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SPECIAL REPORT

Influence of chronic hypoxia on the contributions of non-inactivating and delayed rectifier K currents to the resting potential and tone of rat pulmonary artery smooth muscle

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Exposing rats to chronic hypoxia increased the 4-aminopyridine (4-AP) sensitivity of pulmonary arteries. 1 mM 4-AP caused smooth muscle cell depolarization and contraction in arteries from hypoxic rats, but had little effect in age-matched controls. Chronic hypoxia downregulated delayed rectifier K^+ current ($I_{K(V)}$), which was nearly 50% blocked by 1 mM 4-AP, and non-inactivating K^+ current ($I_{K(N)}$), which was little affected by 1 mM 4-AP. The results suggest that $I_{K(N)}$ determines resting potential in control rats and that its downregulation following hypoxia leads to depolarization, which activates $I_{K(V)}$ and increases its contribution to resting potential. The hypoxia-induced increase in 4-AP sensitivity thus reflects a switch in the major K^+ current determining resting potential, from $I_{K(N)}$ to $I_{K(V)}$. This has important implications for the actions and specificity of pulmonary vasodilator drugs.

Keywords: Pulmonary artery; pulmonary hypertension; chronic hypoxia; K channel; delayed rectifier; K_V; 4-aminopyridine; depolarization; arterial myocyte; non-inactivating K current

Introduction Prolonged exposure to hypoxia causes pulmonary hypertension. This is associated with membrane depolarization, which in rats is thought to result from the downregulation of delayed rectifier K^+ current ($I_{K(V)}$) in pulmonary artery smooth muscle (Smirnov *et al*., 1994; Wang *et al*., 1997). In rabbit pulmonary artery myocytes, acute hypoxia caused depolarization by inhibiting a non-inactivating, voltage gated K^+ current, $I_{K(N)}$, that was pharmacologically distinct from $I_{K(V)}$ (Osipenko *et al*., 1997). $I_{K(N)}$ is the major determinant of resting potential in these cells (Evans *et al*., 1996; Osipenko *et al*., 1997) and its downregulation is predicted to cause depolarization. This study investigated the effects of 2 weeks chronic hypoxia on $I_{K(V)}$ and $I_{K(N)}$ in rat pulmonary artery myocytes, and the roles of these currents in mediating depolarization and contractile responses to the K^+-channel blocking drug, 4-AP.

Methods Male rats, 28–30-day-old (~65g), were maintained for 2 weeks in an hypoxic (10% O_2) environment as previously described (MacLean *et al*., 1996), with age-matched controls maintained in room air. They were killed by sodium pentobarbitone overdose (60 mg. kg^{-1} i.p.) and the first intrapulmonary artery branch removed. Isometric tension was recorded from arterial rings at 37°C as previously described (MacLean *et al*., 1996), using Krebs-bicarbonate solution of composition (mM): NaCl 119, KCl 4.7, $MgSO_4$ 0.6, KH_2PO_4 1.2, $CaCl_2$ 2.5, $NaHCO_3$ 25, glucose 11.1; bubbled with 16% O_2/6% CO_2, balance N_2. Myocytes were isolated and whole-cell, current-clamp and voltage-clamp recordings made as previously described (Evans *et al*., 1996). Recording solutions contained (mM): Bath- NaCl 124, KCl 5, $NaHCO_3$ 15, $CaCl_2$ 1.8, $MgCl_2$ 1, NaH_2PO_4 0.5, KH_2PO_4 0.5, glucose 10, HEPES 15, pH 7.3; Pipette - KCl 130, $MgCl_2$ 1, EGTA 1, HEPES 20, Na_2GTP 0.5, pH 7.2. Glibenclamide (Sigma; 10 μM) and tetraethylammonium chloride (Fluka; 10 mM) were added to the bath when recording K^+ currents to block K_{ATP} and BK_{Ca} channels. Glibenclamide stock (10 mM) was prepared with dimethylsulphoxide, which alone had no effect. 4-AP (Sigma) was dissolved in bath solution, pH corrected, and applied locally to cells from a flow pipe. Membrane potentials were measured as the average zero-current potentials during 30 s periods. Results are quoted as mean ± s.e.m. Data from chronic hypoxic (CH) animals were compared with age-matched controls using a two-tailed, unpaired *t*-test. 4-AP effects were analysed using paired *t*-tests.

Results Intrapulmonary arteries contracted when the extracellular KCl concentration was elevated to 50 mM; contraction amplitudes did not differ significantly between control (335 ± 46 mg, $n=10$) and CH (200 ± 40 mg, $n=6$) vessels. When applied at 1 mM, 4-AP had little effect on control arteries, but was almost as effective as 50 mM KCl at contracting vessels from CH animals (Figure 1a). The results from several experiments (Figure 1b) show a marked increase in 4-AP sensitivity following hypoxia. This was also observed at the single-cell level. Figure 1c shows membrane potentials recorded from control and CH myocytes. In control cells, 1 mM 4-AP produced only a small depolarization of 4 ± 2 mV ($n=18$), although 10 mM depolarized cells to nearly 0 mV. By contrast, in CH cells 4-AP caused substantial depolarization even at 1 mM. 4-AP depolarized cells from CH animals by 11 ± 2 mV ($n=16$) at 1 mM, which was significantly ($P<0.05$) larger than the 4 mV depolarization produced in control cells. The enhanced 4-AP sensitivity was accompanied by a significant shift of the resting potential (Figure 1d) from −46 ± 2 mV ($n=101$) in control cells to −26 ± 2 mV ($n=66$, $P<0.0001$) in CH cells. The depolarized potential of CH myocytes would promote contraction to 4-AP, because it is closer to the threshold for Ca^{2+} channel opening. Thus 1 mM

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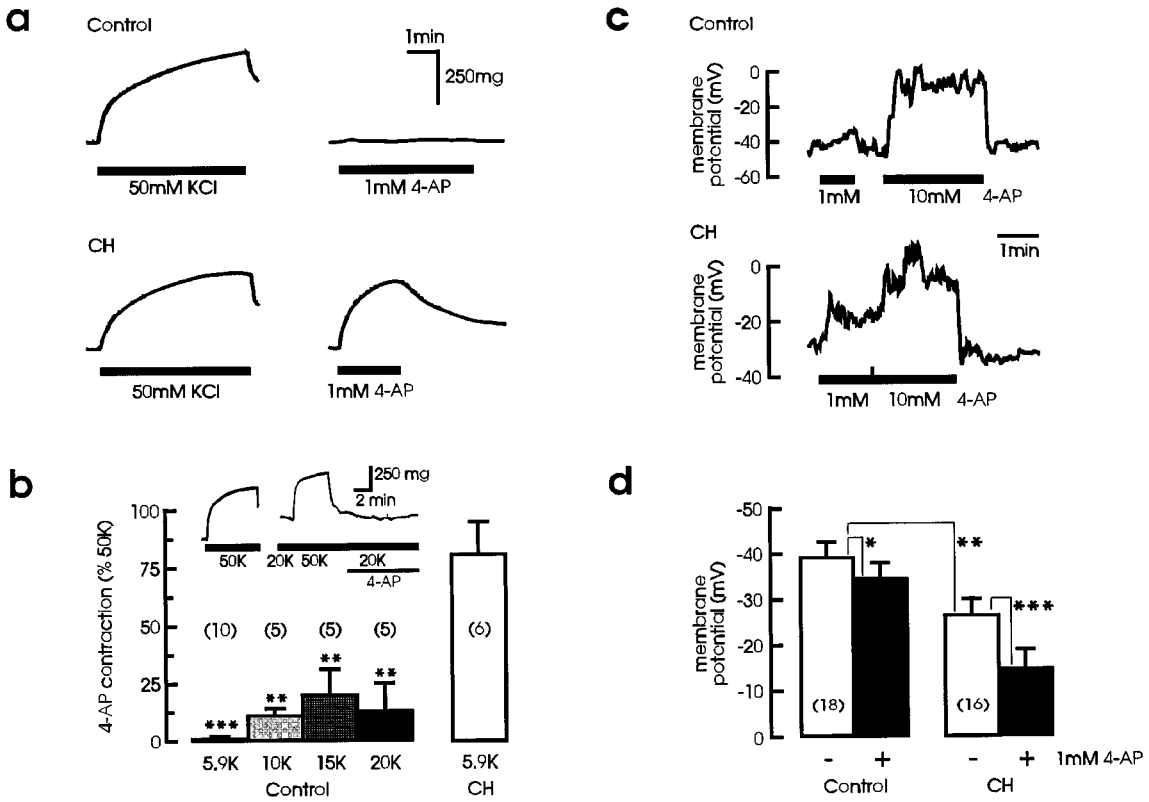

Figure 1 Effects of 4-AP on control and CH pulmonary arteries. (a) Responses of arterial rings to 50 mM KCl or 1 mM 4-AP. (b) Contractions to 1 mM 4-AP, expressed as % of the 50 mM KCl response, in normal Krebs (5.9K) and after elevation of KCl to 10, 15 or 20 mM (protocol inset). $***P<0.001$, $**P<0.01$ compared with CH vessels. (c) Membrane potential responses of myocytes to 4-AP. (d) Membrane potentials in the absence and presence of 1 mM 4-AP, which induced a significantly larger depolarization of CH cells compared with age-matched controls ($P<0.05$). $***P<0.001$, $**P<0.001$, $*P<0.05$. Numbers of vessels or cells shown in parenthesis.

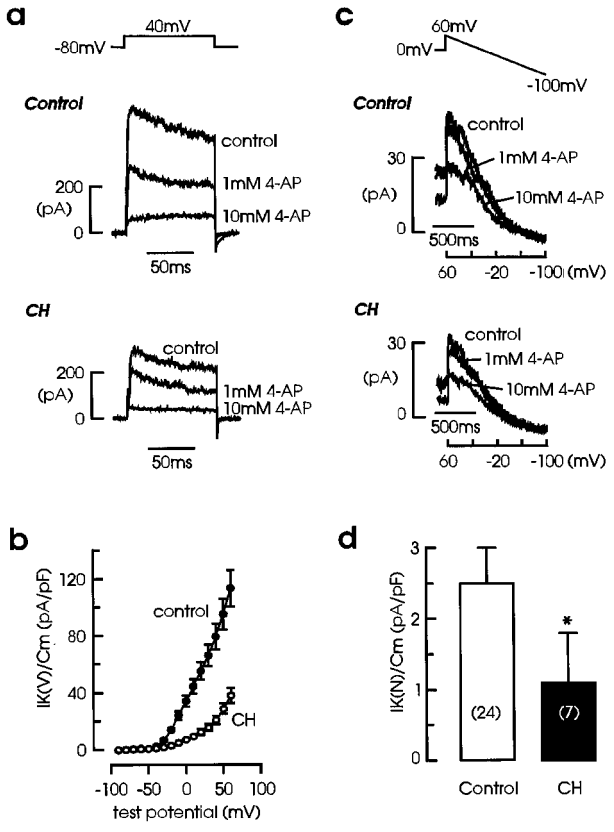

Figure 2 (a) Voltage protocol and $I_{K(V)}$ recorded from control and CH myocytes, in the absence and presence of 4-AP. (b) Current density *versus* voltage relationship for $I_{K(V)}$ in 76 control and 30 CH cells. (c) Voltage protocol and records of $I_{K(N)}$ from control and CH myocytes, in the absence and presence of 4-AP. Cells were clamped at 0 mV for 5 min before applying the voltage ramp. (d) Amplitude of $I_{K(N)}$, measured as the non-inactivating current at 0 mV and normalised against cell capacitance, in 24 control and 7 CH cells. $*P<0.05$ compared with control.

4-AP contracted control arteries that were depolarized by elevating the extracellular $[K^+]$ (equimolar substitution of KCl for NaCl; Figure 1b). However, even at 20 mM K^+, which produces around 15 mV depolarization in intact vessels (Suzuki & Twarog, 1982), the 4-AP-induced contraction was small compared with that in CH vessels (Figure 1b). The larger 4-AP-induced contraction of CH vessels must therefore reflect the increased 4-AP-induced depolarization of CH myocytes.

Figure 2a shows $I_{K(V)}$ recorded from control and CH myocytes, during steps to 40 mV from a holding potential of -80 mV, and its inhibition by 4-AP. $I_{K(V)}$ amplitude, normalised against cell capacitance, is plotted as a function of test potential in Figure 2b. As found before (Smirnov *et al*., 1994), $I_{K(V)}$ was smaller in cells from CH rats, but its 4-AP sensitivity was unchanged; 1 mM 4-AP reduced $I_{K(V)}$ by $39\pm10\%$ ($n=4$) in CH cells and $48\pm4\%$ ($n=10$) in controls, while 10 mM caused $83\pm2\%$ ($n=4$) and $80\pm3\%$ ($n=7$) inhibition in CH and control cells, respectively. As illustrated in Figure 2c, myocytes from control and CH rats displayed a current with properties akin to $I_{K(N)}$ in rabbit cells. Outward current persisted after clamping at 0 mV for >5 min and a subsequent ramp from 60 mV to -100 mV revealed deactivation at negative potentials. $I_{K(N)}$ was also inhibited by 4-AP, but not at 1 mM (Figure 2c). In control cells, 4-AP block of

$I_{K(N)}$ at 0 mV amounted to $66 \pm 5\%$ ($n=5$) at 10 mM, but only $9 \pm 11\%$ ($n=6$) at 1 mM. Similarly, 4-AP reduced $I_{K(N)}$ in CH cells by $67 \pm 18\%$ ($n=3$) at 10 mM and $5 \pm 19\%$ ($n=3$) at 1 mM. Chronic hypoxia was, however, associated with marked downregulation of $I_{K(N)}$ measured at 0 mV (Figure 2d).

Discussion Chronic exposure of rats to an hypoxic environment resulted in downregulation of $I_{K(V)}$ and $I_{K(N)}$ in intrapulmonary artery myocytes, without a change in 4-AP sensitivity. At the same time myocytes became depolarized and both membrane potential and vessel tone increased in sensitivity to 4-AP. Although, as previously suggested (Smirnov *et al*., 1994), downregulation of $I_{K(V)}$ could explain membrane depolarization, it cannot explain the increased 4-AP sensitivity. Downregulation of $I_{K(N)}$ can explain both the depolarization and increased 4-AP sensitivity. The lack of effect of 1 mM 4-AP on $I_{K(N)}$ and membrane potential is consistent with $I_{K(N)}$ playing a prominent role in setting resting potential in control myocytes. The measured potential of -46 mV is within the activation range for $I_{K(N)}$ (Evans *et al*., 1996) but below the threshold for $I_{K(V)}$ (Figure 2b; Evans *et al*., 1996; Patel *et al*., 1997). By causing depolarization, hypoxia-induced downregulation of $I_{K(N)}$ would promote the opening of K_V channels, which would then contribute more to resting potential and increase 4-AP sensitivity. Furthermore, since hypoxia increased 4-AP sensitivity more than elevated $[K^+]$, $I_{K(V)}$ contributed more to resting potential than expected from depolarization alone. This suggests a switch in the dominant K^+ current determining resting potential in CH vessels, from $I_{K(N)}$ to $I_{K(V)}$.

The results suggest a central role for $I_{K(N)}$ in setting myocyte resting potential in healthy pulmonary arteries and in the depolarization and vasoconstriction caused by chronic hypoxia. An understanding of the channels underlying $I_{K(N)}$ could therefore aid the development of therapeutic pulmonary vasodilators. Progress was recently made in cloning a Kv9.3 subunit from rat pulmonary artery, which may form part of an O_2-sensing channel (Patel *et al*., 1997), although its relationship to $I_{K(N)}$ is not known.

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