Reversal of chronic hypoxia-induced alterations in pulmonary artery smooth muscle electromechanical coupling upon air breathing

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Abstract

Objective: Chronic hypoxia (CH) induces selective pulmonary hypertension which is accompanied by structural and functional alterations in the pulmonary vasculature. Little information is available on the regression of CH-induced functional alterations of pulmonary wall. In the present work, we investigated the reversal of CH-induced pulmonary hypertension with a special focus on alterations in the electrophysiological properties of pulmonary artery smooth muscle cells (PAMCs) after normoxia recovery. Methods: Rats were exposed to a hypobaric environment for 3 weeks (CH rats) and then subjected to a normoxic environment for 3 weeks (normoxia-recovery group) and compared with rats maintained in a normoxic environment (control rats). Electrophysiological properties of PAMCs were studied using conventional microelectrodes and patch-clamp technique. Results: CH rats exhibited a threefold increase in pulmonary blood pressure compared to control rats and this increase was fully reversed following 3 weeks of normoxia. PAMCs from CH rats were depolarised (about 20 mV), had an elevated calcium concentration and exhibited a hypersensitivity to 4-aminopyridine (4-AP) of membrane potential as well as the tone of arterial rings compared with tissues from control rats. Whole cell patch-clamp recordings indicated that voltage gated potassium channel currents \( I_{\text{K}} \) and \( I_{\text{K(N)}} \) were decreased in PAMCs from CH rats with a hyper sensitivity of \( I_{\text{K(N)}} \) to 4-AP. CH-induced alterations in electrophysiological properties of PAMCs were also fully reversed after 3 weeks of normoxia recovery. Conclusions: Both the increase in the pulmonary blood pressure and alterations in electrophysiological properties of PASMCs simultaneously reverse after normoxia recovery. This complete reversibility of all of the CH-induced pulmonary vascular alterations suggests that curative treatments for PAHT may now be designed aimed at targeting the very limited key factors implicated in hypoxia sensing. © 2002 Elsevier Science B.V. All rights reserved.

Keywords: Arteries; Hypertension; Hypoxia/anoxia; K-channel; Pulmonary circulation

1. Introduction

In mammalians including humans, chronic exposure to an hypoxic environment induces a selective pulmonary artery hypertension (PAHT) [1]. Similar to that observed as a consequence of generalized alveolar hypoxia, chronic hypoxia (CH)-induced PAHT develops as a consequence of structural and functional alterations in the pulmonary vasculature, including vascular remodeling and increased vasomotor tone [2–4]. This vascular remodeling process has been shown to involve medial smooth muscle cell hypertrophy and hyperplasia, fibroblast proliferation, and matrix protein synthesis [4–6]. Despite their pathophysiologic importance, molecular and cellular mechanisms underlying these phenomena have not been fully elucidated. All of these changes contribute to elevated pulmonary vascular resistance and pulmonary arterial...
pressure, thereby impairing right ventricular ejection, eventually causing right ventricular hypertrophy (RVH) and right heart failure [7].

CH-induced elevated vasomotor tone appears to be related, all along the pulmonary vascular bed (extra and intrapulmonary arteries), to: (1) a membrane depolarisation of pulmonary artery smooth muscle cells (PASMCs) [8–11]; (2) an increase in intracellular calcium concentration [Ca^{2+}] of PASMCs [10,11]; (3) a hypersensitivity to 4-aminopyridine (4-AP), a potent blocker of voltage gated potassium channels (Kv), of both the membrane potential of PASMCs and pulmonary artery tension [8–10]. At the onset (1–2 weeks) of CH-induced PAHT, increase in arterial tone of the main pulmonary artery (MPA) is accompanied by spontaneous and rhythmical contractions which could represent a temporary adaptive process of the pulmonary circulation to CH [10]. Electrophysiological studies have revealed that CH induces a down regulation of voltage-gated potassium channel currents (I_{Kv}) in PASMCs from both animals and humans [12–15]. Moreover, it has been shown that exposure to CH both in vitro and in vivo downregulates mRNA and protein expression of Kv1.1, Kv1.2, Kv1.5 and Kv2.1, that constitute delayed rectifier Kv channels in PASMCs [14,16]. Downregulation of Kv channels by CH could thus explain the observed depolarisation of PASMCs [17]. In rat intrapulmonary arteries, Osipenko et al. [8] have suggested that the CH-induced depolarisation could result from inhibition of a non-inactivating, voltage gated K+ current (I_{K(N)}), partially activated at resting membrane potential in normoxic conditions and poorly sensitive to 4-AP [18]. Down regulation of I_{K(N)} would subsequently lead the membrane potential value to the threshold for the activation of 4-AP-sensitive I_{Kv}, whose contribution to resting potential would thus increase. This phenomenon could be responsible for the increased sensibility to 4-AP of the CH pulmonary artery. Whether or not such a mechanism is occurring all along the pulmonary vascular bed is not known.

Some aspects of CH-induced PAHT are reversed upon recovery under normoxic conditions. For example, RVH, increase in pulmonary tension, structural changes in elastic laminae, muscularisation of distal pulmonary vessels and alteration in the expression of angiotensin II receptors are fully reversed after 2–3 weeks of normoxia recovery [19,20]. However, no detailed study has been performed on the reversal of CH-induced electrophysiological changes in PASMCs. Such information may help to better understand the cellular mechanisms responsible for CH-induced PAHT, especially those underlying CH-induced membrane depolarisation of PASMCs. In addition, such information may also prove useful in designing new therapeutic strategies.

In the present work, we thus have studied, in the rat, the effect of 3 weeks of recovery under a normoxic environment on the pulmonary circulation and PASMCs properties changes induced by 3 weeks of CH exposure. We paid special attention to the reversal of the CH-induced effect on the resting membrane potential using conventional microelectrodes and, on Kv currents using the whole cell patch-clamp technique, in PASMCs from the main pulmonary artery (MPA).

2. Methods

2.1. Chronic hypoxia and pulmonary artery hypertension

Adult male Wistar rats (aged 8–10 weeks, weighing 220 g) were separated into three groups. One group (control or normoxic rats) was housed in room air at a normal atmospheric pressure (101 kPa). Two other groups were exposed for 3 weeks to CH in a hypobaric chamber; in one, experiments were performed within 1 h of removal from the hypoxic chamber (hypoxic group); in the other, experiments were performed once rats recovered under a normoxic environment for 3 weeks (normoxia-recovery group). A duration of 3 weeks for CH exposure as well as for normoxia recovery was chosen on the basis of previous experiments showing that: (1) RVH and PAHT are maximum after 3 weeks of hypoxia and remain consistent upon longer duration of CH [10,21]; (2) regression of structural changes is in relation to the duration of exposure to hypoxia [19]. For CH-exposure, the pressure in the hypobaric chamber was reduced to 0.5 atmosphere (50.5 kPa) using an electrically-driven pump. The chamber was opened for 15–30 min twice a week.

Pulmonary hypertension was assessed by measuring the mean pulmonary artery pressure, the ratio of right ventricle (RV) to left ventricle plus septum (LV+S) weight, and hematocrit. At completion of the exposure, rats were anaesthetised by intraperitoneal injection of 40 mg ethylcarbamate. Pulmonary artery blood pressure was measured, through a 20-cm polyethylene (PE-50, Biotrol) catheter filled with heparinized saline (0.5%). The catheter was inserted in the right jugular vein, then through the right atria and the right ventricle into the pulmonary artery, and attached to a Baxter Uniflow gauge pressure transducer. Pressure was displayed on a HP 78342A strip-chart recorder (Hewlett-Packard, Palo Alto, CA, USA) and analysed with a computer. The investigation was carried out in agreement with the Guide for the Care and Use of Laboratory Animals published by the US National Institute of Health (NIH Publication No. 85-23, revised 1996) and European Directives (86/609/CEE).

2.2. Tissue and cell preparation

At completion of the exposure, the heart and lungs were removed en bloc. The MPA was then dissected under binocular control and, the adventitial and intimal layers were removed. For mechanical and membrane potential measurements, MPA rings (3 mm in length) were prepared.
For study of membrane currents and measurement of $[\text{Ca}^{2+}]$, isolated PASMCs were obtained using an enzymatic dissociation method previously described [22,23]. Briefly, the MPA was cut into small segments which were placed successively in a first (Ca$^{2+}$-free) dissociation solution containing 1 mg/ml bovine serum albumin (BSA) for 10 min at room temperature, then placed in a second dissociation solution at 37 °C containing 1 mg/ml papain and 1 mg/ml dithioerythritol for 18 min and in a third dissociation solution at 37 °C containing 1.6 mg/ml collagenase, 1.6 mg/ml trypsin inhibitor and 0.25 mg/ml elastase for 8 min. Tissues were then replaced in the first dissociation solution for 5 min and were gently agitated using a polished wide-bore Pasteur pipette to release the cells. Cells were stored at 4 °C and used between 2 and 8 h after isolation. Only elongated, smooth and optically refractive cells were used for patch-clamp measurements.

2.3. Recording of mechanical activity

Isometric contraction was measured in rings mounted between two stainless steel clips in vertical 20-ml organ baths of a computerised isolated organ bath system (IOX, EMKA Technologies, Paris, France). Baths were filled with Krebs–Henseleit (KH) solution. The upper stainless steel clip was connected to an isometric force transducer (EMKA Technologies). The rings from control and normoxia recovery and CH rats were set at optimal length by equilibration against a passive load as described previously [8]. Cells were then superfused with a physiological salt solution (PSS) in the presence of 100 nM IbTx in cells voltage clamped at 0 mV for 5 min to inactivate Kv [8,18]. Cells were then

... transmembrane potential was generated by step-clamp amplifier (Axopatch 200B, Axon instruments, USA). Signals were filtered at 1 kHz and digitised at 5 kHz. Peak current elicited at a single membrane potential was defined as the average of 500 sample points encompassing the maximal current point. Trials were performed in triplicate in the same cell and averaged to estimate peak current amplitude. Currents were normalised to cell capacitance and were expressed as picoamperes per picofarad (pA/pF).

Net macroscopic K$^+$ currents were generated by stepwise 10 mV depolarising pulses (400 ms duration; 5 s intervals) from a constant holding potential of −80 mV to +60 mV. Based on their pharmacological and electrophysiological properties, the following K$^+$ current subtypes were identified. The $I_{\text{Ca}}$ current was defined as the voltage between the outward current recorded in the absence and in the presence of 100 nM iberiotoxin (IbTx), a selective blocker of $\text{K}_\text{Ca}$ channel type [25]. The $I_v$ current was defined as the difference between the outward current recorded in the presence of 100 nM IbTx and in the presence of 100 nM IbTx plus 3 mM 4-AP, a blocker of $K_v$ and $K_N$ channels [8]. $I_{\text{Ca}}$ current was recorded in the presence of 100 nM IbTx in cells voltage clamped at 0 mV for 5 min to inactivate $K_v$ [8,18]. Cells were then stepped to +60 mV and immediately depolarised to −100 mV.
mV using a voltage ramp at 0.2 V s\(^{-1}\). Voltage clamp protocols were generated and the data were stored with a computer using a Digidata 1200 interface (Axon Instruments) and PCLAMP 8 software (Axon Instruments). Data were analysed using CLAMPFIT 8 and ORIGIN 6 software (Microcal Software, Northampton, MA, USA).

2.6. \([Ca^{2+}]\), measurements

To assess the dynamic changes in \([Ca^{2+}]\), in individual arterial myocytes, we used the \([Ca^{2+}]\), sensitive fluorescent indo-1. Cells were loaded with indo-1 by incubation in PSS containing 1 \(\mu\)M indo-1 penta-acetoxymethyl ester (indo-1 AM) for 25 min at room temperature and then washed in PSS for 25 min. The coverslip with attached cells was then mounted in a perfusion chamber. The recording system included a Nikon Diaphot inverted microscope fitted with epifluorescence (Nikon, Tokyo, Japan). A single cell, among those on the coverslip, was studied cell was illuminated at 360 nm and counted simultaneously at 405 and 480 nm by two photomultipliers (PI100, Nikon). The fluorescence ratio (405/480) was calculated on-line and displayed with the two voltage signals on a monitor. \([Ca^{2+}]\), was estimated from the 405:480 ratio [26] using a calibration for indo 1 determined within cells [27]. 4-AP was applied to the recorded cell by pressure ejection from a glass pipette located close to the cell.

2.7. Solutions and chemicals

The Krebs–Henseleit (KH) solution had the following composition (in mM): 118.4 NaCl, 4.7 KCl, 2.5 CaCl\(_2\), 1.2 MgSO\(_4\), 1.2 KH\(_2\)PO\(_4\), 25 NaHCO\(_3\), 11.1 d-glucose, pH 7.4 maintained at 37°C and bubbled with a 95% O\(_2\)-5% CO\(_2\) gas mixture. K\(^+\)-rich solution was obtained by substituting an equimolar amount of KCl for NaCl from KH solution. The PSS contained (in mM): NaCl, 138.6; KCl, 5.4; CaCl\(_2\), 1.8; MgCl\(_2\), 1.2; NaH\(_2\)PO\(_4\), 0.33; HEPES, 10 and glucose, 11; pH was adjusted to 7.4 using NaOH. The dissociation solution contained (in mM): NaCl, 145; KCl, 4; MgCl\(_2\), 1; HEPES, 10 and glucose, 10; pH was adjusted to 7.3 using NaOH. For whole cell patch-clamp recordings, the pipette solution contained (in mM): glutamic acid, 125; KCl, 20; Na\(_2\)ATP, 1; CaCl\(_2\), 0.37; MgCl\(_2\), 1; HEPES, 10; EGTA, 1; pH was adjusted to 7.2 using KOH. \(P_{\text{ca}}\) ~ 7 was calculated by a computer program [28].

4-AP, BSA (fraction V), collagenase (type H), dithioerythritol, (type IV), IbTx, indo-1 AM, papain, trypsin inhibitor (type I-S) were from Sigma (St. Quentin Fallavier, France). Stock solutions of 4-AP and IbTx were prepared in distilled water and then diluted in PSS to the appropriate concentration.

2.8. Analysis of data and statistics

Results are expressed as mean±S.E.M. Contractions are expressed as a percentage of K\(^+\)-rich (80 mM) solution-induced contraction. Statistical analysis was performed using MINITAB software (Minitab). Data were compared with one factor ANOVA with posthoc tests or the Mood’ median test when normality test failed (Anderson–Darling test) with posthoc tests as indicated. Homogeneity of variance was tested using Bartlett’s test when the data were normally distributed or using Levene’s test when normality test failed. For some comparisons, the Pearson product moment correlation coefficient between pair of variables was used as indicated. Regarding the number of experiments, \(n\) refers to the number of rings or cells and \(N\) to the number of animals. Differences were considered significant at \(P<0.05\).

3. Results

3.1. Reversal of CH-induced pulmonary hypertension

Three weeks of hypoxic exposure induced PAHT characterized by a significant increase in the mean pulmonary artery pressure from 10.2±2 mmHg (\(N=5\)) to 33±2.1 mmHg (\(N=9\)) in control and CH rats, respectively (\(P<0.05\)). PAHT was accompanied by an increase in the ratio of RV/LV+S weight from 0.3±0.01 (\(N=10\)) to 0.62±0.05 (\(N=7\)) and in the hematocrit from 44±4% (\(N=4\)) to 68±8% (\(N=9\)) (\(P<0.05\), ANOVA and posthoc Dunnett’ test). These increases were totally abolished after 3 weeks of normoxia recovery (Fig. 1) with no significantly difference between control and normoxia-recovery groups (posthoc testing Dunnett’ test). The reversal of the increase in mean pulmonary artery pressure correlated with the variation in the ratio of RV/LV+S weight (\(P<0.05\), Pearson correlation coefficient= 0.743), showing that the decrease in the ratio of RV/LV+S weight after normoxia recovery was linked to the decrease of the mean pulmonary artery pressure.

3.2. Reversal of CH-induced increase in 4-AP sensitivity of MPA mechanical activity

CH increased both efficacy to and potency for 4-AP in MPA rings (Fig. 2). The threshold concentration of 4-AP inducing a contraction was 100 times lower in rings from CH rats than in rings from control rats: 0.03 and 0.3 mM, respectively. The tension induced by 3 and 10 mM 4-AP was increased by 98 and 72% in rings from CH rats compared with rings from control rats, respectively. After 3 weeks of normoxia-recovery, the reversal of this effect was complete (Fig. 2). No significant difference was observed between control and normoxia-recovery rings (posthoc testing Dunnett’ test).
3.3. Reversal of CH-induced depolarization and of CH-induced increase of 4-AP effect on resting membrane potential and \([\text{Ca}^{2+}]_i\)

Fig. 3A shows a typical recording of resting membrane potential in MPA rings obtained from control, CH and normoxia-recovery rats. As previously shown [10], cells in rings from CH rats were depolarized by about 20 mV compared with cells in rings from control rats (Fig. 3B and Table 1). This sustained change in membrane potential was accompanied by a change in the response to 4-AP application. A 1-mM concentration of 4-AP had no effect on membrane potential in rings from control rats, but induced a significant additional depolarization in rings from hypoxic rats (Fig. 3Aab). CH-induced change in both resting membrane potential and 4-AP response was fully reversed after 3 weeks of normoxia-recovery (Fig. 3Ac and Table 1). No difference was observed between cells from control and normoxia-recovery cells \((P>0.05; \text{ANOVA and post-hoc Dunnett’ test)}) (Fig. 3B). The reversal of CH-induced increase in 4-AP response was correlated with the variation in the resting membrane potential \((P<0.05, \text{Pearson correlation coefficient } 0.851\)).

Similarly to the change in membrane potential value, 3 weeks of CH increased both resting \([\text{Ca}^{2+}]_i\) value and \([\text{Ca}^{2+}]_i\) response to 1 mM 4-AP (Fig. 4A and B and Table 1). After 3 weeks of normoxia recovery, resting \([\text{Ca}^{2+}]_i\) value and 4-AP-induced \([\text{Ca}^{2+}]_i\) response returned to control values (Fig. 4C).

3.4. Reversal of CH-induced changes in passive membrane properties of isolated MPA myocytes

Cell capacitance and membrane resistance in isolated MPA myocytes obtained from control, CH and normoxia-recovery rats are indicated in Table 1. No significant difference was observed between mean values of cell capacitance in MPA myocytes obtained from the three groups of rats \((P>0.05, \text{ANOVA})\). Membrane resistance significantly increased in myocytes from CH rats compared with myocytes from control rats and this increase was fully reversed after 3 weeks of normoxia-recovery \((P>0.05, \text{ANOVA, and posthoc Dunnett’ test})\).
Fig. 3. Reversal of CH-induced depolarisation and of CH-induced increase in 4-AP effect on resting membrane potential in MPA rings. (A) Original recordings of resting membrane potential and of the effect of 4-AP in rings from control (a), CH (b) and normoxia-recovery rats (c). Horizontal line indicates the duration of 4-AP application. (B) Mean values for the resting membrane potential and the 4-AP-induced depolarisation (relative value) in control (open columns, \(n=5, N=3\)), CH (closed columns, \(n=11, N=3\)) and normoxia-recovery rats (cross-hatched columns, \(n=15, N=3\)). Data are the mean±S.E.M. *, Significant difference from control (\(P<0.05\)).

3.5. Reversal of CH-induced changes in 4-AP sensitive currents

Fig. 5 shows that \(K_V\) current density was significantly decreased in myocytes from CH rats compared with myocytes from control rats. CH reduced \(K_V\) current density by 89 and 82% at 0 and +40 mV, respectively (\(P<0.05\), ANOVA and posthoc Dunnett’ test). After 3 weeks of normoxia-recovery, \(K_V\) current density was fully restored. It is noteworthy that \(K_V\) current density was null at \(-55\) mV in cells form control rats and slightly negative at \(-30\) mV in cells from CH rats (Fig. 5 insert), the respective resting membrane potential values determined above for these two rat groups. In order to further demonstrate the absence of involvement of \(K_v\) current in the resting membrane potential, the latter was measured

### Table 1
Passive membrane properties, \([Ca^{2+}]\), and 4-aminopyridine (4-AP) response for both resting membrane potential (RMP) and \([Ca^{2+}]\), in pulmonary artery smooth muscle cells from control, CH and normoxia-recovery rats

<table>
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<tr>
<th></th>
<th>Control</th>
<th>CH</th>
<th>Normoxia recovery</th>
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<tbody>
<tr>
<td>RMP (mV)</td>
<td>(-58.1±1.1) ((n=4))</td>
<td>(-35.6±1.2^*) ((n=15))</td>
<td>(-54.6±1.5) ((n=11))</td>
</tr>
<tr>
<td>4-AP (1 mM)-induced</td>
<td>(-12±1) ((n=4))</td>
<td>(-14.9±2.1^*) ((n=7))</td>
<td>(-1.6±0.75) ((n=6))</td>
</tr>
<tr>
<td>depolarisation (mV)</td>
<td></td>
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<tr>
<td>Cell capacitance (pF)</td>
<td>(13.2±2) ((n=13))</td>
<td>(14.6±3.4) ((n=15))</td>
<td>(12.3±1.9) ((n=9))</td>
</tr>
<tr>
<td>Membrane resistance (GΩ)</td>
<td>(2.4±0.4) ((n=12))</td>
<td>(5.3±0.7^*) ((n=12))</td>
<td>(3.04±0.8) ((n=7))</td>
</tr>
<tr>
<td>Resting ([Ca^{2+}]) (nM)</td>
<td>(74.6±12) ((n=12))</td>
<td>(124.3±21^*) ((n=16))</td>
<td>(81.1±7) ((n=9))</td>
</tr>
<tr>
<td>4-AP (1 mM) induced</td>
<td>(80.2±6.1) ((n=12))</td>
<td>(588.2±124^*) ((n=16))</td>
<td>(90.6±22.4) ((n=9))</td>
</tr>
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RMP was measured with conventional microelectrodes from artery rings. Results represent the mean±S.E.M.

* Indicates a significant difference from control (\(P<0.05\)).

Fig. 5. Reversal of CH-induced decrease of $I_{K_v}$ in isolated MPA myocytes. Current density–voltage relations for $I_{K_v}$ in cells from control ($n=6$, $N=6$), CH ($n=6$, $N=5$) and normoxia-recovery rats ($n=6$, $N=3$). Insert shows at a different scale, $I_{K_v}$ alteration by CH and reversal by normoxia recovery at $-30$ mV. $I_{K_v}$ was calculated as the difference between the outward current recorded in the presence of 100 mM IbTx and in the presence of 100 mM IbTx plus 3 mM 4-AP during 400 ms depolarising pulses from a holding potential of $-80$ mV. Current amplitude was normalised against cell capacitance. Results represent the mean±S.E.M. *, Significant difference from control ($P<0.05$).

Fig. 4. Reversal of the CH-induced increase in resting [Ca$^{2+}$]i and 4-AP-induced response. [Ca$^{2+}$]i was measured in isolated MPA myocytes from control (A), CH (B) and normoxia-recovery rats (C). 4-AP (1 mM) was applied near the myocyte for 30 s as indicated by the horizontal line. Note that increase in both resting [Ca$^{2+}$]i, value and 4-AP response (B) was fully reversed after 3 weeks of normoxia recovery (C). Each trace was recorded from a different cell and is typical of 12 cells (A), 16 cells (B) and 9 cells (C).

immediately upon switching from voltage to current clamp ($I=0$ pA) before and just after clamping the membrane at 0 mV for 5 min in order to inactivate $K_v$ currents. This set of experiments was performed in the presence of iberiotoxin (100 nM). The pair value of membrane potential was $-53.6±4$ mV before, $(n=3)$ and $-53.1±4.2$ mV after, $(n=3)$, $-37±2$ mV before, $(n=4)$ and $-32.6±2.7$ mV after, $(n=4)$, $-49±1.6$ mV before, $(n=3)$ and $-48.6±2.3$ mV after, $(n=3)$ in PASMCs from control, CH and normoxia-recovery groups, respectively.

As illustrated in Fig. 6, myocytes from rat MPA displayed a current with properties similar to $I_{K(N)}$ previously observed in rabbit and rat intrapulmonary arteries [8,18]. After inactivation of $K_v$ currents (see Methods), application of a voltage ramp from $-60$ mV to $-100$ mV, revealed a nonlinear dependence on the membrane potential of the non inactivating current. In myocytes from control rats, $I_{K(N)}$ was reduced by 30 and 40% in the presence of 3 mM 4-AP at $-55$ and 0 mV, respectively (Fig. 6A). In myocytes from CH rats, the amplitude of $I_{K(N)}$ was significantly decreased by 20% at $-55$ mV compared to control rats ($P<0.05$, ANOVA and posthoc Dunnett’ test). Normalization of $I_{K(N)}$, measured at 0 mV, to cell capacitance revealed a significant CH-induced decrease in current density (Fig. 6D). Moreover, 4-AP exhibited greater inhibitory effect on the amplitude of $I_{K(N)}$ than in controls: 85 and 100% reduction at $-55$ and 0 mV, respectively (Fig. 6B). Interestingly, $I_{K(N)}$ was significantly reduced by 4-AP at $-30$ mV in cells from CH rats (Fig. 6B) whereas it was not altered at $-55$ mV in cells from control rats (Fig. 6A).

The CH-induced decrease in the maximal amplitude of $I_{K(N)}$ was abolished in myocytes from normoxia-recovery rats. Similarly, the greater inhibitory effect of 4-AP on $I_{K(N)}$ in CH rats was reversed, although not completely, in myocytes from normoxia-recovery rats where 4-AP decreased $I_{K(N)}$ by 40 and 37% at $+55$ and 0 mV, respectively (Fig. 6C).
Fig. 6. Reversal of CH-induced increase to 4-AP sensitivity of $I_{K(N)}$ in isolated MPA myocytes. Typical examples of $I_{K(N)}$ recorded in the presence of 100 nM IbTx and 100 nM IbTx plus 3 mM 4-AP in cell from control (A), CH (B) and normoxia-recovery rats (C). Cells were clamped at 0 mV for 5 min before applying the voltage ramp from +60 mV to −100 mV. (D) Mean amplitude of $I_{K(N)}$ measured at 0 mV was normalised against cell capacitance in cells from control (open column; $n=5$, $N=6$), CH (closed column; $n=4$, $N=5$) and normoxia-recovery rats (cross-hatched column; $n=4$, $N=4$). Results represent the mean ± S.E.M. * Significant difference from control ($P<0.05$).

4. Discussion

The present work conducted in the MPA indicates that CH-induced downregulation of K-channels, membrane depolarization and altered calcium homeostasis in pulmonary vascular smooth muscle are fully reversible upon normal air breathing. This reversal in these electromechanical alterations parallels that of the increase in pulmonary blood pressure and of the hypertrophy of the right ventricle. Finally, the complete reversibility of all of the CH-induced pulmonary vascular alterations suggests that curative treatments may now be designed aimed at targeting the very limited key factors implicated in hypoxia sensing.

Exposure of rats to hypoxia for 3 weeks induced a significant PAHT (a threefold increase in the mean pulmonary blood pressure) accompanied by a RVH and an increase in the hematocrit. This duration of hypoxia exposure was selected on the basis of previous experiments [10] indicating that it corresponds to the full development of RVH and pulmonary vascular remodeling. At the pulmonary vascular level, our data in MPA clearly indicate that PAHT is related to a membrane depolarization, a downregulation of voltage-gated K⁺ channels and an increase in the [Ca²⁺]i of smooth muscle cells. CH-induced PAHT is also characterized by a hypersensitivity to 4-AP of both membrane potential and pulmonary artery tone as previously shown in intrapulmonary arteries [8,9,13]. Cellular and molecular mechanisms underlying CH-induced depolarization and alteration in agonist-induced vasoreactivity [29] in pulmonary artery are not yet fully established. In intrapulmonary arteries, the CH-induced depolarization and the increase in 4-AP sensitivity results from the sequential involvement of both $I_{K(N)}$ and $I_{Kv}$. CH down regulates $I_{K(N)}$, leading the membrane potential to the threshold value for $I_{Kv}$ activation without change in sensitivity to 4-AP of these two currents [8]. Such is not exactly the case in the present study performed in extrapulmonary artery since: (i) $I_{Kv}$ did not appear activated at resting membrane potential in cells from CH rats (Fig. 5B); (ii) the sensitivity to 4-AP of $I_{K(N)}$ was significantly increased after CH exposure; (iii) in cells
from CH rats, 4-AP significantly reduced $I_{K(N)}$ at resting membrane potential i.e. $-30 \text{ mV}$. Taking into account the high membrane resistance value in PASMCs especially in cells from CH rats (5 GΩ), even a moderate decrease in membrane current such as that induced by 4-AP could lead to a significant depolarization. Collectively, these results suggest that, under our experimental conditions, $I_{K(N)}$ is the main target of CH. Both membrane depolarization and the increase sensitivity to 4-AP of arterial tone in MPA essentially result from the dual action of CH on $I_{K(N)}$ i.e. downregulation and increased sensitivity to 4-AP of this current. The mechanism of this increase in 4-AP sensitivity in not known but could involve some structural changes in the channel. At the molecular level, several investigators have attempt to identify the true $O_2$ sensor in PASMCs, by relating $O_2$-sensitive $K$ currents to the expression of genes encoding for $Kv$ subunits and have been reported, so far, conflicting results. It thus has been suggested that $Kv1.2/\text{Kv1.5 and Kv2.1/Kv9.3}$ channel proteins could account for $I_{K(N)}$ and $I_{K_\infty}$ respectively [14,30,31]. Moreover, it has indeed been reported that expression of $Kv1.5$ and $Kv2.1$ channel proteins is decreased by CH [32]. However, a recent study using recombinant channels [33] has shown that $K$ current through $Kv3.1$, but not $Kv1.1$ or $Kv1.2$, is decreased by hypoxia and this effect is retained in excised patches suggesting a direct effect of hypoxia on the channel protein.

In the present work, all of the CH-induced alterations were reversed following recovery for an identical 3 weeks duration of normal air breathing. To the best of our knowledge, this work is the first extensive investigation regarding the reversal of functional aspects of CH-induced PAHT. The fact that both increase in the pulmonary blood pressure and alterations in electrophysiological properties of PASMCs simultaneously reverse after normoxia recovery reinforces the hypothesis of a vascular myogenic origin of CH-induced PAHT. However, to fully support this statement, similar experiments should also be conducted in the intrapulmonary arteries that mainly contribute to pulmonary blood pressure. In addition, this complete reversibility of all of CH-induced pulmonary vascular alterations suggests that curative treatments of CH-induced PAHT may now be designed. Indeed, treatments targeting the limited key factors implicated in hypoxia sensing are likely to allow complete reversal of CH-induced pulmonary vascular alterations as observed in the present study, provided that these phenomena occur at the site of the pulmonary smooth muscle cell. One possibility is related to the fact that hypoxia alters gene expression in cardiovascular tissues [34]. A key factor of transcriptional responses to decrease of $O_2$ partial pressure is hypoxia-inducible factor 1 (HIF-1) [35,36]. HIF-1 activates the transcription of genes encoding several factors involved in the development of PAHT. In this connection, Shimoda et al. [37] have recently shown that a partial deficiency of HIF-1α in mice [(Hif1α(±))] prevents some electrophysiological alterations induced by CH, especially membrane depolarization and reduction in $Kv$ current density. Since $Kv$ downregulation as well as all of CH-induced pulmonary vascular alterations are reversed by normoxia recovery, it will be interesting to investigate the effect of the pharmacological modulation of HIF-1 [36] in PAHT.

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References


