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Involvement of Native TRPC3 Proteins in ATP-Dependent Expression of VCAM-1 and Monocyte Adherence in Coronary Artery Endothelial Cells

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Background—Vascular cell adhesion molecule-1 (VCAM-1) is critical in monocyte recruitment to the endothelium, a key event in development of atherosclerotic lesions. Stimulation of human coronary artery endothelial cells (HCAECs) with ATP positively modulates VCAM-1 expression and function through a mechanism involving Ca^{2+} signaling. We here examined the role of Ca^{2+} influx and native TRPC3 channels in that mechanism.

Methods and Results—Omission of extracellular Ca^{2+} or pretreatment of cells with channel blockers markedly reduced ATP-induced VCAM-1 and monocyte adhesion. Using a siRNA strategy and real-time fluorescence, we found that native TRPC3 proteins contribute to constitutive and ATP-regulated Ca^{2+} influx. ATP-dependent upregulation of VCAM-1 was accompanied by an increase in basal cation entry and TRPC3 expression. Notably, TRPC3 knock-down resulted in a dramatic reduction of ATP-induced VCAM-1 and monocyte adhesion.

Conclusions—These findings indicate that in HCAECs, native TRPC3 proteins form channels that contribute to constitutive and ATP-dependent Ca^{2+} influx, and that TRPC3 expression and function are fundamental to support VCAM-1 expression and monocyte binding. This is the first evidence to date relating native TRPC3 proteins with regulated expression of cell adhesion molecules in coronary endothelium, and suggests a potential pathophysiological role of TRPC3 in coronary artery disease. (***Arterioscler Thromb Vasc Biol*. 2008;28:2049-2055**)

Key Words: TRPC3 ■ VCAM-1 ■ monocyte recruitment ■ Ca^{2+} influx ■ atherogenesis

Recruitment of circulating monocytes to the arterial intima is a crucial event in initiation, progression, and fate of the atherosclerotic lesion. Indeed, monocyte infiltration in the subintima is observed early in atherogenesis, and also at more advanced stages, when plaque infiltration and neovascularization may occur.[1,2] At the molecular level, monocyte adhesion to the vascular wall is secured by the interaction between the integrin $\alpha_4\beta_1$ (Very Late Antigen 4; CD49 days/CD29) expressed on the monocyte and vascular cell adhesion molecule 1 (VCAM-1, CD106) on the endothelial cell.[3] Next, VCAM-1–dependent signaling drives transendothelial migration of the bound monocyte. VCAM-1 is virtually absent in resting endothelium, but its expression is rapidly upregulated in response to proinflammatory and proatherogenic stimuli, particularly in vascular areas prone to lesion development.[3] In vascular endothelium, nucleotides (ie, ATP, UTP) released to the extracellular milieu in response to ischemia, hypoxia, chemical, or mechanical stress are known to exert a strong proinflammatory effect (reviewed in[4]). For instance, ATP stimulates adhesion of neutrophils to pulmonary artery endothelium[5] and promotes release of inflammatory mediators such as interleukin (IL)-6 and monocyte chemoattractant protein-1 in dermal microvascular endothelium.[6] In human coronary artery endothelial cells (HCAECs) ATP induces expression of VCAM-1 and monocyte adhesion through stimulation of $P2Y_2$ receptors,[7] in line with its effect in an in vivo model of neointima hyperplasia.[8] This effect is specific for VCAM-1, as other cell adhesion molecules such as intercellular cell adhesion molecule-1 are not affected.[7] The underlying signaling, although not fully defined, is known to involve transactivation of VEGF receptor (VEGFR) type 2 and stimulation of the small GTPase Rac1.[9] As is the case for several inflammatory mediators acting on vascular beds other than the coronary circulation,[10,11] changes in intracellular Ca^{2+} levels associated to Ca^{2+} release from internal stores also seem to contribute to ATP-induced VCAM-1 in HCAECs.[7,12] However, despite that in these cells stimulation of $P2Y_2$ receptors promotes a robust Ca^{2+} influx, the specific role of Ca^{2+} entry in regulation of VCAM-1 has not been examined. In pilot studies we found that HCAECs express all members of the Canonical Transient Receptor Potential (TRPC) family of channel forming proteins (TRPC1-7, except TRPC2, a pseudogene in humans[13]) and that TRPC3 forms, or is part of, endogenous

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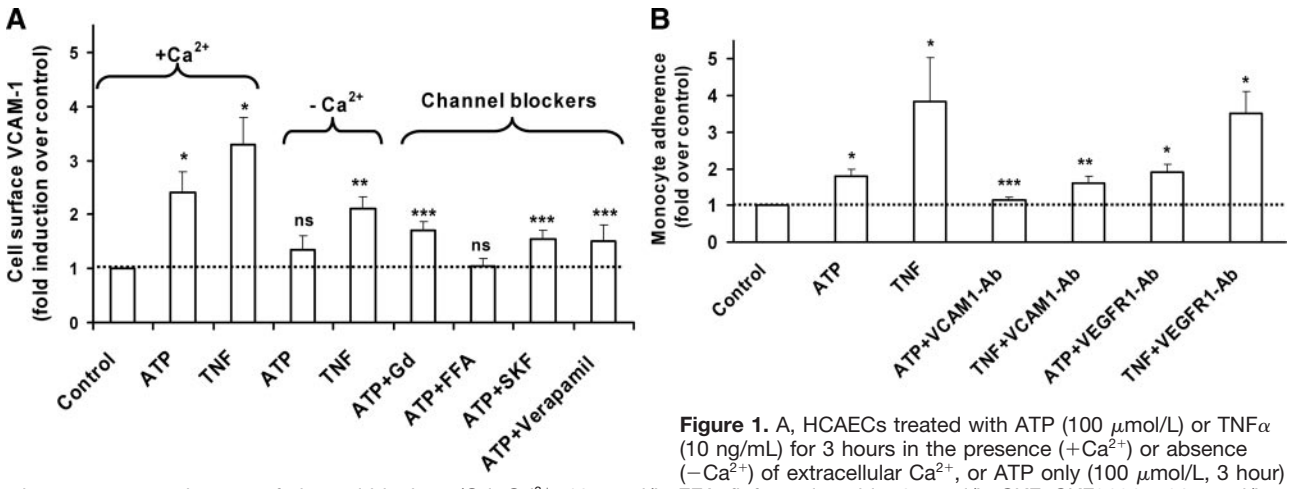

Figure 1. A, HCAECs treated with ATP (100 μmol/L) or TNFα (10 ng/mL) for 3 hours in the presence ($+Ca^{2+}$) or absence ($-Ca^{2+}$) of extracellular Ca^{2+}, or ATP only (100 μmol/L, 3 hour) in the presence or absence of channel blockers (Gd: Gd^{3+}, 10 μmol/L; FFA: flufenamic acid, 50 μmol/L; SKF: SKF96365, 30 μmol/L; verapamil, 50 μmol/L) were processed for ELISA detection of surface VCAM-1. *$P<0.01$; **$P<0.02$; ***$P<0.056$, not quite significant; ns: not significantly different. Neither cell viability nor pH of the medium was altered by the channel blockers at these concentrations. B, HCAECs were treated with ATP (100 μmol/L) or TNFα (10 ng/mL) for 3 hours before evaluation of monocyte adhesion. When indicated, cells were incubated with 10 μg/mL anti–VCAM-1 (VCAM-1-Ab, clone E-10, Santa Cruz) or 10 μg/mL anti-VEGFR1 (VEGFR1-Ab, clone RR9S, Santa Cruz) antibodies 45 minutes before addition of monocytes. *$P<0.03$ respect to control; **$P<0.05$ respect to control and TNFα alone; ***$P<0.06$, not quite significant respect to control, and $P<0.04$ respect to ATP alone.

Ca^{2+}-permeable channels that contribute to ATP-stimulated Ca^{2+} influx.[14] Based on this, in the present work we examined whether Ca^{2+} influx and TRPC3 contribute to the actions of ATP on VCAM-1 expression and monocyte adhesion in HCAECs. Our findings suggest that in these cells those two events depend, to a significant extent, on Ca^{2+} influx, and that native TRPC3 plays a prominent role in the underlying signaling mechanism. These findings underscore a potential novel function of TRPC3 within the context of development and progression of atherosclerotic lesions in coronary artery disease.

Methods

Cells and Transfections

HCAECs (Lonza, Calif) were grown in endothelial basal medium (EBM-2) supplemented with endothelial growth factors and 5% fetal bovine serum (FBS) at 37°C under humidified air (5% CO_2), and used between passages 2 to 10. U937 human monocytic cells (ATCC, Va) were grown in RPMI containing 10% FBS. TRPC3 siRNA (100 nmol/L; Dharmacon) or nonspecific oligonucleotides were delivered to cells with Lipofectamine2000 (Invitrogen) and cells used 48 hours after transfection.

Cell Lysis and Immunoblotting

Cells (≈80% confluence) were made quiescent by replacing growth medium with EBM-2 (10 mmol/L glucose, no serum or growth factors) during 24 hours and processed for SDS-PAGE and immunoblotting as in.[15] Proteins were separated in 10% acrylamide gels, transferred to nitrocellulose membranes, and immunoblotted with anti–VCAM-1 (clone E-10, Santa Cruz), antibodies against TRPC1, 3 to 6 (Alomone Labs), anti-TRPC7 (kindly provided by Dr W. Schilling, Case Western University School of Medicine), or anti-beta actin (Millipore). After incubation with secondary antibodies, immunoreactive bands were visualized by ECL (Amersham), quantified by densitometry within the linear range of the film, and their values normalized against those for β-actin.

Cell ELISA

HCAECs grown to confluence in 96-well plates were made quiescent as described above. After the indicated treatments cells were fixed in 0.5% glutaraldehyde, nonspecific sites blocked with 0.5% bovine serum albumin, and then incubated (1 hour, 37°C) with VCAM-1 monoclonal antibody (R&D Systems) and peroxidase-conjugated antimouse antibody (Amershan). Peroxidase reaction was performed with 3,3′,5,5′-Tetramethylbenzidine (Sigma) and stopped with 2 N HCl within the linear range of color development (10 to 15 minutes). Cell surface VCAM-1 was estimated as optical density at 450 nm after background subtraction (O.D. in the absence of primary antibody).

Monocyte Adhesion

HCAECs grown to confluence in 24-well plates were made quiescent as described above. After indicated treatments calcein-loaded U937 cells were added (50 000 per well) and incubation proceeded for 45 minutes at 37°C. After washes with PBS, bound monocytes were counted (3 fields per well, triplicates/condition). In siRNA experiments, transfected HCAECs were plated onto 24-well plates for 48 hours and then processed as above.

Real-Time Fluorescence

Coverslip-plated cells loaded with the Ca^{2+}-sensitive dye Fura-2 were used to monitor real-time fluorescence changes of intracellular Ca^{2+} or Ba^{2+} on multiple cells with a charge-coupled device (CCD) camera-based imaging system (Intracellular Imaging Inc) as previously described.[15] Measurements were performed at room temperature and treatment conditions were in HEPES-buffered saline solution (HBSS) containing (in mmol/L): 140 NaCl, 4.7 KCl, 1 $MgCl_2$, 10 glucose, 10 HEPES pH 7.4, 2 $CaCl_2$. "Nominally Ca^{2+}-free medium" means HBSS with no Ca^{2+} added (free Ca^{2+} ≈5 μmol/L). In transfection experiments GFP was used as a marker and measurements were performed on GFP^+ cells selected by their green fluorescence (excitation, 485 nm; emission, 520 nm).

Statistical Analysis

Means of cytosolic Ca^{2+}, rates of Ca^{2+}/Ba^{2+} entry, or densitometric values were compared using a 2-tailed t test for two means, using Graph Pad InStat version 3.00 for Windows 95 (Graph Pad Software). Averaged results are from 3 to 5 independent experiments. $P<0.05$ was considered significant.

Results

Treatment of HCAECs with ATP (100 μmol/L) or tumor necrosis factor (TNF) α (10 ng/mL) induced a significant

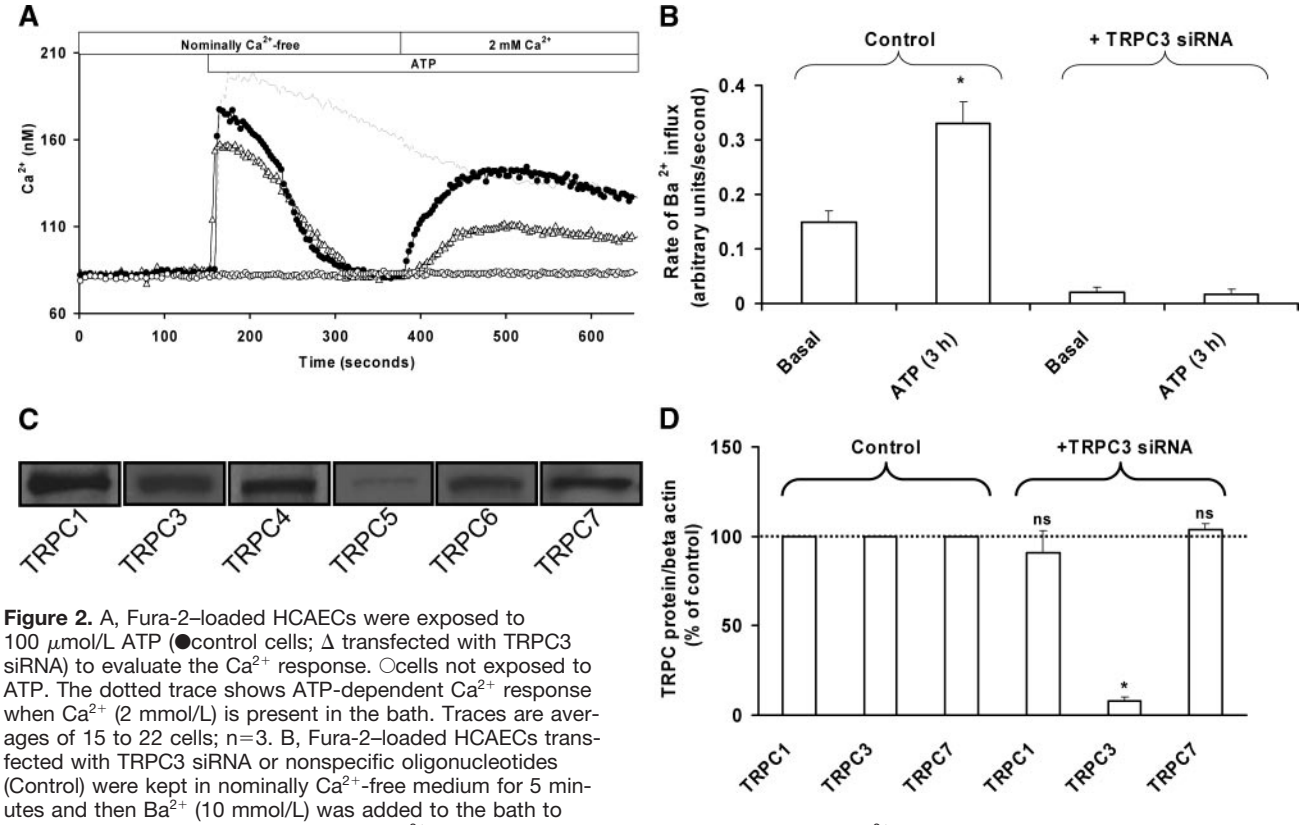

Figure 2. A, Fura-2–loaded HCAECs were exposed to 100 μmol/L ATP (●control cells; Δ transfected with TRPC3 siRNA) to evaluate the Ca^{2+} response. ○cells not exposed to ATP. The dotted trace shows ATP-dependent Ca^{2+} response when Ca^{2+} (2 mmol/L) is present in the bath. Traces are averages of 15 to 22 cells; n=3. B, Fura-2–loaded HCAECs transfected with TRPC3 siRNA or nonspecific oligonucleotides (Control) were kept in nominally Ca^{2+}-free medium for 5 minutes and then Ba^{2+} (10 mmol/L) was added to the bath to evaluate constitutive influx. The rate of Ba^{2+} entry was assessed within 2 minutes after Ba^{2+} addition. *$P<0.01$. C, Representative blots showing expression of TRPC members in HCAECs; molecular weights: ≈97 to 105 kDa. D, Protein expression level for TRPC1, 3, and 7 in control or TRPC3 siRNA-transfected HCAECs. For comparison, normalized densitometric values are expressed as percent of control. *$P<0.0001$; ns: not significantly different; n=3.

increase in the amount of plasma membrane, or pathophysiologically relevant VCAM-1, as evaluated by cell ELISA (Figure 1A). VCAM-1 levels increased as early as 3 hours after treatment and started to decline by 16 to 24 hours (not shown). The effects of ATP and TNFα were also evident in total VCAM-1 protein levels, as evaluated by immunoblot analysis of whole-cell lysates ([7] and not shown) and were translated into augmented monocyte adhesion (Figure 1B). Preincubation of HCAECs with an antibody that recognizes the extracellular domain of VCAM-1 (clone E-10, Santa Cruz) markedly reduced the binding of U937 monocytes, evidencing the contribution of VCAM-1 to the adhesion process (Figure 1B). Blocking VEGFR1 (Flt-1), expressed in HCAECs but not involved in adhesion,[9] did not affect monocyte binding (Figure 1B). To examine whether Ca^{2+} influx played a role in regulated expression of VCAM-1, we treated cells with ATP or TNFα in the presence or absence (nominally Ca^{2+}-free) of extracellular added Ca^{2+}. As shown in Figure 1A, VCAM-1 levels were markedly reduced when Ca^{2+} was omitted in the bath. Whereas TNFα effect was partially reduced (≈30% to 40%), that of ATP was completely abolished. Alternatively, we tested the effect of various Ca^{2+} channel blockers on ATP-induced VCAM-1. The inorganic pore channel blocker gadolinium, the nonselective cation channel blockers SKF96365 and flufenamic acid, and the nondihydropyridine verapamil, all caused a significant reduction of VCAM-1 expression (Figure 1A) at concentrations that markedly reduced ATP-dependent Ca^{2+} influx (inhibition of peak Ca^{2+} influx was: 95±3% with 10 μmol/L gadolinium or 30 μmol/L SKF96365; 85±6% with 50 μmol/L flufenamic acid; 45±15% with 50 μmol/L verapamil; all reductions had at least $P<0.05$ respect to control, n=3 to 4). These chemically unrelated blockers were chosen on the basis of their ability to block a broad spectrum of Ca^{2+}-permeable channels, which includes store-operated and nonstore-operated channels with different degrees of selectivity for Ca^{2+} ([16] and references therein). Importantly, neither treatment with channel blockers nor transfection with siRNA oligonucleotides (see below) altered expression of $P2Y_2$ receptor (not shown). In HCAECs, ATP induces a typical biphasic Ca^{2+} response composed by a transient increase in cytosolic Ca^{2+} attributable to inositol triphosphate (IP_3)-induced Ca^{2+} release from internal stores, which is followed by a robust Ca^{2+} influx phase ([14] and Figure 2A). Both phases operate simultaneously, as indicated by experiments in which cells were challenged with ATP in the presence of extracellular Ca^{2+} (Figure 2A, dotted trace). Neither Ca^{2+} release nor influx were altered by NF279 or MRS2179, P2X and $P2Y_1$ antagonists, respectively[17] (peak Ca^{2+} release and influx were, respectively, 185±20 and 138±10 nmol/L, regardless of the absence or presence of NF279 or MRS2179; n=15 to 22 cells), suggesting that the Ca^{2+} response was mediated by $P2Y_2$ receptors, as is the case for ATP-induced VCAM-1 and monocyte adhesion.[7] Under basal conditions, ie, in the absence of ATP stimulation, Ca^{2+} influx was not detectable (Figure 2A, open circles). However,

the use of Ba^{2+} (10 mmol/L) as a surrogate for Ca^{2+} unmasked the existence of constitutive or nonregulated cation influx (Figure 2B, control basal). The inability to detect constitutive cation influx with 2 mmol/L Ca^{2+} in the bath likely reflects operation of a highly efficient Ca^{2+} buffering system. In line with this, when cells were exposed to higher Ca^{2+} gradients (10 mmol/L in the bath), a significant yet transient Ca^{2+} influx was observed (not shown). Ba^{2+} is not subject to the counteracting actions of such buffering systems, and enters the cell unidirectionally, magnifying any existing basal influx (discussed in[18]).

HCAECs express message for all members of the TRPC family,[19] namely, TRPC1, 3 to 7 (TRPC2, a pseudogene in humans,[13] is not present) and we confirmed expression at the protein level by immunoblot analysis of cell lysates ([14] and Figure 2C). Among all TRPC proteins, TRPC3 forms channels endowed with significant constitutive activity.[18] Using a siRNA approach we examined whether native TRPC3 contributed to constitutive cation influx in HCAECs. The results shown in Figure 2B (+TRPC3 siRNA, basal) indicate that indeed that is the case, as nonregulated Ba^{2+} influx was completely suppressed in cells transfected with siRNA oligonucleotides specific for TRPC3. Knock-down of TRPC3 also caused a significant reduction in both initial rate (2- to 3-fold decrease) and magnitude (≈50% reduction at peak) of ATP-induced Ca^{2+} influx (Figure 2A, open triangles) suggesting that TRPC3 is also an important component of receptor-regulated cation entry. Notably, ATP-dependent upregulation of VCAM-1 was accompanied by a gain in basal cation entry, as evidenced by a more than 2-fold increase in the rate of constitutive Ba^{2+} influx (Figure 2B, control+ATP). This Ba^{2+} influx remained unchanged in the presence of the phospholipace C inhibitor U73122 (not shown), indicating it was genuine nonregulated receptor-independent cation influx. Remarkably, this was correlated with a significant increase in TRPC3 protein levels after 3-hour treatment with ATP (Figure 3). Again, constitutive influx was absent if TRPC3 was knocked-down before treatment with ATP (Figure 2B, ATP+TRPC3 siRNA). No change was observed under these conditions in any of the other TRPC proteins expressed in HCAECs (not shown). In addition, the siRNA protocol targeted TRPC3 in an effective and specific manner, as protein expression levels of TRPC7, a structurally close relative of TRPC3, or the more distantly related member TRPC1, were not altered (Figure 2D).

Because Ca^{2+} influx was necessary for ATP-induced VCAM-1 and TRPC3 contributed to ATP-regulated Ca^{2+} influx, we next examined whether TRPC3 was part of the mechanism underlying ATP-regulated VCAM-1 expression and function. The experiments in Figure 4A show that knock-down of TRPC3 completely reduced ATP-induced VCAM-1. Of importance, VCAM-1 is not the sole cell adhesion molecule mediating monocyte adhesion, whereas Ca^{2+} influx is a critical component of the signaling associated to monocyte adhesion and migration,[20–22] regardless of the adhesion molecules involved.[23–26] Thus, we examined to what extent Ca^{2+} influx or TRPC3 were required for monocyte adhesion to HCAECs. Cells were exposed to ATP in the presence or absence of extracellular added Ca^{2+} or pretreated

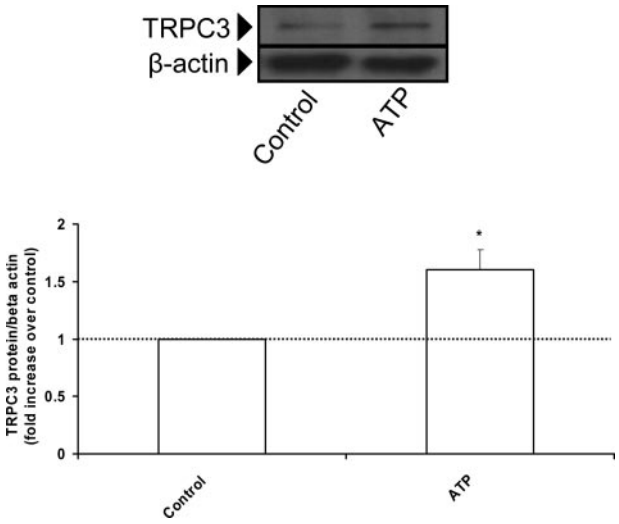

Figure 3. HCAECs were treated with ATP (100 μmol/L, 3 hours) and processed for immunodetection of TRPC3. Bars show average normalized values of densitometric analysis of 5 experiments, expressed as fold induction over control (vehicle-treated cells). *$P<0.04$.

with channel blockers, and monocyte adhesion was evaluated as described in Methods. Alternatively, HCAECs were transfected with TRPC3 siRNA (100 nmol/L) and 48 hours later processed for monocyte binding. In any case, during the incubation with monocytes, Ca^{2+} in the bath was kept at 2 mmol/L, as Ca^{2+} is required for proper interaction between VLA-4 and VCAM-1.[27] As shown in Figure 4B, omission of Ca^{2+} in the bath, or adding channel blockers during treatment with ATP, markedly reduced adhesion of U937 monocytic cells. Notably, TRPC3 knock-down reduced monocyte adhesion to almost the same extent as nominally Ca^{2+}-free conditions.

Discussion

The importance of Ca^{2+} signaling in regulated expression of VCAM-1 has been appreciated in previous studies. For example, changes in intracellular Ca^{2+} associated to Ca^{2+} release from internal stores have been linked to the ability of Substance P and β_2-microglobulin to induce VCAM-1 in microvascular endothelium[10] and synovial fibroblasts,[11] respectively. In HCAECs, Ca^{2+} mobilization has been related to the mechanism by which lipoprotein A and ATP promote VCAM-1 expression.[7,12] Nevertheless, the specific role of Ca^{2+} influx has not been directly examined. Besides, in most instances VCAM-1 expression was evaluated under conditions of strong cytosolic Ca^{2+} buffering, which may prevent a contribution from Ca^{2+} entry if Ca^{2+} microdomains at the channel mouth are perturbed.[28,29] Here we addressed the role of Ca^{2+} influx in ATP-dependent regulation of VCAM-1 in HCAECs. Several important conclusions can be derived from the present findings. That Ca^{2+} influx contributes to the signaling underlying ATP-induced VCAM-1 was first suggested by the observation that maneuvers that prevent Ca^{2+} entry into HCAEC significantly impaired VCAM-1 expression. This was evident not only on the amount of total cellular VCAM-1 protein, but most importantly on the levels of

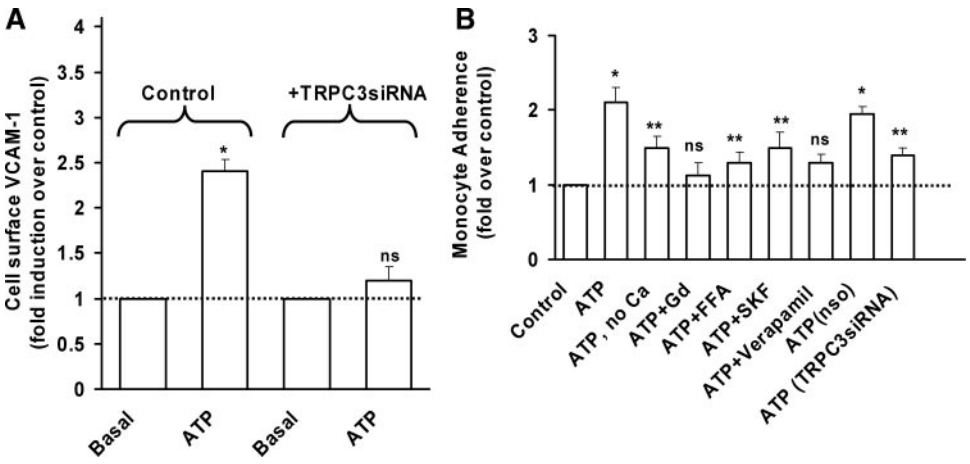

Figure 4. A, HCAECs transfected with nonspecific oligonucleotides (control) or TRPC3 siRNA were treated with ATP (100 μmol/L, 3 hours) and processed for ELISA detection of surface VCAM-1. *$P<0.01$. B, HCAECs were treated with ATP (100 μmol/L, 3 hour) in the presence or absence of extracellular Ca^{2+}, or channel blockers (see legend to Figure 1A for details) or transfected with nonspecific oligonucleotides (nso) or TRPC3 siRNA before ATP treatment, and then monocyte adhesion was evaluated. *$P<0.03$ respect to control; **$P<0.02$ respect to ATP treatment in normal conditions, $P<0.05$ respect to their corresponding control; ns: not significantly different.

plasma membrane resident VCAM-1, which is the pathophysiologically relevant form in terms of its role in monocyte recruitment to the endothelium. This not only indicated that Ca^{2+} influx was necessary, but that Ca^{2+} release from internal stores was not sufficient. This is particularly important when we consider the action of agonists that induce biphasic Ca^{2+} responses, such as ATP (see for instance Figure 2A). ATP-induced VCAM-1 occurs even if cells are exposed shortly (few minutes) to ATP[7]; it was interpreted that the early signaling triggered by ATP, which includes Ca^{2+} release, is sufficient to drive the pathway controlling VCAM-1 expression. If Ca^{2+} release were sufficient, we would have expected that under our conditions ATP would induce full expression of VCAM-1 regardless of Ca^{2+} influx; clearly, this was not the case. Although the extent of contribution from Ca^{2+} release versus Ca^{2+} influx to VCAM-1 expression was not evaluated here, it is possible that different events within the underlying signaling may differentially depend on those two different sources of Ca^{2+}. Because both phases of the Ca^{2+} response occur simultaneously (dotted trace in Figure 2A), as they would under physiological conditions, it is reasonable to speculate that both Ca^{2+} release and influx may be necessary, at least early in the signaling, to trigger a fully operational mechanism leading to transcriptional regulation of VCAM-1 expression. Using a siRNA approach, we showed for the first time that native TRPC3 forms, or is part of, Ca^{2+}-permeable channels that contribute not only to ATP-regulated, but also to constitutive cation influx in HCAECs. Notably, besides its effect on VCAM-1, ATP treatment also increased expression of TRPC3 protein, which was correlated with augmented TRPC3-dependent constitutive cation influx. It should be noted here that after 3 hours of treatment with ATP acute channel stimulation by the nucleotide (Ca^{2+} entry phase in Figure 2A) subsides—in fact, Ca^{2+} levels are back to basal. Thus, at this point Ba^{2+} influx reflects constitutive nonregulated channel function only. This favors the existence of a scenario where augmented expression of TRPC3 protein seems to be translated into more functional channels in the plasma membrane. Strikingly, knock-down of TRPC3 completely suppressed ATP-dependent VCAM-1 expression, in agreement with a clear decrease of ATP-induced monocyte adhesion. TRPC3 has been shown to be sensitive to diverse channel blockers, with relative sensitivities varying considerably depending on expression conditions. In most instances, TRPC3 constitutive and regulated functions are inhibited by micromolar concentrations of gadolinium, SKF96365, flufenamic acid, or verapamil.[13,18,30] Although none of these blockers can be claimed as specific for TRPC3, the observation that all of them markedly reduced both VCAM-1 expression and monocyte binding is in agreement with the view of TRPC3 contributing to those events. Altogether, these findings strongly support the notion that in HCAECs native TRPC3 proteins form, or are part of, endogenous channels that contribute to Ca^{2+} influx after stimulation of purinergic P_2Y_2 receptors, and that TRPC3 is fundamental within the signaling underlying ATP-induced VCAM-1. Studies are underway to determine the nature of the Ca^{2+}-dependent events activated downstream TRPC3-mediated Ca^{2+} influx that participate in regulated expression of VCAM-1. The observation that ATP-induced TRPC3 protein expression is paralleled by augmented constitutive cation influx, raises an important question: does TRPC3 contribute to ATP-induced VCAM-1 through regulated activity, constitutive activity, or both? The reduction in TNFα-induced VCAM-1 when external Ca^{2+} is omitted (Figure 1A) suggests that, besides regulated Ca^{2+} influx, constitutive activity may also play a role, as TNFα does not stimulate Ca^{2+} influx in HCAEC (Smedlund K, Vazquez G, unpublished data, 2008). Additional studies are required to determine the extent of contribution, if any at all, of TRPC3 constitutive function into such mechanism. Interestingly, increased constitutive activity derived from upregulated expression of TRPC3 in vascular smooth muscle has been shown to account for the augmented vasoconstriction in TRPC6 knock-out mice.[31]

Besides TRPC3, other TRPC members are also expressed in HCAECs ([19] and not shown). Functional TRPC channels are thought to be formed by either homo- or hetero-tetrameric arrangements of 4 TRPC proteins (discussed in[13]). Thus, the possibility exists that native channels in HCAECs are formed by either TRPC3 alone, or in association with other TRPCs. Our observations favor the notion that if other TRPCs are part of the native channels contributing to VCAM-1 expression

and function, it must be in combination with TRPC3. Otherwise, homo-tetramers made of TRPC proteins other than TRPC3 would be expected to behave independently, and their contribution to VCAM-1 expression should remain, even after knocking-down TRPC3; nevertheless, TRPC3 siRNA completely abrogated ATP-induced VCAM-1 and monocyte binding.

TRPC channels are now recognized among the most important Ca^{2+}-permeable cation channels in vascular endothelium physiology.[32,33] In addition, it is becoming evident that they are critical players in cardiovascular disease. For instance, TRPC1, 4, and 6 participate in regulation of vascular tone and thus play a role in hypertension.[34–37] TRPC1 and 6 modulate proliferation of vascular smooth muscle cells and may have implications in the pathogenesis of intima hyperplasia.[38,39] Ca^{2+} entry through TRPC3 and 6 promotes cardiac hypertrophy.[40,41] TRPC3 and 5, by yet to be known mechanisms, are upregulated in monocytes from patients with essential hypertension.[42] Our studies represent the first evidence to date suggesting a link between native TRPC3 proteins expressed in coronary artery endothelial cells and cellular and molecular events that are crucial in development of the atherosclerotic lesion. Advances on elucidating molecular and cellular components involved in lesion formation and progression, such as VCAM-1 and its role in monocyte recruitment, have been enthusiastically received in the field as promising new opportunities to develop antiinflammatory therapies for atherosclerosis.[43–45] Therefore, identifying new players within the signaling underlying VCAM-1 expression and function is imperative to develop alternative therapeutic targets for effective treatment of this disease. Within that context, our studies warrant further in vitro and in vivo studies to determine the relevance of TRPC3 in development and progression of coronary artery disease.

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Disclosures

None.

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