Hypoxia activates a background conductance in freshly isolated pulmonary arterial endothelial cells of the rat

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Abstract Utilising the patch-clamp recording technique we have demonstrated for the first time the effects of hypoxia on the background current in pulmonary arterial endothelial cells. Electrophysiological studies revealed the presence of a novel oxygen-sensitive, non-selective cation conductance (I_{NSC}) in these cells. The inward component of I_{NSC} was significantly potentiated by hypoxia. Both the inward and outward components of I_{NSC} were inhibited by both La^{3+} and Gd^{3+}. Hypoxic activation of I_{NSC} may provide an important Ca^{2+} influx pathway essential for the release of a pulmonary-selective vasoconstrictor pivotal to the sustained phase of hypoxic pulmonary vasoconstriction.

Key words: Pulmonary artery; Endothelial cell; Non-selective cation channel; Hypoxia

1. Introduction

Pulmonary arterial blood gas tension in the lungs is optimised by the matching of local perfusion to local ventilation and is achieved by a mechanism known as hypoxic pulmonary vasoconstriction (HPV). It is generally accepted that, in the small pulmonary arteries (200–400 μm diameter), HPV is a biphasic response consisting of phase 1, an initial transient constriction, followed by phase 2, a sustained but slowly developing constriction [1,2]. The endothelium has been shown to be essential for phase 2 of HPV [1,3,4] and many reports also indicate removal of the endothelium attenuates phase 1 of HPV [1,4–6]. The importance of the endothelium in HPV is further compounded by the opinion that phase 2 constriction of HPV is physiologically more relevant than phase 1, since phase 2 is sustained [2].

Recently it has been suggested that phase 2 of HPV may depend upon endothelial release of a vasoconstrictor [1,2,7,8], since, during hypoxia, removal of the endothelium decreases tone to below the level of pretone [1]. It is also known that release of vasoactive mediators by endothelial cells is closely associated with their membrane potential (E_m) and consequently their intracellular Ca^{2+} concentration [9,10], with hyperpolarisation driving entry of extracellular Ca^{2+} [10]. It is therefore interesting to note that phase 2 constriction of HPV has been shown to occur as a result of elevated Ca^{2+} entry [8,11,12], and recently several reports have demonstrated that hypoxia can indeed release vasoconstricting factors from the pulmonary endothelium [7,13,14].

Electrophysiological studies aimed at elucidating the ion channels responsible for maintaining the E_m of pulmonary arterial endothelial cells (PAEC) have mainly been confined to cultured cell models and those from larger conduit arteries [15,16]. These are not ideal models with which to study the ions channels of endothelial cells of small pulmonary arteries since it has been shown that main pulmonary arteries respond to hypoxia with a transient constriction followed by full relaxation [17–20], indicating the absence of the mechanism(s) responsible for phase 2 HPV within the endothelium of these arteries. Differences are also known to exist between the electrophysiological profile of cells from cultured and freshly isolated preparations, where changes or loss in channel expression is thought to occur [21–23].

We have previously shown that endothelial cells freshly isolated from small pulmonary arteries possess a K^+ conductance [24]. However, the nature of the background currents of these pulmonary endothelial cells, freshly isolated from resistance arteries, is unknown although a non-selective cation channel has been reported in pulmonary endothelial cell lines [16]. This channel is thought to represent an important Ca^{2+} influx pathway, with influx driven by hyperpolarisation [15,25,26], that may in turn influence release of vasoactive mediators.

The aim of the present study was to identify the nature of the background currents in endothelial cells freshly isolated from small pulmonary arteries of the rat. Hypoxia is thought to cause a rise in intracellular Ca^{2+} in these cells coupled to release of a pulmonary-selective vasoactive mediator that is essential for phase 2 of HPV. Since these currents may underlie the oxygen-sensing mechanisms present in the endothelium of small pulmonary arteries that initiate sustained HPV, it was important to determine the hypoxia sensitivity of these currents.

2. Materials and methods

2.1. Cell isolation

Male Wistar rats (~200 g; Harlan, UK) were killed by an overdose of i.p. sodium pentobarbitone (Rhone Merieux, Ireland). Following removal and cleaning, small pulmonary arteries (200–400 μm in diam-

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Abbreviations: DCB, 2′,4′-dichlorobenzamil; E_m, membrane potential; HEPES, *N*-2-hydroxyethylpiperazine-*N*-2-ethanesulfonic acid; HPV, hypoxic pulmonary vasoconstriction; I_{-90}, mean current at −90 mV; I_{NSC}, non-selective cation current; *I–V*, current–voltage; PAEC, pulmonary artery endothelial cells

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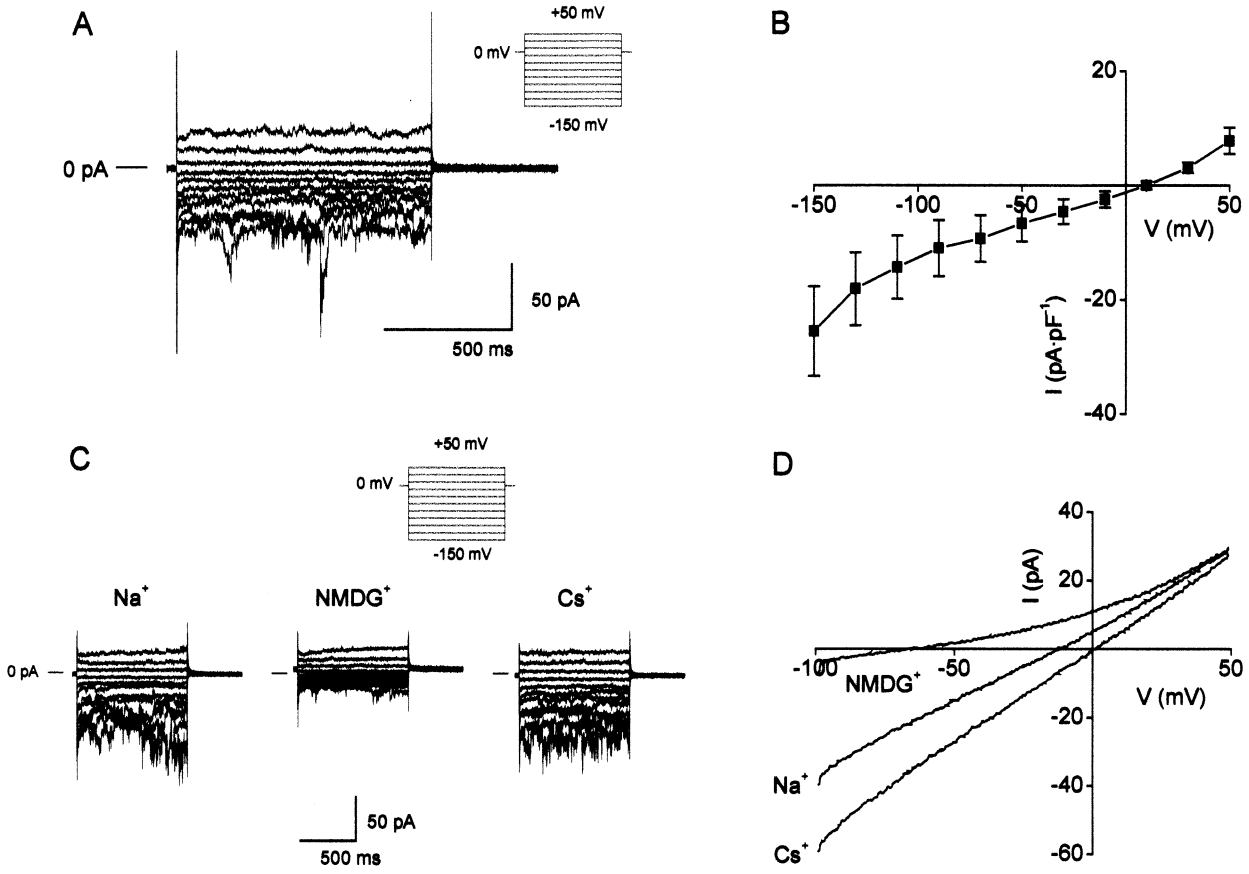

Fig. 1. A–D: Background currents recorded in PAEC under K^+-free, perforated-patch conditions. A: A typical family of currents recorded in response to voltage steps from −150 mV to +50 mV from a holding potential of 0 mV (see inset), which displayed little time-dependent activation and inactivation during the course of the voltage step. In the example shown here, spontaneous transient inward currents appear to be superimposed on the whole-cell membrane currents at more hyperpolarised potentials. B: Mean steady-state *I–V* relationship recorded for the background current (pA pF^{-1}; $n=7$). Note the mild inward rectification at very hyperpolarised potentials. C: Effects of extracellular cation substitution on the background currents. Typical families of currents evoked by voltage steps from −150 mV to +50 mV in 20 mV increments from a holding potential of 0 mV, measured in a Na^+ extracellular solution (left panel). Ionic substitution of extracellular Na^+ for $NMDG^+$ caused a marked decrease in inward current (centre panel), while replacement with Cs^+ induced a small increase in inward current (right panel). However, ionic substitution of the major extracellular cation had no effect on the outward component of the background current. D: Typical *I–V* relationships derived from depolarising voltage ramps from −100 mV to +50 mV recorded in an Na^+ extracellular solution and after ionic substitution of extracellular Na^+ for either $NMDG^+$ or Cs^+.

eter) were transferred to phosphate-buffered saline (PBS) (Life Technologies Ltd., UK) containing 0.2 mg ml^{-1} dithiothreitol and 0.2 mg ml^{-1} papain (from *Carica papaya* latex). The vessels were then agitated in an oscillating water bath at 37°C for 20 min. Bovine serum albumin (BSA) (2.0 mg ml^{-1}) was then added. Single PAEC were released by gently triturating with a fire-polished Pasteur pipette. Cells were stored at 4°C and were viable for 3–4 h.

2.2. Electrophysiology

To investigate the electrophysiological characteristics of individual PAEC, E_m and membrane currents were recorded utilising the perforated-patch configuration [27] of the patch-clamp technique [28], using an Axopatch 200B patch-clamp amplifier (Axon Instruments Inc., USA). Patch pipettes were fabricated from borosilicate glass capillaries (Harvard Apparatus, UK) using a vertical microelectrode puller (PP-83, Narishige Scientific Instruments, Japan) and fire-polished. Fire-polished patch pipettes had resistances of 5–7 MΩ. Data were filtered at 5 kHz, digitised at 24 kHz and recorded off-line with a modified DAT recorder (Sony DTC-A8, Sony Corp., Japan). Data were analysed using pClamp software (version 6.04, Axon Instruments Inc.).

In order to investigate the nature of the background currents, cells were perfused at room temperature (~22°C) with a K^+-free bath solution that consisted of (in mM): 145 NaCl, 0.5 $MgCl_2$, 1.5 $CaCl_2$, 5.5 D-glucose, 10 *N*-2-hydroxyethylpiperazine-*N*-2-ethanesulfonic acid (HEPES); pH 7.4 (NaOH) and dialysed with a K^+-free patch pipette solution that contained (in mM): 140 CsCl, 1 $MgCl_2$, 2.3 $CaCl_2$, 5 ethylene glycol-bis(β-aminoethylether)-*N*,*N*,*N*′,*N*′-tetraacetic acid (EGTA), 10 HEPES and 240 μg ml^{-1} amphotericin B (stock, 20 mg ml^{-1} in dimethyl sulfoxide); pH 7.3 (CsOH). To determine the ionic permeability of the inward component of the background currents, extracellular Na^+ was replaced with equimolar Cs^+ or $NMDG^+$. The PO_2 of the extracellular bathing solution was monitored using an O_2-sensitive microelectrode (ISO_2, World Precision Instruments, UK). Hypoxic solutions were achieved by bubbling bath solutions with 100% N_2 to achieve a PO_2 of ~25 mmHg. 2′,4′-Dichlorobenzamil (DCB) was obtained from Biomol Research Laboratories Inc., USA and dissolved in dimethyl sulfoxide as a 50 mM stock. All other reagents were purchased from BDH-Merck Ltd., UK or Sigma-Aldrich Company Ltd., UK.

For voltage step experiments, cells were maintained at a holding potential of 0 mV and membrane currents were recorded in response to 1000 ms voltage steps from −150 mV to +50 mV in 20 mV increments. The mean steady-state current, measured as the mean current during the last 100 ms of each voltage step, was used to construct a current–voltage (*I–V*) relationship. For voltage ramp experiments cells were voltage-clamped at −50 mV and subjected to a ramp protocol applied at a frequency of 0.2 Hz which consisted of an initial hyperpolarising ramp (−0.71 V s^{-1}) to −100 mV, followed by a depolarising ramp (0.75 V s^{-1}) to +50 mV, ending with a repolarising ramp

(-0.71 V s^{-1}) back to the holding potential. *I–V* relationships were constructed using the mean currents activated from three consecutive depolarising voltage ramps from -100 mV to +50 mV. Comparison of currents before and after ion substitution or application of drugs was made using the mean current amplitude from three consecutive voltage ramps at -90 mV (I_{-90}). I_{-90} were normalised either to cell capacitance or to the peak outward inward current at -100 mV. Data are expressed as mean ± standard error of the mean. Statistical comparisons were made using an unpaired Student's *t*-test, with differences considered significant at $P < 0.05$.

3. Results

To investigate the nature of the background currents present in PAEC, perforated-patch voltage-clamp recordings were carried out with a Cs^+ pipette solution and K^+-free bath solutions to eliminate K^+ currents. Membrane currents elicited in response to voltage steps demonstrate the absence of both time-dependent activation and inactivation during the course of the voltage steps (Fig. 1A). However, examination of the mean steady-state *I–V* relationship indicates very mild inward rectification in addition to the presence of erratic spontaneous transient inward currents superimposed on the whole-cell membrane currents at more hyperpolarised potentials (Fig. 1B).

In order to examine the nature of the charge carrier of this background current, extracellular Na^+ was substituted with equimolar concentrations of either $NMDG^+$ or Cs^+. Ionic substitution of extracellular Na^+ with $NMDG^+$ dramatically reduced the magnitude of the inward component of background currents while replacement of Na^+ with Cs^+ caused a small increase in the magnitude of the inward component of background currents evoked by both voltage steps and voltage ramps (Fig. 1C and D). Ionic substitution of Na^+ for $NMDG^+$ or Cs^+ caused changes in the I_{-90} of voltage ramp-induced currents from -13.94 ± 3.77 pA pF^{-1} ($n = 8$) to -4.68 ± 1.47 pA pF^{-1} ($n = 8$; $P < 0.05$) and -16.01 ± 4.44 pA pF^{-1} ($n = 4$), respectively. Furthermore, ionic substitution of Na^+ for $NMDG^+$ or Cs^+ also significantly shifted the experimental reversal potential from -7.22 ± 1.12 mV ($n = 17$) to -37.38 ± 6.88 mV ($n = 8$; $P < 0.0001$) and -1.11 ± 0.90 mV ($n = 7$; $P < 0.005$), respectively. Since the outward current is of similar magnitude under each condition,

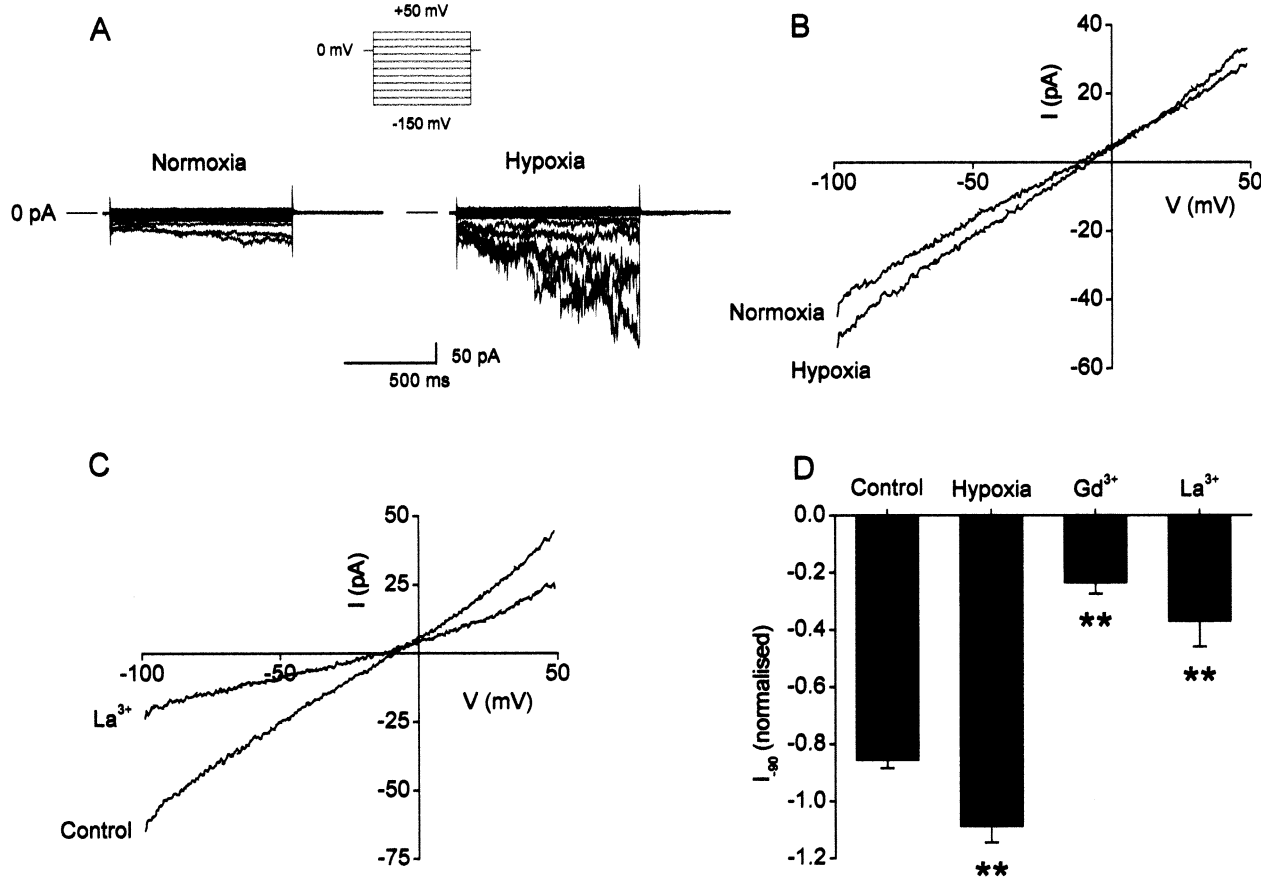

Fig. 2. Effects of hypoxia and trivalent cations on the I_{NSC} recorded from PAEC under K^+-free perforated-patch conditions. A: Voltage steps from -150 mV to +50 mV in 20 mV increments from a holding potential of 0 mV induced a typical family of membrane currents (left panel), which were significantly potentiated in the presence of hypoxia ($PO_2 \sim 25$ mmHg). Note the erratic nature and appearance of time-dependent activation of these currents at more hyperpolarised potentials. B: Typical examples of *I–V* relationships derived from depolarising voltage ramps from -100 mV to +50 mV under normoxic ($PO_2 \sim 150$ mmHg) and hypoxic conditions. Note the increase in the magnitude of inward currents upon application of hypoxia. C: Representative examples of *I–V* relationships derived from depolarising voltage ramps from -100 mV to +50 mV in the absence and presence of 100 μM La^{3+}. Note the inhibition of both the inward and outward components of the background currents in the presence of 100 μM La^{3+}. D: Data summarising the effects of hypoxia ($n = 4$), 100 μM Gd^{3+} ($n = 3$) and 100 μM La^{3+} ($n = 3$) on the I_{-90} (normalised to peak inward current) when compared to control conditions ($n = 10$). ** Indicates $P < 0.01$ when compared to control.

this would indicate that it is movement of extracellular cations into the cell that is responsible for the inward component of the background current. Together, the change in amplitude of inward currents and the shifts in experimental reversal potential indicate that the background current may be carried by a non-selective cation channel with a permeability sequence of $Cs^+ > Na^+ \gg NMDG^+$.

Perforated-patch experiments were carried out to determine whether hypoxia had any effects on the non-selective cation currents (I_{NSC}) identified in PAEC. In response to voltage steps, membrane currents were markedly potentiated under hypoxic conditions ($PO_2 \sim 25$ mmHg) when compared to currents under normoxic conditions ($PO_2 \sim 150$ mmHg; Fig. 2A). The example shown here shows a family of background currents that appear more erratic at hyperpolarised potentials. Upon application of hypoxia, the inward components of background currents were augmented, displayed some time-dependent activation and became very erratic at more hyperpolarised potentials (Fig. 2A). A typical example of an *I–V* relationship derived from depolarising voltage ramps demonstrates that membrane currents are activated under hypoxic conditions in endothelial cells freshly isolated from small pulmonary arteries (Fig. 2B). The mean I_{-90} of voltage ramp-induced currents (normalised to peak inward current) was significantly increased by $\sim 30\%$ under hypoxic conditions when compared to those under normoxic conditions (Fig. 2D). In order to confirm that the hypoxia-activated background current was due to activation of I_{NSC} and not a Na^+/Ca^{2+} exchanger, experiments were carried out in the presence of DCB, a selective Na^+/Ca^{2+} exchange inhibitor [29,30]. DCB (50 μM) had no significant effect on the I_{-90} of the I_{NSC} ($n = 4$) and also failed to attenuate the hypoxic activation of I_{NSC} ($n = 3$; data not shown) when compared to control conditions. These data indicate that Na^+/Ca^{2+} exchange was not responsible for the hypoxia-activated background current.

Trivalent cations such as Gd^{3+} and La^{3+} have been shown to inhibit I_{NSC} [31–33] and La^{3+} has also been shown to inhibit the endothelium-dependent phase of HPV [34]. It was therefore important to determine the effects of both Gd^{3+} and La^{3+} on the I_{NSC} identified in PAEC. Perforated-patch recordings of I_{NSC} evoked in response to voltage ramps were significantly attenuated by the addition of either 100 μM La^{3+} or Gd^{3+}. An *I–V* relationship constructed from depolarising voltage ramps demonstrates attenuation of both the inward and outward components of the I_{NSC} in the presence of 100 μM La^{3+} (Fig. 2C). The I_{-90} (normalised to peak inward current) of voltage ramp-induced currents were attenuated by Gd^{3+} and La^{3+} by $\sim 70\%$ and $\sim 60\%$, respectively (Fig. 2D). Together these results suggest that the hypoxia-sensitive I_{NSC} identified in PAEC may be inhibited by the trivalent cations La^{3+} or Gd^{3+}. The identification of this novel hypoxia-sensitive I_{NSC} may have important implications for elucidating the oxygen-sensing mechanisms underlying the endothelium-dependent phase of HPV.

4. Discussion

This study provides the first observation of a hypoxia-activated I_{NSC} in endothelial cells of small pulmonary arteries; those vessels that are responsible for mediating HPV. This finding is of major importance since the endothelium of these small arteries is essential for the physiologically important, sustained phase of HPV and may help to elucidate the oxygen-sensing mechanisms which underlie this response.

Under K^+-free conditions, extracellular cationic substitution experiments revealed that the background currents of the PAEC recorded within this study possessed a permeability sequence of $Cs^+ > Na^+ \gg NMDG^+$ similar to that of I_{NSC} present in other cell types [10,16,35]. In addition, the electrophysiological profile of the I_{NSC} of PAEC displayed little voltage-dependent activation and inactivation, a finding also consistent with the properties of other I_{NSC} [35]. In contrast to other I_{NSC}, voltage step experiments revealed spontaneous transient inward currents that were superimposed on the whole-cell currents at hyperpolarised potentials and also showed some weak inward rectification. Weak inward rectification has been observed in currents recorded from endothelial cells isolated from intrapulmonary arteries [36] and guinea-pig endocardial cells [37] and has also been observed in vascular endothelial cells after activation by mechanical stretch [38,39]. Why the I_{NSC} observed in this study demonstrate weak inward rectification is unclear. However, it is possible that a component of stretch-induced I_{NSC} may be activated under our conditions [39].

The inward component of the I_{NSC} identified in PAEC was significantly potentiated after the application of hypoxia. The augmentation of this novel hypoxia-sensitive current may have important implications for the role of the endothelium in the control and release of the vasoactive mediators responsible for phase 2 of HPV. The hypoxia-sensitive I_{NSC} identified in PAEC may therefore provide an important Ca^{2+} influx pathway since the I_{NSC} identified in other preparations have been shown to provide Ca^{2+} entry pathways [33,40]. In support of the existence of a hypoxia-activated Ca^{2+} influx pathway in these cells are the observations that cytosolic free calcium is increased upon application of hypoxia in PAEC [41,42]. Consistent with this notion, the I_{NSC} identified in PAEC could be attenuated by the trivalent cations La^{3+} and Gd^{3+}, which have both previously been shown to block I_{NSC} of endothelial cells of the human umbilical vein [40] and guinea-pig endocardium [33]. Similar concentrations of La^{3+} to those used in this study have been reported to inhibit the endothelium-dependent, second phase of HPV [34]. It is therefore possible that the hypoxia-activated I_{NSC} is responsible for the La^{3+}-sensitive Ca^{2+} influx pathway essential for the sustained elevation of Ca^{2+} required for phase 2 of HPV. This sustained rise in the intracellular Ca^{2+} concentration may in turn lead to release of endothelium-derived constricting factors [13,14], or indeed endothelin-1 which has been implicated in HPV [43,44], in order to maintain pulmonary vasoconstriction.

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References

[1] Leach, R.M., Robertson, T.P., Twort, C.H. and Ward, J.P.T. (1994) Am. J. Physiol. 266, L223–231.
[2] Ward, J.P.T. and Aaronson, P.I. (1999) Respir. Physiol. 115, 261–271.
[3] Demiryurek, A.T., Wadsworth, R.M., Kane, K.A. and Peacock, A.J. (1993) Am. Rev. Respir. Dis. 147, 283–290.

[4] Hoshino, Y., Morrison, K.J. and Vanhoutte, P.M. (1994) Am. J. Physiol. 267, L120–127.
[5] Ward, J.P.T. and Robertson, T.P. (1995) Exp. Physiol. 80, 793–801.
[6] Turner, J.L. and Kozlowski, R.Z. (1997) Exp. Physiol. 82, 629–645.
[7] Kovitz, K.L., Aleskowitch, T.D., Sylvester, J.T. and Flavahan, N.A. (1993) Am. J. Physiol. 265, H1139–1148.
[8] Robertson, T.P., Aaronson, P.I. and Ward, J.P.T. (1995) Am. J. Physiol. 268, H301–307.
[9] Adams, D.J., Barakeh, J., Laskey, R. and Van Breemen, C. (1989) FASEB J. 3, 2389–2400.
[10] Nilius, B., Viana, F. and Droogmans, G. (1997) Annu. Rev. Physiol. 59, 145–170.
[11] Salvaterra, C.G. and Goldman, W.F. (1993) Am. J. Physiol. 264, L323–328.
[12] Vadula, M.S., Kleinman, J.G. and Madden, J.A. (1993) Am. J. Physiol. 265, L591–597.
[13] Gaine, S.P., Hales, M.A. and Flavahan, N.A. (1998) Am. J. Physiol. 271, L657–664.
[14] Robertson, T.P., Ward, J.P.T. and Aaronson, P.I. (2001) Cardiovasc. Res. 50, 145–150.
[15] Johns, A., Lategan, T.W., Lodge, N.J., Ryan, U.S., Van Breemen, C. and Adams, D.J. (1987) Tissue Cell 19, 733–745.
[16] Voets, T., Droogmans, G. and Nilius, B. (1996) J. Physiol. 497, 95–107.
[17] Rodman, D.M., Yamaguchi, T., Hasunuma, K., O'Brien, R.F. and McMurty, I.F. (1990) Am. J. Physiol. 258, L207–214.
[18] Greenberg, B. and Kishiyama, S. (1993) Am. J. Physiol. 265, H1712–1720.
[19] Ogawa, Y., Kawabe, J., Onodera, S., Tobise, K., Morita, K., Harada, T., Hirayama, T. and Takeda, A. (1993) Jpn. Circ. J. 57, 228–236.
[20] Archer, S.L., Huang, J.M., Reeve, H.L., Hampl, V., Tolarová, S., Michelakis, E. and Weir, E.K. (1996) Circ. Res. 78, 431–442.
[21] Chung-Welch, N., Shepro, D., Dunham, B. and Hechtman, H.B. (1988) J. Cell Physiol. 135, 224–234.
[22] Tracey, W.R. and Peach, M.J. (1992) Circ. Res. 70, 234–240.
[23] Von Beckerath, N., Dittrich, M., Klieber, H.G. and Daut, J. (1996) J. Physiol. 491, 357–365.
[24] Hogg, D.S., Albarwani, S., Davies, A.R.L. and Kozlowski, R.Z. (1999) Biochem. Biophys. Res. Commun. 263, 405–409.
[25] Cannell, M.B. and Sage, S.O. (1989) J. Physiol. 419, 555–568.
[26] Laskey, R.E., Adams, D.J., Johns, A., Rubanyi, G.M. and Van Breemen, C. (1990) J. Biol. Chem. 265, 2613–2619.
[27] Horn, R. and Marty, A. (1988) J. Gen. Physiol. 92, 145–159.
[28] Hamill, O.P., Marty, A., Neher, E., Sakmann, B. and Sigworth, F.J. (1981) Pflügers Arch. 391, 85–100.
[29] Kleyman, T.R. and Cragoe, E.J. (1988) J. Membr. Biol. 105, 1–21.
[30] Danaceau, J.P. and Lucero, M.T. (2000) J. Gen. Physiol. 115, 759–768.
[31] Ling, B.N. and O'Neill, W.C. (1992) Am. J. Physiol. 263, H1827–1838.
[32] Hescheler, J. and Schultz, G. (1993) in: Nonselective Cation Channels: Pharmacology, Physiology and Biophysics (Siemen, D. and Hescheler, J., Eds.), pp. 26–43, Birkhauser Verlag, Basel.
[33] Manabe, K., Takano, M. and Noma, A. (1995) J. Physiol. 487, 407–419.
[34] Robertson, T.P., Hague, D., Aaronson, P.I. and Ward, J.P.T. (2000) J. Physiol. 525, 669–680.
[35] Bae, Y.M., Park, M.K., Lee, S.H., Ho, W.K. and Earm, Y.E. (1999) J. Physiol. 514, 747–758.
[36] Yamamoto, Y., Chen, G., Miwa, K. and Suzuki, H. (1992) J. Physiol. 450, 395–408.
[37] Manabe, K., Ito, H., Matsuda, H., Noma, A. and Shibata, Y. (1995) J. Physiol. 484, 41–52.
[38] Yang, X.C. and Sachs, F. (1990) J. Physiol. 431, 103–122.
[39] Popp, R., Hoyer, J., Meyer, J., Galla, H.J. and Gögelein, H. (1992) J. Physiol. 454, 435–449.
[40] Kamouchi, M., Mamin, A., Droogmans, G. and Nilius, B. (1999) J. Membr. Biol. 169, 29–38.
[41] Hu, Q.H. and Wang, D.X. (1993) J. Tongji Med. Univ. 13, 14–17.
[42] Hampl, V., Cornfield, D.N., Cowan, N.J. and Archer, S.L. (1995) Eur. Respir. J. 8, 515–522.
[43] Helset, E., Kjæve, J., Bjertnæs, L. and Lundberg, J.M. (1995) Scand. J. Clin. Lab. Invest. 55, 369–376.
[44] Jones, R.D. and Morice, A.H. (1998) Pulm. Pharmacol. Ther. 11, 177–181.