<sub>3</sub> pathway [111]. It is possible that cADPR primarily influences bradykinin-induced coronary vasodilation *via* its Ca<sup>2+</sup> release and consequent NO production. Taken together, we conclude that cADPR serves as an intracellular second messenger in mediating bradykinin-induced Ca<sup>2+</sup> 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<sup>2+</sup> 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<sup>2+</sup> release from the SR *via* RyRs and involves in the regulation of CICR and consequent Ca<sup>2+</sup> waves, producing global Ca<sup>2+</sup> increase within these cells. cADPR activates RyRs by binding to FKBP12.6 and results in dissociation of this accessory protein from RyRs, whereby Ca<sup>2+</sup> from the SR was enhanced. There is considerable evidence showing that this cADPR-mediated Ca<sup>2+</sup> signaling plays a critical role in Ca<sup>2+</sup> release and vasoconstrictor response to different agonist, oxidative stress, cell membrane depolarization, and Ca<sup>2+</sup> influx. Moreover, decrease in this cADPR-mediated Ca<sup>2+</sup> 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.

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