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DIFFERENTIAL K^+ CHANNEL DISTRIBUTION IN SMOOTH MUSCLE CELLS ISOLATED FROM THE PULMONARY ARTERIAL TREE OF THE RAT

Sulayma Albarwani*, Gideon Heinert*, Joanne L. Turner and Roland Z. Kozlowski#

University Department of Pharmacology, Mansfield Road, Oxford, OX1 3QT and

*University Laboratory of Physiology, Parks Road, Oxford, OX1 3PT, England, U.K.

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Intracellular photorelease of Ca^{2+} demonstrated the presence of Ca^{2+}-activated K^+ channels in smooth muscle cells isolated from different locations of the rat pulmonary arterial tree. However, cell-free patch studies revealed marked differences in K^+ channel distribution. In the main pulmonary artery the most frequently observed K^+ channel was a ~245pS conductance Ca^{2+}- and ATP-activated ($K_{Ca,ATP}$) channel. In small pulmonary arteries two K^+ channel types predominated: the $K_{Ca,ATP}$ channel and a ~185pS conductance K^+ channel insensitive to intracellular Ca^{2+}, ATP and voltage. This difference in K^+ channel distribution may highlight a more complex regulatory mechanism for controlling membrane potential in small pulmonary arteries, reflecting their physiologically more important role in governing pulmonary vascular reactivity.

In recent years the cellular mechanisms underlying hypoxic vasoconstriction (HPV) have been the subject of considerable interest. The finding that single smooth muscle cells (1) and endothelium denuded pulmonary arteries (2,3) constrict in response to hypoxia suggests that the smooth muscle itself may sense changes in oxygen tension. Evidence is accumulating to suggest that K^+ channels may be the focus for changes in oxygen tension. Indeed, electrophysiological experiments have demonstrated depolarisation of pulmonary arterial smooth muscle cells by hypoxia (4,5). Furthermore, hypoxia has been reported to reversibly inhibit K^+ currents in these cells although there is some dispute as to whether the K^+ channel (or channels) affected are Ca^{2+}-activated (5,6) or Ca^{2+}-insensitive (7). The majority of electrophysiological studies at a single cell level have been carried out on pulmonary arterial smooth muscle cells isolated from the main pulmonary artery (from a variety of species). Few experiments have been performed on small pulmonary arteries found within the lungs actually responsible for controlling pulmonary vascular reactivity and HPV. We have therefore examined the relative distribution of K^+ channels in the pulmonary arterial tree of the rat.

To whom all correspondence should be addressed. FAX: 44 865 721853.

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Materials and Methods

Male albino Wistar rats (200-250g) were killed by an overdose of intraperitoneal Euthatol (pentobarbitone sodium B.P.; Rhone Merieux, Ireland) and cervically dislocated. Following an injection of heparin (500 units) into the left and right ventricles of the heart, the main pulmonary artery and small pulmonary arteries 100-400μm in diameter were removed. Throughout the procedure the lungs were bathed in saline solution containing (in mM): 119 NaCl, 4.7 KCl, 1.18 KH_2PO_4, 1.17 $MgSO_4$, 5.5 glucose, 25 $NaHCO_3$, 10 HEPES (N-2-hydroxyethylpiperazine-N-2-ethanesulfonic acid) adjusted to pH 7.4 with NaOH. Arteries were placed in a papain (papaya latex; 1.5 mg/ml) and dithiothreitol (DTT; 1 mg/ml) containing dissociation medium (DM) which consisted of (in mM): 128 NaCl, 5.4 KCl, 4.16 $NaHCO_3$, 0.35 Na_2HPO_4, 0.95 KH_2PO_4, 10 glucose, 2.9 sucrose, 10 HEPES, pH 7.3 with NaOH. Following incubation for 1 hour at 4°C, the tissue was agitated at 37°C for 6 minutes before being transferred to DM containing collagenase (type VIII; Sigma; 1.5mg/ml) for a further 6 minutes at 37°C. The tissue was then washed in saline and single pulmonary arterial smooth muscle cells were isolated by trituration of the treated tissue using a Pasteur pipette.

Single channel and whole-cell recordings were performed at a temperature of ~22°C using the patch-clamp recording technique (8). Single channel currents were detected using a Axopatch-1C patch-clamp amplifier (Axon Instruments, Foster City, CA, USA) and recorded onto digital audio tape (DTC 1000ES, Sony, Japan) at a frequency of 10 kHz for subsequent off-line analysis. Prior to analysis single channel currents were filtered at 1 kHz and subsequently sampled at 5 kHz using a CED 1401 analogue-to-digital converter (Cambridge Electronic Design, Cambridge, U.K). Analysis was performed using a 386 microcomputer and software developed by CED (Patch and Voltage Clamp System v.6, Cambridge, U.K.).

The extracellular membrane surface of cell-free patches and whole-cells was bathed in a solution which consisted of (mM): NaCl 135, KCl 5, $CaCl_2$ 1, $MgCl_2$ 1, HEPES 10; pH 7.4 with NaOH, although for whole-cell recordings $CaCl_2$ was omitted. For ion substitution experiments the NaCl in this solution was substituted with 140mM KCl thus keeping the monovalent ion concentration constant. The intracellular membrane surface of cell-free patches was bathed in solution which consisted of (mM): 140 KCl, 1 $MgCl_2$, 10 HEPES; pH 7.3 with KOH. To buffer the free Ca^{2+} in this solution different proportions of $CaCl_2$ and EGTA were used (in mM): 10μM Ca^{2+}, 5 $CaCl_2$ and 4 EGTA; 1μM Ca^{2+}, 4.5 $CaCl_2$ and 5 EGTA; 0.1μM Ca^{2+}, 2.3 $CaCl_2$ and 5 EGTA; 10nM Ca^{2+} 0.4 $CaCl_2$ and 5 EGTA. On addition of ATP (500μM) to this solution the concentration of $MgCl_2$ was raised to 1.5mM to compensate for chelation of Mg^{2+} ions. To examine the effects of elevating the intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$) on outward whole-cell currents, flash photolysis experiments using nitr-5 were conducted as described previously (9). For these experiments cells were dialysed with 140 KCl, 1 $MgCl_2$, 2 nitr-5 (tetrasodium salt), 1 $CaCl_2$, 10 HEPES; pH 7.3 with KOH. All experiments were performed at room temperature 21-24°C. ATP (adenosine-5'-triphosphate) dipotassium salt, ethylene glycol-bis[ß-aminoethyl ether] N,N,N',N',-tetraacetic acid (EGTA), HEPES, collagenase, papain, DTT were all purchased from Sigma (Poole, Dorset, UK). Nitr-5 was purchased from Calbiochem-Novabiochem (Nottingham, UK).

Data are presented as changes in open state probability (P_o) of single channels or as a change in the number of functional channels (N_f) in the patch by multiplied by their P_o.

Results

Intracellular photorelease of Ca^{2+}, by photolyis of its caged derivative nitr-5, caused a marked increase in outward current in smooth muscle cells isolated from the pulmonary

arterial tree of the rat (Figure 1a), indicating the existence of Ca^{2+}-activated K^+ channels in these cells. The capacitance of cells isolated from the small pulmonary arteries was 9.74±0.86pF (mean ± standard error mean, throughout; n=5) while the maximum current density of the outward Ca^{2+}-activated K^+ current at +30mV was 157±64pA/pF (n=5). Similar results were obtained using cells isolated from the main pulmonary artery which had a cell capacitance of 11.80±0.79pF (n=9) and a Ca^{2+}-activated outward current density of 141±66pA/pF (n=9) at +30mV.

Consistent with these results cell-free patches isolated from both the small and the main pulmonary arteries exhibited Ca^{2+}-activated channels. Such channels, with a conductance of ~245pS, also activated by ATP ($K_{Ca,ATP}$ channels), were observed in

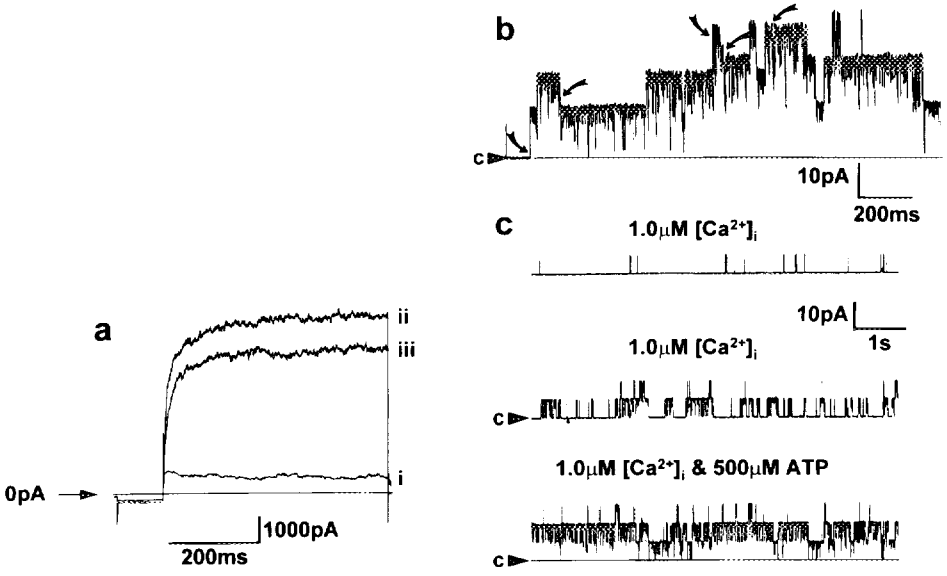

Figure 1.

(a) Raising intracellular Ca^{2+} by flash photolysis of caged Ca^{2+} enhances the outward current in smooth muscle cells isolated from the pulmonary arterial tree of the rat, on this occasion from a small pulmonary artery. Outward currents were activated from a holding potential of -50 mV by stepping to -100 mV for 100ms and then to +30 mV for a further 500ms at a frequency of 0.2Hz. The currents shown were recorded immediately before (i), 5s (ii) and 10s (iii) after the flash (F). For composition of solutions see Materials and Methods.

(b) Single channel currents recorded from an inside-out membrane patch excised from a small pulmonary arterial smooth muscle cell. The patch was held at a membrane potential of +30mV in the presence of 0.1μM Ca^{2+} bathing the intracellular membrane surface. It is clear that this patch probably contains at least three types of channel indicated by arrows (see text for details).

(c) Single $K_{Ca,ATP}$ channel currents recorded from an inside-out patch (excised from a small pulmonary arterial smooth muscle cell) held at a membrane potential of +40mV in symmetrical 140mM KCl. The intracellular aspect of the patch was initially superfused with a solution containing 0.1μM Ca^{2+}; upon increasing this to 1.0μM there was a marked increase in channel activity ($N_f.P_o$ increased from 0.02 to 0.5). Addition of 500μM ATP in the presence of 1mM Mg^{2+} caused a further increase in channel activity ($N_f.P_o$ = 1.6).

virtually all cell-free (inside- and outside-out; n=33) patches from the main pulmonary artery. Openings of other types of channel were rarely seen. Note, we have been previously characterised the biophysical properties of $K_{Ca,ATP}$ channels and their sensitivity to ATP and other nucleotides (10,11). In marked contrast to the main pulmonary artery a variety of channels with different conductances, or apparent conductance states, were frequently observed in inside-out membrane patches isolated from small pulmonary arterial smooth muscle cells (Figure 1b). $K_{Ca,ATP}$ channels were present in only ~50% of inside-out patches isolated from these vessels. However, consistent with findings in the main pulmonary artery (11) raising the $[Ca^{2+}]_i$ from 0.1μM to 1.0μM increased $N_f.P_o$ of $K_{Ca,ATP}$ channels from 0.02±0.01 to 0.49±0.8 (n=3), while application of 500μM ATP (405μM Mg-ATP) further increased $N_f.P_o$ to 300±20% (n=3). Typical examples of the activating actions of $[Ca^{2+}]_i$ and ATP are shown in Figure 1c.

In contrast to the main pulmonary artery there was a second equally abundant class of K^+ channel in isolated patches from small vessels under our conditions. These channels were insensitive to voltage across a wide range of test potentials (-70mV to +70mV; Figure 2a) with a mean P_o across this potential range of 0.90±0.07 (n=6). Due to their insensitivity to voltage, and other intracellular charged intermediates (see below) they are referred to as K_{ins} ($K_{insensitive}$) channels. The K^+ selectivity of K_{ins} channels was studied by changing the solution bathing the extracellular surface of outside-out membrane patches from one containing 140mM KCl to one containing 135mM NaCl and 5mM KCl. The solution bathing the intracellular surface contained ~1nM Ca^{2+} to prevent activation of $K_{Ca,ATP}$ channels.

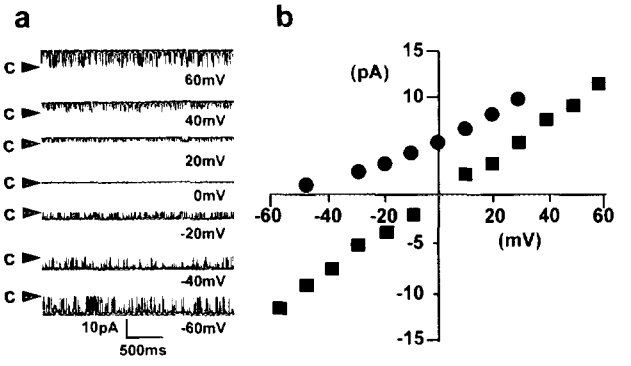

Figure 2.

(a) Single voltage-insensitive K^+ channel currents (K_{ins}) recorded from an inside-out patch excised from a small pulmonary arterial smooth muscle cell. The patch was bathed in symmetrical 140mM KCl. The solution bathing the intracellular membrane surface contained 1nM Ca^{2+}. Note that the channels spent most of their time in an open state regardless of the holding potential. The P_o values are as follows: +60, 0.93; +40, 0.91; +20, 0.90; -20, 0.92; -40, 0.91; -60, 0.89. The closed state in this and all subsequent single channel records is indicated by the letter C.

(b) Current-voltage relationship for K_{ins} channels. Squares represent single channel currents recorded in symmetrical 140mM KCl under the conditions described in (a) while circles represent currents recorded in the presence of 135mM NaCl and 5mM KCl extracellularly.

In symmetrical 140mM KCl reversal occurred at 0mV and outward currents were observed at positive membrane potentials and inward currents at negative membrane potentials. Upon replacing the extracellular solution with Na^+ rich solution outward currents were observed at 0mV and reversal occurred between -70 to -80mV, suggesting a high K^+ selectivity (Figure 2b). Upon constructing current-voltage relationships for this channel, it was found to have a conductance of 185±10pS (n=5) in symmetrical 140mM KCl when measured between 0 and +60mV and 110±15pS in a quasiphysiological cation gradient (determined by measuring the slope between 0 and +30mV. Extrapolation of the I-V curve indicated that reversal of the current occurred at -73mV, a value close to the theoretical reversed potential (~84.5mV) derived using the Nernst equation (Figure 2b). This observation suggests that K_{ins} channels may be partly permeable to Na^+ ions. The permeability ratio (α) for Na^+ ions to K^+ ions was found to be 0.018; indicating an approximate 50 fold selectivity for K^+ ions. Under conditions of symmetrical 140mM K^+ assuming a conductance of ~185pS a value of $3.46 \times 10^{-13} cms^{-1}$ was obtained for the P_K predicted using by Goldman Hodgkin Katz constant field theory (12,13).

The $[Ca^{2+}]_i$ sensitivity of K_{ins} channels was examined by applying 10nM, 0.1μM, 1 μM and 10μM $[Ca^{2+}]_i$ solutions to the intracellular surface of the inside-out membrane patch held at a membrane potential of +40mV. Channel activity was found not to be affected by varying $[Ca^{2+}]_i$ concentration over this range (Figure 3a). The mean P_o

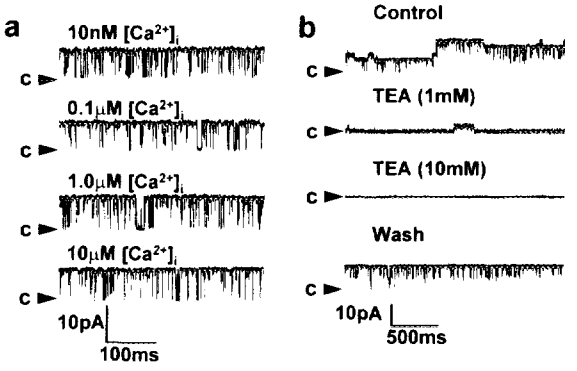

Figure 3.

(a) K_{ins} channel current recorded from an inside-out membrane patch excised from a smooth muscle cell isolated form a small pulmonary artery in symmetrical 140mM KCl at a membrane potential of +40mV. Upon increasing the concentration of Ca^{2+} bathing the intracellular membrane surface form 10nM to 10μM there was little change in channel activity. The P_o values are as follows: 10nM, 0.93; 0.1μM, 0.92; 1.0μM, 0.94; 10μM, 0.96.

(b) K_{ins} channel currents recorded from an inside-out membrane patch excised from a small pulmonary arterial tissue smooth muscle cell at a membrane potential of +40mV in symmetrical 140mM KCl. The intracellular solution contained 1nM Ca^{2+}. Intracellularly applied TEA (1mM) reversibly inhibited the activity of K_{ins} channels but did not abolish the activity of a smaller unidentified channel also present in the patch. Upon application of 10mM TEA there was a reduction in the 'noise' of the record, indicating the presence of other low conductance TEA sensitive channels.

determined from five patches was 0.93±0.03, 0.92±0.03, 0.91±0.05 and 0.93±0.04 in $[Ca^{2+}]_i$ of 10nM, 0.1μM, 1μM and 10μM, respectively; indicating that the channel was insensitive to Ca^{2+}. In contrast to $K_{Ca,ATP}$ channels, K_{ins} channels were found to be insensitive to ATP. The mean P_o in the absence and presence of ATP (500μM) was 0.92±0.06 and 0.89±0.07 (n=3), respectively. K_{ins} channels were unaffected by 1 and 10mM 4-AP when applied to the intracellular aspect of the membrane patches, although low concentrations of TEA (1mM) completely abolished their activity (Figure 3b).

Discussion

These results represent the first recordings of single K^+ channel from smooth muscle cells isolated from small pulmonary arteries of the rat. It is clear that several different types of K^+ channel are present in these cells which appear to possess a quite different K^+ channel profile to cells isolated from the main pulmonary artery. The physiological role of K_{ins} channels has yet to be determined although they may be involved in regulating the membrane potential of these cells since it can be predicated that they will be active under normoxic conditions at resting levels of Ca^{2+} and ATP, intracellularly. It has already been suggested that $K_{Ca,ATP}$ channels represent a potential link between cellular metabolism, K^+ channel homeostasis and hypoxia (11). This notion is supported by their existence in small pulmonary arteries (their presence had, to date, only been demonstrated in the main pulmonary artery (10,11)) and by the recent finding of Yuan and co-workers (14) who show that the glycolosis inhibitor, 2-deoxy-D-glucose, causes inhibition of K^+ currents in rat pulmonary arterial smooth muscle cells.

The observation that cells isolated from the main pulmonary artery have a very different K^+ channel profile to those of the small pulmonary artery has two important implications. Firstly, it may highlight a more complex regulatory mechanism for the control of membrane potential reflecting their physiologically more important role in governing pulmonary vascular reactivity. Secondly, it may be an important consideration to those using rat main pulmonary arteries to elucidate the cellular mechanisms underlying HPV.

Acknowledgments

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References

1. Madden, J. A., Vadula, M. S. and Kurup, V. P. (1992) Am. J. Physiol. 263. L384-L393.
2. Yuan, X-J., Tod, M. L., Rubin, L. J. and Blaustein, M. P. (1990) Am. J. Physiol. 259. H281-H289.
3. Leach, R.M., Twort, C.H.C, Cameron, I.R. and Ward, J.P.T. (1992) Am. J. Physiol. 266. L223-L231.

4. Madden, J. A. Dawson, C. A. and Harder, D. R. (1985) J. Appl. Physiol. 59. 113-118.
5. Post, J M., Hume, J. R., Archer, S. L., Weir, E. K. (1992) Am. J. Physiol. 262. C882-C890.
6. Post, J. M., Weir, E. K. Archer, S.L. and Hume J. R. (1993) In Ion flux in pulmonary vascular control (Weir, E. K. Hume, J. R. and Reeves, J. T. Eds), pp. 189-204, Plenum Press.
7. Yuan, X-J., Goldman, W. F., Tod, M. L., Rubin, L. J. and Blaustein, M. P. (1993) Am. J. Physiol. 264. L116-L123.
8. Hamill, O.P., Marty, A., Neher, E., Sakmann, B. and Sigworth, F.J. (1981) Pfluegers Arch 391. 85-100.
9. Powell, T., Noma, A., Shioya, T. and Kozlowski, R.Z. (1993) J. Physiol. 472. 45-53.
10. Robertson, B. E., Corry, P. R., Nye, P. C. G. and Kozlowski, R. Z. (1992) Pfluegers. Arch. 421. 94-96.
11. Albarwani, S., Robertson, B. E., Nye, P. C. G. and Kozlowski, R. Z. (1994) Pfluegers. Arch. 428. 446-454.
12. Goldman, D.E. (1943) J. Gen. Physiol. 27. 37-60.
13. Hodgkin, A.L. and Katz, B. (1949) J. Physiol. 108. 37-77.
14. Yuan, X-J, Tod, M.L., Rubin, L.J. and Blaustein M.P. (1994) Am. J. Physiol. 267. L52-L63.