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H_2O_2 regulates recombinant Ca^{2+} channel α_{1C} subunits but does not mediate their sensitivity to acute hypoxia

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Abstract

Acute hypoxic inhibition of the pore-forming α_{1C} subunit of the L-type Ca^{2+} channel mediates hypoxic arterial vasodilatation, a physiological response which matches tissue O_2 demand and supply in the systemic vasculature. In numerous O_2-sensing cell types, reactive O_2 species (ROS) have been proposed as mediators linking lowered O_2 levels with the appropriate cellular response. In this study, we examined the roles of H_2O_2 and NADPH oxidase as mediators of hypoxic inhibition of recombinant α_{1C} subunits. Human cardiac L-type Ca^{2+} channel α_{1C} subunits were stably expressed in HEK 293 cells. Ca^{2+} currents were recorded using the whole-cell configuration of the patch-clamp technique. Bath application of 100 μM H_2O_2 significantly enhanced depolarisation-evoked Ca^{2+} currents in a voltage-dependent manner, while dialysis with 1000 U ml^{-1} catalase reduced these currents. In the presence of catalase, hypoxic inhibition of Ca^{2+} currents was not significantly different compared to non-dialysed controls. The NADPH oxidase inhibitors diphenylene iodonium (10 μM) and phenylarsine oxide (5 μM) were without effect on either basal Ca^{2+} currents or responses to hypoxia. Thus, endogenous production of H_2O_2 regulates the α_{1C} subunit. However, neither suppression of H_2O_2 levels nor inhibition of NADPH oxidase is involved in O_2-dependent regulation of the Ca^{2+} channel.

Keywords: Ca^{2+} channel; α_{1C} subunit; HEK 293; Acute hypoxia; NADPH oxidase; H_2O_2

Hypoxic regulation of ion channels is a vital link in the physiological matching of cellular O_2 demand with supply. Since the initial demonstration of O_2-sensitive K^+ channels in chemosensory carotid body type I cells [1], numerous studies have demonstrated K^+ channels to be O_2-sensitive in a host of other cell types (for review see [2,3]). In the systemic and proximal pulmonary vasculature, Ca^{2+} channels also respond to an acute hypoxic stimulus, and Franco-Obregon and Lopez-Barneo [4,5] provided the initial demonstration of O_2-sensitive L-type Ca^{2+} channels in isolated rabbit vascular myocytes. Inhibition of these channels, and the ensuing reduction in intracellular Ca^{2+} levels, is thought to underlie hypoxic arterial vasodilatation, a physiological mechanism which enhances blood flow to O_2-deprived systemic, cerebral, and coronary tissues during ischaemia or increased metabolic need [6–8]. Although the full role of channel auxiliary subunits [9] has yet to be elucidated, hypoxic regulation of the major pore-forming α_{1C} subunit underlies the O_2-sensitivity of the L-type channel, since Ca^{2+} channel activity was reduced when HEK 293 cells expressing this subunit alone were exposed to acute hypoxia [10].

Several mechanisms have been proposed to underlie acute hypoxic regulation of ion channels, and redox modulation via intracellular mediators has received much attention to date [11]. It has been proposed that acute hypoxic inhibition of K^+ channels occurs via changes in cellular redox status, such that cysteine residues on channel proteins are susceptible to redox modulation, leading to structural changes which alter ion flux. One theory suggests that hypoxia enhances the formation of reduced forms of regulatory intracellular redox couples such as oxidised/reduced glutathione (GSSG/GSH), and/or NAD(P)/NAD(P)H [12], arising from changes in the activity of enzymes such as NADPH oxidase [13–16]. In support of this, GSH inhibited K^+ channels in both the carotid body [17] and pulmonary VSM [18,19]. In addition, glutathione (GSSG) potentiated K_{Ca} channels in

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pulmonary VSM [20]. Support for the involvement of NADPH oxidase, although not universal, comes from studies examining mouse airway chemoreceptor cells in which the $gp91^{phox}$ subunit of the oxidase was selectively knocked out [21], an intervention which ablated the inhibition of K^+ current by acute hypoxia. Consistent with this involvement, pharmacological disruption of NADPH oxidase function diminished responses to hypoxia in airway chemoreceptors and their immortalised counterpart H146 cells [21,22].

Despite the wealth of data concerning K^+ channel regulation during hypoxia, there is little information on the alterations in cellular metabolism that alter Ca^{2+} channel activity, although specific oxidising and reducing agents altered recombinant Ca^{2+} channel function and hypoxia sensitivity [23]. However in cardiac myocytes H_2O_2 enhanced Ca^{2+} currents [24] and the hypoxia-induced increase in sensitivity of these currents to β-adrenergic stimulation [25] were mimicked by catalase and attenuated by H_2O_2 [26]. Given this possible role for H_2O_2 in mediating these effects on Ca^{2+} current stimulation, we hypothesised that this reactive oxygen species acts as a mediator of acute, direct hypoxic regulation of current flow through the α_{1C} subunit [10]. In support of this hypothesis we found that H_2O_2 enhanced, and catalase decreased, depolarisation-induced Ca^{2+} currents. However, following cell dialysis with catalase, hypoxia still caused a robust decrease in Ca^{2+} current. Further, inhibition of H_2O_2-producing NADPH oxidase was without effect on hypoxic inhibition of Ca^{2+} currents. Thus although H_2O_2 regulated the α_{1C} subunit and modulated the response of Ca^{2+} current to isoproterenol [26], neither regulation of H_2O_2 production nor NADPH oxidase plays a role in the acute hypoxic regulation of the Ca^{2+} channel.

Methods

Stable transfection of HEK 293 cells. Experiments were conducted in HEK 293 cells stably expressing the human cardiac L-type Ca^{2+} channel α_{1C} subunit [27]. The α_{1C} subunit cDNA clone was a kind gift from Dr. Gyula Varadi (University of Cincinnati). After splitting the previous day and seeding at ~60% confluency, wild-type HEK 293 cells were transfected with 3 μg pCDNA3.1-α_{1C} using ExGen 500 (Fermentas, Burlington, ON, Canada), according to the manufacturer's instructions. Three days post-transfection, the medium was replaced with one containing 400 mg/ml G418 (Invitrogen). Selection was applied for 2 weeks, after which time individual colonies could be visualised using an inverted microscope (Zeiss) and phase-contrast objectives. Colonies were picked and seeded in wells of a 96-well plate and allowed to reach confluency, after which they were transferred to 35 mm dishes for further culture and for examination of Ca^{2+} currents. Of the ~60 clones screened, >50% were positive for Ca^{2+} channel activity, and a single clone was identified for further study based on the number of cells within the clone expressing current and the size of these currents. This clone was sub-cloned to ensure a pure population of stably transfected cells. G418 selection was continued throughout the cloning process and in all subsequent sub-culturing.

Culture of HEK 293 cells. Cells were grown in minimum essential medium with Earle's salts and L-glutamine (Gibco, Paisley, UK), containing 9% (v/v) fetal calf serum (Globepharm, Esher, Surrey, UK), 1% (v/v) non-essential amino acids, gentamicin (50 mg L^{-1}), 10,000 U L^{-1} penicillin G, 10 mg L^{-1} streptomycin, 0.25 mg L^{-1} amphotericin, and 400 mg L^{-1} G418 (all Gibco) at 37 °C in a humidified atmosphere of air/CO_2 (19:1). Cells were cultured in 35 mm dishes and split twice per week at a ratio of 1:5. For electrophysiological studies, cells were split at least 24 h prior to experimentation and plated in 35 mm dishes at a confluency of ~25%.

Electrophysiology. Pieces of coverslip with attached cells were transferred to a continually perfused (approximately 2 ml min^{-1}) recording chamber and whole-cell patch-clamp recordings [28] were made using patch pipettes of resistance 3–7 MΩ. Cells were perfused with a solution composed of (in mM): NaCl, 95; CsCl, 5; $MgCl_2$, 0.6; $BaCl_2$ 20; Hepes, 5; D-glucose, 10; and TEA-Cl, 20 (21–24 °C, pH adjusted to 7.4 with NaOH) and patch electrodes were filled with a solution of composition (in mM): CsCl, 120; TEA-Cl, 20; $MgCl_2$, 2; EGTA, 10; Hepes, 10; and ATP, 2 (pH adjusted to 7.2 with CsOH). Cells were voltage-clamped at −80 mV, and whole-cell currents were evoked by step depolarising the membrane to various test potentials for 100 ms at a frequency of 0.1 Hz. All recordings were made at room temperature (22 ± 2 °C).

Current traces were filtered at 5 kHz, digitised at 10 kHz, and stored on a PC for later analysis. Capacitative transients were minimised by analogue means (residual transients have been truncated for illustrative purposes) and corrections for leak current were made off-line by the appropriate scaling and subtraction of the average leak current evoked by small hyperpolarising and depolarising steps (⩽5 mV). Current amplitudes were measured at their peaks during each step depolarisation. All analysis and voltage protocols were performed using a Multiclamp 700 amplifier in combination with a Digidata 1322 A interface and pCLAMP 9.0 software (Axon Instruments). Results are expressed as means ± SEM, and statistical comparisons were made using paired and unpaired Student's *t* tests as appropriate.

Hypoxia was produced by bubbling the extracellular perfusate with 100% N_2 gas for >30 min prior to experimentation. Bath PO_2 was measured using a depolarised (−600 mV) carbon fibre electrode, and was always stable at ~10 mm Hg within 30–45 s of exchanging solution. Bubbling with N_2 caused no change in the pH of the perfusate.

Drug solutions. Drug solutions were prepared by dissolving in the intracellular or extracellular perfusate to the required concentration, and pH was adjusted as necessary. Hydrogen peroxide, catalase, diphenylene iodonium (DPI), and phenylarsine oxide (PAO) were obtained from Sigma (Mississauga, ON, Canada). To avoid breakdown, H_2O_2 was stored at 4 °C prior to use and added to the perfusate immediately (<1 min) prior to making recordings. When catalase was included in the intracellular solution, cells were dialysed via the patch pipette for at least 15 min prior to making recordings. Given the MW of catalase (60 kDa; [29]) and the access resistance (<10 mΩ), calculations as described by [30] demonstrate this to be a sufficient time period to allow dialysis of the enzyme into the cell through the patch pipette. Solutions containing catalase were kept on ice prior to use. DPI and PAO were made as 10 mM stock solutions in DMSO before dilution into the extracellular perfusate. 1:1000 DMSO was without effect on Ca^{2+} channel currents (not shown).

Results

H_2O_2 regulates Ca^{2+} currents in HEK 293 cells expressing α_{1C} subunits

All experiments were carried out in HEK 293 cells stably expressing the hHT isoform of the human cardiac L-type Ca^{2+} channel α_{1C} subunit [27,31]. Since H_2O_2

was proposed as a mediator of acute hypoxic signalling onto K^+ channels in vascular smooth muscle [32] and regulated the hypoxia sensitivity of β-adrenergic stimulation of L-type Ca^{2+} channel currents [26], we initially examined the responsiveness to bath-applied H_2O_2 of depolarisation-evoked currents through the α_{1C} subunit. In 5 cells examined, 100 μM H_2O_2 caused a reversible enhancement of Ca^{2+} current amplitudes (Fig. 1A). This enhancement was voltage-dependent, such that currents were increased by H_2O_2 at test potentials below which currents were maximal, while currents were not affected at more depolarised potentials (Fig. 1A and inset). In some cases, due to the shift in the *I–V* relationship, the effect of H_2O_2 at the more depolarised potentials was to cause slight inhibition. At a test potential of 0 mV (holding potential −80 mV), currents were increased from -4.3 ± 1.1 pA/pF under control conditions to -7.4 ± 1.2 pA/pF in the presence of H_2O_2 ($n = 5$; $P < 0.05$, paired Student's *t* test). At a test potential of +30 mV, currents were -4.7 ± 1.3 pA/pF (control) and -4.0 ± 0.3 pA/pF (H_2O_2), values not significantly different ($n = 5$; $P > 0.05$, paired Students' *t* test).

To further investigate the regulation of the α_{1C} subunit by H_2O_2, we dialysed cells with 1000 U ml^{-1} catalase to reduce endogenous H_2O_2 levels. Cells were dialysed for at least 15 min prior to obtaining recordings, to allow for diffusion of catalase from the patch electrode into the cell (see Methods). Control cells were dialysed for 15 min with catalase-free intracellular solution. After dialysis, depolarisation-evoked currents were significantly reduced in magnitude compared to those evoked in cells dialysed with a catalase-free solution (Fig. 1B). For example, at a test potential of 0 mV, mean currents were -4.8 ± 1.0 pA/pF ($n = 5$) in control cells, and -3.3 ± 0.9 pA/pF ($n = 5$) in cells dialysed with catalase ($P < 0.05$, unpaired Student's *t* test). The reduction of Ca^{2+} current amplitudes due to catalase was voltage-dependent, such that inhibition was most prominent at potentials in the hyperpolarising direction to those at which maximal currents were obtained. At a test potential of −10 mV (20 mV negative to potential at which the mean peak current was observed; Fig. 1B), currents were inhibited by ∼42%, while at +30 mV (20 mV positive to peak potential), currents were reduced by ∼28%.

Catalase does not prevent hypoxic inhibition of α_{1C} subunits

Since in the present study H_2O_2 regulated α_{1C} subunit activity and was previously shown to regulate the sensitivity to hypoxia of β-adrenergic signaling onto Ca^{2+} channels [26], we investigated whether changes in H_2O_2 levels mediated the inhibition of α_{1C} subunit activity which occurs during acute hypoxia. Similar to non-dialysed cells (Fig. 2A), following dialysis with 1000 U ml^{-1} catalase, acute hypoxia still caused robust inhibition of Ca^{2+} channel currents (Fig. 2B) in 5 cells examined. At a test potential of 0 mV, the mean (±SEM) magnitude of the O_2-sensitive Ca^{2+} current ($ICaO_2$) was -1.4 ± 0.2 pA/pF ($n = 5$), a value not significantly different from that seen in cells dialysed with catalase-free intracellular solution (-1.6 ± 0.5 pA/pF, $n = 5$; $P > 0.05$. unpaired Students' *t* test). Thus, although H_2O_2 regulated the α_{1C} subunit, and there existed a basal production of H_2O_2 which stimulated Ca^{2+} channel activity, altered production of H_2O_2 does not underlie the response of this channel to acute hypoxia.

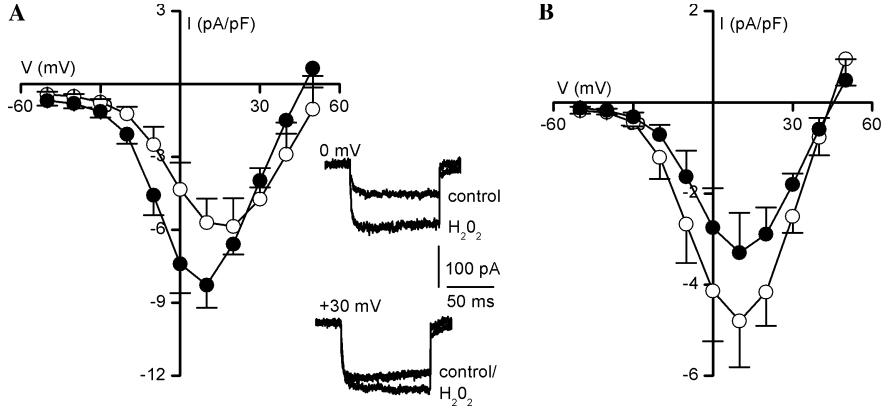

Fig. 1. H_2O_2 regulates recombinant Ca^{2+} currents. (A) current–voltage (*I–V*) relationships demonstrating the enhancing effect of 100 μM H_2O_2 on Ca^{2+} currents through recombinant α_{1C} subunits. Each point shows the mean (±SEM; $n = 5$) peak current evoked by step depolarising cells for 100 ms to the indicated test potential (holding potential of −80 mV, frequency 0.1 Hz) prior to (○) and during (●) the bath application of H_2O_2. Note the voltage-dependency of the effect of H_2O_2, such that current enhancement was maximal at test potentials below which currents were maximal. Inset, individual current records obtained in the same cell prior to (control) and during the application of H_2O_2, as indicated. Currents were evoked by step depolarising to test potentials of 0 mV (upper) and +30 mV (lower), and demonstrate the voltage-dependency of the effects of H_2O_2. (B) *I–V* relationships obtained in cells dialysed for 15 min via the patch pipette with normal intracellular solution (○; $n = 6$) or one containing 1000 U ml^{-1} catalase (●; $n = 6$).

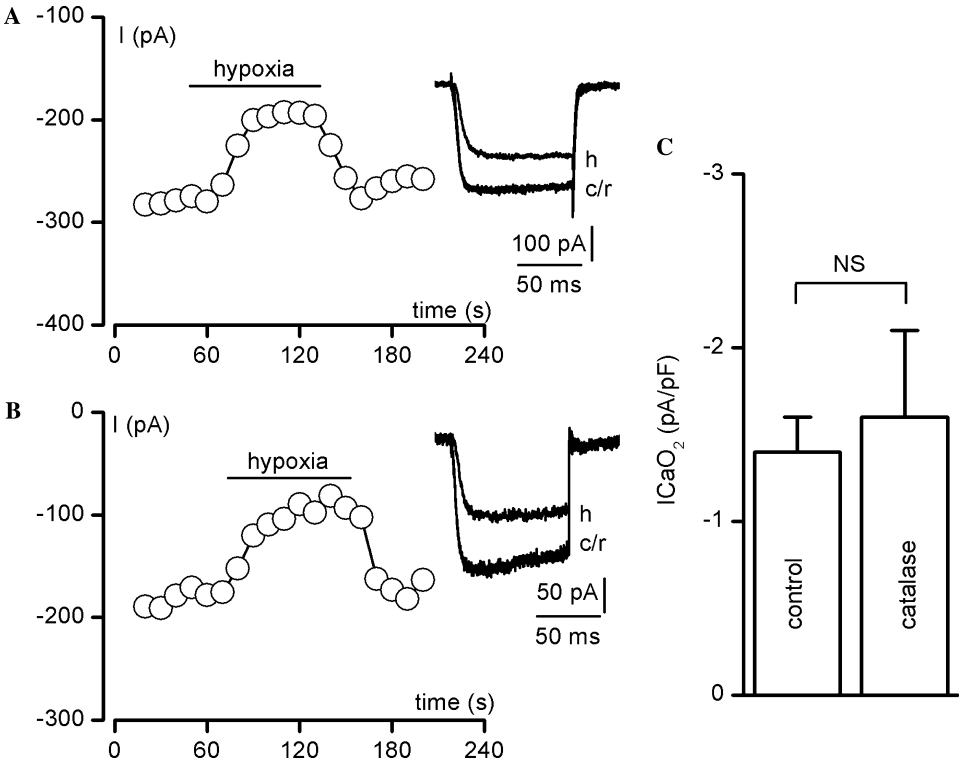

Fig. 2. Altered production of H_2O_2 does not underlie acute hypoxic regulation of the Ca^{2+} channel. (A,B) Example time-series recordings showing the effect of acute hypoxia (PO_2 ~10 mm Hg, horizontal bars) on peak depolarisation-evoked Ca^{2+} currents in HEK 293 cells stably expressing human cardiac L-type Ca^{2+} channel α_{1C} subunits. Recordings were made in cells dialysed for 15 min either with normal intracellular solution (A, typical of five such recordings) or with intracellular solution containing 1000 U ml^{-1} catalase (B, typical of five recordings). Currents were evoked by step depolarising cells to 0 mV from a holding potential of −80 mV for 100 ms every 10 s. (C) Mean (±SEM) magnitude of the O_2-sensitive Ca^{2+} current ($ICaO_2$) was obtained from recordings such as those represented in (A) and (B), by subtracting current amplitudes evoked in hypoxia from those previously obtained in normoxia in the same cell. Data are presented in pA/pF following normalisation for cell size by dividing current by the cell capacitance. Data were obtained from five recordings (control cells) and five recordings (catalase dialysed cells). NS, not significant, Student's unpaired *t* test.

Inhibition of NADPH oxidase does not affect hypoxic regulation of α_{1C} subunits

NADPH oxidase has been proposed to act as an O_2-sensing enzyme in numerous cell types [33]. To test the hypothesis that this enzyme functions as a regulator of recombinant O_2-dependent Ca^{2+} currents we examined the effects of bath application of two distinct inhibitors of the oxidase on both basal Ca^{2+} currents and responses to acute hypoxia. Bath application of 10 μM diphenylene iodonium (DPI) was without effect on evoked Ca^{2+} currents in 5 cells tested. Furthermore, the magnitude of the response to acute hypoxia was 2.3 ± 0.7 pA/pF ($n = 6$) under control conditions and 1.9 ± 0.5 pA/pF in the presence of DPI ($n = 5$; $P > 0.05$, paired Students' *t* test; see Figs. 3A, B, and D). Similarly, 5 μM phenylarsine oxide (PAO) was without effect on Ca^{2+} currents, and the magnitude of hypoxic inhibition (-1.5 ± 0.3 pA/pF) ($n = 5$) was not significantly altered in the presence of the inhibitor (-1.8 ± 0.4 pA/pF, $n = 5$; $P > 0.05$, paired Students' *t* test; see Figs. 3A, C, and D). Even at a higher concentration of PAO (10 μM), hypoxia still caused a robust inhibition of Ca^{2+} channel currents (data not shown).

Discussion

Acute hypoxia is a well-described regulator of plasmalemmal ion channels, a function of which is to mediate physiological responses designed to maintain a supply of O_2 commensurate with O_2 demand. In the systemic, cerebral, and coronary vasculature, a lowering of O_2 levels initiates hypoxic arterial vasodilatation, a physiological process which assists in maintaining the supply of blood to O_2-deprived tissues in response to hypoxia and/or increased O_2 utilisation [6–8]. This effect is mediated, at least in part, by the closure of voltage-gated Ca^{2+} channels in smooth muscle cell membranes [4,5] and more specifically by hypoxic inhibition of the pore-forming α_{1C} subunit [10]. Although hypoxic regulation may involve specific redox-sensitive cysteine residues on this subunit [23], there is little information concerning the cellular events which transduce a low O_2

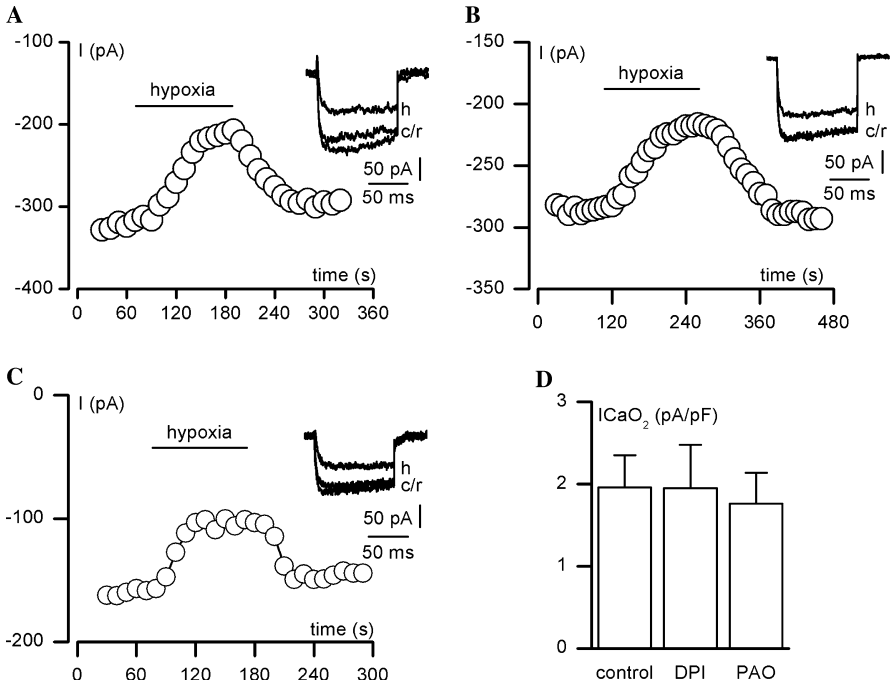

Fig. 3. Regulation of NADPH oxidase does not underlie hypoxic regulation of the α_{1C} subunit. (A–C) Example time-series recordings demonstrating the inhibitory effect of acute hypoxia ($PO_2 \sim 10$ mm Hg) on Ca^{2+} channel currents in HEK 293 cells stably expressing human cardiac L-type Ca^{2+} channel α_{1C} subunits. The effects of hypoxia were examined (A) under control conditions, (B) during exposure to the NADPH oxidase inhibitor diphenylene iodonium (DPI, 10 μM; typical of five such recordings), and (C) during exposure to a further inhibitor of the oxidase, phenylarsine oxide (PAO, 5 μM; typical of five such recordings). Both DPI and PAO were without effect on the ability of hypoxia to inhibit Ca^{2+} channel activity. Currents were evoked by step depolarising cells to 0 mV from a holding potential of −80 mV for 100 ms every 10 s. Inset shows individual current records obtained from the corresponding time-series recording in normoxia (c), during hypoxia (h), and following return to normoxia (r). (D) Magnitude of $ICaO_2$ (see Fig. 2 legend for explanation) under control conditions and in the presence of DPI and PAO. Data were plotted as means (±SEM) from 11 control recordings and from five recordings for both DPI and PAO. No significant differences were seen between control and test groups.

signal into a messenger which can regulate Ca^{2+} activity. This is despite the wealth of available information concerning the intracellular mechanisms underlying acute hypoxic regulation of K^+ channels in the cardiovascular system, amongst other tissues.

In several O_2-sensing cell types, including immortalised lung neuroepithelial cells [22] and ductus arteriosus smooth muscle [32], H_2O_2 has been proposed as an intracellular mediator of the hypoxic signal. Initially, we examined responses of expressed Ca^{2+} currents to bath-applied H_2O_2 and saw significant enhancement of Ca^{2+} current due to this exogenously applied reactive O_2 species (ROS). Interestingly, the effects of H_2O_2 were voltage-dependent, which bore a striking resemblance to the effects of acute hypoxia on both the native Ca^{2+} channel [4,5] and recombinant α_{1C} subunits [10]. To examine the role of basal, endogenous production of H_2O_2 in regulating Ca^{2+} channel activity, we examined Ca^{2+} channel currents in cells in which catalase was introduced into cells via the patch pipette for a sufficient length of time to allow the enzyme to dialyse into the cell [29,30]. Following this intervention current amplitudes were decreased when compared to cells which were dialysed without catalase. This was consistent with a constitutive production of H_2O_2 which activated the Ca^{2+} channel. Like the effects of H_2O_2 itself, inhibition of Ca^{2+} currents by catalase-induced removal of H_2O_2 was also moderately voltage-dependent, with inhibition most prominent at test potentials below or at which maximal currents were evoked.

These data are consistent with the hypothesis that H_2O_2 is produced during normoxia, and that this production is suppressed during hypoxia such that Ca^{2+} channel activity is inhibited. Such a hypothesis is supported by fluorescence measurements of intracellular H_2O_2 levels in H146 cells [22] and cardiac myocytes [26]. Similarly, amplex red-reactive H_2O_2 was decreased during hypoxia in human ductus arteriosus [32]. In H146 cells basal production of H_2O_2 induced basal K^+ channel tone during normoxia, while in hypoxia H_2O_2 production, and K^+ channel activity, were suppressed [22]. To test this hypothesis we exposed HEK 293 cells to acute hypoxia following dialysis with catalase, to ablate intracellular H_2O_2 levels and inhibit the ability of the cell to alter H_2O_2 levels during hypoxia. In these studies, we used catalase concentrations higher than those previously

shown to regulate the sensitivity of the L-type channel to isoproterenol [26], and as such at concentrations expected to exert sufficient cellular effects to alter hypoxic regulation should H_2O_2 production be involved. The ability of catalase to reduce the size of Ca^{2+} currents in the dialysis studies indeed demonstrated the ability of catalase to exert intracellular effects. However, in all cases hypoxia caused a rapid and reversible inhibition of Ca^{2+} channel currents in the presence of catalase, the degree of which was not dissimilar to that observed in cells dialysed with catalase-free solution. Thus although HEK 293 cells produced H_2O_2 during normoxia, such production was not altered during exposure to acute hypoxia and does not mediate hypoxic inhibition of this channel.

In numerous cell types, including isolated and model lung neuroepithelial cells [21,22] and carotid body type I cells [13], hypoxic regulation of ion channel activity is thought to involve hypoxic regulation of NADPH oxidase, a superoxide-(O_2^-)-producing enzyme from which H_2O_2 forms via the action of intracellular superoxide dismutase. HEK 293 cells express NOX4, an isoform of the catalytic gp91(phox) subunit of NADPH oxidase which possesses the ability to produce O_2^- and which is sensitive to the inhibitor diphenylene iodonium (DPI; [34]). However, we found no evidence of a role for this oxidase in hypoxic inhibition of the Ca^{2+} channel. At bath-applied concentrations of both DPI and phenylarsine oxide (PAO) equal to or exceeding those which have been shown to cause modulation of ion channel function and suppression of responses to acute hypoxia [22] we saw no effect on hypoxic inhibition of the Ca^{2+} channel. Furthermore, the lack of effect of DPI and PAO on Ca^{2+} currents per se suggests that NADPH oxidase does not regulate the Ca^{2+} channel under basal conditions. Interestingly, DPI has been proposed to directly block Ca^{2+} currents in rat carotid body (CB) type I cells [35]. In the rat CB, Ca^{2+} current is carried almost exclusively by L-type channels [36,37], and taken with the present data concerning the lack of effect of DPI in cells expressing the α_{1C} subunit may suggest the involvement of channel auxiliary subunits in responses to DPI.

In summary, H_2O_2 regulates basal Ca^{2+} channel activity via an interaction with the pore-forming α_{1C} subunit. However, neither altered production of H_2O_2 nor regulation of the H_2O_2-producing NADPH oxidase is involved in acute hypoxic regulation of this channel. Thus, although altered H_2O_2 production during hypoxia can alter certain aspects of Ca^{2+} channel function such at its responsiveness to stimulation by β-adrenergic agonists [26], regulation of the levels of this reactive O_2 species does not underlie the direct, acute response of this channel to hypoxia. Further studies are required to elucidate the intracellular mechanisms which underlie O_2-dependent regulation of the Ca^{2+} channel.

Acknowledgments

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