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SHORT COMMUNICATION

Hypoxic Modulation of Ca^{2+} Signaling in Human Venous and Arterial Endothelial Cells

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Abstract Our understanding of vascular endothelial cell physiology is based on studies of endothelial cells cultured from various vascular beds of different species for varying periods of time. Systematic analysis of the properties of endothelial cells from different parts of the vasculature is lacking. Here, we compare Ca^{2+} homeostasis in primary cultures of endothelial cells from human internal mammary artery and saphenous vein and how this is modified by hypoxia, an inevitable consequence of bypass grafting (2.5% O_2, 24 h). Basal $[Ca^{2+}]_i$ and store depletion-mediated Ca^{2+} entry were significantly different between the two cell types, yet agonist (ATP)–mediated mobilization from endoplasmic reticulum stores was similar. Hypoxia potentiated agonist-evoked responses in arterial, but not venous, cells but augmented store depletion-mediated Ca^{2+} entry only in venous cells. Clearly, Ca^{2+} signaling and its remodeling by hypoxia are strikingly different in arterial vs. venous endothelial cells. Our data have important implications for the interpretation of data obtained from endothelial cells of varying sources.

Keywords Ca^{2+} · Hypoxia · Endothelium · Human tissue · Vein · Artery · Ca^{2+} influx · Ca^{2+} store

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Introduction

Endothelial cells play a vital role in vascular functions such as coagulation, inflammation, vessel permeability, angiogenesis, lipid metabolism and vascular tone (Cai and Harrison 2000; Galley and Webster 2004; Li and Shah 2004; Harrison and Cai 2003). Although a continuum, endothelial cells in different parts of the vasculature exist under very different conditions. For example, endothelial cells in arteries are exposed to high, pulsatile pressures and relatively high levels of oxygenation, whereas venous cells are exposed to much lower pressures and oxygen tensions. This diversity of location doubtless places differing demands on endothelial cells throughout the vasculature, so it is highly unlikely that any data obtained from one specific type of endothelial cell (e.g., the commonly used human umbilical vein endothelium or bovine aortic endothelium) reflect the behavior of all endothelial cells (Klein et al. 1994; Tan et al. 2004). This issue extends to studies in endothelial cell lines, which are now recognized as varying significantly from their natural counterparts (Scoumanne et al. 2002; Unger et al. 2002).

Many endothelial cell functions require Ca^{2+} as an intracellular second messenger (Tiruppathi et al. 2002; Hoebel et al. 1997; Nilius and Droogmans 2001; Tran et al. 2000). For example, formation of nitric oxide is Ca^{2+} dependent (Yao and Huang 2003). Thus, Ca^{2+} homeostasis must be tightly regulated in order for endothelial cells to respond appropriately to various cues. Like other nonexcitable cells, rises of cytosolic $[Ca^{2+}]$ can be induced by release from internal stores (primarily the endoplasmic reticulum [ER]) following application of agonists capable of generating inositol 1,4,5-trisphosphate (IP_3), and this store depletion can in turn trigger Ca^{2+} influx across the plasma membrane via capacitative Ca^{2+} entry (Tiruppathi

et al. 2002; Bishara et al. 2002; Fiorio and Munaron 2001; Nilius & Droogmans, 2001). Restoration of cytosolic Ca^{2+} levels after such activity involves reuptake of Ca^{2+} into organelles as well as extrusion across the plasma membrane utilizing the Na^{+}–Ca^{2+} exchanger (e.g., Sage et al. 1991) and Ca^{2+}-ATPase (e.g., Liu et al. 1996).

Ca^{2+} levels within excitable cells can be regulated by oxygen tension via modulation of ion channels (e.g., Peers 1997; Lopez-Barneo et al. 2001). The effects of hypoxia in nonexcitable cells have received comparatively little attention, but evidence is accumulating to indicate that, as in excitable cells, a close relationship exists between O_2 levels and $[Ca^{2+}]_i$. Thus, various studies have indicated that hypoxia causes, e.g., a sustained rise of $[Ca^{2+}]_i$ (Arnould et al. 1992) or triggers oscillations of $[Ca^{2+}]_i$ following reoxygenation (Hu and Ziegelstein 2000; Kimura et al. 2000). At present, however, there has been no systematic or comparative analysis of the relationship between O_2 levels and $[Ca^{2+}]_i$ in endothelial cells from different vascular beds. This is of fundamental importance since hypoxia is a primary cue in Ca^{2+}-dependent endothelial responses associated with a variety of clinically important situations, such as proliferation (Schafer et al. 2003), inflammation-induced increases of vessel permeability (Pearlstein et al. 2002) and release of nitric oxide (Yao and Huang 2003), the last being of particular importance in coronary artery bypass grafting (Pearson et al. 1998). Furthermore, the hypoxia of blood stasis can precipitate formation of varicoses (Michiels et al. 1993; Michiels et al. 1997). In the present study, we therefore examined Ca^{2+} homeostasis and how this is modified by chronic hypoxia in primary endothelial cells from human internal mammary artery and saphenous vein, two tissues of clinical importance as they are used for coronary artery bypass grafting (Nwasokwa 1995).

Materials and Methods

Isolation and Culture of Endothelial Cells

The isolation of primary cultures of endothelial cells was adapted from previously described methods (Budd et al. 1991). Samples of internal mammary artery or saphenous vein were collected from patients undergoing elective coronary bypass grafting, following local ethical permission and informed, written patient consent. Tissue from a total of 37 patients with coronary artery disease and no comorbidity was used, 10 female (27%) and 27 male (73%). Age ranged 44–79 years, with a median of 67.

Individual samples, ranging 10–30 mm in length, were opened longitudinally and pinned, lumen uppermost, onto silicone elastomer–coated 60-mm Petri dishes using A1 Minuten pins (Carolina Biological, Burlington, NC). The tissue sample was then incubated in 1 mg/ml Type II collagenase (Worthington Biochemicals, Lorne Laboratories, Reading, UK) dissolved in Medium 199 (Sigma, Poole, UK) (37°C, 15 min). The collagenase solution was collected along with 2× 10-ml wash solution (MEM supplemented with 5% FCS and 1% antibiotic/antimycotic), which was used to detach any residual endothelial cells from the tissue. The suspension was centrifuged for 6 min at 600×*g*, the supernatant removed and the pellet resuspended in 25-ml wash solution and recentrifuged. The supernatant was once again removed, and the final pellet was resuspended in 4 ml of complete endothelial culture medium (M199) supplemented with 20% FCS, 1% penicillin–streptomycin, 1% glucose, 1 M HEPES (GIBCO-BRL, Paisley, UK), heparin (5 U/ml; Leo Laboratories, Princes Risborough, UK), endothelial growth factor (15 μg/ml) and pyruvate (1 μM; Sigma-Aldrich, Poole, UK). This was then plated into a 25-cm^2 flask and maintained in a humidified incubator at 37°C (95% air, 5% CO_2). Two days following plating, cells received a full medium change to remove nonadherent cells. Culture medium was then half changed every 2–3 days, resulting in a confluent flask within 2–3 weeks. This was designated passage 0; cells were subcultured using trypsin and used for experiments up to passage 3. Exposure of cells to chronic hypoxia was performed by transferring them to an incubator gassed with 2.5% O_2, 5% CO_2 and 92.5% N_2 for 24 h prior to study. Wherever studied, effects of hypoxia were compared with results observed in normoxia on cells from the same patient at the same passage.

Measurement of $[Ca^{2+}]_i$

Cells were plated onto glass coverslips in 24-well culture plates and grown to approximately 80% confluence. Coverslips onto which cells had grown were incubated in 2 ml of culture medium containing 4 μM Fura-2AM (Molecular Probes, Cambridge, UK) for 40 min at 37°C in the dark, then left to deesterify for 15 min in control solution. Fragments of coverslips were then transferred into an 80-μl recording chamber mounted on the stage of an inverted microscope, where cells were continuously perfused under gravity at a rate of ca. 5 ml/min. Control perfusate was composed of (in mM) NaCl 135, KCl 5, $MgSO_4$ 1.2, $CaCl_2$ 2.5, HEPES 5 and glucose 10 (pH 7.4, osmolarity adjusted to 300 mOsm with sucrose, 21–24°C). Nominally Ca^{2+}-free perfusate contained 1 mM EGTA and no added Ca^{2+}. $[Ca^{2+}]_i$ was determined ratiometrically using an Improvision monochromator-based imaging system (Openlab Image Processing & Vision, Coventry, UK), alternating excitation 340 and 380 nm (0.2 Hz), emission 510 nm. Regions of interest were used to restrict data collection to individual cells. All imaging was controlled by

Improvision software, including Openlab 2.2.5. Solutions were exchanged via a gravity-fed perfusion system, and all experiments were conducted at 21–24°C. Since calibration of Ca^{2+} signals can introduce artefactual inaccuracy (Duchen 1992), data are presented as ratio units. However, to avoid unexpected differences in the ability of Fura-2 to report $[Ca^{2+}]_i$ in the two cell types under investigation, a preliminary calibration yielded similar R_{max} and R_{min} values (1.754 ± 0.011 and 0.333 ± 0.011 for arterial cells [$n = 5$ determinations] and 1.833 ± 0.014 and 0.338 ± 0.013 for venous cells [$n = 5$]), and there was no obvious subcellular compartmentalization of the dye. Where relevant, results are expressed as means ± SEM, together with example traces, and statistical comparisons were made using unpaired Student's *t*-tests. All mean data were obtained from the number of individual cells or recordings indicated. In each case these were collected from up to eight, typically three or four, different patients.

Measurement of Membrane Potential

The whole-cell configuration of the patch-clamp technique was used to monitor membrane potential. Following formation of a gigaseal and rupture of the patch of membrane within the patch tip required to gain access to the cell interior, the amplifier was switched to current clamp ($I = 0$) mode to determine membrane potential. For these recordings, cells were perfused with a solution containing (in mM) 135 NaCl, 5 KCl, 1.2 $MgCl_2$, 5 HEPES, 2.5 $CaCl_2$ and 10 D-glucose (pH 7.4 with KOH, 21–24°C). Patch pipettes (resistance 4–7 MΩ) were filled with an intracellular solution consisting of (in mM) 10 NaCl, 117 KCl, 2 $MgCl_2$, 11 HEPES, 11 EGTA, 1 $CaCl_2$ and 2 Na_2ATP (pH 7.2 with KOH). Data were acquired and digitalized through Digidata 1322A in combination with an Axopatch 200B amplifier and Clampex 9 software (all Molecular Devices, Foster City, CA), sampled at 2 kHz and filtered at 1 kHz.

Results

Resting $[Ca^{2+}]_i$ was monitored in both internal mammary arterial endothelial cells (hereafter referred to as "arterial endothelial cells" [AECs]) and saphenous vein endothelial cells ("venous endothelial cells" [VECs]), while cells were perfused with a solution containing 2.5 mM Ca^{2+} (Fig. 1a). Resting $[Ca^{2+}]_i$ was significantly higher in AECs compared with VECs (Fig. 1a, b). When the perfusate was exchanged for one which was nominally Ca^{2+}-free (Ca^{2+} replaced with 1 mM EGTA), resting $[Ca^{2+}]_i$ levels fell in both cell types (Fig. 1a), an effect which was statistically significant (Fig. 1b). Thus, the equilibrium between Ca^{2+} influx vs. efflux and intracellular uptake, which determines resting cytosolic $[Ca^{2+}]$, was similarly altered by removing external Ca^{2+}, but perhaps more importantly, the factors regulating this equilibrium are clearly different in endothelial cells from the arterial and venous tissues employed.

As described under "Introduction," numerous agonists evoke endothelium-dependent vascular effects by causing a rise of $[Ca^{2+}]_i$. This typically occurs via activation of a G protein–coupled receptor, leading to the generation of IP_3 and activation of ER IP_3 receptors, which allow Ca^{2+} to flow from the ER to the cytosol. This emptying of Ca^{2+} stores in turn triggers Ca^{2+} entry (termed "capacitative" or "store depletion–mediated" Ca^{2+} entry). Ca^{2+} mobilization can be temporally isolated from Ca^{2+} influx by applying agonists in the absence of extracellular Ca^{2+}. Figure 1c shows $[Ca^{2+}]_i$ responses measured in representative AECs and VECs during application of a maximally effective concentration of ATP (10 μM). The time course of responses appeared different between the two cell types, being more transient in nature in AECs than VECs. However, peak responses were not significantly different. Indeed, full concentration–response relationships determined in the two cell types were remarkably similar (Fig. 1d), yielding EC_{50} values of 1.13 μM for AECs and 1.27 μM for VECs.

Following agonist-evoked mobilization of Ca^{2+} from internal stores, Ca^{2+} entry pathways are activated in endothelial and many other cell types. Figure 2a shows the mean time courses of rises of $[Ca^{2+}]_i$ caused by addition of 2.5 mM Ca^{2+} to the perfusate in cells which had previously been exposed to 10 μM ATP in the absence of extracellular Ca^{2+}. Clearly, store-operated Ca^{2+} entry is observed in both cell types, but it was significantly greater ($P < 0.001$, unpaired *t*-test) in AECs compared with VECs. One possible explanation for this difference is that stores were less completely discharged in VECs, leading to a smaller rise of $[Ca^{2+}]_i$ when Ca^{2+} was restored to the perfusate. To address this, we exposed cells to 1 μM thapsigargin (which causes complete ER store depletion through inhibition of the Ca^{2+} ATPase responsible for reuptake into the ER) in Ca^{2+}-free solution for 20 min prior to recording $[Ca^{2+}]_i$. Subsequent rises of $[Ca^{2+}]_i$ on reexposure to Ca^{2+}-containing perfusate are plotted in Fig. 2b. In both cell types the responses were larger than those observed following ATP application (Fig. 2a), but the difference between the two cell types remained striking and significant ($P < 0.001$). Thus, compared with VECs, AECs appear to display a greater Ca^{2+} influx following store depletion regardless of the method of store emptying. To confirm that the rises of $[Ca^{2+}]_i$ being monitored were indeed attributable to store depletion, we removed and then replaced extracellular Ca^{2+} without mobilizing intracellular stores. Figure 1c indicates that, on return of Ca^{2+} to the perfusate, the resultant changes of $[Ca^{2+}]_i$ were negligible in both AECs and VECs. The greater magnitude of store

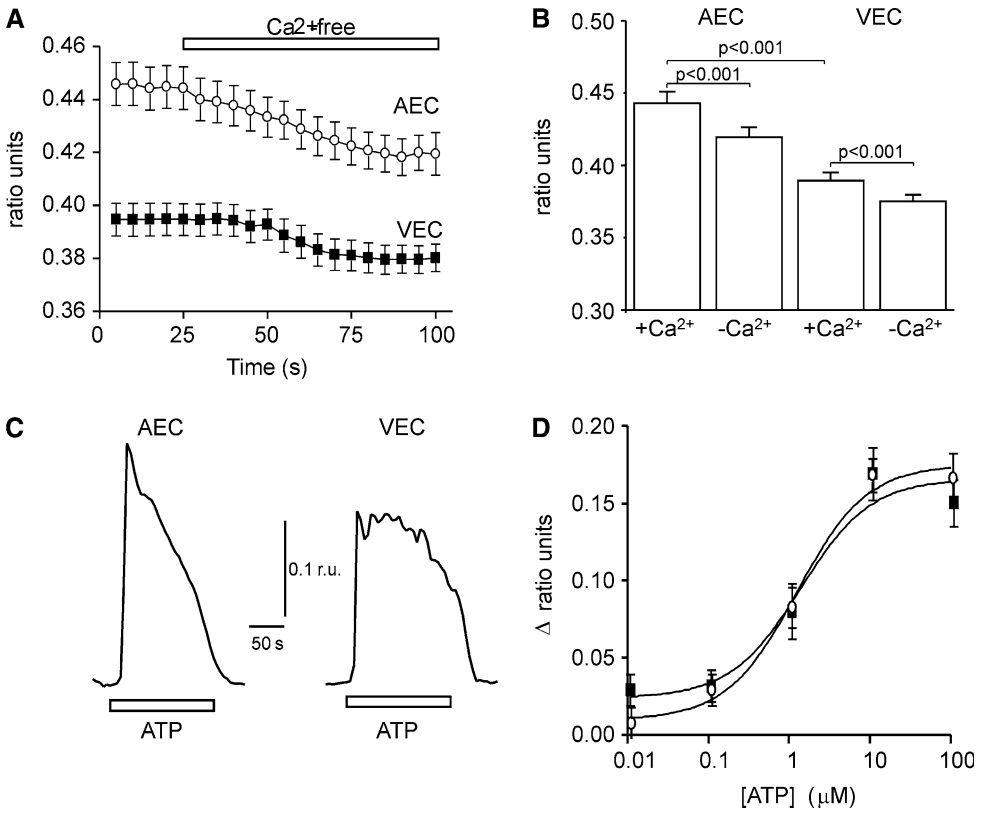

Fig. 1 **a** Mean (±SEM, bars) measurements of $[Ca^{2+}]_i$ taken from internal mammary AECs (*open symbols*, $n = 28$ recordings from seven patients: five male, two female) and saphenous VECs (*filled symbols*, $n = 28$ recordings from eight patients: six male, two female). For the period indicated by the *horizontal bar*, Ca^{2+} was removed from the external perfusate and replaced with 1 mM EGTA. Data were averaged every 5 s to construct the graph. **b** Mean (±SEM) basal $[Ca^{2+}]_i$ in AECs ($n = 28$) and VECs ($n = 28$) from experiments as in **a** in the presence (*open bars*) and absence (*shaded bars*) of extracellular Ca^{2+}. Statistical significance was determined using *t*-tests (paired between conditions, unpaired between tissues). **c** Rises of $[Ca^{2+}]_i$ evoked by exposure of cells to 10 μM ATP (applied for period indicated by *horizontal bar* in each case) recorded in AECs and VECs. Extracellular Ca^{2+} was absent throughout (replaced with 1 mM EGTA). Scale bars apply to both traces. **d** Mean (±SEM) concentration–response relationships obtained under conditions as in **c** for AECs (*open symbols*, EC_{50} 1.13 μM) and VECs (*closed symbols*, EC_{50} 1.27 μM). Each point is the mean from eight to 10 individual recordings (three to eight patients in each case)

depletion–mediated Ca^{2+} entry seen in AECs compared with VECs could be attributable to an increased driving force for Ca^{2+} entry. To examine this possibility, we monitored membrane potential in the two cell types. As indicated in Fig. 2d, membrane potential was not significantly different between AECs (-50.4 ± 6.2 m*V*, $n = 13$) and VECs (-47.3 ± 5.0 m*V*, $n = 10$).

To examine any potential remodeling of Ca^{2+} signaling by chronic hypoxia (CH), we compared responses evoked as in Figs. 1 and 2 between cells cultured under normoxic vs. CH (2.5% O_2, 24 h) conditions. Such treatment had no significant effects on resting $[Ca^{2+}]_i$. Thus, in AECs, basal levels were 0.464 ± 0.010 ratio units in Ca^{2+}-containing perfusate, which fell to 0.446 ± 0.014 ratio units ($n = 42$, from 14 patients: 10 male, four female). For VECs, basal levels were 0.399 ± 0.007 ratio units in Ca^{2+}-containing perfusate, which fell to 0.389 ± 0.007 ratio units ($n = 3{,}042$, from 10 patients: eight male, two female). Ca^{2+} mobilization from ER stores by exposure of cells to 10 μM ATP is show in Fig. 3. For AECs, responses to ATP remained more transient in appearance (Fig. 3a) than were seen in VECs (Fig. 3b) regardless of O_2 levels. However, CH produced a significantly greater ATP-evoked response in AECs (Fig. 3a) compared with normoxically cultured AECs. This effect of hypoxia appeared to be dependent on the source of cells since ATP-evoked responses in VECs were unaffected by an identical period of CH (Fig. 3b). The augmentation of the ATP-evoked rise of $[Ca^{2+}]_i$ seen in AECs could be attributable to an increase in store size. To investigate this, we monitored rises of $[Ca^{2+}]_i$ evoked by cyclopiazonic acid (CPA, a reversible inhibitor of ER Ca^{2+} ATPase) in the absence of extracellular Ca^{2+}. As indicated in Fig. 3c, store size, as determined by responses to CPA, were unaffected by hypoxia and, indeed, were not significantly different between AECs and VECs. Interestingly, if we compare responses to CPA with those evoked by ATP, our findings indicate that ATP mobilizes a greater fraction of the CPA-sensitive pool in AECs and that this

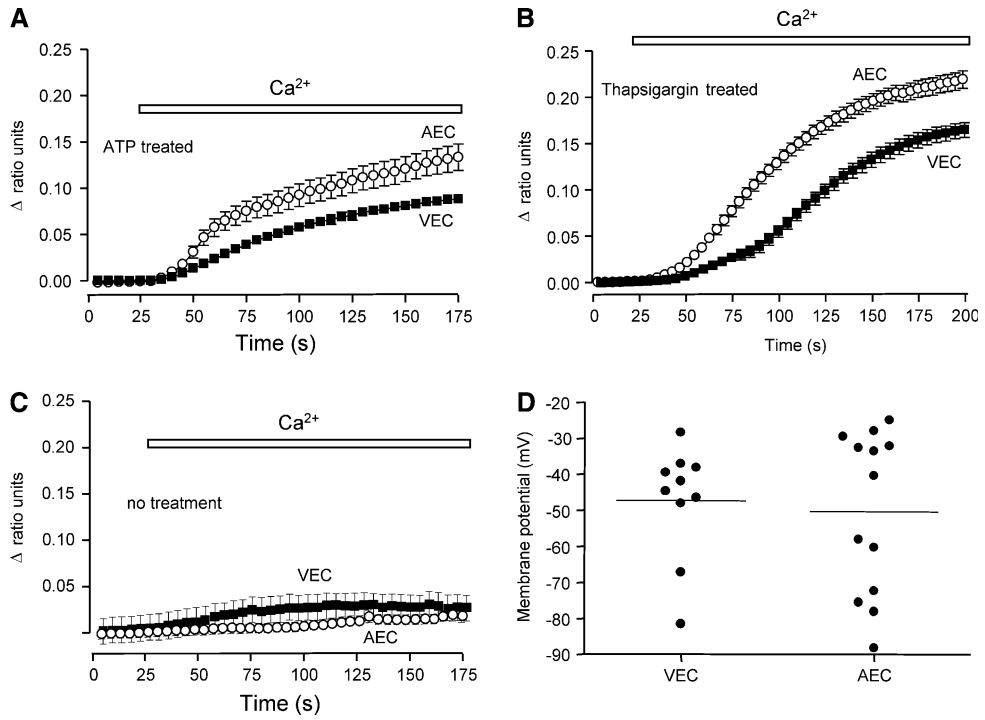

Fig. 2 **a** Mean (±SEM) time courses of rises of $[Ca^{2+}]_i$ observed when Ca^{2+} was replaced in the perfusate (for the period indicated by the *horizontal bars*) in AECs (*open symbols*, $n = 60$ from 14 patients: 11 male and three female) and VECs (*filled symbols*, $n = 78$ from 16 patients: 13 male and three female). Cells were previously exposed to 10 μM ATP in the absence of extracellular Ca^{2+}. **b** Like **a**, except that stores were initially depleted by exposure of cells to 1 μM thapsigargin for 20 min in the absence of extracellular Ca^{2+} ($n = 48$ AECs from nine patients [seven male, two female] and 63 VECs from 14 patients [12 male, two female]). **c** Like **a**, except that no intervention was applied to modulate Ca^{2+} stores ($n = 8$ AECs from three patients [two male, one female] and $n = 10$ VEC recordings from four patients [three male, one female]). **d** Scatterplot of membrane potentials obtained by whole-cell patch-clamp recordings in AECs (three patients: two male, one female) and VECs (three patients: two male, one female). Each point is an individual cell's value. Bars indicate mean values for each cell group

fraction is increased by hypoxia, whereas in VECs ATP mobilizes a slightly smaller fraction of the CPA-sensitive Ca^{2+} pool and this is unaltered by hypoxia.

Following maximal store depletion by exposure of cells to 1 μM thapsigargin for 20 min, the effects of CH on store depletion–mediated Ca^{2+} influx were measured in the two endothelial cell types. As shown in Fig. 4a, CH was without effect on the maximal rises of $[Ca^{2+}]_i$ observed in AECs following addition of Ca^{2+} to the perfusate, although there was a trend for responses to show a slight delay compared with those seen in normoxically cultured cells. In marked contrast, store depletion–mediated Ca^{2+} entry was significantly increased by CH in VECs without any obvious change in the time course of influx (Fig. 4b). These data collectively show that Ca^{2+} signaling in AECs and VECs adapts to CH but in markedly different ways.

Discussion

The present study has revealed a number of issues concerning endothelial Ca^{2+} homeostasis which are likely to be of physiological and potentially clinical importance. Our work presents the first direct comparison of human endothelial cell Ca^{2+} signaling in primary cultures of cells from two different vascular sites: the internal mammary artery and the saphenous vein. As previously described for vascular endothelial cells of other origins (Aromolaran and Blatter 2005), the vasoactive agent ATP was able to mobilize Ca^{2+} from an intracellular pool, presumably via the generation of IP_3 and subsequent activation of its receptors on the ER. We found that the sensitivity of Ca^{2+} mobilization in response to exogenous ATP was indistinguishable between AECs and VECs (Fig. 1). However, all other aspects of Ca^{2+} signaling examined were strikingly different in the two tissues. Thus, compared with VECs, basal Ca^{2+} levels in AECs were significantly higher (Fig. 1), as was store depletion–mediated Ca^{2+} influx following treatment with either ATP or thapsigargin (Fig. 2). One possible explanation for this is that AECs might have a more hyperpolarized membrane potential than VECs, therefore providing more driving force for Ca^{2+} influx. However, if this were the explanation for such differences, basal cytosolic Ca^{2+} levels in the absence of extracellular

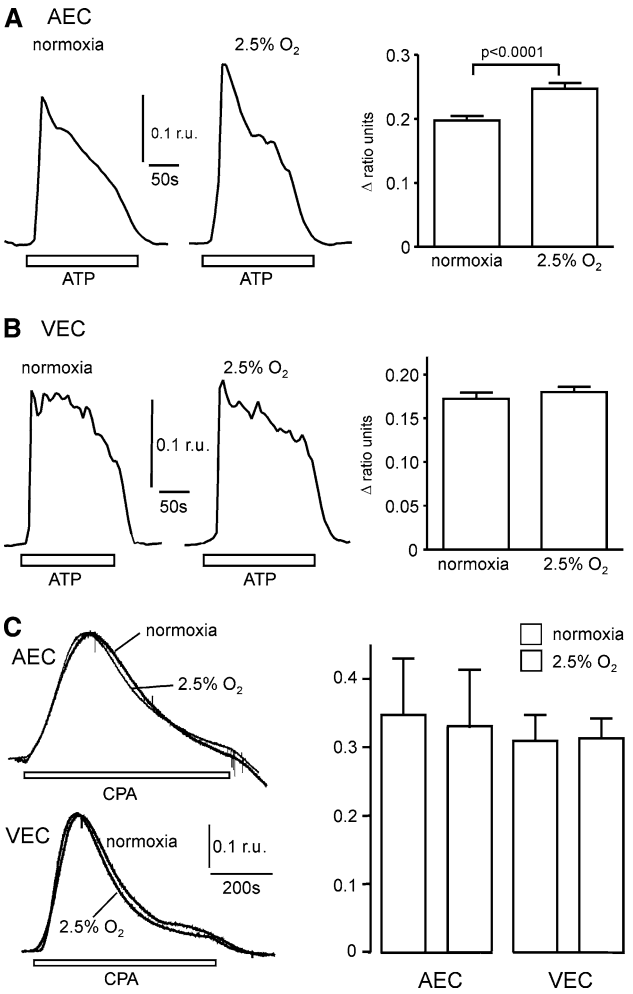

Fig. 3 **a** Rises of $[Ca^{2+}]_i$ evoked by exposure of normoxic and hypoxic (2.5% O_2) cultured AECs to 10 μM ATP (applied for period indicated by *horizontal bar* in each case). Extracellular Ca^{2+} was absent throughout (replaced with 1 mM EGTA). Scale bars apply to both traces. Bar graph shows mean (±SEM) peak responses evoked by 10 μM ATP in normoxic (*open bar*, $n = 51$ from 12 patients: 10 male and two female) and hypoxic (*shaded bar*, $n = 47$ from the same 12 patients) cells. Statistical significance was determined using unpaired *t*-test. **b** Like **a**, except data were obtained from VECs ($n = 48$ normoxic and 60 hypoxic recordings from 16 patients [13 male, three female]). **c** *Left* Traces showing rises of $[Ca^{2+}]_i$ evoked in the absence of extracellular Ca^{2+} in AECs (*upper*) and VECs (*lower*) in response to CPA (10 μM). Data were obtained from normoxically and hypoxially maintained cells, as indicated. *Right* Mean (±SEM) peak responses evoked by 10 μM CPA in normoxic and hypoxic AECs ($n = 10$ and 11, respectively; three patients: two male, one female) and normoxic and hypoxic VECs ($n = 12$ for each group; three patients: two male, one female)

Ca^{2+} would not have remained so different between the two cell types (Fig. 1), and as shown in Fig. 2d, membrane potentials were similar in the two groups. Instead, we can at present only speculate that there exist differences in the expression or activity of the various pumps, channels and transporters which collectively determine transmembrane Ca^{2+} movements in these two cell types.

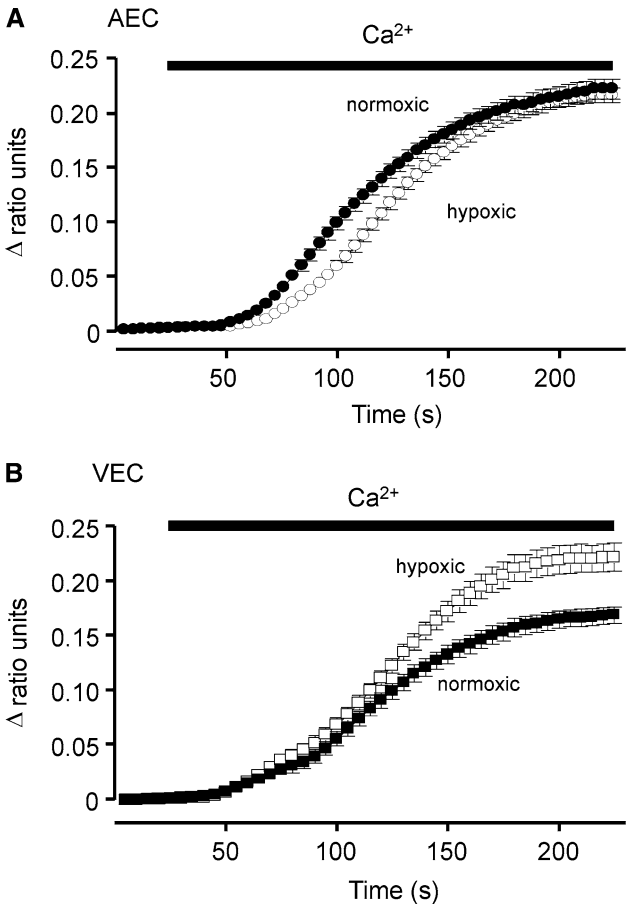

Fig. 4 **a** Mean (±SEM) time courses of rises of $[Ca^{2+}]_i$ observed when Ca^{2+} was replaced in the perfusate (for the period indicated by the *horizontal bars*) in AECs cultured normoxically (*solid symbols*, $n = 48$ from nine patients [seven male, two female]) or hypoxically (2.5% O_2, $n = 49$, *open symbols*, from the same nine patients). Intracellular stores were previously depleted by exposure to 1 μM thapsigargin for 20 min in the absence of extracellular Ca^{2+}. **b** Like **a**, except that data were obtained from VECs ($n = 63$ normoxic and 83 hypoxic recordings from 14 patients [12 male, two female]). Normoxic data in each case were replotted from Fig. 2a, b

One of the fundamental differences facing VECs and AECs in vivo is the O_2 levels to which they are exposed. For this reason, we speculated that they might respond differently to an imposed change of O_2 levels. It should be noted that our experimental paradigm was to compare normoxia with a hypoxic level of 2.5% O_2. In reality, the cells would experience lower local levels of O_2 under both normoxic and hypoxic conditions due to diffusional gradients and O_2 consumption. Indeed, a previous study has indicated that endothelial cells maintained in an atmosphere of air and 5% CO_2 have a local pO_2 of ca. 80 mm Hg (Metzen et al. 1995). Thus, any experimentally imposed O_2 level likely leads to a greater degree of hypoxia. Notwithstanding this, we found differential adaptation to hypoxia in AECs and VECs, yet clear remodeling of Ca^{2+} homeostasis was observed in both cell types. Thus, for AECs Ca^{2+} mobilization by

supramaximal levels of ATP evoked larger responses in cells maintained under hypoxic conditions (Fig. 3a), yet responses remained unchanged in VECs. This effect was observed despite the fact that the CPA-sensitive intracellular Ca^{2+} pool was not significantly different between the two cell populations and was unaffected by hypoxia (Fig. 3c). Also noteworthy, and in contrast to the effects of hypoxia on pulmonary smooth muscle (Bonnet et al. 2001), there was no change in the rate of recovery of $[Ca^{2+}]_i$ following store mobilization by either ATP or CPA (Fig. 3). By contrast, store depletion–mediated Ca^{2+} entry was largely unchanged in AECs yet was markedly enhanced in VECs. The present study does not provide mechanistic explanations for such effects of hypoxia. However, exploration of changes in expression or activity of the numerous proteins involved in Ca^{2+} homeostasis caused by hypoxia is clearly a worthy future aim. Hypoxia is a primary feature of numerous cardiorespiratory disorders, such as lung diseases (e.g., emphysema, pulmonary hypertension) in which ventilation perfusion matching is compromised and can lead to marked remodeling of numerous cell functions, including endothelial Ca^{2+} homeostasis (e.g., Abdallah et al. 2007; Millar et al. 2007). The concept of hypoxic remodeling of Ca^{2+} homeostasis is not restricted to the endothelium, and clear evidence of altered Ca^{2+} homeostasis caused by chronic hypoxia is found in, e.g., the vasculature (Bonnet et al. 2001; Paffett and Walker 2007), arterial chemoreceptors (Powell 2007), astrocytes (Smith et al. 2003) and neurons (Peers et al. 2005; Webster et al. 2006). However, no clear pattern of response to hypoxia is common to each tissue type studied. Our present data indicate that physiological Ca^{2+} homeostasis in human vascular endothelial cells and its remodeling by hypoxia are very much dependent on where in the circulation the cells were harvested from. Since Ca^{2+} homeostasis itself is fundamental to the physiology of the endothelium (Dedkova and Blatter 2002; Nilius et al. 2003; Galley and Webster 2004), these findings are likely to have important implications for determining the role and importance of endothelial function in both the laboratory and clinical settings.

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