Characterization of slowly inactivating Kvα current in rabbit pulmonary neuroepithelial bodies: effects of hypoxia and nicotine

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Fu XW, Nurse C, Cutz E. Characterization of slowly inactivating Kvα currents in rabbit pulmonary neuroepithelial bodies: effects of hypoxia and nicotine. Am J Physiol Lung Cell Mol Physiol 293: L892–L902, 2007. First published July 20, 2007; doi:10.1152/ajplung.00098.2007.—Pulmonary neuroepithelial bodies (NEB) form innervated cell clusters that express voltage-activated currents and function as airway O2 sensors. We investigated A-type (NEB) form innervated cell clusters that express voltage-activated currents and function as airway O2 sensors. We investigated A-type channels in NEB cells using neonatal rabbit lung slice preparation. The whole cell K+ current was slowly inactivating with activation threshold of ~-30 mV. This current was blocked ~27% by blood-depressing substance I (BDS-I; 3 μM), a selective blocker of Kv3.4 subunit, and reduced ~20% by tetraethylammonium (TEA; 100 μM). The BDS-I-sensitive component had an average peak value of 189 ± 14 pA and showed fast inactivation kinetics that could be fitted by one-component exponential function with a time constant of (τ1) 77 ± 10 ms. This K+ slowly inactivating current was also blocked by heteropodatoxin-2 (HpTx-2; 0.2 μM), a blocker of Kv4 subunit. The HpTx-2-sensitive current had an average peak value of 234 ± 23 pA with a time constant (τ1) 82 ± 11 ms. Hypoxia (Po2 = 15–20 mmHg) inhibited the slowly inactivating K+ current by ~47%, during voltage steps from ~30 to +30 mV, and no further inhibition occurred when TEA was combined with hypoxia. Nicotine at concentrations of 50 and 100 μM suppressed the slowly inactivating K+ current by ~24% and ~40%, respectively. This suppression was not reversed by mecamylamine suggesting a direct effect of nicotine on these K+ channels. In situ hybridization experiments detected expression of mRNAs for Kv3.4 and Kv4.3 subunits, while double-label immunofluorescence confirmed membrane localization of respective channel proteins in NEB cells. These studies suggest that the hypoxia-sensitive current in NEB cells is carried by slowly inactivating A-type K+ channels, which underlie their oxygen-sensitive potassium currents, and that exposure to nicotine may directly affect their function, contributing to smoking-related lung disease.

Kv 3.4 and Kv4.3 currents; oxygen sensitive

The activity of voltage-dependent Kv channels regulates the threshold, shape, duration, and frequency of action potentials in a wide variety of excitable cells. Transient K+ currents form a group of voltage-dependent K+ currents characterized by their rapid activation and inactivation upon depolarization (26). Several transient K+ channel subunits have been cloned, among them Kv4.1, Kv4.2, and Kv4.3 of the Shal family, Kv3.3 and Kv3.4 of the Shaw family, and Kv1.4 of the Shaker-related subfamily. These channel subunits are differentially modulated by second-messenger systems (16) or by oxidation (26, 28). Kv3 and Kv4 channels are also found in nonfast-spiking neurons and other cells, such as alveolar epithelial cells (19), suggesting that they perform functions other than sustain rapid firing (25).

Airway epithelium of mammalian lungs contains pulmonary neuroepithelial bodies (NEBs) composed of amine [serotonin (5-HT)] and peptide (i.e., bombesin)-producing cells that are extensively innervated by vagal sensory fibers derived from the nodose ganglion (18). These cells are thought to function as airway oxygen sensors involved in autonomic control of breathing, especially during the neonatal period (4). Exposure to acute hypoxia in vivo or in vitro causes release of 5-HT from NEB cells (11, 18). Studies on NEB cells both in culture and lung slices have shown that they express a membrane-delimited O2 sensor and that inhibition of K+ channel activity plays an important role in the initiation of hypoxia chemotransduction (10, 37, 38). In NEB cells, voltage-dependent K+ currents have been described in whole cell recordings and include a Kv3.3a slowly inactivating current (37) and a noninactivating, outward delayed rectifier K+ current by 10.2±0.3 on October 29, 2017 http://ajplung.physiology.org/ Downloaded from

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METHODS

In all studies, we used lung tissue from neonatal (day 1–7) New Zealand White rabbits of both sexes. The rabbits were euthanized by an intraperitoneal injection of Euthanol (100 mg/kg, Bimeda-MTC, Cambridge, Ontario, Canada) and the lungs were removed. All experiments were approved by the local ethics committee in accordance with institutional guidelines for animal care.

In Situ Hybridization

To study the expression of Kv3.4 and Kv4.3 mRNA in NEB cells, we used nonisotopic in situ hybridization method with oligonucleotide probes (GeneDetect.com Limited, Auckland, New Zealand) followed by 5-HT immunofluorescence labeling to identify NEB cells using protocols similar to our previous studies (8, 37). The antisense probes were synthesized using the cDNA clones containing fragments of rabbit Kv3.4 5'-CGATGGGATGCTCTTGAAG-3' (AF933545) and Kv4.3 5'-TTTGTCTCAGTCTCGTGTC-3' (Kv4.3-1) (AF988445) (29). The probes were labeled with 5'-fluorescein phosphoramidite (DNA Synthesis Facility, HSC, Toronto, Canada). The labeled single-strand probes were hybridized to the cell mRNAs under high-stringency conditions, which allowed the probes to bind selectively to their corresponding mRNA (22).

Lung tissues were fixed in 4% paraformaldehyde and embedded in paraffin. The sections were cut at 6 μm deparaffinized, rehydrated, and postfixed for 10 min in 4% paraformaldehyde. They were then washed with PBS, treated with 10 μg/ml RNase-free proteinase K in DEPC-treated TE buffer (0.1% diethylpyrocarbonate; 100 mM Tris·HCl, 50 mM EDTA; pH 8.0) for 20 min. After being rinsed with PBS, sections were treated with 0.1 M triethanolamine, and acetylated with 0.005% acetic anhydride, before incubation with prehybridization buffer (hybridization buffer without probe) for 2 h at 37°C. Sections were then rinsed in 2× SSC solution, followed by incubation at 37°C for 40 h in hybridization solution containing fluorescent-labeled oligonucleotide probes 0.4 μg/ml. The sections were rinsed in a descending series of SSC (sodium chloride-sodium citrate in buffer, 2× SSC, 1× SSC, 0.25× SSC) containing 10 mM DTT. Under high-stringency incubation conditions, the sections were exposed to 1× SSC and 10 mM DTT at 37°C for 15 min. The specificity of the binding of the probes was demonstrated by absence of signal when the corresponding “sense” probe was used. As a positive control, Kv oligonucleotide probes were tested on sections of rabbit brain. Samples were covered with Vectashield Mounting Medium (Vector Laboratories, Burlington, Ontario, Canada) before viewing under an Olympus BX60 microscope (Carsen Group, Ontario, Canada). RSimage software (Roper Scientific, Tucson, AZ) was used for image acquisition and Adobe Photoshop 6.0 software was used to merge the images of double immunostaining and to edit the image size.

Immunofluorescence

For immunohistochemical studies to localize Kv3.4 and Kv4.3 epitopes in NEB cells, we used double-label immunofluorescence method on 7-μm cryostat sections of lung tissue fixed in 4% paraformaldehyde. The sections were washed in PBS before incubation in blocking solution containing either 10% normal rabbit or goat serum (depending on the animal species in which the primary or secondary antibody was produced) for 1 h, followed by overnight incubation at 4°C in a cocktail of primary antisera. The primary antibodies used included: 1) anti-Kv3.4 (lot number AN-02; 1:100 dilution), a rabbit polyclonal antibody raised against a purified peptide, corresponding to residues 177–195 of rat Kv3.4 (Alomone Laboratories, Jerusalem, Israel), 2) anti-Kv4.3 (lot number AN-05; 1:100 dilution), a rabbit polyclonal antibody raised against peptide residues 451–467 of human Kv4.3 (Alomone Laboratories), and 3) rat monoclonal anti-5-HT antibody (1:100 dilution, Medicorp, Montreal, Canada), as a marker of NEB cells. To visualize Kv3.4 and Kv4.3 subunits, Texas red-conjugated secondary goat anti-rabbit IgG (1:400 dilution, Jackson Immunoresearch Laboratories, West Grove, PA) was used. Colocalization of 5-HT with Kv3.4 and Kv4.3 in NEB cells was investigated using FITC-conjugated rabbit anti-rat IgG (1:300 dilution, Jackson Immunoresearch Laboratories) to visualize 5-HT immunofluorescence. Secondary antibodies were diluted in PBS containing 0.7% BSA and 10% normal goat or rabbit serum to block nonspecific binding. The sections were covered with Vectashield Mounting Medium (Vector Laboratories) before viewing under an Olympus BX60 microscope (Carsen Group). As positive controls for Kv3.4 and Kv4.3 subunits, we used a frozen section of rat and rabbit brain tissues fixed in 4% paraformaldehyde according to immunostaining protocol recommended by the manufacturer. As negative controls, we used two approaches, in the first the primary antibody was omitted, and in the second the primary anti-Kv antibody was preincubated with respective peptide antigen (0.3 mg/ml) for 2 h before application on lung sections.

Electrophysiological Methods

For electrophysiological studies, the lungs were cut into 4-mm² blocks and embedded in 2% agarose (FMC Bioproducts, Rockland, ME). Sectioning was performed with tissue immersed in ice-cold Krebs solution that had the following composition (in mM): 140 NaCl, 3 KCl, 1.8 CaCl₂, 1 MgCl₂, 5 HEPES, and 5 glucose; pH 7.3 adjusted with NaOH. Transverse lung slices (200–300 μm) were cut with a Vibrating Blade Microtome (Leica Instruments GmbH, Nussloch, Germany).

For electrophysiological recordings, the lung slices were transferred to a recording chamber mounted on the stage of a Nikon microscope (Optiphot-2UD, Nikon, Tokyo, Japan). To identify NEB cells, lung slices were incubated with the vital dye, neutral red, neutral red (0.02 mg/ml) for 15 min at 37°C as previously described (10). Ionic currents were recorded at room temperature (20–25°C) using the whole cell configuration of the patch-clamp technique (13). An internal pipette solution with the following composition was used (in mM): 30 KCl, 100 potassium glutonate, 1 MgCl₂, 4 Mg-ATP, 5 EGTA, 10 HEPES; pH adjusted to 7.25 with KOH. To isolate inward currents, pipette solution with the following composition was used (in mM): 130 CsCl, 1 CaCl₂, 2 MgCl₂, 10 EGTA, 10 HEPES, and 4 MgATP; pH adjusted to 7.2 with CsOH. Patch pipettes were made from borosilicate glass (1.5-mm outer diameter, World Precision Instruments, Sarasota, FL) double-pulled (Narishige PP-83) to resistances ~2.5–3.5 MΩ when filled with the internal solution. The access resistance was ~5–15 MΩ. The composition of the bath solution was (in mM) 140 NaCl, 3 KCl, 2.5 CaCl₂, 1.2 MgCl₂, 5 HEPES, and 10 glucose; pH 7.35–7.4. The chamber, which had a volume of 1 ml, was perfused continuously with solution at a rate of 6–7 ml/min. Hypoxia solution was prepared by bubbling 95% N₂ and the level of PO₂ of solution in the recording chamber varied between 15–20 mmHg.

The Kv3.4-specific blocker, a venom of sea anemone Anemonia sulcata, known as blood-depressing substance 1 (BDS-1) and Kv4.3-specific blocker, a spider venom toxin heteropodatoxin-2 (HpTx-2), were obtained from Alomone Laboratories. Nicotine, mecamylamine, and tetraethylammonium (TEA) were obtained from Sigma (Oakville, ON, Canada). Drugs were applied to the perfusate chamber and delivery to the cells was controlled by separate valves.

An Axopatch 200B amplifier (Axon Instruments, Foster, CA) was used to record whole cell currents under voltage clamp. The data were filtered at 2 kHz. Records were digitized with a Digidata 1200 A/D interface, driven by pClamp 9.0, Clampex software. An online P/4 protocol was used when leak subtraction was performed. Data analyses were performed with the Clampfit and results are expressed as means ± SE. Tests for statistical significance were performed using Student’s two-tailed t-test with the level of significance set to P < 0.05.
RESULTS

Detection of mRNAs for Transient Kv Channel Transcripts in Rabbit NEB Cells

To verify the presence of Kv3.4 and Kv4.3 Kv channel transcripts in NEB cells, we carried out in situ hybridization experiments using sections of rabbit neonatal lung. In these experiments, the Kv3.4-specific antisense oligonucleotide probe yielded a strong signal in the apical cytoplasm of airway epithelial cells (Fig. 1A), whereas the signal was greatly reduced or absent in the “sense” control (Fig. 1C). Colocalization experiments confirmed Kv3.4 mRNA expression in NEB cells, identified by positive 5-HT immunofluorescence (Fig. 1B). Similar experiments using the Kv4.3-specific oligonucleotide probe confirmed expression of Kv4.3 in 5-HT-positive NEB cells and also in the airway epithelial cells (Fig. 1, D and E), while the sense control was negative (Fig. 1F). The same experiment was repeated four times. Thus NEB cells in neonatal rabbit lung express mRNA for both Kv3.4 and Kv4.3 subunits.

Immunofluorescence Localization of Kv3.4 and Kv4.3 Proteins in NEB Cells

The expression of Kv3.4 and Kv4.3 protein in NEB cells was examined using antisera specific for the Kv3.4 and Kv4.3, respectively. In NEB cells, both Kv3.4 and Kv4.3 immunoreactivity was expressed on the plasma membrane and submembrane location (Fig. 2, A and D). The staining pattern was similar to that previously reported for glomus cells of rabbit carotid body using peroxidase immunocytochemistry method on paraffin sections (29). To confirm Kv3.4 and Kv4.3 protein expression in 5-HT-positive NEB cells, we used double-label immunofluorescence. As shown in Fig. 2, A–F, there was colocalization of Kv3.4 or Kv4.3 immunofluorescence with 5-HT-positive signal, indicating that NEB cells express Kv3.4 and Kv4.3 subunits at the protein level. Negative controls confirmed the specificity of immunostaining for both Kv3.4 and Kv4.3 antibodies. No positive signal was observed on sections where primary antibody was omitted or on sections preincubated with respective peptide antigen (data not shown).

Electrophysiological Characterization of Kv A-Type Current in Rabbit NEB Cells

Kv 3.4 fast transient outward current in NEB cells. Voltage-dependent K⁺ currents were studied in rabbit lung NEB cells. After establishing the whole cell configuration, slowly inactivating outward currents were elicited during 1-s depolarizing voltage steps (15-mV intervals) following a 250-ms hyperpolarizing prepulse to −90 mV to remove inactivation (15, 31, 32), as illustrated in Fig. 3A, inset (holding potential = −60 mV). Out of 286 NEB cells sampled, 85 (~30%) responded to voltage command evoking Kv A-type current. These A-type inactivating K⁺ currents were apparent during step potentials between −30 and +30 mV as shown for a typical NEB in Fig. 3A; the corresponding current-voltage (I-V) relationship is shown in Fig. 3E. The average peak value of this Kv current at +30 mV was 694 ± 13 pA (n = 5). After application of 3 μM BDS-I, a selective blocker of Kv3.4 current, the peak current was reduced to 505 ± 22 pA (P < 0.05, n = 5), corresponding to an inhibition of ~27% (Fig. 3, B and C). The slowly

Fig. 1. Detection of Kv3.4 and Kv4.3 subunit transcripts in pulmonary neuroepithelial body (NEB) cells by in situ hybridization. Expression of mRNA for Kv3.4 and Kv4.3 in apical cytoplasm of airway epithelial cells (A and D), including NEB cells (B and E) identified by immunostaining for serotonin (5-HT; red) in the same sections. C and F: control “sense” probes for Kv3.4 and Kv4.3 yielded negative signal in both airway epithelial cells and NEB. Calibration bar represents 30 μm.
inactivating current was fitted by one component exponential function with time constant \( \tau \) of 569 ± 19 ms in control conditions and 427 ± 70 ms during exposure to BDS-I \((P < 0.05, n = 4)\), corresponding to a reduction of \( \sim 25\% \) (Fig. 3F). The BDS-I-sensitive difference current (Fig. 3G), obtained by subtracting the current recorded in the presence of BDS-I (Fig. 3B) from the control current (Fig. 3A), is plotted against voltage in the I-V relationship shown in Fig. 3E. The BDS-I-sensitive current had an average peak value of 189 ± 14 pA \((n = 5)\) and showed fast inactivation that could be fitted by exponential functional with time constant \( \tau \) of 77 ± 10 ms \((n = 4)\). The sea anemone peptide BDS-I was the first reported specific blocker identified for the rapidly inactivating Kv3.4 channel (7, 29).

Kv3 channels have been shown to be sensitive TEA in the micromolar range in rat hippocampal granule cells and in mouse carotid body chemoreceptors (25, 26). We therefore tested the effects of TEA on the slowly inactivating K\(^+\) current in NEB cells (Fig. 3G). The peak current was reduced by \( \sim 19 \pm 2\% \) by 100 \( \mu \)M and \( \sim 61 \pm 10\% \) by 10 mM TEA \((n = 4)\). The fact that a slowly inactivating component was still detectable following application of 10 mM TEA suggests other Kv subunits may contribute to the total inactivating current. It should be noted that these NEB cells also contain a Ca\(^{2+}\)-dependent K\(^+\) current which is also sensitive to TEA (10).

**Kv 4.3 fast transient outward current in NEB cells.** We next tested whether Kv4.3 also contributed to the fast transient outward K\(^+\) current in NEB cells. The spider venom HpTx-2 blocks Kv4.2 channels expressed in *Xenopus laevis* oocytes in a voltage-dependent manner (30), as well as other members of the Kv4 family (2). Figure 4, A and E, shows control example traces and I-V relationship of the slowly inactivating K\(^+\) current recorded in NEB cells. The average peak K\(^+\) current at \( \pm 30\) mV decreased from a control value of 1,016 ± 56 pA \((n = 5)\) to 769 ± 52 pA \((n = 5, P < 0.05)\) after application of 0.2 \( \mu \)M HpTx-2, corresponding to a reduction by \( \sim 24\% \) (Fig. 4F). The HpTx-2-sensitive difference current (Fig. 4D) is plotted against voltage in the I-V relationship shown in Fig. 4E. The HpTx-2-sensitive current had an average peak value of 234 ± 23 pA \((n = 5)\) and the time constant \( \tau \) for the fast inactivating current was 82 ± 11 ms \((n = 4)\). A time series plot of the reversible inhibition of the peak slowly inactivating K\(^+\) current by 0.2 \( \mu \)M HpTx-2 is shown for a NEB cell in Fig. 4G. This inhibitory effect of HpTx-2 on the slowly inactivating current, together with the presence of the Kv4.3 mRNA and protein, suggested that NEB cells express functional Kv 4.3 subunits. In HEK293 cells transfected with a short isoform of human Kv 4.3 (S-hKv4.3) cDNA, the Kv4.3 current was partially inhibited by 4-aminopyridine (4-AP) (3). In NEB cells, 4-AP (2 mM) also reduced the Kv A-type current by \( \sim 20\% \) (data not shown).

Taken together, these pharmacological data suggest that both Kv3.4 and Kv4.3 subunits contribute to the inactivating K\(^+\) current expressed in NEB cells.
Hypoxia Suppresses Kv A-Type Currents in NEB Cells

Previous studies on NEB cells in fetal lung cultures and in fresh fetal lung tissue slices have shown that hypoxia chemotransduction mediated, in part, by inhibition of a Ca-dependent K⁺ as well as a delayed rectifier K⁺ current (8, 10, 38). To test whether the slowly inactivating Kv currents in NEB cells are also hypoxia sensitive, we monitored K⁺ currents before and after exposure to acute hypoxia (P O₂ ≈ 20 mmHg). This stimulus resulted in a rapid and reversible reduction of both the transient peak and sustained components of Kv currents in NEB cells (Fig. 5, A–C). This reduction of the peak and sustained components of current amplitudes was significant at +30 mV test potential (Fig. 5F). Peak current before and during hypoxia was 646 ± 29 and 324 ± 25 pA, respectively (P < 0.01, n = 10), corresponding to a reduction ∼49%. The I-V curve of the mean (±SE) peak transient current elicited in 10 cells before and during hypoxia is shown in Fig. 5E. The sustained current measured at a test potential of +30 mV near the end of 1 s depolarizing step was 366 ± 46 and 118 ± 23 pA, before and during hypoxia, respectively (P < 0.01, n = 10), corresponding to a reduction of ∼67% (Fig. 5F). The hypoxia-sensitive difference Kv current components, isolated by subtracting the remaining currents in hypoxia (Fig. 5B) from the control currents in normoxia (Fig. 5A), are shown in Fig. 5D at different voltage steps. At +30 mV test potential, the peak current is 319 ± 14 pA (n = 8), and the corresponding I-V curve is shown in Fig. 5E. We also tested the effects of TEA on the slowly inactivating K⁺ current under hypoxic conditions. The peak currents before and after 10 mM TEA were 622 ± 32 and 412 ± 41 pA, respectively, at a test potential of +30 mV (n = 4, P < 0.05; Fig. 5G). In the presence of TEA, hypoxia solution failed to reduce the residual K⁺ current (Fig. 5G).

To assess the relationship between the O₂ and BDS-1- or HpTx-2-sensitive currents in NEB cells, the Kv slowly inactivating current was first evoked by a depolarizing step from −90 to 30 mV. The current amplitude was reduced by applying 3 µM BDS-1 plus 0.2 µM HpTx (Fig. 6, Aa and b). However, subsequent addition of hypoxia failed to reduce the residual A-type current (Fig. 6Ac). The control current, 3 µM BDS-1 plus 0.2 µM HpTx, and residual current after hypoxia+BDS-1+HpTx-2 were 563.1, 394 ± 33, and 386.2 ± 69 pA (n = 3; for latter two, P > 0.05), respectively. The I-V relationship is shown in Fig. 6B. These findings suggest that the BDS-1- and...
HpTx-2-sensitive slowly inactivating Kv currents contribute to hypoxia chemosensitivity in rabbit neonatal NEB cells.

**Effects of Nicotine on Inward Current and Slowly Inactivating Kv Currents in NEB Cells**

We previously showed that NEB cells in hamster lung express functional heteromeric \( \alpha 3 \beta 2, \alpha 4 \beta 2, \) and \( \alpha 7 \) nicotinic acetylcholine receptors (9). Since there may be species variations, in the present study we tested the effects of nicotine and expression of functional nAChR’s in NEB cells of rabbit neonatal lung. Application of 50 \( \mu \)M nicotine evoked transient inward current at the holding membrane potential of \(-60 \) mV, followed by a rapid desensitization of the response (Fig. 7). The mean peak amplitude of current was \(-556.9 \pm 37.2 \) pA \((n = 4)\). The inward current response induced by 50 \( \mu \)M nicotine was reversibly suppressed by 10 \( \mu \)M mecamylamine (Fig. 7; \( n = 4 \)). These data indicate that NEBs in rabbit neonatal lung express functional nAChR are quite sensitive to mecamylamine. In addition, since nicotine can have also direct effects on certain Kv channels, we tested whether nicotine can block slowly inactivating Kv channels in NEB cells. Indeed, nicotine (50 \( \mu \)M) reversibly inhibited the slowly inactivating Kv in NEB cells as exemplified in Fig. 8, A, B, and D; the corresponding I-V curve is shown in Fig. 8F. The nicotine-sensitive difference current isolated by subtracting the remaining currents in nicotine (Fig. 8B) from the control currents (Fig. 8A) is shown for each voltage step in Fig. 8D, and the corresponding I-V curve is shown in Fig. 8F. This suggests that nicotine not only inhibited Kv A-type current but also decreased delayed rectifier K⁺ current. We also tested whether the inhibitory effect of nicotine on Kv current could be prevented by the nicotinic receptor antagonist, mecamylamine. A
A summary of the concentration-dependent inhibitory effects of nicotine on peak Kv current is shown in Fig. 8 at three different concentrations (n = 4–8 for each group). As exemplified in Fig. 8E, application of 10 μM mecamylamine failed to reverse the inhibitory effect of 50 μM nicotine on fast transient slowly inactivating Kv current (n = 3). Similar results have been reported for cloned Kv 4.3 currents expressed in X. laevis oocytes (36). These studies suggest that nicotine may directly inhibit the Kv A-type current in NEB cells.

**DISCUSSION**

In the present study, we report electrophysiological and pharmacological characterization of Kv3.4 and Kv4.3 A-type currents in NEB cells of neonatal rabbit lung. Our in situ hybridization studies revealed that NEB cells expressed mRNAs for Kv3.4 and Kv4.3 subunits and antibodies raised against specific epitopes of Kv3.4 and Kv4.3 channels showed expression of the corresponding channel protein subunits in the plasma membrane of NEB cells. In addition, electrophysiological and pharmacological studies confirmed that voltage-gated fast transient channels Kv 3.4 and Kv 4.3 were sensitive to both hypoxia and nicotine.

Sea anemone venom toxin, BDS-I, is known for its ability to block a variety of K⁺ channels expressed in X. laevis oocytes. The specificity of BDS-I for the channels of the Shaw subfamily was first analyzed by assaying its possible action on different members of the K⁺ channel family including Shaw (Kv3.1, Kv3.3, Kv3.4), Shaker (Kv1.2, Kv1.3, Kv1.4, and Kv1.5), Shab (Kv2.1 and Kv2.2), and Shal (Kv4.2 and Kv4.3). None of these cloned channels expressed in X. laevis oocytes was significantly affected by BDS-I except the Shaw family. Among the Shaw family members, only one Kv3.4 was extensively inhibited by BDS-I (7). Kv3 currents are activated specifically during action potential repolarization (27). In rab-
bit NEB cells, the fast transient A-type $K^+$ current component showed pharmacological properties that were similar to the cloned Kv3.4 channels. This current was sensitive to micro-molar concentrations of BDS-I, and to TEA (0.1–10 mM), and had an activation threshold of $-30$ mV. The A-type current we observed in NEB cells is not a pure A-type current as described in neurons (26, 27). It is likely contaminated with slowly inactivating Kv current and/or delayed rectifier $K^+$ current accounting for recordings shown in Figs. 3–6 that are “slower inactivating” compared with neurons.

It has been reported that cloned Kv4.3 channel has two isoforms, long and short, with similar kinetic properties (6). Both long and short forms of the channel are expressed in the human brain, but the long form is found in the heart and other organs (26, 35). Our in situ hybridization studies showed that the long form (Kv4.3-l) mRNA was expressed in rabbit NEB cells, and in voltage-clamp experiments the A-type Kv current was also blocked by spider venom toxin, HpTx-2, as previously reported for rabbit chemoreceptor cells (29). In NEB cells, 4-AP also inhibited the A-type $K^+$ current (data not shown).

Fig. 6. Effects of hypoxia and combination with BDS-1 and HpTx-2 on slowly inactivating $K^+$ current. A: Kv current was evoked by depolarizing step potential in control Krebs solution (a). Kv current was reduced by perfusing 3 μM BDS-1 and 2 μM HpTx-2 (b), and Kv current was not altered by further exposure to hypoxia. B: $I-V$ relationship was plotted under control condition (●), after perfusing 3 μM BDS-I and 0.2 μM HpTx-2 (○), hypoxia solution plus 3 μM BDS-I and 0.2 μM HpTx-2 (△).

Fig. 7. Effects of nicotine on whole cell current in rabbit NEB cells. A: application of 50 μM nicotine evoked an inward current. B: perfusion of 10 μM mecamylamine (Mec) for 5 min and then application of 50 μM nicotine plus 10 μM Mec resulted in diminished nicotine response. C: 50 μM nicotine evoked inward current after washout of Mec.
shown), a result that is reminiscent of the short form of human Kv4.3 (S-hKv4.3) channels heterologously expressed in HEK293 (3). The similarity of the effects of HpTx-2 and BDS-I on A-type inactivating Kv current raises the possibility that Kv4.3 and Kv3.4 subunits could contribute to a hetero-
meric O2-sensitive K\(^{+}\)/channel in NEB cells. However, further
studies are required to address the issue whether these subunits
form homomeric and heteromeric channels in native NEB
cells.

We previously reported that the O2-sensitive noninactivating
outward K\(^{+}\)/current in NEB cells was reversibly inhibited
(40%) by hypoxia in both NEB cell cultures and lung slices
(10, 38). This O2-sensitive, voltage-gated K\(^{+}\)/current was
blocked (40%) by TEA or 4-AP. The hypoxia-sensitive K\(^{+}\)/currents in NEB cells include a Ca\(^{2+}\)-independent \([\text{Ik}^{(v)}]\) and a
Ca\(^{2+}\)-dependent \([\text{Ik}^{(Ca)}]\), representing 45 and 55%, respec-
tively, of the O2-sensitive K\(^{+}\)/current (10). In NEB cells, both
Kv3.4 and Kv4.3 subunits contribute to the slowly inactivating
K\(^{+}\)current. Hypoxia inhibited the peak A-type current by
49%, and the sustained current by 67%. The slowly inac-
tivating K\(^{+}\)current was blocked 34% by TEA, and no further
inhibition occurred in the presence of TEA plus hypoxia,
suggesting that TEA-sensitive A-type K\(^{+}\)current corresponds
to O2-sensitive K\(^{+}\)current in NEBs. We previously reported
that mRNAs for both hydrogen peroxide (H\(_2\)O\(_2\))-sensitive
voltage-gated K\(^{+}\)/channel subunit (KH\(_2\)O\(_2\)3.3a and mem-
brane components of the O2 sensor, i.e., NADPH oxidase
(gp91phox and P22phox) are coexpressed in the NEB cells of fetal
rabbit and neonatal human lungs as well as related small cell
lung carcinoma cell lines (37). The K\(^{+}\)/currents in cultured
NEB cells exhibited inactivating properties similar to Kv3.3a
transcripts expressed in the X. laevis oocyte model (34). These
studies provided strong evidence in support of the “membrane
model” of O2 sensing in NEBs where the K\(^{+}\)/channel is closely
associated with an O2-sensing NADPH oxidase complex, and
the interaction occurs via a membrane-delimited pathway.

Fig. 8. Effects of nicotine on slowly inactivating K\(^{+}\)/current. A: K\(^{+}\)/current evoked by depolarizing steps from −90 to +30 mV in control Krebs solution.
B: K\(^{+}\)/current was reduced by 50 μM nicotine. C: washout of 50 μM nicotine caused a recovery of the K\(^{+}\)/current. D: nicotine-sensitive K\(^{+}\)/current was obtained by subtracting currents in B from those in A. E: K\(^{+}\)/current recordings at +30 mV, during application of 50 μM nicotine, in absence and presence of antagonist 20 μM Mec. F: I-V relationship for K\(^{+}\)/current in control condition (●), after perfusion 50 μM nicotine (○), and nicotine-sensitive K\(^{+}\)/current in D (△). G: percent
of inhibition of nicotine on K\(^{+}\)/currents, at test potential +30 mV, n = 4–8 for each group, P < 0.05.
neurons, but this action was blocked by the nicotinic antagonist mecamylamine. Similarly, in cloned Kv4.3 current, nicotine had a concentration-dependent inhibitory effect on fast transient slowly inactivating K⁺ current (Ito) phenotypes and distribution of fast-inactivating potassium channel alpha subunits in ferret left ventricular myocytes. J Gen Physiol 113: 581–600, 1999.


EFFECTS OF HYPOXIA AND NICOTINE ON KVα CURRENT IN NEB CELLS


