Two-Pore Domain K Channel, TASK-1, in Pulmonary Artery Smooth Muscle Cells

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Abstract—Pulmonary vascular tone is strongly influenced by the resting membrane potential of smooth muscle cells, depolarization promoting Ca^{2+} influx, and contraction. The resting potential is determined largely by the activity of K+-selective ion channels, the molecular nature of which has been debated for some time. In this study, we provide strong evidence that the two-pore domain K+ channel, TASK-1, mediates a noninactivating, background K+ current (I_{KN}), which sets the resting membrane potential in rabbit pulmonary artery smooth muscle cells (PASMCs). TASK-1 mRNA was found to be present in PASMCs, and the membranes of PASMCs contained TASK-1 protein. Both I_{KN} and the resting potential were found to be exquisitely sensitive to extracellular pH, acidosis inhibiting the current and causing depolarization. Moreover, I_{KN} and the resting potential were enhanced by halothane (1 mmol/L), inhibited by Zn^{2+} (100 to 200 μmol/L) and anandamide (10 μmol/L), but insensitive to cytoplasmic Ca^{2+}. These properties are all diagnostic of TASK-1 channels and add to previously identified features of I_{KN} that are shared with TASK-1, such as inhibition by hypoxia, low sensitivity to 4-aminopyridine and quinine and insensitivity to tetraethylammonium ions. It is therefore concluded that TASK-1 channels are major contributors to the resting potential in pulmonary artery smooth muscle. They are likely to play an important role in mediating pulmonary vascular responses to changes in extracellular pH, and they could be responsible for the modulatory effects of pH on hypoxic pulmonary vasoconstriction. (Circ Res. 2003; 93:●●●●●.)

Key Words: two-pore domain K channel ▪ pulmonary artery myocyte ▪ smooth muscle ▪ resting potential

The membrane potential of pulmonary artery smooth muscle cells (PASMCs) is an important factor in the control of pulmonary vascular tone. This is reflected in the vasoconstrictor effect of agents causing membrane depolarization and the vasodilator action of drugs causing hyperpolarization. There is general agreement that the resting potential of PASMCs is determined largely by a noninactivating K+ conductance (I_{KN}) that is sensitive to inhibition by 4-aminopyridine, albeit at relatively high (>1 mmol/L) concentrations. Thus, both the resting potential and I_{KN} are abolished when pipette K+ is replaced with equimolar Cs+, whereas equimolar substitution of Cs+ for extracellular K+ has little effect. In addition, both the resting potential and I_{KN} display low sensitivity to the drugs quinine and Ba^{2+} and are insensitive to tetraethylammonium ions, glibenclamide, and a range of Ca^{2+}-activated K+-channel blockers. This pharmacological profile is reminiscent of the two-pore domain K+ channel, TASK-1, which was recently shown to act as a background K+ conductance regulating resting potential in neuronal, cardiac, and adrenal glomerulosa cells. Because an important characteristic of the resting conductance in PASMCs is that it is inhibited by hypoxia, leading to membrane depolarization and Ca^{2+} influx, we were especially interested in the finding that hypoxia inhibits recombinant TASK-1 channels. Moreover, channels with TASK-like properties have been found to underlie oxygen-sensing K+ currents in chemosensitive cells of the carotid body, airways, and central nervous system. This raises the possibility that TASK-1 channels might be the molecular correlate of I_{KN} and be responsible for setting the resting membrane potential in PASMCs. Indeed, mammalian lung has been shown to express mRNA for TASK-1, although it is unclear in which of the many cell types present in lung it is located.

A defining characteristic of TASK-1 channels is their high sensitivity to changes in extracellular pH. Although specific and selective inhibitors of TASK-1 channels have yet to be identified, the channels do display a distinctive pharmacological profile. Thus, in addition to the drug sensitivities described above, TASK-1 channels are characteristically activated by volatile anesthetics like halothane and inhibited by low concentrations of anandamide and Zn^{2+}. These properties were exploited in the present study to investigate the expression of TASK-1 channels in rabbit PASMCs and their...
contribution to the background K⁺ current, \( I_{\text{KN}} \), and the resting membrane potential. The results provide the first demonstration of an important functional role for a two-pore domain K⁺ channel in vascular smooth muscle.

**Materials and Methods**

New Zealand White rabbits (2 to 3 kg) were euthanized by sodium pentobarbital overdose (80 mg/kg IV), according to the UK Animals (Scientific Procedures) Act, 1986. The main pulmonary artery and its right and left branches were dissected free and placed in physiological salt solution (PSS) of the following composition (in mmol/L): NaCl 124, KCl 5, CaCl₂ 1.8, MgCl₂ 1, NaH₂PO₄ 0.5, KH₂PO₄ 0.5, glucose 10, 5-N-2-hydroxyethylpiperazine-N-2-ethanesulphonic acid (HEPES) 15; adjusted to pH 7.3 with NaOH. Smooth muscle cells were dissociated by overnight incubation with papain as previously described, or by incubating 1-mm strips of artery for 30 minutes at 37°C with 12 mg collagenase (Sigma type IA), 1 mg protease (Sigma type XXIV), 10 mg trypsin inhibitor (Sigma), and 10 mg bovine serum albumin (BSA) in 5 mL dissociation medium (DM) of the following composition (in mmol/L): NaCl 110, KCl 5, NaHCO₃ 15, CaCl₂ 0.16, MgCl₂ 2, NaH₂PO₄ 0.5, KH₂PO₄ 0.5, glucose 10, HEPES 15, phenol red 0.04, ethylenediaminetetraacetic acid (EDTA) 0.49, taurine 10; adjusted to pH 7.0 with NaOH. Resting potential and \( I_{\text{KN}} \) amplitude were not significantly different in cells obtained with either method.

Reverse transcription (RT) PCR was performed on total RNA extracted from suspensions of rabbit PASMCs as previously described, or single PASMCs harvested under microscope control and aspirated directly into the RT-PCR mix. Four sets of primer pairs (PP) were designed against conserved sequences in mouse (GenBank accession No. AB013345), rat (AB048823), and human (NM_002246) TASK-1 channels. They were as follows: PP1, 5'-TCCATGTGCTCGTGCCTCTG-3', 5'-AGCTCCTGCGCTT-CATGAGG-3'; PP2, 5'-GGCAAGGTGTTCTGCATGTTCTACG-3', 5'-AAGCCGATGGTGGTGAGGGTG-3'; PP3, 5'-CCTTCTACTTCGCCATCACCGTCATC-3', 5'-CATGGTCATGGACACCTCG-3'; PP4, 5'-TCATCGTGTGCACCTTCACCTACCTG-3', 5'-CATGGTCATGGACACCTCG-3'. PP2, PP3, and PP4 target overlapping regions of the TASK-1 template (see Figure 1C). Cycle parameters were typically 95°C for 5 minutes, followed by 20 to 35 cycles at 95°C for 1 minute, 52°C to 60°C for 30 seconds, and 68°C for 2 to 4 minutes. Reverse transcriptase was omitted from control cDNA reactions. Products were resolved by agarose gel electrophoresis, purified, and verified by sequencing.

Immunofluorescence was performed as previously described, using two different antibodies directed against unique domains in TASK-1, one at the amino terminus (Santa Cruz Biotechnology) and the other at residues 252 to 269 in the carboxy terminal end of the protein (Alomone Labs). Cells adhered to glass coverslips were fixed in 4% paraformaldehyde in phosphate buffered saline (PBS) for 10 minutes then permeabilized with 0.1% Triton X-100 in PBS for 5 minutes. After blocking with 1% BSA for 1 hour, cells were
incubated with anti-TASK1 antibody (1:100 dilution) for 1 hour then probed for 1 hour with a secondary antibody followed to fluorescent Alexa 488, or a biotinylated secondary antibody followed by streptavidin-Alexa 488. Duplicates were processed without primary antibody for controls. Fluorescence was imaged at wavelengths >500 nm with a BioRad Radiance 2000MP confocal microscope, using the 488-nm line of an argon laser and a Nikon ×60 (N.A. 1.0) objective.

For electrophysiological experiments, cells were superfused at ~0.5 mL/min at room temperature with PSS supplemented with 10 mmol/L tetraethylammonium chloride (TEA) and 10 μmol/L glibenclamide to block BK Ca and KATP channels, respectively. The whole-cell patch-clamp technique was used as previously described (Gurney et al. Resting K Channels in Pulmonary Artery (3)).

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The pH of the PSS was set with 1 mol/L NaOH. The order of the change in pH was randomized for each experiment and had no effect on the results. Halothane was dissolved in dimethylsulphoxide (DMSO) to 1 mmol/L, diluted in PSS, and used immediately. Other drug solutions were prepared from frozen aliquots. Glibenclamide (10 mmol/L) was dissolved in DMSO and anandamide (10 mmol/L) in 50% ethanol in water. Effects of these drugs were determined by comparison with vehicle controls. Other drugs were dissolved in deionized water. Data are expressed as mean ± SEM of n cells. Statistical comparisons used one-way analysis of variance (ANOVA), with probability values corrected by Tukey’s pairwise comparison, or Student’s paired or unpaired t test as appropriate. A value of P<0.05 was considered significant.

**Results**

**Expression of TASK-1 Channels in PASMCs**

We first established the presence of TASK-1 mRNA in rabbit PASMCs. RT-PCR experiments with four specific primer pairs for TASK-1 indicated that mRNA for the channel is present in suspensions of cells isolated from pulmonary arteries (Figure 1A). These results were reproduced in separate RT-PCR reactions on RNA from at least three rabbits. Although the suspensions contained predominantly smooth muscle cells, they were almost certainly contaminated with endothelial and other cells. To verify the smooth muscle origin of the amplified mRNA, further RT-PCR experiments were performed on single, identified PASMCs. Figure 1B shows reproducible amplification of a PCR product of the predicted size from a single PASMC, using primer pair P3. Immunocytochemistry provided further evidence for TASK-1 expression in PASMCs. The presence of TASK-1 protein was established with two anti–TASK-1 antibodies directed against either the amino or carboxy terminal regions of the protein. Both antibodies resulted in fluorescent labeling in PASMCs from at least six separate preparations (Figures 2A and 2B). In contrast, staining was absent from control cells treated in the same way, but without exposure to TASK-1 antibody (Figures 2C and 2D). Confocal microscopy of cells stained with both antibodies showed the presence of TASK-1 staining at the surface membrane and in the cytoplasm.

**K+ Current Modulation by pH**

A defining hallmark of the TASK family of channels is their sensitivity to extracellular pH (pH). As shown in Figure 3A, varying the pH of the extracellular solution caused reversible
changes in the resting membrane potential of isolated rabbit pulmonary artery myocytes. The relationship between resting potential and pH0 is illustrated in Figure 3B, where the resting potentials measured at pH 6.3, 7.3, and 8.3 were significantly different (P<0.001, ANOVA). Increasing pH0 from 7.3 to 8.3 caused membrane hyperpolarization of 8 ± 2 mV (n=8), whereas acidification to pH 6.3 depolarized cells by 20 ± 5 mV (n=5). These changes in membrane potential were reflected in the effects of pH0 on the amplitude of the noninactivating K+ current, I\text{Kn}, recorded at 0 mV (Figure 3C). In 10 cells, the mean amplitude of the current measured at pH 6.3 was 48 ± 7% of that measured at pH 7.3, whereas the current at pH 8.3 was 63 ± 14% of that at pH 7.3. The modulation of I\text{Kn} and resting potential by pH0 was usually apparent within seconds of changing the perfusion solution, consistent with a direct effect of pH0 on the channels rather than mediation by slow changes in intracellular pH. Figure 3D illustrates the effect of pH0 across the full voltage range over which I\text{Kn} is resolved. From the holding potential of 0 mV, the voltage was stepped to 60 mV and then ramped to −10 mV over a period of 1 second. The current during the ramp reflects I\text{Kn} in parallel with a nonspecific “leak” current and reverses direction at the resting potential of the cell. The effect of pH0 was restricted to membrane potentials of −60 mV or above, where I\text{Kn} is active. It had little effect at more negative potentials where leak current predominates. As a consequence of modulating outward current, pH0 also influenced the reversal potential of the current recorded during voltage ramps. Consistent with the depolarizing effect of acidification, a positive shift of 5 ± 2 mV (n=3) resulted when pH0 was reduced from 7.3 to 6.8, whereas increasing pH0 to 7.8 caused a shift of −9 ± 3 mV (n=3). Figure 3E shows the pH0 dependence of I\text{Kn} measured at 0 mV. Fitting a Boltzmann relationship to the data indicates that the pH0 giving 50% inhibition (pH0.5) was pH 7.3.

Varying the extracellular pH also modulated I\text{KV}, the K+ current activated during short (250 ms) depolarizing steps applied from a holding potential of −80 mV. This is illustrated in Figure 4A where the main effect appears to be on current inactivation, which was accelerated by acidification and slowed by alkalinization. Thus, at pH 8.3, the peak amplitude of I\text{KV} at 40 mV, seen as an early transient component that has been likened to A-current, 26 was unchanged compared with that at pH 7.3 (106 ± 12%, n=3). In contrast, the sustained component of current measured at 200 ms in the same cells, attributed to delayed rectifier channels, 26 was significantly enhanced at pH 8.3 compared with pH 7.3 (147 ± 12%, n=3, P<0.05). Reducing the pH from 7.3 to 6.3 suppressed both components of current, although inhibition of the sustained component (56 ± 13%, n=3, P<0.01) was usually more pronounced than the early peak current (42 ± 12%, n=3, P<0.05). As shown in Figure 4B, the effect of pH0 on the sustained I\text{KV} appeared within a few seconds of changing the solution and was fully reversible. Modulation was apparent across the voltage range over which I\text{KV} was activated, with similar percentage changes measured at all potentials (Figure 4C). It is clear from Figure 4C that acidification reduced the current at −40 mV, implying that inhibition of I\text{KV} could have contributed to its depolarizing effect.

Modulation by Halothane, Anandamide, and Zn2+

Another marker of TASK channels is their sensitivity to volatile anesthetics, such as halothane, which cause facilitation of channel activity. As illustrated in Figure 5A, 1 mMol/L halothane caused facilitation of the nonactivating current at 0 mV in 12 out of 14 cells. The mean facilitation observed was 19 ± 4% (n=14), halothane significantly increasing I\text{Kn} at 0 mV from 15 ± 3 to 18 ± 4 pA (n=14, P<0.01). This effect was fully reversible, although it sometimes took a few minutes for the current to return to the control level after washout. Consistent with the involvement of TASK-1 channels in this facilitation, 28 it was blocked at acid pH0 (Figure 5B). Thus, when cells were bathed in PSS at

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Figure 3. Modulation of membrane potential and I\text{Kn} by pH. A. Increasing the extracellular pH (pH0) from 7.3 to 8.3 hyperpolarized the cell membrane while reducing the pH to 6.3 caused depolarization. B. Histogram showing mean resting membrane potential ±SEM at different pH0. P=0.00001 by ANOVA. C. Membrane current recorded at 0 mV when the PSS was buffered at pH 7.3, following acidification to pH 6.3 and after increasing pH0 to 8.3. D. Records of I\text{Kn} at different pH0 during 1-second voltage ramps from 60 to −100 mV. E. Relationship between pH0 and mean membrane current recorded at 0 mV (n=5). These changes in membrane potential were reflected in the effects of pH0 on the amplitude of the noninactivating K+ current, I\text{Kn}, recorded at 0 mV (Figure 3C). In 10 cells, the mean amplitude of the current measured at pH 6.3 was 48 ± 7% of that measured at pH 7.3, whereas the current at pH 8.3 was 63 ± 14% of that at pH 7.3. The modulation of I\text{Kn} and resting potential by pH0 was usually apparent within seconds of changing the perfusion solution, consistent with a direct effect of pH0 on the channels rather than mediation by slow changes in intracellular pH. Figure 3D illustrates the effect of pH0 across the full voltage range over which I\text{Kn} is resolved. From the holding potential of 0 mV, the voltage was stepped to 60 mV and then ramped to −10 mV over a period of 1 second. The current during the ramp reflects I\text{Kn} in parallel with a nonspecific “leak” current and reverses direction at the resting potential of the cell. The effect of pH0 was restricted to membrane potentials of −60 mV or above, where I\text{Kn} is active. It had little effect at more negative potentials where leak current predominates. As a consequence of modulating outward current, pH0 also influenced the reversal potential of the current recorded during voltage ramps. Consistent with the depolarizing effect of acidification, a positive shift of 5 ± 2 mV (n=3) resulted when pH0 was reduced from 7.3 to 6.8, whereas increasing pH0 to 7.8 caused a shift of −9 ± 3 mV (n=3). Figure 3E shows the pH0 dependence of I\text{Kn} measured at 0 mV. Fitting a Boltzmann relationship to the data indicates that the pH0 giving 50% inhibition (pH0.5) was pH 7.3.

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pH 6.3, the mean current was 4±3 pA before and 3±3 pA (n=9) after applying halothane (1 mmol/L). The stimulatory effect of halothane was restricted to potentials positive of 60 mV, with no effect on the leak current at more negative potentials (Figure 5C). In five of the cells studied at pH 7.3, the facilitation caused by halothane was transient. Thus in the continuous presence of halothane, the response in these cells appeared biphasic, and in some the current eventually declined to below the control level (not shown). This may reflect an additional inhibitory effect of halothane, because a small inhibition was observed in the two cells that failed to show facilitation in response to halothane. The mean reduction in current amplitude amounted to 16±6% of the maximum (facilitated) current (n=7). The inhibitory effect became more pronounced at higher halothane concentrations. Thus at 2 or 5 mmol/L halothane, facilitation was observed in only three of eight cells and inhibition, amounting to a decrease in the current amplitude of 37±9% (n=8), was predominant. The effects of halothane on the resting membrane potential mirrored its effects on $I_{K_N}$; up to 5 mmol/L halothane had no consistent effect on the amplitude of the transient or sustained $I_{K_N}$, measured over a range of test potentials (Figure 5E).

Although the effects of pH and the facilitatory action of halothane on membrane potential and $I_{K_N}$ are indicative of a possible role for TASK channels, these agents discriminate poorly among different members of the TASK family and even other K channels. One agent that is able to inhibit TASK-1 at concentrations well below those affecting other TASK channels is Zn$^{2+}$25,29 As illustrated in Figure 6A, $I_{K_N}$ recorded at 0 mV was reversibly inhibited by Zn$^{2+}$ at relatively low concentrations in 11 out of 13 cells tested. At 100 µmol/L, Zn$^{2+}$ reduced the current by 32±11% (n=6; $P<0.05$) and at 200 µmol/L inhibition increased to 53±10% (n=6; $P<0.05$). This effect of Zn$^{2+}$ was restricted to membrane potentials above 60 mV, where $I_{K_N}$ is active (Figure 6B). As shown in Figure 6C, the inhibition of $I_{K_N}$ was associated with 10 mV depolarization of the resting potential from 37±4 mV to 27±6 mV (n=3). Unfortunately, the effect of Zn$^{2+}$ was not selective, because as illustrated in Figure 6D (inset), it also inhibited the transient component of $I_{K_N}$, effectively slowing the rate of activation of $K_N$ current. Except at steps to −20 mV, Zn$^{2+}$ (200 µmol/L) did not, however, alter the sustained component of $I_{K_N}$ (Figure 6C).

The endocannabinoid anandamide was recently shown to be a direct and selective blocker of TASK-1 channels, low
micromolar concentrations inhibiting these channels but not other TASK or two-pore domain channels. Figure 6E shows the effect of 10 μmol/L anandamide on the noninactivating current recorded at 0 mV. At this concentration, anandamide reduced $I_{KN}$ on average by 25 ± 3% (n = 8) from 13 ± 4 to 10 ± 3 pA ($P < 0.05$), although in two cells it had no measurable effect.

$I_{KN}$ Lacks Sensitivity to Cytoplasmic Ca$^{2+}$

Studies on recombinant TASK-1 channels have shown that intracellular Ca$^{2+}$ has no effect on their activity, yet there is evidence that Ca$^{2+}$ inhibits the resting $K^+$ conductance in dog PASMCs. To test whether or not $I_{KN}$ in rabbit is a Ca$^{2+}$ sensitive conductance, we first studied the effect on the current of increasing Ca$^{2+}$ influx with the Ca$^{2+}$ ionophore, A23187. This often induced inward current at negative potentials and increased outward current at positive potentials, probably due to the activation of $Cl^-$ channels by the rise in submembrane [Ca$^{2+}$]. To avoid this and its potential interference with $I_{KN}$, the experiments were repeated using low $Cl^-$ (10 mmol/L) PSS, and pipette solutions and measurements were confined to 0 mV, the $Cl^-$ equilibrium potential. At a concentration evoking contraction of rabbit PASMCs, A23187 (1 μmol/L) had no clear effect on the amplitude of $I_{KN}$ measured at 0 mV in these conditions (Figure 7A, n = 3). In three other cells studied in normal recording solutions, the removal of extracellular Ca$^{2+}$ also failed to change the amplitude of $I_{KN}$ at 0 mV (Figure 7B). This was despite the presence of 1 mmol/L EGTA in the bath solution to ensure that extracellular Ca$^{2+}$ was buffered to a minimal level. An alternative method used to modulate the extracellular Ca$^{2+}$ concentration was to vary the nature and concentration of the Ca$^{2+}$ chelator used in the internal, pipette solution. The amplitude of $I_{KN}$ measured at 0 mV was not found to differ significantly when the pipette contained 0.05

Figure 5. Effects of halothane on resting potential and $K^+$ currents. A, Facilitation of the noninactivating current recorded at 0 mV when halothane (1 mmol/L) was applied at pH 7.3. B, When applied at pH 6.3, halothane (1 mmol/L) did not facilitate the current. C, $I_{KN}$ during voltage ramps (protocol inset) before and during facilitation induced by halothane (5 mmol/L). D, Effect of halothane (1 mmol/L) on resting potential (same cell as A). E, Current versus voltage relationship for sustained $I_{KV}$ before and after applying halothane (5 mmol/L; n = 3); voltage protocol inset.
to 0.1, 1 or 5 mmol/L EGTA, or 10 mmol/L BAPTA (Figure 7C).

**Discussion**

We have demonstrated that the noninactivating K⁺ current, $I_{KN}$, previously shown to underlie the resting potential of rabbit PASMCs,⁷ has all the properties expected for a background K⁺ channel belonging to the TASK family of two-pore domain channels. Pharmacological characterization previously demonstrated that $I_{KN}$ is insensitive to block by TEA, glibenclamide, Cs⁺, and a range of K Ca channel blockers and is inhibited only at high concentrations of 4-aminopyridine and quinine.⁷,⁸ The present study shows that $I_{KN}$ is additionally sensitive to pHₐ₅₀, halothane, Zn²⁺, and anandamide, but not to intracellular Ca²⁺. Thus, the pharmacological profile of $I_{KN}$ and the resting potential of PASMCs closely matches that of heterologously expressed TASK-1 channels.²¹,²² The pHₐ₅₀ at which 50% of $I_{KN}$ was inhibited, pH 7.3, is the same as that found for TASK-1 channels.²² Furthermore, the high sensitivity of $I_{KN}$ to inhibition by Zn²⁺ and anandamide strongly implicates TASK-1 as a major contributor to the current.²⁴,²⁵ The finding that pHₐ₅₀ and Zn²⁺ also modulated $I_{KV}$ is consistent with a role for $I_{KV}$ in their effects on membrane potential. It is unlikely to be a major role though, because our previous studies showed that $I_{KV}$ (both the transient and sustained components) contributes little to the normal maintenance of resting potential.⁷ The lack of effect of halothane on $I_{KV}$, although producing membrane hyperpolarization and facilitation of $I_{KN}$, provides further evidence to link resting potential regulation to $I_{KN}$ and TASK-1 channels, rather than $I_{KV}$. Previous studies showed that mRNA for TASK channels is expressed in the lung.²¹ We now extend these observations to show that TASK-1 mRNA and protein are expressed in PASMCs. Moreover, antibody staining showed TASK-1 expression at the surface membrane, compatible with a functional role in mediating membrane K⁺ currents.

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**Figure 6.** Zn²⁺ and anandamide inhibit $I_{KN}$ and membrane potential. A, Effect of 100 μmol/L Zn²⁺ on the noninactivating current recorded at 0 mV. B, Records of $I_{KN}$ in control conditions and in the presence of 200 μmol/L Zn²⁺. Current was markedly depressed at potentials above −60 mV, but not at more negative potentials. C, Effect of 200 μmol/L Zn²⁺ on the resting potential. D, Relationship between test potential and sustained $I_{KV}$, activated by 250-ms depolarizing steps from a holding potential of −80 mV (protocol inset right), before and after applying 200 μmol/L Zn²⁺. Zn²⁺ had little effect on the sustained component, but suppressed the transient component of $I_{KV}$ (inset top). *P<0.05. E, Inhibition of $I_{KN}$ at 0 mV by 10 μmol/L anandamide.
The sensitivity of $I_{KN}$ to small changes in pH means that extracellular acidification depolarizes PASMCs, thereby facilitating voltage-gated Ca$^{2+}$ entry and contraction. Thus, modulation of $I_{KN}$ and membrane potential may contribute to acidosis-induced pulmonary vasoconstriction and the well-known pulmonary vasodilator effect of alkalosis, which is inhibited by 10 mmol/L 4-aminopyridine and by manipulations that suppress the transmembrane K$^{+}$/H$^{+}$ gradient and cause depolarization. Because $I_{KN}$ inhibition and membrane depolarization may contribute to the vasoconstrictor response of pulmonary arteries to hypoxia, $I_{KN}$-dependent modulation of $I_{KN}$ may also explain the stimulatory effect of acidosis and inhibitory effect of alkalosis on hypoxic pulmonary vasoconstriction. The exquisite sensitivity of $I_{KN}$ to changes in pH suggests that pH-dependent modulation of $I_{KN}$ plays a functional role in physiological conditions, but it may become particularly important in pathological conditions where substantial changes in pH can occur.

The finding that acidosis inhibited the facilitation caused by halothane supports the hypothesis that halothane was acting on pH-sensitive TASK-1 channels. It is important to note, however, that the effects of halothane, Zn$^{2+}$, and anandamide on $I_{KN}$ and membrane potential were not always as expected for modulation of TASK-1 channels. The failure of a small proportion of cells to respond to these agents suggests that the expression of TASK-1 channels may vary among PASMCs. Variable expression of K$^{+}$ channels has been reported before and is thought to reflect phenotypic diversity among PASMCs. The inhibitory effect of halothane seen in some PASMCs seemed to obscure the facilitation at high concentrations and probably limited it at lower concentrations. This effect could be due to a separate action on additional channels that contribute to $I_{KN}$. Halothane was recently found to inhibit an O$_2$-sensitive background K$^{+}$ current in glossopharyngeal neurons, which has a pharmacological profile compatible with THIK, another family of two-pore domain K$^{+}$ channels. Like $I_{KN}$, this current was insensitive to TEA and inhibited by millimolar quinidine, but it was only weakly modulated by pH, and not inhibited by 5 mmol/L 4-aminopyridine. It is therefore interesting that although acidosis inhibited $I_{KN}$, it did not completely abolish it, and previous studies have shown that maximally effective concentrations of 4-aminopyridine only partially inhibit $I_{KN}$. Thus several observations suggest that $I_{KN}$ may reflect the activity of more than a single population of K$^{+}$ channels with differential sensitivity to pH and pharmacological agents, and that the relative expression of these channels may vary among PASMCs. This is consistent with other cell types, where multiple K$^{+}$ channels contribute toward the background K$^{+}$ conductance that controls the resting potential.

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References


30. Post JM, Gelband CH, Hume JR. 


