Functional Consequences of Polyamine Synthesis Inhibition by DFMO: Cellular Mechanisms for DFMO-mediated Ototoxicity

Liping Nie¹, Weihong Feng¹, Rodney Diaz¹, Michael A Gratton², Karen Jo Doyle¹, and Ebenezer N. Yamoah¹

¹Center for Neuroscience, Dept. of Otolaryngology, University of California, Davis 1544 Newton Ct. Davis, CA 95616
²Department of Otorhinolaryngology, University of Pennsylvania, 3400 Spruce St. Philadelphia, PA 19014

Key words: Inward rectifier K⁺ channels, Voltage clamp, Inner ear, Stria vascularis, Endocochlear potential, Hearing loss.

Abbreviated title: Kir4.1 in the inner ear.
Number of text pages: 14
Number of figures: 4
Words in abstract: 170
Words in introduction: 500
Words in discussion: 1130

Proof and correspondence to: Ebenezer N. Yamoah
Center for Neuroscience
Department of Otolaryngology
University of California, Davis
1544 Newton Ct.
Davis, CA. 95616
Tel: 530-754-6630
Fax: 530-754-7183
Email: enyamoah@ucdavis.edu

Acknowledgment
This was supported by grants to LN (Deafness Research Foundation), and ENY (NIH-DC007592, DC004523). MAG was supported by DC006442.
ABSTRACT

L-α-difluoromethylornithine (DFMO) is a chemo-preventive agent for colon cancer in clinical trials. Yet, the drug produces an across-frequency elevation of the hearing threshold, suggesting that DFMO may affect a common trait along the cochlear spiral. The mechanism for the ototoxic effects of DFMO remains uncertain. The cochlear duct is exclusively endowed with endocochlear potential (EP). EP is a requisite for normal sound transduction as it provides the electromotive force that determines the magnitude of the receptor potential of hair cells. EP is generated by the high throughput of K+ across cells of the stria vascularis (StV), conferred partly by the activity of Kir4.1 channels. Here, we show that the ototoxicity of DFMO may be mediated by alteration of the inward rectification of Kir4.1 channels, resulting in a marked reduction in EP. These findings are surprising given that the present model for EP generation asserts that Kir4.1 confers the outflow of K+ in the StV. We have proposed an alternative model. These findings should also enable the rational design of new pharmaceuticals devoid of the untoward effect of DFMO.
INTRODUCTION

L-α-difluoromethylornithine (DFMO) is an irreversible inhibitor of ornithine decarboxylase (ODC), a key enzyme in the synthesis of polyamines. Inhibition of polyamine synthesis suppresses carcinogen-induced epithelial cancers in animal models (1), which has raised the possibility that DFMO can be used effectively as a chemo-preventive agent for colon cancer. However, the therapeutic potential of DFMO is hindered by its unaccounted mechanisms of ototoxicity. DFMO decreases the level of polyamines in the cochlea (2,3), and causes an across-frequency hearing loss (4), suggesting that the drug may alter a fundamental feature of cochlear function.

The acute sensitivity of the auditory system is conferred partly by the baseline spontaneous activity of hair cells. Moreover, to overcome the intrinsic membrane noise, the sensitivity of hair cells is dependent on the endocochlear potential (EP > 80 mV). Thus, EP is indispensable for normal sound transduction (5,6). EP is generated by the high throughput of outward K$^+$ flux across cells of the stria vascularis (StV) into the scala media by K$^+$ transporters, pumps, and channels together with Cl$^-$ channels (7-12). A congenital deficiency in intermediate cells (IC), melanocytes forming the middle cellular layer of the StV, results in a reduction in EP and an increase in the hearing threshold (13,14). For this reason, as well as the fact that ICs express Kir4.1, it has been asserted that the Kir4.1 channel is responsible for the high throughput of outward K$^+$ movement across the IC membrane that leads to generation of EP (15-17). Consistent with this assertion is the evidence that Ba$^{2+}$, a non-specific blocker of Kir channels, decreases the magnitude of EP (15,18,19). Indeed, null deletion of the channel resulted in marked reduction in EP as well as a decreased concentration of endolymphatic K$^+$ (17,20,21). Despite the circumstantial evidence, it is uncertain how an inward rectifier channel can produce...
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a robust outward K$^+$ efflux to generate EP. It is conceivable, albeit unlikely, that the Kir4.1 channels in the StV may be specialized to confer a tissue-specific function. Alternatively, the channel may not be a major player in K$^+$ extrusion from ICs into the interstitial space (IS) but may contribute towards the removal of K$^+$ from the IS.

The inward rectification of Kir channels is caused by both blockage of the outward current by cytoplasmic Mg$^{2+}$, and by intrinsic polyamines (21-26). We surmised that DFMO-induced ototoxicity is mediated through inhibition of polyamine synthesis, consequently altering inward rectification of inner ear-specific Kir4.1. Thus, we cloned mouse cochlear lateral wall-specific Kir4.1 and analyzed the phenotypic features of the channel both with and without DFMO in order to determine the effects of the drug on EP. It was reassuring to observe that DFMO reduced the inward rectification of cochlear lateral wall Kir4.1. However, it was startling to determine that the drug produces a marked reduction in EP. These results indicate that although Kir4.1 channels are involved in the generation of EP, the existing model for the generation of EP should be refined. The underlying cellular mechanisms for the contribution of Kir4.1 to EP generation are reevaluated and the possible mechanism of DFMO-mediated ototoxicity is outlined.

MATERIALS and METHODS

Polymerase chain reaction (PCR) and cDNA cloning

Total RNA was purified from 12-week old mouse inner ear StV using Trizol reagent following the protocol suggested by the manufacturer (Invitrogen, Palo Alto, CA). RNA (∼1 µg) was then used as a template to synthesize cDNA using SuperScript II reverse transcriptase. Aliquots of this cDNA were used as a DNA template for PCR. The sequences of the 5’- and 3’- primers for
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amplification of the Kir4.1 channel were derived from the mouse brain sequence (Genbank Access No. AF322631) as follows: 5’-GCCGCCACCATGACGTCGGTCGCTAAG-3’ and 5’-AGGATATCAGACGTTGCTG-3’, respectively. PCR was performed according to the following schedule: pre-denature at 94°C for 3 min, followed by 30 cycles at 94°C for 30 sec, 54°C for 30 sec, and 72°C for 1 min. The PCR products were recovered after agarose gel separation and subcloned into a pCRII-TOPO vector (Invitrogen, Palo Alto, CA). Four clones were selected and checked by the digestion of internal BglII and HindIII sites. The cDNA sequences were determined by the chain termination method. A representative clone, pKJ-E3, was analyzed using the Xenopus oocyte expression system.

Expression in heterologous systems

For expression in the Xenopus oocyte system, the cDNA fragment containing the complete open reading frame (ORF) of the Kir4.1 potassium channel was subcloned into the vector, pNLE, which included the 5’ and 3’ untranslated regions of a Xenopus β-globin gene (27). From the resulting expression plasmid, RNAs of Kir4.1 were transcribed in vitro using T7 RNA polymerase and injected into stage V-VI oocytes as described (28).

Electrophysiological Recordings

Two-electrode voltage clamp experiments were carried out with a commercially available amplifier (Warner Instrument Corp., Hamden, CT) with microelectrodes, which were filled with 3 M KCl. Oocytes were bathed in a solution that contained 96 mM NaCl, 2 mM KCl, 1 mM MgCl₂, 1.8 mM CaCl₂, 5 mM HEPES (N-[2-hydroxyethyl] piperazine-N’-[2-ethanesulfonic acid]) and 1 mM niflumic acid to block endogenous chloride currents in oocytes (pH adjusted to 7.6 with NaOH). The current was activated using different voltage-clamped protocols as
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described in the results section. Only the oocytes expressing robust Kir4.1 currents were selected for single-channel recordings.

Single-channel recordings were performed in the cell-attached and excised-patch configurations using a patch-clamp amplifier, Axopatch 200B (Axon Instrument, Union City, CA), which was interfaced with a personal computer by way of an analog-to-digital converter. Whereas the whole-cell recordings were acquired using Pclamp software (Axon Instr.), the single-channel data was collected with custom-written software (Q program). Single-channel recordings of membrane patches were held at -40 mV and stepped to different depolarizing test pulses at frequencies from 0.2-0.5 Hz. Current traces were amplified and filtered at 1 kHz and digitized at 5 kHz. Patch pipettes were fabricated from borosilicate glass capillaries (World Precision Instruments, FL) on a four-step horizontal Flaming-Brown microelectrode puller (Sutter Instrument, CA) and coated with Sylgard 184™ (Dow-Corning Corp., Midland, MI) to within 100 µm of the tip and fire-polished before use. All chemicals were obtained from Sigma unless otherwise noted. Endogenous Cl⁻ currents were blocked with 1 mM niflumic acid in the recording pipette and in the bath solution. The bath solution contained (mM): 90 NaCl, 25 TEA-Cl, 5 4-AP, 5 CaCl₂, 3 glucose, and 5 HEPES (pH 7.4). All experiments were performed at room temperature (~21°C).

Data Analysis

For single channel recordings, leakage and capacitative transient currents were subtracted by fitting a smooth template to null traces. Leak-subtracted current recordings were idealized using a half-height criterion (29). Transitions between closed and open levels were determined by using a threshold detection algorithm, which required that two data points exist above the half mean amplitude of the single-unit opening. The computer-detected openings were confirmed by
visual inspection, and sweeps with excessive noise were discarded. Amplitude histograms at a given test potential were generated and then fitted to a single Gaussian distribution using a Levenberg-Marquardt algorithm to obtain the mean and standard deviation. At least five voltage steps and their corresponding single-channel currents were used to determine the unitary conductance. Single-channel current-voltage relations were fitted by linear least-square regression lines, and single-channel conductances were obtained from the slope of the regression lines. Idealized records were used to construct ensemble-averaged currents, open probability, and histograms for the distributions of open and closed intervals. Curve fits and data analyses were performed using Origin software (MicroCal Inc., Northampton, MA). Where appropriate, pooled data are presented as means ± S.D.

**Endocochlear potential measurement**

Mice were anesthetized with 20% urethane (0.01 ml/g). A tracheal cannula was inserted, and the bulla was opened using a retroauricular approach. A small hole was made in the bony wall of the cochlea over the basal turn. A glass micropipette electrode filled with 150 mM KCl was advanced through the hole while secured to a hand-controlled micromanipulator. The electrode was connected to a high-impedance DC amplifier. The reference electrode (silver/silver chloride pellet) was placed under the dorsal skin. The recording electrode passed through the spiral ligament of the lateral wall into the scala media. The potential difference (mV) between the scala media and the reference electrode was recorded.

**Auditory brainstem responses (ABR) measurement**

Mice were anesthetized with avertin, and auditory brainstem response (ABR) waveforms were recorded as previously described (30,31). Briefly, a ground needle electrode and recording needle were placed subcutaneously in the scalp, and a calibrated electrostatic speaker coupled to
a hollow ear bar was placed inside the pinna. Broadband clicks and pure tones (8, 16, and 32 kHz) were presented to the animal’s ear in 10 dB increments, starting from 0 dB to 100 dB SPL. The ABR sweeps were computer-averaged (time-locked with onset of 128-1024 stimuli, at 20/s) from the continuous electroencephalographic activity. The threshold of hearing was determined as the lowest intensity of sound required to elicit a characteristic waveform.

RESULTS

The Kir4.1 channel has been cloned from the brain (32). However, given the presumed specific role of the channel in the inner ear, we performed RT-PCR using the primers related to mouse brain Kir4.1 and cDNA from mouse inner ear lateral wall to amplify StV-specific Kir4.1. A single band with the expected size, 1155 bp, was obtained. The PCR product was cloned into a pCRII-TOPO vector and sequenced. We identified the Kir4.1 channel cDNA from the StV that contained a complete ORF (Genbank Access No. AY374423), encoding a putative protein with 379 amino acids (aa). Analysis of the aa sequences of putative proteins from Kir4.1 cDNAs revealed high similarities between StV-specific and other cloned Kir4.1 channels (15,32-35), and bears the signature of an intermediate inward rectifier (Fig. 1), which gave the assurance that the channel is likely to be subjected to partial polyamine block and hence influenced by ODC (Fig 1).

The phenotypic features of Kir4.1 expressed in Xenopus oocytes are illustrated in Figure 2. The current rectifies inwardly, showing stunted growth at depolarized step potentials. By contrast, current magnitude was robust in response to hyperpolarized step potentials from a holding potential of -40 mV (Fig. 2A). Consistent with a K⁺-selective channel, the whole-cell current-voltage relations shifted to depolarized potentials (Fig. 2B), and the estimated reversal
potentials ($E_{rev}$) were close to the calculated Nernst potential as the bath $K^+$ was elevated systematically (Fig. 2C). The current was blocked by $Ba^{2+}$ with a half blocking concentration at $\sim 30 \pm 10 \ \mu M$, binding to $\sim 20\%$ of the functional electrical field of the channel (Figs. 2E & F). These results were in accord with previous reports (32,34,36). Moreover, the resting potential of oocytes expressing Kir4.1 was controlled exclusively by the $Ba^{2+}$-sensitive current, indicating that the channel serves as a background current that clamps the membrane potential close to the net resting potential of the oocytes (Fig. 2D).

To determine the effects of DFMO on the cloned Kir4.1 channels, *Xenopus* oocytes were injected with RNA and incubated in ND-96 solution containing 500 $\mu M$ DFMO for 24 hours. Experiments were also performed by injecting DFMO directly into oocytes. The ensuing whole-cell currents were compared to currents from control oocytes (Figs. 3B & C). In accord with previous findings from Kir2.3 and other Kir channels, DFMO and other inhibitors of ODC produced a marked increase in the outward component of the current (37,38). Moreover, modification of Kir4.1 channel currents by DFMO did not alter the amplitude of the inward component of the current, nor was there a rightward shift in the apparent $E_{rev}$. As expected, oocytes treated with DFMO were more efficient in restoring the resting membrane potential than untreated cells (Fig. 3A). Similar results were obtained when DFMO was injected directly into oocytes expressing the Kir4.1 channels.

We next sought to evaluate the effects of DFMO on the Kir4.1 channels by recording single channel fluctuations. Under the cell-attached configuration and at a holding potential of -40 mV, single-channel events were observed at negative voltage steps. However, at positive step voltages, the openings were minute and remained unresolved (Figs. 3D & 3E). By contrast, in excised patches, depolarized voltage steps elicited robust single channel openings. Consistent
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with the whole-cell current records, cell-attached patches from DFMO-treated oocytes had single-channel openings at depolarized potentials and the probability of channel openings increased substantially in the excised-patch mode (data not shown). The single channel conductance obtained for the Kir4.1 channels (29.1 ± 5.3 pS, n = 7) was in accord with reports from the channel cloned from other systems (39), giving further assurance that the identified Kir4.1 in the StV is not a unique channel.

To ensure the high throughput of K+ across the ICs of the StV, the K+ concentration in the IS should be kept at relatively low levels. Inward rectification of Kir4.1 in the ICs may promote removal of K+ from the IS. Alternatively, like many other inward rectifier currents, the role of Kir channels in ICs may optimize the activity of an unidentified outward current by setting the membrane potential to a level that generates EP. Thus, we surmised that modification of the inward rectification of Kir4.1 in vivo should alter EP and hearing threshold. ABRs were analyzed in DFMO-treated (1 mg/g body weight/day for 4 weeks: Dolye, 2001) CBA mice and compared to their age-matched sham-controls to determine the sound pressure levels at which typical ABR waveforms are seen. The control mice exhibited the characteristic ABR waveform beginning at sound pressure levels of ~40, 30, 15, and 30 dB using broadband clicks, and pure tones of 8, 16, and 32 kHz stimuli (Fig. 4). The DFMO-treated mice yielded elevated thresholds for broadband clicks and the three pure pips tested. The generation of across-frequency elevation of hearing threshold by DFMO raised the possibility that the drug may affect a common trait along the cochlear spiral. Measurement of EP in DFMO-treated and sham-control mice suggested that the ototoxic effects of the drug are mediated at least partly through reduction of EP (Fig. 4B). The relation between increased hearing threshold and a decline in EP (0.8 dB/1
mV) was consistent with previous reports on several animal models, which demonstrated a correlation between hearing loss and EP (40-43).

DISCUSSION

DFMO is, potentially, an efficient cancer-preventive drug. Yet, its insidious ototoxic side effect hinders its use as a mainstream cancer remedy. We have identified, cloned (Genbank Access No. AY374423), and functionally expressed an inner ear lateral wall Kir4.1 channel that may serve as a target for the underlying mechanism for DFMO-induced ototoxicity. We report that the Kir4.1 channel in the lateral wall resembles and has properties that are consistent with an intermediate inward rectifier channel. Furthermore, in contrast to the suggestion that the channel may confer high outward throughput of K+ across ICs into the IS in the StV (17,44), we propose that Kir4.1 in the ICs (16) may control the cells’ membrane potential and operate to reduce the K+ concentration in the IS. This action would serve to promote the steep K+ gradient necessary to generate EP. An increase in the outward component of Kir4.1 current by DFMO is likely to render the gradient shallow, reducing the flux of K+ and consequently resulting in a drop in EP. Alternatively, as an upshot of DFMO-induced reduction in inward rectification, the channel may clamp the IC membrane closed to the Erev of K+, which would produce a similar outcome. Finally, the findings of this report transcend the outline of mechanisms for DFMO-induced ototoxicity. The data suggests that an unidentified outward K+ channel current that confers extrusion of K+ across the ICs should be sought, thus leading to a new understanding of the mechanisms for EP generation.

The rationale for the inhibition of ODC by DFMO as a cancer chemo-preventive strategy is underpinned by the evidence that the enzyme is transactivated by the c-myc oncogene in certain cell/tissue types and cooperates with the ras oncogene in malignant transformation of
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epithelial tissues (45,46). Thus, DFMO is a cancer chemo-preventive drug that is currently used in clinical trials for the prevention of various types of cancer. Its major toxicity is reversible sensorineural hearing loss that has been found in adult humans at moderate doses (47) and in developing animals at low doses (48). Previous studies have demonstrated that the organ of Corti and the lateral wall of rat inner ear have the highest ODC activity, which is consistent with the distribution of polyamines (49,50) and the suggestion that ODC may play a developmental role in the cochlea. Although the effects of polyamines in suppressing the outward component of Kir currents in other systems have been shown as a biophysical phenomenon (51), until now the functional and clinical significance has not been apparent. Moreover, the production of across-frequency hearing loss by DFMO (48) raises the possibility that the drug may alter a common trait along the cochlear spiral. It has been demonstrated that DFMO decreases the level of polyamines in the cochlea (2,3). The fact that the drug alters the inward rectification of StV Kir4.1 was not surprising. However, it was startling to observe that the DFMO-induced suppression of inward rectification reduced EP.

EP is generated across cells in the StV to produce a composite of the main driving force for sensory transduction (5,6). It has been demonstrated that the high throughput of K+ across ICs membrane yields EP (14,52,53). A combination of immunohistochemistry (16,54), pharmacological (44), and null mutant mice experiments (17) have resulted in the assertion that Kir4.1 channels residing at membranes of intermediate cells in the StV confer the extrusion of K+ to polarize the membrane. Marcus et al., (2002) noted the paradox that the present model possesses because of the inward rectification of the channel. However, given the paucity of data, they offer no plausible explanation for the inconsistency in the model.
The Kir channel family is a superfamily of channel proteins that play important roles in maintaining the resting membrane potential and in the secretion as well as absorption of K\(^+\) ions across cell membranes (55). They are tetramers with each subunit consisting of cytoplasmic N- and C-termini, two transmembrane domains, and a pore-forming region. The Kir channel family consists of more than 20 members, which can be classified into seven major subfamilies (56,57). It has been demonstrated that two negatively charged aas (Fig. 1) are crucial for the inward rectification of Kir channels. One is in the second transmembrane segment (21,23,24), and the other in the cytoplasmic C-terminal domain (25,26). Channels such as Kir2.1-2.4, with two negatively charged aas in these positions, confer strong rectification (24,58), whereas those with neutral or positively charged aas produce weak rectification e.g. Kir1.1 (Fig. 1, also (26,33,59). Kir4.1 has one negatively charged residue (E158) and a neutral residue (G210). As expected, it shows an intermediate rectification (Fig. 2).

It is conceivable that modulation of Kir4.1 in the StV may yield sufficient outward flux of K\(^+\) to generate EP. For example, Kir5.1 has been found to form heteromeric channels with Kir4.1 in the renal tubular epithelia, retina, and brainstem (60-64). Compared to the homomeric Kir4.1 channel, the heteromeric Kir4.1-Kir5.1 channel has a large conductance and distinct kinetics. It has been suggested that Kir5.1 may be responsible for physiological modulation of functional Kir channels in the kidney (60-62). Although modulation of Kir4.1 channels by other channels or second messengers remains a viable option, an alternative to the present model for the function of the channel in the generation of EP is made apparent by the effects of suppression of inward rectification by DFMO.

We propose that the main role of Kir4.1 in the ICs of StV is to contribute towards the maintenance of the membrane potential, decrease the K\(^+\) concentration in the IS in order to
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improve the function of other outward K⁺ channels, and confer a steep K⁺ gradient between ICs and the IS. This model neither challenges nor refutes the prevailing evidence that Kir4.1 is involved in the generation of EP (65). It is implicit in our present replica that null deletion of the Kir4.1 channels will alter the resting membrane potential of ICs to more positive values, which will produce a crippling effect on K⁺ extrusion resulting in a reduction in EP. A DFMO-induced increase in outward K⁺ is expected to produce a similar outcome by rendering the K⁺ gradient between the IS and ICs shallow. These findings suggest that other outward K⁺ currents may be responsible for the high throughput of K⁺ ions across IC membranes. Recently, a membrane protein with the signature sequence of TWIK-1 K⁺ channels (66-69) has been identified in the StV. The TWIK-1 channel has weak inward rectifying properties when expressed in Xenopus oocytes and may serve as a background channel (70). In addition, although preliminary, the StV-specific K⁺ channel, MERG1a, has been identified and is poised to contribute to K⁺ flux (71). Thus, EP may be produced and maintained by a cadre of K⁺ channels in the membrane of ICs and MCs in conjunction with K⁺ transporters and pumps.

REFERENCES

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Figure Legend

Figure 1.

Inhibition of mammalian polyamine metabolism by DFMO. Ornithine decarboxylase (ODC), the key enzyme in the mammalian polyamine biosynthetic pathway (left panel), is inhibited by DFMO. DFMO is an analogue of ornithine. The inward rectification of Kir channels is caused by extracellular Mg$^{2+}$ and intrinsic polyamines. The right panel shows a schematic diagram of the Kir channel; M1 and M2 are two transmembrane domains. Two amino acids (aa) that are critical for the inward rectification of Kir channels are indicated with blue dots. One is located in the M2 domain and the other is in the C-terminus. At the bottom of the right panel are the different aa residues of Kir channels that are responsible for the inward rectification (highlighted in blue). N, asparagine; G, glycine; D, aspartic acid; E, glutamic acid; S, serine and Q, glutamine.

Figure 2.

Inner ear-specific inward rectifier K$^+$ channel (Kir4.1) currents. A. Examples of inner ear-specific inward rectifier K$^+$ channel (Kir4.1) currents expressed in Xenopus oocytes. Traces were recorded by applying hyperpolarizing and depolarizing voltage steps (step potentials were
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from -120 to 30 mV from a holding potential of -40 mV). **B.** The I-V relations of the sustained current plotted as a function of voltage. Changes in the I-V as the external K⁺ concentration was altered from 1 to 30 mM. **C.** A plot of the reversal potential versus the external K⁺. Shown in the inset are instantaneous current traces used to generate the plot. **D.** Changes in the resting membrane potential of *Xenopus* oocytes upon application of increasing concentrations of Ba²⁺. The resting potential changed to more depolarized potentials with increasing concentrations of Ba²⁺. **E.** The effect of Ba²⁺ on the inward rectifier current was reversible. The dose response relation of the effect of Ba²⁺ indicated that the half blocking concentration was ~ 30 ± 10 µM Ba²⁺. A plot of the natural logarithm of the function, \(I_c/I_b - 1\), *versus* voltage gives a slope which indicates that Ba²⁺ binds to ~20% of the functional electrical field of the channel. \(I_c\) represents the control current and \(I_b\) is the current after Ba²⁺ block.

Figure 3.

**Effects of DFMO on inward rectification of Kir4.1 channel currents.** **A.** Continuous perfusion of a bath solution containing 20 mM K⁺ produced an expected membrane depolarization. The time course of recovery after washout (arrow) with control saline (ND 96) was faster (\(\tau = 2.3 \pm 1.8\) min, \(n = 6\)) in DFMO-treated than control (\(\tau = 12 \pm 4.7\) min, \(n = 6\)) oocytes. **B.** Kir4.1 current traces after incubation of *Xenopus* oocytes in 500 µM DFMO. **C.** The corresponding current-voltage relation shows that inward rectification of control oocytes was substantially reduced (\(n = 9\)). Single-channel Kir4.1 currents in *Xenopus* oocytes. **D.** Representative and consecutive single-channel traces recorded in a cell-attached patch using pipette [K⁺] = 120 mM. The bath solution contained 120 mM K⁺ and the resting potential of
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oocytes was ~0 mV. The holding potential was -40 mV; the step potentials are indicated. Example of amplitude histograms used to generate the I-V relation as shown in E is depicted as inset. E. The single-channel conductances of control and DFMO-treated oocytes were 29.1 ± 5.3 pS and 31.2 ± 3.6 (n = 7), respectively.

Figure 4.

Effects of DFMO on hearing threshold and endocochlear potential in mice. A. Average ABR threshold for saline-injected control and DFMO-treated 12-week old mice (n = 6) using clicks and pure tones at 8, 16, and 32 KHz. There were significant increases (p < 0.05) in the hearing thresholds of the DFMO-treated mice as compared to age-matched saline-injected controls. B. In addition, DFMO-treated mice had a significant reduction in endocochlear potential (EP (in mV) at the basal turn of the cochlea, control = 87 ± 9; DFMO-treated = 49 ± 14: apical turn, control = 83 ± 5; DFMO-treated = 45 ± 8 mV:  p < 0.05). The correlation between the reduction in EP and increased hearing threshold is striking.
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Figure 1.
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Figure 2.
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Figure 3.
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Figure 4.

A  ABR Thresholds

B  Endocochlear Potentials

* p < 0.05
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*J. Biol. Chem.* published online February 17, 2005

Access the most updated version of this article at doi: [10.1074/jbc.M409856200](http://doi.org/10.1074/jbc.M409856200)

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