Air bubble contact with endothelial cells in vitro induces calcium influx and IP3-dependent release of calcium stores

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Sobolewski P, Kandel J, Klinger AL, Eckmann DM. Air bubble contact with endothelial cells in vitro induces calcium influx and IP3-dependent release of calcium stores. Am J Physiol Cell Physiol 301: C679–C686, 2011. First published June 1, 2011; doi:10.1152/ajpcell.00046.2011.—Gas embolism is a serious complication of decompression events and clinical procedures, but the mechanism of resulting injury remains unclear. Previous work has demonstrated that contact between air microbubbles and endothelial cells causes a rapid intracellular calcium transient and can lead to cell death. Here we examined the mechanism responsible for the calcium rise. Single air microbubbles (50–150 μm), trapped at the tip of a micropipette, were micromanipulated into contact with individual human umbilical vein endothelial cells (HUVECs) loaded with Fluo-4 (a fluorescent calcium indicator). Changes in intracellular calcium were then recorded via epifluorescence microscopy. First, we confirmed that HUVECs rapidly respond to air bubble contact with a calcium transient. Next, we examined the involvement of extracellular calcium influx by conducting experiments in low calcium buffer, which markedly attenuated the response, or by pretreating cells with stretch-activated channel blockers (gadolinium chloride or ruthenium red), which abolished the response. Finally, we tested the role of intracellular calcium release by pretreating cells with an inositol 1,4,5-trisphosphate (IP3) receptor blocker (xestospongin C) or phospholipase C inhibitor (neomycin sulfate), which eliminated the response in 64% and 67% of cases, respectively. Collectively, our results lead us to conclude that air bubble contact with endothelial cells causes an influx of calcium through a stretch-activated channel, such as a transient receptor potential vanilloid family member, triggering the release of calcium from intracellular stores via the IP3 pathway.

GAS EMBOLISM is a serious complication of surgery, diving, and aviation (45). Depending on their size and rate of delivery, air bubbles can circulate, be deposited into, and cause damage in the microcirculation of any organ, obstruct blood vessels, or air-lock the heart (31). Great strides have been made in preventing macroscopic gas embolism from occurring during surgery (3), but the risk of microembolism remains and the vast majority of microemboli, as small as 3 μm, are gaseous (1). This is particularly true for cardiac procedures utilizing cardiopulmonary bypass (CPB), and the incidence of cognitive deficit following such surgeries is high (3). Similarly, decompression illness is also caused by the intravascular formation of gaseous microemboli (46). Gas microemboli have vascular sequelae that include endothelial cell damage or dysfunction, platelet activation, and leukocyte adhesion (45, 46). Furthermore, gas microemboli that did not obstruct blood flow caused changes in both cerebral blood flow and depressed neural function in a rabbit model (25). However, the consequences of air bubble-endothelial cell contact and the mechanisms of microvascular injury resulting from gas microembolism have been little studied (28) outside of computer modeling (32, 41). Likewise, despite gas embolism being a well-documented problem, there are very few clinical treatment options available aside from prevention. The “gold standard” hyperbaric oxygen therapy is both limited in effectiveness and potentially difficult and dangerous to administer, while the availability of pharmacological therapies has been limited (31).

Thus, to develop novel preventive or therapeutic approaches to treating gas embolism injury, it is necessary to understand the signaling pathways evoked by air bubbles in the microvasculature. Our group has developed a platform that enables us to examine the consequences of endothelial cell interactions with air microbubbles (28). Our method involves the generation of physiological-sized micro air bubbles, manipulation of these air bubbles into contact with single endothelial cells, and subsequent recording of the cellular response in real time using phase contrast and epifluorescence microscopy. Previous work in our laboratory (28) has shown that an intracellular calcium transient is elicited by bubble-cell contact, and that this transient was associated with lethality. Here, we focus on the mechanism responsible for the intracellular calcium increase following air bubble contact with endothelial cells, because calcium is known to be a crucial regulator of many endothelial cell functions including nitric oxide production (14), barrier function (44), and mitochondrial function (11).

MATERIALS AND METHODS

Cell culture and dye loading. As an in vitro cell culture model, human umbilical cord vein endothelial cells (HUVECs) (27, 33) were obtained from Lifeline Cell Technology (Walkersville, MD) and cultured in VascuLife VEGF Cell Culture Media (Lifeline Cell Technology). Media samples were checked for mycoplasma contamination using MycoAlert Kit (Lonza, Rockland, ME). Cells between passage 2 and 6 were plated in BD Primera 35-mm cell culture dishes (BD, Franklin Lakes, NJ) ~48 h before planned experiments at a density of ~3,000 cells/cm2. Cells were dye loaded with the calcium-sensitive dye 1 μmol/l Fluo-4 AM (Invitrogen, Carlsbad, CA) plus 0.005% Pluronic F-127 (Invitrogen) for 15 min at room temperature, then washed three times and incubated for an additional 15 min while protected from light to allow for deesterification. All experiments were carried out at room temperature in recording HBSS (pH 7.4 with 1.3 mmol/l CaCl2, 0.9 mmol/l MgCl2, 2 mmol/l glutamine, 0.1 g/l heparin, 5.6 mmol/l glucose, and 1% FBS). An alternate low calcium recording HBSS was prepared by diluting recording HBSS 50-fold with calcium-free HBSS to examine the role of extracellular calcium.

Pharmacological agents. To determine the contributions of various pathways and cellular components to the intracellular calcium re-
spontaneous response elicited by bubble-cell contact, dye-loaded HUVECs were treated with various pharmacological agents. Unless otherwise noted, the agents were handled per manufacturer’s instructions, prepared as 100× stock in the vehicle noted, and were applied individually at room temperature 20 min before bubble experiments, with no removal or wash step after exposure. Briefly, to examine the role of mechanosensitive channels, cells were pretreated with 25 μmol/l gadolinium chloride (prepared in recording HBSS; Sigma Aldrich, St. Louis, MO) or 10 mmol/l neomycin (prepared as positive controls. The dosages selected were the maximum doses tolerated by cells while maintaining solubility with no more than 1% vehicle, and, as a result, did not always yield complete inhibition. For example, the 20-min pretreatment with 1 μmol/l xestospongin C resulted in 75% of cells not responding to stimulus with the 10 μmol/l ATP, a positive control.

Air bubble-cell contact experiments. Air bubble-cell contact experiments were performed in similar fashion to previous work by Kobayashi et al. (28), with additional refinements. Drummond Nanoliter micropipettes (Drummond Scientific, Broomall, PA) were pulled using a Sutter model P-97 micropipette puller (Sutter Instruments, Novato, CA) and ground at a 32.5 degree angle using Narishige EG-44 grinder (Narishige, East Meadow, NY) to a diameter of 30–40 μm. A micropipette was then mounted on a Nanoject II injector (Drummond Scientific), backfilled alternately with recording HBSS and air, and manipulated with a PPM500 micromanipulator (WPI, Sarasota, FL). The micropipette tip was oriented such that the orifice was parallel to cell culture dish bottom, immersed in the HBSS recording buffer, and positioned ~700 μm above the cells. Air was expelled using the Nanoject II until a bubble formed at the tip of the micropipette, followed by gradual aspiration until the bubble was 50–150 μm in diameter (Fig. 1). The bubble was then manipulated into position 100–200 μm above the perinuclear region of an isolated target cell (see Fig. 1D) and then lowered at a rate of ~5 μm/s (z-axis manipulation only) until contact was observed in the phase contrast image capture. After bubble-cell contact, the bubble was gently lifted and manipulated out of the field of view, and the recording was continued for a total of 6 min.

Microscopy and image analysis. Cells were imaged using a SensiCam QE camera (The Cooke, Romulus, MI) (2×2 binning, 688×520) attached to Olympus IX70 microscope (Olympus, Melville, NY) with an Olympus LUCPlanFL N×40 0.6 numerical aperture objective (Olympus) and Photofluor light source (89 North, Burlington, VT). Computer control of the microscope was facilitated by LUDL programmable filter wheels, shutters, and focus control (Ludl Electronic Products, Hawthorne, NY) and both phase contrast (10 μm exposure) and fluorescent images (100 μm exposure) were collected every 1 s using IPL 3.7 software (BD, Rockville, MD) and saved as image stacks. ImageJ software (NIH, Bethesda, MD) was used to analyze the collected images. First, regions of interest (ROI) for background fluorescence (cell-free region) and around the nucleus were defined (Fig. 1D). Next, the Time Series Analyzer plugin was used to calculate the mean fluorescence intensity of the ROIs at each time point. Fluorescence ratio (Fp/Fn) was then calculated for each time point as: \( Fp = \frac{(F - F_{bg})}{(F_{bg} - F_0)} \), where \( F \) is intensity of the nuclear ROI, \( F_{bg} \) is the intensity of a background ROI, and \( F_0 \) intensity of the nuclear ROI before bubble impact. Typical \( F_0 \) was 65 to 75 arbitrary fluorescence units compared with typical \( F_{bg} \) of 55 to 65, with the exception of dishes containing neomycin, which contributed an additional ~15 units of autofluorescence. A cell was considered a “responder” if the \( F_F \) exceeded 1.5 within 100 s of bubble-cell contact. SigmaPlot (Systat, San Jose, CA) was used for data plotting and statistical analysis. Where appropriate, data are reported as means ± SD. When comparing the peak response elicited by bubble-cell contact under two different conditions, unpaired Student’s t-test was used to test significance, with \( P < 0.05 \) being considered significant.

RESULTS

HUVECs respond to bubble contact with a calcium transient. After contact with an air bubble, individual HUVECs respond with a calcium transient that typically achieves a maximum \( F_F \) of 5.9 ± 1.5 (\( n = 21 \)) within 10–20 s postcontact and then decays over 30–120 s, returning to baseline [see Fig. 2 for a montage of fluorescence micrographs (A) and representative trace (B)]. [By comparison, stimulation of the cells with 10 μmol/l ATP results in a \( F_F \) of 8.1 ± 0.6 (\( n = 6 \)) and 2 μmol/l ionomycin, a calcium ionophore, results in a \( F_F \) of >12.] Spatially, the calcium transient originates at the bubble contact point and rapidly spreads throughout the cell. Brief, perinuclear bubble-cell contact does not result in any discernable change in morphology over the course of 6 min recordings.

Extracellular Ca, entering via calcium channel, is necessary for bubble-cell contact response. To determine the origin of the calcium transient, air bubble contact experiments were conducted with low calcium recording HBSS (~26 μmol/l calcium), resulting in 6 of 13 cells (46%) not responding to bubble contact (see Fig. 2B for representative traces of responding cells). Furthermore, the peak of the calcium response in the responding cells was significantly reduced, 3.7 ± 1.7 (\( n = 7 \), \( P < 0.004 \)) compared with the normal recording HBSS experiments (Fig. 3A).

After the finding that extracellular calcium concentration affected the response of the cell-to-bubble contact, we used pharmacological interventions to examine the role of calcium channels, which may be responsible for the calcium influx from the extracellular medium to the intracellular region. After dye loading, HUVEC cells were incubated at room temperature for 20 min with recording HBSS containing 25 μmol/l gadolinium chloride (Gd), a nonspecific stretch-activated channel (SAC) inhibitor (8), or 1 μmol/l RuR, a transient receptor potential (TRP) vanilloid (TRPV) family of channel inhibitor (48). Both inhibitors had a marked effect (see Fig. 2 for representative traces of responding cells), with Gd resulting in 7 of 10 cells (70%) not responding to bubble contact and RuR abolishing the calcium response in 11 of 12 cells (91%) (Fig. 3B). As a positive control, cells were treated with 10 μmol/l ATP, resulting in a normal calcium response. The role of SACs was further examined by disrupting the actin cytoskeleton with 100 nmol/l cytochalasin D (20 min, room temperature, disruption was confirmed via phalloidin staining), which resulted in all nine cells (100%) not responding to bubble contact (Fig. 3B). Again, the response of these cells to ATP was not affected in this case. Collectively, these findings strongly suggest that bubble contact causes extracellular calcium to enter via a calcium channel, such as a mechanosensitive TRPV family member, and that this entry is necessary for the generation of the calcium transient.
Intracellular Ca release via IP3 receptor is also necessary for cell response to air bubble contact. We next examined the possible role of calcium release from intracellular stores as a result of bubble contact. In endothelial cells, calcium is primarily stored in the endoplasmic reticulum (ER) and released via IP3 receptor (IP3R) calcium channels (43). To block this pathway, we pretreated HUVECs for 20 min with either 1 μmol/l xestospongin C, an IP3R blocker (18), or 10 mmol/l neomycin, which binds phosphatidylinositol 4,5-bisphosphate (PIP2) (17), thus inhibiting phospholipase C (PLC). Both inhibitors had a marked effect (see Fig. 4 for representative traces of responding cells). Treatment with xestospongin C resulted in 9 of 14 cells (64%) not responding to bubble contact, whereas neomycin abolished the response in 6 of 9 cells (66%) (Fig. 5). These results strongly suggest that the release of intracellular calcium stores is also necessary for the generation of the calcium transient following bubble contact, and that the influx of extracellular calcium alone is not sufficient to produce a detectable calcium transient.

**DISCUSSION**

To develop effective preventative or therapeutic approaches to treating gas embolism, we require a greater understanding of the molecular signaling events elicited in endothelial cells by contact with air bubbles. The initial experiments of Kobayashi et al. (28) identified an increase in intracellular calcium as an early response to bubble-cell contact and associated this increase with cell injury. Those findings, combined with the fact that intracellular calcium is a crucial regulator of many endo-
Our studies with HUVECs confirm the findings of Kobayashi et al. (28) that large calcium transients result from air bubble-cell contact. We selected HUVECs for this study to be able to more readily compare our results to existing endothelial cell biology research (33). We find that HUVECs respond with calcium transients that are similar to those observed in bovine aortic endothelial cells (BAECs) used by Kobayashi et al. (28), with the primary difference being that we observe cells rapidly returning to precontact baseline calcium levels, within 2 min, as opposed to a more gradual reduction in cytosolic calcium levels. However, this temporal difference may be due to differences between the two endothelial cell lines or arise from changes in stimulation technique or length of recording window. Thus further work is required to determine whether endothelial cells from different vascular beds respond differently to air bubble contact. Like Kobayashi, we attempted to minimize cell-cell signaling and cross-talk by studying individual, isolated cells. However, in contrast to Kobayashi, our experiments involved only single, brief, nonlethal/injurious bubble stimulations in an attempt to isolate the response to

Fig. 2. A: montage of fluorescence micrographs illustrating the spatial and temporal progression of the calcium transient following air bubble contact. The time stamps indicate time relative to bubble impact and the location indicated by the white circle. B: representative traces of fluorescence signal (F_R) obtained from HUVEC loaded with Fluo-4 dye responding to air bubble contact at t = 0, under control recording HBSS, low calcium recording HBSS, and following pretreatment with calcium channel blockers gadolinium chloride (Gd, 25 μmol/l) or ruthenium red (RuR, 1 μmol/l).

Fig. 3. A: comparison of maximum F_R in HUVECs responding to bubble contact in recording HBSS (n = 21) or low calcium recording HBSS (n = 7). *P < 0.005. B: percentage of HUVECs responding to bubble contact under control conditions (recording HBSS), following 20 min pretreatment with calcium channel blockers Gd (25 μmol/l) or RuR (1 μmol/l) or after disruption of the actin cytoskeleton (CytoD, 100 nmol/l).
bubble contact from other pathways (such as apoptosis and/or necrosis). Furthermore, we refined the experimental technique to allow us to generate bubbles of uniform size, thus reducing variability in the contact area between air bubbles and cells. Our current technique also allows us to manipulate bubbles with a controlled rate of descent, thus reducing the role of any fluid shear component and minimizing the range of the force of impact and the degree of cellular deformation incurred. As a result of our efforts to minimize these potential confounding effects, the intracellular calcium transients we observe following air bubble contact under controlled conditions (recording HBSS) are very reproducible, with all 21 cells tested responding to bubble contact with a relative standard deviation of 26%. Thus, by carefully standardizing the bubble-related factors, we are confident that we were able to isolate the cell response to bubble contact, allowing us to use pharmacological interventions to help identify the signaling cascade behind this process. However, we acknowledge that variations in bubble parameters, including size and rate of descent, are likely to have an effect on the cellular response and may warrant additional investigation in future work.

Having essentially reproduced the basic findings of Kobayashi et al. (28) with another endothelial cell line, we proceeded to examine the role that extracellular calcium plays in generating the observed calcium transients. First, we confirmed the dependence of the air bubble contact response on extracellular calcium by lowering the extracellular calcium concentration 50-fold and observing reduced calcium signaling by HUVECs in response to air bubble contact. Next, we investigated how the extracellular calcium may be entering the cell following bubble-cell contact. We used two different cation channel blockers to determine whether extracellular calcium was entering via a channel. We first tested the effect of gadolinium, a nonspecific SAC inhibitor (8), on the bubble contact response. We found that this blocker had a marked effect, abolishing the calcium transient response of the cell to bubble contact in all but three cells. The lack of efficacy of gadolinium in these few experiments was likely due to the well-documented ability of various inorganic anions, such as the phosphate and bicarbonate present in our recording HBSS, to bind gadolinium, which can result in false negatives (8). To confirm our gadolinium results, we also used RuR, a TRPV family of channel inhibitor (48), which also consistently and completely inhibited the intracellular calcium signal in all but one cell. Furthermore, because actin has been associated with the function of SACs (26) and TRPV channels specifically (7), we confirmed our findings by pretreating cells with cytochalasin D, which disrupted the actin cytoskeleton and abolished the intracellular calcium response upon bubble air contact in all cells. Collectively, these results indicate that the entry of intracellular calcium through a TRPV channel is necessary for the generation of the calcium transients by bubble-cell contact. TRPV channels are a subfamily of the TRP cation channels that are gated by esoteric stimuli such as light, heat, osmolarity, stretch, or various chemical species (38). Four of the six known TRPV channels are expressed by endothelial cells, with TRPV1, TRPV3, and TRPV4 having known functional roles (6). Of particular interest is the possible involvement of TRPV4 in the response to bubble contact, as calcium entry through this channel has been shown to play a role in both vascular physiology [shear-dependent nitric oxide production and vasodilation (24, 29)] as well as pathology [ventilator-induced lung edema (22)]. Furthermore, exogenous activation of TRPV4 using a chemical agonist has been shown to cause microcirculatory collapse and vascular leak (47). This introduces the possibility that inhibitors of TRPV channels may have potential as pharmacological therapies for gas embolism.

The involvement of a TRPV channel in the response to air bubble contact is a key difference between the way cells respond to a mechanical stimulus, such as touching with a micropipette (12, 40) and contact with an air bubble. Briefly, Sigurdson et al. (40) found that the intracellular calcium transient resultant from micropipette stimulation required some extracellular calcium, and Diamond et al. (12) demonstrated that the transients could be blocked by cytochalasin B or manoolide, a phospholipase A and PLC inhibitor. However, the calcium signal was not affected by lowering the extracellular calcium concentration and was not blocked by gadolinium (40). Whereas the mechanical deformation in our experiments is similar to those of Sigurdson and Diamond, an air bubble is...
substantially different from a glass pipette tip in that it presents an air-liquid interface that can interact with biological macromolecules (30). At present, the mechanism of activation of TRPV channels, including TRPV4, in response to mechanical stimuli remains unclear (10): they may be activated directly or indirectly through a mechanosensitive partner, possibly via a second messenger. Ongoing work has demonstrated that proteins can adsorb to air-water interfaces (30) and that this adsorption can cause conformational changes, including protein denaturation (19, 20). The precise interactions between any specific protein and the air-liquid interface depend on the nature and structure of the protein (20, 35), as well as the presence of other species, including other proteins or surfactants (30). The endothelial cell surface is rich in biomolecules, both membrane bound and adsorbed (37). We hypothesize that the air-liquid interface presented by an air bubble is capable of directly interacting with endothelial cell surface biomolecules (TRPV channels and/or partner sensor molecules), causing a conformational change, resulting in a calcium influx via TRPV channel opening. Possible sensor partners include the mechanosensors implicated in sensing fluid shear stress, such as components of the glycocalyx (42), cell adhesion molecules (16), G proteins (21), or G protein-coupled receptors (9). Our conclusions are supported by the findings of Kobayashi that protein (5% BSA) and a surfactant (0.1% wt/vol Pluronic F-127) were protective in BAEC cells touched with bubbles, reducing both the peak of the calcium and lethality (compared with plain buffer). Protein present in the media will interact with both the bubble, adsorbing to the surface (23), as well as with the glycocalyx (2), effectively thickening the endothelial surface layer (37). Likewise, Pluronic F-127 has been shown to be capable of out-competing proteins at the air-liquid interface, minimizing protein adsorption (30). Both of these effects may serve to minimize direct interactions between the air bubble and membrane-associated proteins, such as a TRPV channel or partner sensor proteins, thus minimizing channel opening and calcium transient generation. Thus our findings support the concept of surfactant therapy for gas embolism (13).

While the influx of calcium through a channel is necessary to generate the observed intracellular calcium transients following bubble-cell contact, our results show that it alone is not sufficient to do so. The primary pathway responsible for calcium release in endothelial cells is the IP3-dependent release of ER calcium stores. Briefly, IP3 is produced by the enzymatic hydrolysis of PIP2, a membrane-associated phospholipid, by PLC. Once produced, IP3 can bind to the IP3 receptor at the ER, opening the calcium channel (43). We examined the contribution of intracellular calcium to the signal by either inhibiting the activity of PLC with neomycin, which binds PIP2 (17), or blocking the IP3 receptor using xestospongin C (18). Both of these inhibitors had similar and marked effects, abolishing the calcium transients in response to air bubble contact in about two-thirds of cells. Importantly, specifically blocking IP3 receptors alone, as opposed to PLC inhibition, should unmask any other PLC-dependent processes such as diacylglycerol- or IP3-dependent TRP calcium channel opening (38). However, both treatments resulted in similar results, leading us to conclude that IP3 receptor-mediated intracellular release of calcium from the ER is necessary and is the primary component of the observed calcium transients evoked by air bubble contact. The incomplete inhibition observed with xestospongin C and neomycin is likely due to dosage limitations, as we observed similar inhibition rates in

![Diagram](http://ajpcell.physiology.org/)

Fig. 6. Illustration of proposed mechanism by which air bubble contact elicits a calcium transient in HUVECs, with pharmacological interventions indicated by blunt arrows. (For clarity, the glycocalyx and endothelial surface layer have been not been drawn.) 1) Calcium influx following air bubble contact induced channel opening. [The left side of the illustration demonstrates possible direct interaction between air bubble and calcium channel, leading to channel opening and calcium influx, while the right side of the illustration demonstrates the interaction of the air bubble with a sensor protein (labeled “?”), which then causes to the calcium channel to open.] 2) PLC activation caused or facilitated by local increase in calcium, resulting in IP3 production. 3) IP3-dependent release of endoplasmic reticulum calcium stores.
response to the ATP-positive control. In the case of both inhibitors, we used the highest dose that we could expose the cells to without exceeding 1% DMSO vehicle, in the case of xestospongin C, or exceeding the solubility in aqueous media, in the case of neomycin. In pilot studies, we tested two alternative PLC inhibitors edelfosine and U73122, which also abolished the calcium response to air bubble contact (data not shown). However, these compounds were poorly tolerated by our cells. The use of an alternative IP3 receptor inhibitor 2-aminoethoxydiphenyl borate to confirm the xestospongin C results was considered; however, this antagonist also modulates TRP channels, which our earlier results had already determined to play an important role in the cellular response to air bubble contact.

Because our prior experiments had shown the importance of an extracellular calcium influx, we hypothesize that air bubble contact causes an initial calcium influx, resulting in a localized increase in calcium at the plasma membrane, sufficient to activate or enable the activation of PLC, which requires calcium for catalytic function (39). It is likely that we cannot detect the calcium influx alone due to the brief air bubble-contact resulting in a small, localized influx and the actions of the plasma membrane calcium ATPases and ER calcium ATPases, which serve to maintain cytosolic calcium homeostasis (43). Several isoforms of PLC are expressed by endothelial cells (15), but of particular relevance here are isoforms β and δ. In the presence of calcium, PLCβ is activated by G proteins (39), which may be involved in the transduction of air bubble contact. Alternatively, PLC isoform δ can be stimulated directly by physiological calcium levels (0.1–10 μmol/l) (4), such as those resulting from air bubble contact. Our findings appear similar to those of Diamond et al. (12), who found that the calcium transient resultant from micropipette stimulation could be eliminated by inhibiting phospholipase A and PLC, while the addition of arachidonic acid had no effect, suggesting PLC activation as the dominant pathway. However, our results with channel blockers and IP3 receptor blockade suggest that air bubble contact triggers channel opening first, followed by PLC activation. Thus air bubble and micropipette contact differ in way the responses are triggered, but there appears to be a commonality in the ultimate intracellular source of the calcium transients. Furthermore, endothelial cells also generate IP3 in response to shear stress (34) and this can be inhibited by neomycin (36). Given the importance of the IP3 pathway in the response to such a variety of stimuli, and the fact that it appears to be downstream of the initial trigger, it may not be a particularly attractive target for pharmacological intervention specific to gas embolism injury.

We have examined both the role of extracellular calcium influx and intracellular calcium release through a carefully considered series of experiments utilizing various pharmacological agents alone and in combination as a mechanistic means of revealing the critical components of HUVEC calcium signaling in response to bubble contact as can occur during intravascular gas embolism. It is our interpretation that endothelial cell mechanostimulation resulting from bubble contact causes the following sequence of events (Fig. 6): 1) contact between the air-liquid interface presented by the air bubble and the endothelial surface layer causes calcium channel opening (directly or indirectly) followed by calcium entry into the cell; 2) the localized increase in calcium concentration activates or facilitates the activation PLC, causing the hydrolysis of PIP2 and releasing IP3; and 3) the IP3 activates IP3R calcium channels, causing the release of calcium from ER stores, resulting in the large calcium transient we observe. The subsequent signaling caused by this calcium transient is likely to be responsible for the cell injury caused by air bubbles (28), thus mitigating this cellular response to air bubble contact is an attractive target for therapeutic intervention. Our work here clarifies the molecular pathways involved and suggests that further examination of the air-liquid-cell interface is most likely to yield a novel therapeutic target, particularly for preventive care.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

REFERENCES

AIR BUBBLES, CALCIUM, AND ENDOTHELIAL CELLS


