CD38/cyclic ADP-ribose signaling: role in the regulation of calcium homeostasis in airway smooth muscle

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Deshpande, Deepak A., Thomas A. White, Soner Dogan, Timothy F. Walseth, Reynold A. Panettieri, and Mathur S. Kannan. CD38/cyclic ADP-ribose signaling: role in the regulation of calcium homeostasis in airway smooth muscle. Am J Physiol Lung Cell Mol Physiol 288: L773–L788, 2005; doi:10.1152/ajplung.00217.2004.—The contractility of airway smooth muscle cells is dependent on dynamic changes in the concentration of intracellular calcium. Alterations in the processes involved in the regulation of intracellular calcium concentration contribute to the pathogenesis of airway diseases such as asthma. Recent studies have identified cyclic ADP-ribose as a calcium-mobilizing second messenger in airway smooth muscle cells, and modulation of the pathway involved in its metabolism results in altered calcium homeostasis and may contribute to airway hyperresponsiveness. In this review, we describe the basic mechanisms underlying the dynamics of calcium regulation and the role of CD38/cADPR, a novel pathway, in the context of airway smooth muscle function and its contribution to airway diseases such as asthma.

ryanodine receptor; calcium oscillations; inflammatory cytokines; muscarinic receptors; 12.6-kDa FK506-binding protein

Cyclical ADP-Ribose (cADPR), a nucleotide metabolite, mobilizes calcium from the intracellular stores through ryanodine receptors (RyRs). CD38, a membrane-bound glycoprotein, catalyzes the synthesis and degradation of cADPR. Studies from the laboratory and others have demonstrated that CD38/cADPR-mediated calcium signaling and calcium release through RyR channels play a vital role in the regulation of calcium homeostasis in airway smooth muscle cells. Furthermore, the role of CD38/cADPR-mediated calcium signaling has been investigated in disease conditions such as asthma, diabetes, and pulmonary vasoconstriction. The review describes the evidence for the physiological and pathophysiological role of CD38/cADPR/RyR signaling in airway smooth muscle cells.

Dynamic Calcium Regulation in Airway Smooth Muscle Cells

Airway smooth muscle contraction requires elevation of intracellular calcium. Under basal conditions, the intracellular calcium concentration ([Ca2+]i) in smooth muscle cells ranges from 100 to 200 nM (149, 150). Upon activation of airway smooth muscle by an agonist such as acetylcholine (ACh), there is a biphasic intracellular calcium response, an initial transient rise in calcium, followed by a decline to a steady-state level that remains above the basal concentration (125, 146, 148). This biphasic pattern in the global cellular calcium concentration results from influx from the extracellular space and release from intracellular stores, principally the sarcoplasmic reticulum (SR). In airway smooth muscle cells, previous studies have shown that the initial peak of the intracellular calcium response to ACh primarily reflects release from the SR, since blocking calcium influx does not inhibit this response (11, 123). The steady-state level of the intracellular calcium response has been attributed primarily to calcium influx from the extracellular space (124, 146, 148).

Several recent studies have investigated the temporal and spatial dynamics of intracellular calcium responses in airway smooth muscle cells during agonist stimulation (113, 129, 136). Using real-time confocal imaging, with its enhanced spatial and temporal characteristics, investigators have shown that exposure of airway smooth muscle cells to ACh induces regenerative and propagating intracellular calcium oscillations (113, 129, 136). The absolute amplitude and fall time of these intracellular calcium oscillations are inversely correlated to basal [Ca2+]i (136). However, the frequency and rise time of the oscillations are directly correlated to basal [Ca2+]i. In the absence of extracellular calcium, intracellular calcium oscillations are initiated, but not sustained. These observations indicate that calcium influx is only necessary to maintain these oscillations. In the presence of thapsigargin, an inhibitor of sarcoplasmic-endoplasmic reticulum calcium ATPase (SERCA) (114), there is a significant elevation of basal intracellular calcium and subsequent exposure to ACh results in attenuated intracellular calcium oscillations. The conclusion from
studies using real-time measurements that the steady-state phase of intracellular calcium oscillations is not mediated by calcium influx apparently contradicts previous conclusions. However, the findings are consistent with the hypothesis that calcium reuptake into the SR and influx of extracellular calcium are necessary for repletion of calcium stores and for the maintenance of intracellular calcium oscillations.

During maintained intracellular calcium oscillations in the presence of ACh, exposure to caffeine results in a transient intracellular calcium response and transient inhibition of intracellular calcium oscillations. Ryanodine, an antagonist of the RyR (117), abolishes intracellular calcium oscillations in response to ACh. These observations indicate that ACh-induced intracellular calcium oscillations involve repetitive calcium release and reuptake from RyR channels in the SR and that the SR calcium pool involved in ACh-induced oscillations and caffeine response may be common. However, the nature of the interaction between ACh and caffeine is not clear from these studies. Later investigations have shown that the β-NAD metabolite cADPR mediates calcium release through RyR channels in airway smooth muscle cells (see MECHANISMS OF cADPR-INDUCED CALCIUM RELEASE) (138).

Another interesting observation of studies involving real-time measurements of intracellular calcium is that the peak amplitude of the global (mean) intracellular calcium response in ACh-stimulated airway smooth muscle cells increases with agonist concentration (139). Furthermore, when intracellular calcium oscillations were measured within localized regions of a cell, these oscillations displayed agonist concentration-dependent modulation of frequency and propagation velocity. In addition, the oscillation frequency and propagation velocity appear to depend on the basal level of intracellular calcium, since the latter increased at higher agonist concentrations. Furthermore, the frequency of intracellular calcium oscillations during ACh stimulation is also augmented by the addition of cADPR, through a mechanism involving elevation of the basal calcium concentration (138).

The fact that the amplitude of the oscillations and therefore perhaps the SR pool that underlies these oscillations varies at different regions of a cell also suggests heterogeneity in terms of capacity and distribution of calcium stores, i.e., the SR. These results also reflect the following concepts: 1) the basal intracellular calcium level in an airway smooth muscle cell is a reflection of the balance between calcium influx from and efflux into the extracellular space, and calcium release and reuptake from the SR; 2) the SR calcium content within localized regions of the cell determines the absolute amplitude of calcium oscillations within that region; 3) the sensitivity of SR calcium release determines the frequency and propagation velocity of intracellular calcium oscillations; and 4) cADPR, by its capacity to increase the basal intracellular calcium levels, regulates the frequency and propagation velocity of intracellular calcium oscillations and therefore the integrated calcium response of an airway smooth muscle cell.

CALCIUM STORES AND CALCIUM-MOBILIZING SECOND MESSENGERS

In airway smooth muscle cells, the release of calcium from intracellular stores occurs through channels coupled to inositol 1,4,5-trisphosphate receptors (IP3Rs) and RyR (10, 11, 26, 85, 150). IP3R-mediated calcium release has been well documented in airway smooth muscle cells (26, 113, 150). Stimulation of airway smooth muscle cells with contractile agonists whose receptors are coupled to Gαq-type G proteins leads to activation of phospholipase C-β (PLC-β), which in turn initiates the phosphatidylinositol turnover and the production of IP3 and 1,2-diacylglycerol in airway smooth muscle cells (16, 49, 63, 152). IP3 formed in airway smooth muscle cells upon agonist stimulation mediates release of calcium from SR through IP3Rs, resulting in the elevation of [Ca2+]i. It is widely believed that in smooth muscle cells, IP3Rs mediate the initial phase of the biphasic calcium response (11, 112). In fact, inhibition of IP3 generation by exposure to an antagonist of PLC-β prevents initiation of ACh-induced intracellular calcium response. The IP3R channel is a homotetramer consisting of four subunits of ~260-kDa molecular mass each (134). The IP3R channel is regulated by calcium and by calmodulin (134). Activation of IP3Rs during agonist stimulation is critical for the initiation of calcium oscillations in airway smooth muscle cells. However, once initiated the oscillations are maintained in the presence of heparin, an IP3 antagonist. In addition to IP3Rs, calcium mobilization through RyR channels also contributes to agonist induced elevation in [Ca2+]i (85, 93, 115).

In airway smooth muscle cells, the role of RyR channels and the mechanisms involved in the activation of RyR channels has been investigated. Porcine airway smooth muscle cells express both RyR2 and RyR3 isoforms of the RyR (85), whereas human airway smooth muscle cells express RyR3 isoform of the RyR (77). In airway smooth muscle cells permeabilized with β-escin, ACh-induced intracellular calcium oscillations are sensitive to inhibition by ruthenium red, an RyR antagonist (138). In addition, the ACh-induced intracellular calcium oscillations are inhibited by ryanodine and caffeine. These results indicate that activation of RyR channels in the SR is involved in ACh-induced intracellular calcium oscillations in airway smooth muscle cells.

An observation of the studies related to agonist-induced intracellular calcium oscillations in airway smooth muscle cells is that these oscillations appear to originate in a certain localized region of the cell and propagate (139). The propagating nature of these oscillations also indicates that calcium release most likely occurs within localized regions, which then stimulates SR calcium release from adjacent regions. This calcium-induced calcium release (CICR) resulting in propagating intracellular calcium oscillations involves activation of RyR channels. However, the origin of calcium oscillations within localized regions of the cell may also reflect heterogeneity of RyR channel distribution within the airway smooth muscle cell. It may also reflect the sensitivity of the CICR process to calcium and second messengers involved in calcium release.

UNITARY CALCIUM RELEASE EVENTS IN AIRWAY SMOOTH MUSCLE CELLS: CALCIUM SPARKS

Recent studies in skeletal muscle fibers (91, 97, 163), cardiac myocytes (22, 133), and airway (131), vascular (127), and bladder (80) smooth muscle cells have described calcium sparks, which are spontaneous and localized calcium transients. These calcium sparks appear to represent unitary calcium release events and involve RyR channels in the SR. The amplitude and frequency of the calcium sparks most likely reflect respectively the number of RyR channels and their
kinetics. The synchronous activity of several RyR channels within localized regions of a cell may serve as the initiating site for propagating calcium oscillations, either occurring spontaneously or triggered in response to an agonist. The incidence of calcium sparks in airway smooth muscle cells increases in the presence of low concentrations of ryanodine or caffeine (131). Spark activity is unaffected when cells are maintained in calcium-free medium, suggesting intracellular release as the source of the sparks. Within individual airway smooth muscle cells, there are regions of high spark incidence, and exposure to ACh appears to trigger repetitive propagating calcium oscillations originating at these sites. These results support the conclusion that calcium sparks in airway smooth muscle cells represent calcium release through RyR channels and agonist-induced calcium oscillations reflect the integration of calcium release events through RyR channels.

Regardless of the underlying mechanisms involved in the initiation of calcium sparks, the functional role of calcium sparks in airway smooth muscle cells is not clearly understood. In vascular smooth muscle cells, previous studies by Nelson et al. (127) have clearly shown that calcium sparks are involved in the control of resting membrane potential. The occurrence of calcium sparks close to the plasma membrane in vascular smooth muscle cells appears to favor release of high concentration of calcium in a localized region subjacent to the plasma membrane. This results in the activation of calcium-activated potassium channels and membrane hyperpolarization, which may lead to vascular smooth muscle cell relaxation. In airway smooth muscle cells, there is no evidence that calcium-activated potassium channels contribute to membrane potential (92). On the other hand, in airway smooth muscle cells, calcium sparks, by acting as trigger sites for the initiation of spontaneous or agonist-elicted propagating intracellular calcium oscillations, may contribute to contractility. The subtype(s) of RyRs involved in the initiation of calcium sparks in airway smooth muscle cells have not been described. However, in a recent study, Ji et al. (80) have described calcium sparks in bladder smooth muscle cells obtained from wild-type mice and in 12.6-kDa FK506-binding protein null (FKBP12.6−/−) and RyR3−/− mice. The frequency, amplitude, and kinetics of spontaneous and evoked calcium sparks were altered in cells isolated from the FKBP12.6−/− mice. In smooth muscle cells isolated from RyR3−/− mice, there were normal calcium sparks and CICR. Because there is evidence that FKBP12.6 proteins selectively associate with RyR2 channels (160, 174) (see MECHANISMS OF cADPR-INDUCED CALCIUM RELEASE below), the authors concluded that calcium release through RyR2 channels contributes to the formation of spontaneous and evoked calcium sparks in bladder smooth muscle cells. The preponderance of RyR channels in porcine airway smooth muscle cells being RyR2 (85), calcium sparks in this smooth muscle may also arise due to activation of these channels, although conclusive evidence for this has not been obtained. Whether cADPR mediates the spontaneous or evoked spark activity in the airway smooth muscle cells also needs to be established.

MECHANISMS OF ACTIVATION OF THE CALCIUM RELEASE CHANNELS

In a variety of cell types activation of IP3R channels exhibits a bell-shaped dependence on calcium concentration (15, 44, 45, 119). The RyR channel on the other hand is activated by very low calcium concentrations, low pH, millimolar ATP, and millimolar concentrations of caffeine and inhibited by high concentrations of ryanodine, ruthenium red, and millimolar concentrations of calcium and magnesium. However, an endogenous ligand for the RyR channels has remained elusive. In addition, the elevation of intracellular calcium in the presence of ACh activates RyR channels through CICR. Furthermore, CICR can be modulated by additional factors. It is postulated that cADPR is one of the potential candidate molecules that is involved in the activation of RyRs and modulation of CICR (52, 101). Figure 1 summarizes the interplay of various mechanisms involved in the elevation of intracellular calcium during agonist activation of airway smooth muscle cells.

CICR: AN ENDOGENOUS CALCIUM-RELEASING MESSENGER

cADPR is the cyclized derivative of NAD metabolism (25, 105) and found in a variety of cell types (23, 24, 30, 36, 42, 51, 61, 65, 75, 76) (see MECHANISMS OF cADPR-INDUCED CALCIUM RELEASE). Recent studies in smooth muscle cells have provided evidence that calcium release through RyR channels is sensitive not only by calcium but also by cADPR (12, 81, 94, 95, 167). The observation by Clapper et al. (25) that addition of NAD to sea urchin egg microsomes results in calcium release led to the discovery of cADPR. The role of cADPR in intracellular calcium regulation has been investigated in a variety of smooth muscles.

MECHANISMS OF cADPR-INDUCED CALCIUM RELEASE

cADPR mobilizes calcium through RyRs and contributes to CICR. In sea urchin egg microsomes, calcium release by cADPR was identified to be distinct from that of IP3 (29) and sensitive to regulation by ryanodine channel modulators such as ryanodine and caffeine, demonstrating that cADPR mediates calcium release through RyR channels (52, 59, 101). Furthermore, it was identified that cADPR-mediated calcium release contributes to the potentiation of CICR mechanism through RyR (52, 166). This conclusion is supported by the observations that the intracellular calcium responses in sea urchin eggs to both calcium and CICR modulators, but not to IP3, were inhibited by procaine and ruthenium red, inhibitors of CICR. It was also demonstrated that SR Ca2+ release induced by cADPR increases as [Ca2+]i is increased (28, 132, 173), suggesting that the cADPR-induced calcium release is secondary to other mechanisms of calcium elevation in the cells.

cADPR-mediated calcium release through RyRs may involve direct activation of RyRs by cADPR or indirect activation through binding to accessory proteins (111). The direct activation of RyRs by cADPR has been demonstrated in RyRs reconstituted from coronary artery smooth muscle. However, there is little evidence for a direct activation of RyRs by cADPR in intact cells. The interpretation that cADPR activates RyR channels indirectly to bring about calcium release is supported by recent observations that several accessory proteins may be involved in the actions of cADPR. In this context, FKBP have been implicated in the actions of cADPR in some cell types, including airway and bladder smooth muscles (80, 167). FKBP12.6, a 12.6-kDa protein, has been shown to form complexes with the RyR2 isoform and regulate calcium release
Fig. 1. Intracellular mechanisms of calcium release in airway smooth muscle cell. The model describes 2 principal intracellular pathways of calcium mobilization in airway smooth muscle cells. Ligand (agonist) binding to its cognate G protein-coupled receptor results in production of inositol 1,4,5-trisphosphate (IP_3), which causes sarcoplasmic reticulum (SR) calcium release. Calcium-induced calcium release (CICR) is brought about by activation of ryanodine receptor (RyR) channels by calcium and cyclic ADP-ribose (cADPR). Cellular activity, i.e., contraction, requires elevation of intracellular calcium. 8-Bromo-cADPR (8-Br-cADPR) inhibits calcium release by cADPR through RyR channels. The mechanism by which stimulation of the G protein-coupled receptor causes activation of CD38 and production of cADPR is not clearly understood as is the process by which extracellularly generated cADPR enters the cell to bring about intracellular calcium release. ADP R, ADP-ribose; PLC-β, phospholipase-β; DAG, 1,2-diacylglycerol; PIP_2, phosphatidylinositol 4,5-bisphosphate; IP_3R, IP3 receptor; N, N-terminal end; A, B, and Y, α, β, γ subunits of G protein.

through these channels (46, 159, 160, 167). In smooth muscle cells, specific association between FKBP12.6 and RyR2, but not RyR1 or RyR3 proteins, has been described (80). Dissociation of FKBP from RyR channels is apparently involved in the activation of the channels.

The evidence for the involvement of FKBP in calcium release through RyRs came from several observations. FK506, an immunosuppressant known as tacrolimus, induces calcium release in pancreatic islets and in the presence of FK506; cADPR is incapable of inducing calcium release in the presence of FK506 (128). Initial evidence for the role of FKBP12.6 in cADPR-mediated calcium release in smooth muscle came from studies using coronary arterial smooth muscle (157). Using reconstituted RyR channels from coronary arterial smooth muscle on lipid bilayers, the authors measured the open probability of the channels upon exposure to cADPR. The open probability of RyR channels was increased by cADPR; however, the ability of cADPR to open the RyRs was abolished upon pretreatment with FK506. In airway smooth muscle, pretreatment with FK506 results in the loss of cADPR-induced calcium release and cADPR does not elicit calcium responses in airway myocytes obtained from FKBP12.6 knockout (KO) mice (167). Studies from our laboratory using RT-PCR analysis of FKBP in airway smooth muscle cells isolated from porcine trachea revealed the expression of FKBP12.6 (Deshpande DA, Kannan MS, and Walseth TF, unpublished observation). Because the predominant form of RyRs expressed in airway smooth muscle is RyR2 (85), we predict that FKBP12.6/RyR2 plays a role in the regulation of cADPR-induced calcium release in airway smooth muscle. These observations collectively suggest a role for FKBP12.6/RyR2 interaction in the regulation of cADPR-mediated calcium release in airway smooth muscle cells.

The role of other accessory proteins such as calmodulin has been investigated in the process of cADPR-mediated calcium release. The extent of SR Ca^{2+} release by cADPR through RyR channels is also influenced by calmodulin. For example, calmodulin is necessary for cADPR-induced Ca^{2+} release in sea urchin eggs and pancreatic β-cells (8, 156, 158). In sea urchin egg microsomes, calmodulin has been shown to directly interact with RyR channels to enhance cADPR-induced Ca^{2+} release by several orders of magnitude (106, 107). In pancreatic islet cells, a calmodulin kinase II has been shown to phosphorylate RyR and augment cADPR-induced Ca^{2+} release (153).

In summary, evidence has been presented that cADPR mobilizes calcium from SR through RyRs, either directly or indirectly through some accessory proteins. The concept of “cADPR-binding sites” or “cADPR receptors” needs further investigation, particularly in airway smooth muscle cells. Figure 2 describes the various activation mechanisms by which cADPR brings about SR calcium release in cells. The model is consistent with direct activation of RyR channels in the SR to cause calcium release as well as indirect activation involving FKBP12.6 and calmodulin. The effects of calmodulin can be direct or through phosphorylation of RyRs through a calmodulin kinase.

ADP RIBOSYL CYCLASE AND CD38

cADPR is synthesized from β-NAD, and the reaction is catalyzed by ADP-ribosyl cyclase. Lee and Aarhus (103, 104) identified ADP-ribosyl cyclase, the enzyme that converts β-NAD to cADPR, in the sea urchin eggs. An NAD-metabolizing enzyme initially purified from Aplysia ovotestis is a soluble protein of ~30-kDa molecular mass (65). ADP-ribosyl cyclases have also been purified from Aplysia kurodai (79) and the unicellular protist Euglena gracilis (116) and found to be similar to that isolated from Aplysia californica. However, the ADP-ribosyl cyclase isolated from E. gracilis is a membrane-bound protein of ~40-kDa. Subsequently the sequence comparison of Aplysia cyclase and biochemical analysis revealed that CD38, a membrane-bound lymphocyte antigen, possesses...
ADP-ribosyl cyclase activity and is considered as the mammalian homolog of the ADP-ribosyl cyclase (108, 151). Many studies have confirmed that ADP-ribosyl cyclase activity (145) and CD38 are present in both immune and nonimmune cells from mammalian and nonmammalian species (102). CD38, the mammalian homolog of ADP-ribosyl cyclase, also possesses cADPR hydrolase activity that catalyzes the hydrolysis of cADPR to ADP-ribose (ADPR) (55, 76, 155). CD38 is a 45-kDa protein and is associated with the cell membrane (76, 90, 128). Subsequent studies demonstrated that ADP-ribosyl cyclase and cADPR hydrolase activities are also associated with another membrane-bound protein, bone marrow stromal cell surface antigen (BST)-1 or CD157 in mammals (71). BST-1 was identified to be homologous to CD38. On the basis of the production of cADPR, Aplysia cyclases, CD38, and CD157 are grouped as "cyclases," CD38 and CD157 possess both ADP-ribosyl cyclase and cADPR hydrolase enzyme activities and are grouped as bifunctional enzymes. Both ADP-ribosyl cyclase and CD38 are classified as multifunctional enzymes, as these enzymes can utilize multiple substrates such as NAD and NADP to produce their respective products, cADPR and NAADP (102).

Although CD38 is a membrane-bound glycoprotein with the catalytic activity being associated with the extracellular domain (57, 109), recent studies have identified the presence of ADP-ribosyl cyclase activity in the intracellular organelles such as the nuclear membrane (89). Characterization of subcellular fractions from airway smooth muscle revealed that the ADP-ribosyl cyclase and cADPR hydrolase activities are primarily associated with the plasma membrane fraction (168). Furthermore, CD38 is the predominant form of cyclase found in mammals in a variety of cell types (23). A recent study using CD38-deficient mice demonstrated the presence of non-CD38-dependent production of cADPR in the brain tissue (19). This enzyme appears to be regulated by G proteins and does not possess cADPR hydrolase activity. A detailed structural and functional characterization of this novel cyclase needs to be determined.

Determining the structural details of CD38 has improved the understanding of the role of CD38 in a variety of cell types. cDNA cloning and analysis revealed that CD38 consists of an amino-terminal cytoplasmic tail of 23 amino acids, a transmembrane region of 21 amino acids, and an extracellular region of 256 amino acids (102). Comparative analysis of CD38 sequences from rat, mouse, and human has revealed the presence of 12 cysteine residues in the CD38 sequence compared with 10 found in Aplysia cyclase. By site-directed mutagenesis, it has been determined that the two additional cysteine residues in the CD38 are critical for cADPR hydrolase activity (162). Crystal structure of ADP-ribosyl cyclase and homology modeling of CD38 reveal the presence of disulfide linkages between the cysteine residues (102). More recent studies identified the amino acid residues that are critical in Aplysia cyclase for ADP-ribosyl cyclase as well as in CD38 for ADP-ribosyl cyclase and cADPR hydrolase enzyme activities (121, 122, 178).

In conclusion, on the basis of the enzyme activities, there appears to be multiple enzymes involved in the synthesis of cADPR. In airway smooth muscle cells, the evidence supports a plasma membrane-associated enzyme that is an integral part of CD38, with very little, if any, evidence for localization of enzyme activities in other subcellular fractions. Figure 3 describes the metabolism of cADPR in most mammalian cell types. In these cells, CD38 is truly a bifunctional protein with both ADP-ribosyl cyclase and cADPR hydrolase activities, involved in the conversion of β-NAD to cADPR and the hydrolysis of cADPR to ADPR, respectively.

CD38 GENE ORGANIZATION AND REGULATION OF EXPRESSION

The gene encoding CD38 (denoted as cd38) has been localized on chromosomes 4 and 5 in humans and mouse,
Fig. 3. cADPR metabolism in airway smooth muscle cell. CD38, a 45-kDa transmembrane glycoprotein, is a bifunctional protein and has both ADP-ribosyl cyclase and cADPR hydrolase activities. In airway smooth muscle cells, CD38 is associated with the plasma membrane. ADP-ribosyl cyclase converts β-NAD to cADPR. cADPR is converted to ADPR by cADPR hydrolase.

respectively (42, 43). CD38 is ubiquitously expressed in a variety of cell types, and its expression is highly regulated. There are multiple levels at which CD38 expression can be modulated, and regulation at the transcriptional level is one of the key determinants of CD38 expression. cd38 comprises eight exons with no TATA or CAAT box sequences in the 5′-flanking region (126). A GC-rich region has been identified that may act as the promoter region in the regulation of CD38 expression. Several hormones, cytokines, retinoic acid, cAMP, and vitamin D3 are known to modulate the expression of CD38. In several of these factors, regulation of CD38 expression by retinoic acid has been the most characterized. The results reveal the presence of a retinoic acid response element in the first intron of the cd38. The mechanisms by which other agents regulate the expression of CD38 need to be ascertained. However, analysis of promoter region of CD38 gene reveals the presence of binding sites for transcription factors such as PEA-3, CP-2, and PuF (43). In addition, four immunoglobulin gene E box enhancer motifs and binding sites for other elements such as T-cell transcription factor TCF-1α, nuclear factor interleukin (IL)-6, and interferon (IFN) response factor-1 have been identified (43), suggesting the precise regulation of CD38 expression by cytokines. Furthermore, modulation of CD38 expression by IFN-α and -γ, IL-4, and IL-7 has been demonstrated (118) as well as by cytokines such as IL-1β, TNF-α, and IL-13 (32, 33). TNF-α-mediated augmentation of CD38 expression has been further confirmed by two independent studies recently (9, 21). In addition, a CpG island and Sp1 transcription factor binding site have been identified in the first exon and 5′ of the first intron, suggesting that methylation of cd38 may play a role in the process of CD38 expression (43).

Hormones such as estrogen and glucocorticoids are implicated in the regulation of CD38 expression. A glucocorticoid response element and a half palindromic motif of estrogen binding have been identified in the promoter region of CD38 (43). A study from our laboratory demonstrated the upregulation of CD38 expression by estrogen in uterine smooth muscle (36). In conclusion, CD38 expression is regulated at multiple levels and a variety of factors influence its expression. Aberrant changes in the expression of CD38 by these factors may result in pathological conditions.

CD38/cADPR SIGNALING IN [Ca2+]i, REGULATION IN SMOOTH MUSCLE CELLS

Multiples lines of evidence support the second messenger role for CD38/cADPR in the regulation of [Ca2+]i, in a variety of smooth muscles. Kuemmerle and Makhlouf (94) demonstrated that addition of cADPR to permeabilized longitudinal smooth muscle cells of the intestine results in a concentration-dependent increase in [Ca2+]i, and contraction. In airway smooth muscle, the role of cADPR in mediating calcium release has been investigated (138). Addition of cADPR to permeabilized porcine airway smooth muscle cells resulted in a concentration-dependent increase in [Ca2+]i, which is inhibited by 8-amino-cADPR, a cADPR antagonist, and by ruthe

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well as contraction of smooth muscle isolated from bovine trachea.

In a study involving permeabilized smooth muscle cells obtained from porcine coronary vessels, it was demonstrated that addition of cADPR resulted in the release of calcium from intracellular stores (81). Using reconstituted RyRs from coronary artery smooth muscle, Li et al. (111) have demonstrated that cADPR mobilizes calcium through RyR channels. In another recent study, Ge et al. (54) demonstrated the role of cADPR-mediated calcium release in agonist-induced calcium elevation and contractility of coronary arterial smooth muscle. In smooth muscle from seminiferous tubules, Barone and coworkers (12) demonstrated the role of cADPR in the calcium and contractile responses to endothelin (ET)-1. With freshly isolated cells and microsomes obtained from the myometrium, it has been shown that cADPR contributes to calcium and contractile responses elicited by oxytocin (9).

The studies described above point to the role of CD38 and cADPR in the regulation of intracellular calcium and contraction of smooth muscle. However, the precise mechanisms by which agonist activation results in the recruitment of the CD38/cADPR signaling pathway are not clearly understood. Furthermore, how the extracellularly generated cADPR brings about intracellular calcium release is also not well understood. Detailed investigations in this regard have been carried out by De Flora’s group. In this regard, a recent study by Guida et al. (60) provides evidence for nucleoside transporters mediating influx of extracellular cADPR in 3T3 murine fibroblasts. Whether such transporters are expressed in smooth muscle cells and involved in cADPR influx remains to be determined.

**cADPR as a Second Messenger in Airway Smooth Muscle Cells: Criteria**

Several lines of evidence have confirmed the calcium-mobilizing activity of cADPR and the role of CD38 in regulating the metabolism of cADPR. A great deal of interest lies in understanding whether cADPR is a second messenger and contributes to the regulation of intracellular calcium responses to agonists, similar to the PLC/IP3 signaling. In this context, several criteria need to be considered before cADPR can be defined as a potential second messenger molecule.

First, the enzymatic pathways for the synthesis and degradation of the second messenger molecule should be expressed endogenously in the cells. In the context of cADPR, the enzymes ADP-ribosyl cyclase and cADPR hydrolase, catalyzing the synthesis and degradation of cADPR, respectively, are expressed ubiquitously in a variety of cell types (102, 104), including airway smooth muscle cells (168).

Second, agonist stimulation should lead to higher levels of the second messenger molecule. In this context, several studies have demonstrated modulation of ADP-ribosyl cyclase activity and cADPR levels upon stimulation with agonists. In addition, metabolic stimuli such as glucose and NAD/NADH ratio have been shown to increase the production of cADPR in the islet cells of the pancreas (154) and pulmonary vascular smooth muscle (34), respectively. These studies demonstrate that multiple mechanisms can modulate CD38/cADPR signaling in cells.

Third, addition of a second messenger molecule to the cells should mimic the effect of agonists. Incubation of permeabilized cells, microsomal fractions with cADPR, microinjection of cADPR into intact cells, or flash photolysis of caged cADPR resulted in release of calcium from intracellular stores (27, 47, 141). In permeabilized airway smooth muscle cells, addition of cADPR elicits intracellular calcium responses (138). These studies provide evidence for the ability of cADPR to mimic the effects of agonist stimulation.

Fourth, antagonists of the second messengers should block the effect of the second messenger. Studies from our laboratory have demonstrated that in airway smooth muscle cells the intracellular calcium responses to ACh, bradykinin, thrombin, and ET-1 are attenuated by 8-Br-cADPR, a cADPR antagonist (7, 33, 169). Similar findings have been reported by others using a variety of cell types and agonists (6, 9, 12, 18).

Lastly, the second messenger should have binding sites in the target cell type. In this context, binding of cADPR to RyRs either directly or indirectly still needs to be determined. However, evidence seems to point to the involvement of accessory proteins such as FKBP12.6, calmodulin, and other proteins (see mechanisms of cADPR-induced calcium release above on the role of accessory proteins). In airway smooth muscle, studies have demonstrated the role of FKBP12.6 in the regulation of calcium release by cADPR from SR through RyRs (167). Even though there is no conclusive evidence for the presence of cADPR-binding proteins, the data obtained thus far demonstrate that cADPR mobilizes calcium from SR through RyRs by a yet undetermined mechanism.

**Recruitment of the CD38/cADPR Signaling Pathway**

Agents such as ACh, bradykinin, thrombin, ET-1 (7, 32, 33, 138, 143, 169), cholecystokinin (18), angiotensin (70), isoproterenol (69), ATP (17), nitric oxide (NO) (171), glucose (155), and oxytocin (9) utilize CD38/cADPR-mediated calcium release upon stimulation of specific cells. These agonists predominantly utilize IP3-mediated calcium release to elevate intracellular calcium. However, the overall calcium response induced by these agonists is attenuated by cADPR antagonist in a concentration-dependent fashion, suggesting the contribution of cADPR-mediated calcium release in the regulation of intracellular calcium. Even though it has been established that multiple agonists activate CD38 and produce cADPR in different cell types, the mechanisms involved in the activation have not been established. On the basis of the data accumulated in the literature, it appears that CD38/cADPR signaling is a common pathway of intracellular calcium regulation for a variety of agonists. However, a recent study from our laboratory determined that the utilization of CD38/cADPR-mediated calcium release in porcine airway smooth muscle cells is agonist specific (169). In this study, we showed that pretreating the cells with 8-Br-cADPR, the membrane-permeant cADPR antagonist, inhibits the calcium responses to ACh and ET-1, but not to histamine. Because the intracellular calcium responses were measured in the presence of lanthanum (which blocks calcium efflux and influx), as well as in the absence of extracellular calcium, the observed responses to all three agonists examined are clearly attributable to release from SR and not influx from the extracellular space. We also showed that a high concentration of ryanodine inhibited ET-1-induced intracellular calcium responses, and in the presence of ryanodine, there was no further attenuation of the calcium responses by...
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The intracellular calcium response to histamine was not inhibited by ryanodine. Thus it appears that the component of the intracellular calcium response that is sensitive to inhibition by ryanodine is mediated by cADPR in airway smooth muscle cells. These findings support the hypothesis that utilization of CD38/cADPR signaling in airway smooth muscle cells upon agonist stimulation results from activation of specific G protein-coupled receptors. In human airway smooth muscle cells on the other hand, we demonstrated that histamine-elicited calcium responses were inhibited by 8-Br-cADPR (33). The observed differences in the utilization of CD38/cADPR pathway in human airway smooth muscle cells compared with that in porcine airway smooth muscle cells could be due to species specificity of recruitment of this pathway.

In airway smooth muscle cells, agonists such as ACh, ET-1, and histamine induce intracellular calcium elevation and contraction. Although the responses to these agonists require activation of Gaq-type G proteins, receptors coupled to other G protein subtypes have also been described (41, 72, 144). In airway smooth muscle, intracellular calcium response to ACh is largely due to stimulation of PLC-β by Gaq through M3 muscarinic receptors (144). In addition, ACh also acts via Gai1-type G proteins coupled through M2 muscarinic receptors, which contributes to the intracellular calcium response. Because multiple agonists regulate intracellular calcium responses in airway smooth muscle cells, the question arises as to whether recruitment of the CD38/cADPR signaling pathway occurs with stimulation by specific receptor subtypes. There is evidence to support the hypothesis that activation of the CD38/cADPR pathway also occurs in a receptor subtype-specific fashion. In this context, a study from our laboratory demonstrated that CD38/cADPR pathway is coupled to M2 muscarinic receptors in porcine airway smooth muscle cells, although activation of M1 muscarinic receptors is critical for the initiation of the [Ca2+]i response (169). Studies carried out using smooth muscle cells isolated from seminiferous tubules revealed a similar phenomenon in the context of ET-1 stimulation (12). The authors demonstrated that the ETB subtype of receptors is coupled exclusively to CD38/cADPR pathway, whereas the ETA subtype is coupled to both IP3 and cADPR pathways. Similarly, in arterial smooth muscle cells, the CD38/cADPR pathway is coupled to the M1 type of muscarinic receptors (54). These studies demonstrate the coupling of CD38/cADPR signaling to specific receptors and/or subtypes of receptors. However, the precise mechanism involved in this specificity is not clearly understood. The differential recruitment of the CD38/cADPR signaling pathway by agonists suggests that different G proteins may be involved in the activation process. The fact that a specific subtype of muscarinic receptor (i.e., M2) is involved in the recruitment of the CD38/cADPR pathway in airway smooth muscle cells supports this hypothesis. This M2 muscarinic receptor subtype-specific recruitment of the CD38/cADPR signaling in the regulation of intracellular calcium in airway smooth muscle may have pathophysiological consequences. In support of this conclusion are the following observations from recent studies demonstrating the potential role of muscarinic receptors in airway responsiveness. Exposure of airway smooth muscle to the inflammatory cytokine TNF-α results in increased expression of both Gaq1- and Gai1-type G proteins (73, 74), and glucocorticoids decrease the expression of muscarinic receptors (39). Furthermore, M2 muscarinic receptors contribute to airway smooth muscle contraction (72), and their expression is significantly augmented during asthma (40). The increased M2 muscarinic receptor expression is associated with a greater inhibition of forskolin-stimulated adenyl cyclase activity by the M2-selective agent oxotremorine. The increased expression of M2 muscarinic receptors along with Gaq1 may allow for greater intracellular calcium mobilization in airway smooth muscle cells through the CD38/cADPR pathway during muscarinic activation. Furthermore, TNF-α and other inflammatory cytokines are also known to increase the expression of CD38 in airway smooth muscle cells (see ROLE OF CD38/cADPR SIGNALING IN AHR). Together, the changes in the expression of CD38 and the receptors for contractile agonists and G proteins that are associated with these receptors could result in altered calcium homeostasis, leading to airway hyperresponsiveness (AHR).

Involvement of G proteins in the process of CD38 activation and cADPR production has been identified under a variety of experimental conditions. In the longitudinal smooth muscle of the small intestine, stimulation of the cells with cholecystokinin resulted in an increase in the cADPR levels (94). Pretreatment with GDP-βS, an inhibitor of G protein, abolished the cholecystokinin-induced cADPR elevation, suggesting the involvement of G proteins in the activation of CD38 (47, 94, 95). Studies by Higashida et al. (67–70) provided further evidence for the involvement of G proteins. The authors demonstrated that treatment of cardiac myocytes with isoproterenol or angiotensin resulted in the increased ADP-ribosyl cyclase activity, which could be mimicked by the addition of GTP or GTP-γS and inhibited by pretreatment with cholera toxin and GDP-βS. In another study, they demonstrated the involvement of G proteins using NG108, a neuronal cell line (68). They overexpressed different subtypes of muscarinic receptors (M1, M3 coupled to Gaq-type G proteins; and M2, M4 coupled to Gai-type G proteins) in NG108 cells and stimulated the transformed cells with a muscarinic agonist. Upon ACh stimulation of cells overexpressing M1 and M3 receptors, ADP-ribosyl cyclase activity increased, and pretreating the cells with cholera toxin abolished this increase. On the other hand, exposing M2 and M4 transformed cells to ACh resulted in a decrease in the ADP-ribosyl cyclase activity, and this inhibition was abolished upon treatment with pertussis toxin. These findings provide the experimental basis for the involvement of G proteins in the recruitment of the CD38/cADPR pathway. Studies from our laboratory also revealed the role of G proteins in the activation of CD38/cADPR pathway in airway smooth muscle cells. Stimulation of airway smooth muscle cells with ACh resulted in an increase in the cADPR levels, and pertussis toxin inhibited this response, demonstrating the involvement of the Gaq1-type G proteins in the activation of CD38/cADPR signaling (Deshpande DA, Kannan MS and Walseth TF; unpublished observations). Together, these studies suggest the involvement of G proteins in the activation of CD38/cADPR signaling. However, none of the studies have demonstrated the nature of the interaction between G proteins and CD38, i.e., whether CD38 is directly coupled to G proteins or involves intermediary proteins or pathways for activation.

In the context of the interaction between G proteins and CD38/cADPR signaling, several studies have attempted to identify some potential mechanisms. For example, in the lon-
gitudinal smooth muscle from the intestine, the mechanisms involved in cholecystokinin-induced cADPR production have been investigated (94, 95). The increase in cADPR production is cholecystokinin concentration dependent and inhibited by a receptor antagonist and by nifedipine, which blocks Ca\(^{2+}\) influx through voltage-gated calcium channels. These results suggest that Ca\(^{2+}\) influx upon agonist stimulation is a requirement for the increased cADPR levels. The mechanism by which Ca\(^{2+}\) influx leads to this increase was not elucidated in this study. However, in airway smooth muscle, calcium-dependent activation of ADP-ribosyl cyclase is an unlikely mechanism for cADPR production, since all the agonists examined in our study raised intracellular calcium levels, but the responses to histamine were unaffected by the cADPR antagonist (169). Presumably, exposure of airway smooth muscle cells to histamine does not result in cADPR production, although this needs to be ascertained.

We propose a model of intracellular calcium mobilization in airway smooth muscle cells by different agonists through the CD38/cADPR signaling pathway and mechanisms independent of this pathway (Fig. 4). The model is consistent with the findings that activation of specific receptors and/or subtypes of receptors leads to the recruitment of CD38 in airway smooth muscle cells. Although the implication that the underlying mechanism involves activation of G\(\alpha_i\)-type G proteins, the details as to how the G protein activation leads to CD38 activation are not yet clear.

**OTHER MECHANISMS OF REGULATION OF ADP-RIBOSYL CYCLASE ACTIVITY**

*Phosphorylation and ADP-ribosylation.* The role of specific amino acids in the activation of G protein-coupled receptors and their coupling to specific signaling molecules has been well documented (176, 177). In the context of activation of ADP-ribosyl cyclase, mechanisms involving phosphorylation, ADP ribosylation, etc. have been studied. In a study in adrenal chromaffin cells, Morita et al. (120) demonstrated enhanced synthesis of cADPR upon stimulation with ACh. Activation of ADP-ribosyl cyclase was dependent on Ca\(^{2+}\) influx and subsequent activation by cAMP of cAMP-dependent kinase (PKA). They concluded that PKA-dependent phosphorylation of ADP-ribosyl cyclase led to an increase in ADP-ribosyl cyclase activity. Other investigators have described regulation of CD38 activity by ADP-ribosylation in lymphocytes. ADP-ribosylation of specific amino acid residues on CD38 by an ecto-mono-ADP-ribosyltransferase leads to alteration in enzyme activities associated with CD38 (64). ADP-ribosylation of a specific cysteine residue results in inhibition of cADPR hydrolase activity, whereas ADP-ribosylation of a specific arginine residue results in loss of both ADP-ribosyl cyclase and cADPR hydrolase activities. It is not clear whether such modifications occur under physiological conditions.

Gallione and colleagues (53) were the first to suggest that an extracellular stimulus results in the activation of ADP-ribosyl cyclase with a subsequent increase in intracellular cADPR levels. In intact sea urchin eggs, exposure to a cell permeant analog of cGMP resulted in the activation of ADP-ribosyl cyclase, an increase in cADPR production, and the subsequent release of intracellular Ca\(^{2+}\) from ryanodine-sensitive calcium channels. A cGMP-dependent kinase (PKG) inhibitor was effective in preventing the cGMP-dependent Ca\(^{2+}\) release, suggesting an indirect mechanism of activation of ADP-ribosyl cyclase, presumably via a phosphorylation event.

*S-nitrosylation.* NO is the predominant inhibitory neurotransmitter released from intrinsic nonadrenergic, noncholinergic nerves within the airways of many species (13, 14, 82–84). The mechanisms by which NO causes airway smooth muscle relaxation are complex and include both cGMP-dependent and -independent pathways (1). NO, through cGMP generation, has a profound effect on inhibition of calcium release from the SR during agonist stimulation of airway smooth muscle cells (86, 137). Because SR calcium release during stimulation of airway smooth muscle cells by many agonists involves activation of RyR channels and cADPR, we hypothesized that part of the inhibitory effect of NO on calcium release may involve modulation of the CD38/cADPR signaling pathway. In microsomal membranes isolated from airway smooth muscle, incubation with NO donors caused a concentration-dependent decrease in ADP-ribosyl cyclase activity (170). The NO donors had no significant effects on cADPR hydrolase activity. The inhibition of ADP-ribosyl cyclase activity by the NO donors was reversed by the NO scavenger carboxy-2-phenyl-4,4,5,5-tetramethylimidazoline-1-oxyl-3-oxide and prevented by prior incubation with reduced glutathione. These results suggest S-nitrosylation of CD38 as a possible mechanism for the inhibition of ADP-ribosyl cyclase activity. N-ethylmaleimide, which covalently modifies protein sulfhydryl groups and makes them incapable of nitrosylation, also caused a pronounced inhibition of ADP-ribosyl cyclase, but not cADPR hydrolase, activity. cGMP by itself had no

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**Figure 4.** Model depicting the CD38/cADPR signaling pathway of intracellular Ca\(^{2+}\) regulation in airway smooth muscle. In airway smooth muscle, recruitment of the CD38/cADPR signaling pathway is agonist specific. Activation of histamine receptors, M\(_3\) muscarinic receptors (M\(_3\)ACh R), or ETA receptors (ETA R) coupled to G\(_{\alpha_i}\)-type G proteins results in activation of PLC and IP\(_3\) production, leading to Ca\(^{2+}\) release through IP\(_3\)-R, ETA, ETB, and M\(_3\)ACh R. Activation of ETA R coupled to G\(_{\alpha_i}\)-type G proteins results in stimulation of CD38, with the subsequent production of cADPR and Ca\(^{2+}\) release through RyR. 8-Br-cADPR inhibits Ca\(^{2+}\) release through RyR, but not IP\(_3\)-R, channels.

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**CD38/cADPR SIGNALING IN ASM CELLS**

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effect on ADP-ribosyl cyclase activity in the isolated airway smooth muscle microsomes. These observations provide a novel mechanism of regulation of ADP-ribosyl cyclase activity in airway smooth muscle by NO through a cGMP-independent pathway involving S-nitrosylation of thiols. The lack of an effect on cADPR hydrolase activity, which is part of a single molecule, i.e., CD38, suggests differential regulation of CD38 by S-nitrosylation. Whether specific cysteine residues are involved in this differential regulation of CD38 by NO needs to be established.

The effects of NO have been examined in other systems in terms of regulation of ADP-ribosyl cyclase activity. For example, in sea urchin eggs, extracellularly applied NO resulted in the elevation of cADPR levels and mobilization of intracellular Ca^{2+} (53), unlike the effects on airway smooth muscle microsomes. The Ca^{2+}-mobilizing actions of NO were inhibited by the cADPR antagonist, 8-amino-cADPR, and nicotinamide, an inhibitor of ADP-ribosyl cyclase. The actions of NO were also mimicked by cGMP and inhibited by a PKG inhibitor. NO also elevates cADPR levels in sea urchin egg homogenates. Combined these results demonstrate a mechanism of intracellular Ca^{2+} mobilization by NO via a signaling pathway involving cGMP and cADPR. Subsequently, Graeff et al. (58) provided evidence for two forms of ADP-ribosyl cyclases in sea urchin eggs, a soluble and a membrane-bound form. The soluble form of ADP-ribosyl cyclase is stimulated by cGMP, whereas a membrane-bound form is not. Furthermore, activation of the soluble ADP-ribosyl cyclase by cGMP was dependent on ATP, suggesting stimulation was mediated by PKG, which is consistent with evidence that protein kinase inhibitors block cGMP-activation of the cADPR pathway.

**CD38 gene expression.** Another mode of CD38 regulation has been described at the level of gene expression in myometrial smooth muscle. Estrogen administration to gonadectomized rats resulted in an increase in ADP-ribosyl cyclase activity in myometrial smooth muscle (24). Later, Dogan et al. (35, 36) demonstrated that this estrogen-induced increase in ADP-ribosyl cyclase activity is due to increased expression of CD38. An interesting finding of these studies is that while ADP-ribosyl cyclase activity increased with an increase in CD38 expression, cADPR hydrolase activity remained unchanged. This suggests a differential regulation of CD38 possibly through posttranslational modification of the protein. Retinoic acid and triiodothyronine, hormones that are involved in cell differentiation and growth, are reported to increase ADP-ribosyl cyclase activity in rat vascular smooth muscle cells grown in primary culture (31). Retinoic acid has also been found to stimulate CD38 expression in renal LLC-PK1 cells as well as myeloid HL-60 cells (118). Umar and colleagues (164) demonstrated that, in addition to a typical monomeric form of CD38, retinoic acid differentiation of these cells also results in a posttranslational modification of CD38 resulting in a high-molecular-mass (~190 kDa), cross-linked form of CD38 (p190). Purified p190 had at least a threefold increase in ADP-ribosyl cyclase activity and a 2.5-fold decrease in cADPR hydrolase activity. This suggests an additional mechanism by which cells may alter CD38 metabolic activities to favor a net production of cADPR over ADPR.

**PATHOLOGICAL ROLE OF CD38/cADPR SIGNALING**

The role of CD38/cADPR signaling in pathological conditions has been investigated in situations such as diabetes (87, 88, 130), hypoxia-induced vasoconstriction (34), and more recently in AHR (32, 33). These conditions could arise due to changes in the expression of CD38 and/ or changes in the enzymatic activities associated with CD38, leading to alterations in the production of cADPR and calcium homeostasis.

CD38 through cADPR production plays a key role in the regulation of [Ca^{2+}]_{i} in the islet cells of pancreas, the source for insulin production (130). Recent investigations from Okamoto’s group have established that CD38 knockout (KO) mice are glucose intolerant and develop diabetes. Transgenic mice overexpressing CD38 produce increased amounts of insulin (87, 88, 130). Furthermore, the diabetic phenotype of CD38 KO mice can be rescued by cross-breeding with mice overexpressing CD38, demonstrating a key role for CD38-dependent cADPR in the development of the diabetic phenotype. Genetic analysis of type 2 diabetic patients in Japan revealed a missense mutation in the CD38 gene (130). This mutation led to an amino acid substitution in which Arg140 was replaced by Trp, and this resulted in a lower ADP-ribosyl cyclase and cADPR hydrolase enzyme activities associated with CD38. The lack of cADPR production in the islet cells of these patients could have resulted in the poor insulin secretion and development of diabetes. In addition, anti-CD38 antibodies have been found in Japanese and Caucasian diabetic patients (78, 140). Incubation of isolated rat pancreatic islet cells with these sera resulted in a very low insulin secretion, demonstrating the direct effect of these antibodies on the insulin secretion upon stimulation with glucose. These studies together demonstrate that either a change in the expression of CD38 or alterations in the enzyme activities associated with CD38 leads to development of diabetes.

The contribution of the CD38/cADPR signaling has also been investigated in hypoxia-induced pulmonary vasoconstriction (34). Hypoxia induces vasoconstriction in pulmonary vessels leading to the development of pulmonary hypertension and right heart failure. The changes in vascular smooth muscle contractility are responsible for such an effect, and in this context cADPR-mediated calcium release has been shown to play a key role. In these studies, Evans’s group demonstrated that hypoxia leads to increased cADPR hydrolase enzyme activity and thus increases cADPR levels in the pulmonary vascular smooth muscle leading to augmented [Ca^{2+}]_{i} levels and vasoconstriction. 8-Br-cADPR was able to block hypoxia-induced pulmonary vasoconstriction in situ. The proposed mechanism underlying the pathogenesis involves changes in the NAD/NADH ratio during hypoxia. NADH levels increase during hypoxia, which reduces cADPR hydrolase activity whereby cADPR accumulates in the lung tissue. The authors referred to CD38 as a “redox sensor.” These elegant studies demonstrated a role of CD38/cADPR in the pathogenesis of pulmonary vasoconstriction.

**ROLE OF CD38/cADPR SIGNALING IN AHR**

Recent investigations from our laboratory have demonstrated that CD38/cADPR-mediated calcium signaling contributes to AHR, a hallmark of asthma (32, 33). Asthma is a chronic airway disease, and several lines of evidence suggest...
that inflammatory changes in the resident cells of the airways result in the development of asthma symptoms (37, 99, 143). One of the unique changes observed during airway inflammation is AHR, in which the asthmatic patients or allergen-sensitized and challenged animals demonstrate exaggerated responses to contractile agents such as methacholine, histamine, and bradykinin and decreased responses to relaxant agents such as β2-adrenoceptor agonists (37, 147). Recent studies have identified a key role for cytokines such as IL-4, IL-5, IL-9, IL-13, IL-18, IFN-γ, and TNF-α in the pathogenesis of asthma (38). These cytokines directly act on the resident cells of the airways to bring about structural and functional changes (165). Airway smooth muscle cells express adhesion molecules such as ICAM, VCAM, and CD44 that help in binding to the activated T cells (50) and receptors for various cytokines, suggesting a direct action of cytokines on the smooth muscle (3, 100).

Cytokines such as TNF-α, IL-1β, IL-13, and IL-4 bind directly to the receptors expressed on the airway smooth muscle cells and activate specific signaling pathways resulting in alterations of function (5). Exposure of tracheal rings obtained from guinea pig (20, 135) and mice (161) and isolated smooth muscle cells (4) to inflammatory cytokines resulted in modulation of the responsiveness of airway smooth muscle to a variety of contractile and relaxant agents. Because calcium is a common second messenger molecule for a variety of contractile agonists in airway smooth muscle, we determined the effect of these cytokines on the intracellular calcium responses. In recent studies from our laboratory, we demonstrated that the intracellular calcium responses to ACh, bradykinin, and thrombin were significantly higher in human airway smooth muscle cells treated with inflammatory cytokines (32, 33).

Studies have also demonstrated that inflammatory cytokines alter the expression and activity of signaling molecules. Hakonarson and coworkers (62) determined changes in the gene expression profile in human airway smooth muscle cells exposed to the inflammatory cytokines TNF-α and IL-1β. Analysis of expression data revealed changes in the expression of genes encoding a variety of transcription factors, cytokines and chemokines, structural proteins, and most importantly signaling molecules. Evidence for changes in the signaling molecules in airway smooth muscle cells has also come from two other independent studies by Hotta et al. (73, 74) and Amrani et al. (2). In these studies, the authors demonstrated that TNF-α treatment increases the expression of G_{out} and G_{out}-type of G proteins in human airway smooth muscle cells. Functional changes in the signaling mechanisms were reflected as higher inositol turnover in cells upon treatment with TNF-α. In a related study, Yang et al. (175) demonstrated that exposure of airway smooth muscle cells to IL-1β resulted in increased phosphoinositide hydrolysis following stimulation with bradykinin.

We have extended these studies and determined the effect of inflammatory cytokines on CD38/cADPR signaling in human airway smooth muscle cells (33). The findings revealed that inflammatory cytokines upregulate CD38 expression. The augmented CD38 expression is associated with increased ADP-ribosyl cyclase activity. Furthermore, the cell-permeant cADPR antagonist 8-Br-cADPR attenuated the augmented intracellular calcium responses in cells treated with the inflammatory cytokines. Increased CD38 expression and ADP-ribosyl cyclase activity may result in increased cADPR production upon agonist stimulation and contribute to augmented intracellular calcium responses in airway smooth muscle cells. These findings indicate that inflammatory cytokines, in addition to their effects on other signaling molecules, alter calcium homeostasis by modulating the expression and activity of CD38/cADPR signaling in airway smooth muscle cells. Figure 5 is a model describing the role of the CD38/cADPR signaling in cytokine-induced airway smooth muscle hyperresponsiveness.

Recent studies have also shown that IL-13, a Th2 cytokine, plays a key role in the pathogenesis of asthma (142, 172). IL-13 also has direct effects on airway smooth muscle cell function through the IL-13Rα2-IL-4Rα-STAT6 pathway (38, 66, 96, 98). Lee and coworkers (110) demonstrated changes in the transcriptional activity in airway smooth muscle cells following treatment with IL-13. The study identified upregulation of a variety of genes encoding signaling or second messenger molecules, contractile proteins, and signaling effectors in airway smooth muscle cells. In a recent study, we demonstrated that exposure of murine tracheal rings to IL-13 results in an augmented contractile response to ACh (161). In addition, the intracellular calcium responses to ACh, bradykinin, and thrombin were significantly higher in airway smooth muscle cells treated with IL-13 compared with controls. The findings also indicate that IL-13-induced altered calcium homeostasis contributes to the altered contractility of airways. We have also examined the contribution of CD38/cADPR signaling to IL-13-induced alterations in calcium homeostasis in human airway smooth muscle cells (33). Exposure of human airway smooth muscle cells to IL-13 resulted in increased CD38 expression, ADP-ribosyl cyclase activity, and levels of cADPR. Furthermore, IL-13 treatment resulted in augmented
intracellular calcium responses to bradykinin, thrombin, and histamine, and 8-Br-cADPR attenuated these responses. These findings support the hypothesis that modulation of CD38/cADPR signaling by cytokines may lead to the structural and functional changes in the properties of airway smooth muscle, leading to the asthmatic phenotype.

**SUMMARY**

The calcium-mobilizing activity of cADPR contributes to agonist-induced intracellular calcium responses in airway smooth muscle. CD38 is the primary source for cADPR synthesis in airway smooth muscle. Studies thus far have confirmed that cADPR is a potential second messenger for a variety of contactile agonists in airway smooth muscle cells similar to IP3. The recruitment of the CD38/cADPR signaling pathway in airway smooth muscle is agonist receptor and subtype specific. In airway smooth muscle cells, the intracellular calcium response following stimulation of M2 muscarinic receptors appears to arise from activation of the CD38/cADPR signaling pathway, suggesting a role for Gα-activated G proteins. Furthermore, this calcium-regulating pathway contributes to calcium homeostasis in airway smooth muscle induced by inflammatory and Th2 cytokines, suggesting a role in the pathogenesis of AHR. Future investigations must address the following questions: 1) delineation of the mechanisms by which CD38 activation results from stimulation of G protein-coupled receptors; 2) how extracellularly synthesized cADPR brings about its intracellular effects; 3) how cADPR interacts with the RyR to mobilize calcium from the SR; 4) the role of accessory proteins such as FKBP12.6 and modulations of intracellular calcium-release channels by calmodulin. J Membr Biol 273: 1–10, 2002.

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