**Potassium Ion Channel Openers, Maxipost and Retigabine, Protect Against Peripheral Salicylate
Ototoxicity in Rats**

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Abstract

Sodium Salicylate (SS) reliably induces a sensorineural hearing loss and tinnitus when administered in high doses. Recent animal modeled studies indicate that potassium channel openers such as Maxipost and Retigabine (RTG) can block SS- or noise-induced tinnitus respectively; however, the origins and mechanisms are poorly understood. Since SS blocks the same potassium channels that Maxipost and RTG open, we postulated that these drugs might influence peripheral auditory function. To test this hypothesis Maxipost or RTG were administered alone or in combination with SS in rats. When administered alone, Maxipost and RTG had no effect on distortion product otoacoustic emissions (DPOAE) or compound action potentials (CAPs). However when Maxipost or RTG were administered with SS, Maxipost prevented the SS-reduced CAP amplitudes at high frequencies (≥ 20 kHz) and RTG prevented SS-reduced CAP amplitudes at low frequencies (≤ 8 kHz). These results suggest that Maxipost and RTG can protect against peripheral damage and therefore reduce the incidence of tinnitus.

Key words: Sodium salicylate, potassium channels openers, Maxipost, Retigabine, otoacoustic emissions, compound action potential, ototoxicity, and tinnitus

39 **Introduction**

40 High-dose sodium salicylate (SS), the active ingredient in aspirin, reliably induces temporary
41 sensorineural hearing loss, tinnitus (Bauer, Brozoski, Rojas, Boley, & Wyder, 1999; Jastreboff, Brennan,
42 Coleman, & Sasaki, 1988; Lobarinas, Sun, Cushing, & Salvi, 2004; Yang et al., 2007) and hyperacusis
43 (Chen, Radziwon, Kashanian, Manohar, & Salvi, 2014). Consequently, SS is frequently used to
44 investigate peripheral and central mechanisms of hearing loss, tinnitus and hyperacusis in animal models.
45 However, despite being used in auditory research for more than 50 years SS's mechanisms of action are
46 still not fully understood.

47 SS has a profound influence on the cochlea, including a reduction in OHC electromotility and
48 potassium currents. A significant reduction in distortion product otoacoustic emissions (DPOAEs)
49 accompanies a high-dose SS injection in rats (Chen et al., 2010; Sheppard, Hayes, Chen, Ralli, & Salvi,
50 2014; Stolzberg, Chen, Allman, & Salvi, 2011). This reduction has been reported as frequency-specific
51 (Stolzberg et al., 2011). DPOAEs arise from prestin-mediated outer hair cell (OHC) electromotility,
52 supported in part by the +80 mV endocochlear potential and high potassium concentration in endolymph
53 (Liberman et al., 2002; Schmiedt, Lang, Okamura, & Schulte, 2002). SS competitively suppresses the
54 binding of chloride to anion binding sites on the motor protein prestin (Oliver et al., 2001; Rybalchenko
55 & Santos-Sacchi, 2003), thereby reducing the electromotility of OHCs. SS also blocks potassium (K⁺)
56 currents on cochlear sensory cells. Within inner hair cells (IHCs), SS blocks the outward K⁺ current ($I_{K,f}$),
57 but does not affect the inward potassium current ($I_{K,n}$) in guinea-pigs (Kimitsuki et al., 2011). Based on its
58 sensitivity to iberiotoxin and charybdotoxin (BK blockers), the K⁺ current $I_{K,f}$ represents the large
59 conductance Ca²⁺- and voltage-activated BK channels (Kros & Crawford, 1990; Kros, Ruppersberg, &
60 Rusch, 1998). Within OHCs, SS blocks the K⁺ channel KCNQ4 (Kv7.4) in guinea-pig, which has been
61 suggested as the molecular correlate of the potassium current $I_{K,n}$ (Housley & Ashmore, 1992; Wu, Lv,
62 Kim, Yamoah, & Nuttall, 2010). K⁺ channels have different expression levels along the tonotopic
63 gradient of the mouse and rat cochlea (Beisel, Nelson, Delimont, & Fritzsche, 2000; Engel et al., 2006;

Hafidi, Beurg, & Dulon, 2005; Kharkovets et al., 2000; Wersinger, McLean, Fuchs, & Pyott, 2010). Therefore, SS blockage of BK or Kv7.4 could contribute to a frequency-dependent reduction in compound action potentials (CAPs) or DPOAEs.

The K⁺ ion channel openers BMS-204352 (Maxipost) and Retigabine (RTG) were originally developed for stroke (Gribkoff, Starrett, Dworetzky, et al., 2001) and epilepsy (Rostock et al., 1996), respectively. However, they also have anxiolytic (Korsgaard et al., 2005), anti-nociceptive (Blackburn-Munro & Jensen, 2003; Nodera, Spieker, Sung, & Rutkove, 2011), and neuroprotective properties (Jensen, 2002; Rundfeldt, 1997), resulting from Kv7 and BK K⁺ channel activation. These potassium channels are present in the CNS (Brenner et al., 2005; Hansen et al., 2006; Korsgaard et al., 2005; Sausbier et al., 2006) and on cochlear hair cells of mice and rats with varying expression along the tonotopic gradient (Beisel et al., 2000; Hafidi et al., 2005; Kharkovets et al., 2006). Mutations in four of five known Kv7 potassium channel subunits cause human-inherited diseases, some of which are characterized by central hyperactivity and peripheral deafness (Maljevic, Wuttke, Seebohm, & Lerche, 2010). Since tinnitus has been hypothesized as a central hyperactivity disorder, potassium channel openers have been evaluated for their efficacy in suppressing SS- and noise-induced tinnitus in both mice and rats (Li, Choi, & Tzounopoulos, 2013; Lobarinas et al., 2011). Maxipost eliminates SS-induced tinnitus in rats (Lobarinas et al., 2011), while RTG prevents noise-induced tinnitus in mice (Li et al., 2013). However, these mechanisms are not well understood.

In the present study, we characterize the influence that Maxipost and RTG have on the peripheral auditory system alone and in combination with SS using a rat animal model. Since Kv7 and BK channels are abundantly expressed in the peripheral system of murine species, and are blocked by SS, we hypothesized that Maxipost and/or RTG would enhance the gross cochlear potentials alone or in combination with SS. Alterations occurring in the periphery as a result of these drugs can elucidate the role of potassium channels in cochlear function and SS-induced ototoxicity.

1. Materials and Methods

1.1 Subjects

Seventy-seven male Sprague-Dawley rats (250-500g, Charles River) between the ages of 2-5 months were used in this study. Animals were housed two per cage, provided free access to food and water, and kept on a 12/12 h light-dark cycle. For CAP measurements (n=48), animals were randomly assigned into eight groups: Control (n=6), vehicles (Saline n=6, or dimethyl sulfoxide (DMSO, n=6), SS (n=6), Maxipost (n=6), RTG (n=6), Maxipost + SS (n=6), and RTG + SS (n=6). For DPOAE measurements (n=29), animals were randomly assigned to seven groups: vehicles (Saline n=4, or DMSO n=6), SS (n=3), Maxipost (n=4), RTG (n=4), Maxipost + SS (n=4), and RTG + SS (n=4).

1.2 Compound Action Potentials

Rats were anesthetized with a ketamine (60 mg/kg) and xylazine (6mg/kg) injection intraperitoneally (i.p.). Body temperature was maintained at 37 °C using a homoeothermic blanket (Harvard Apparatus, Holliston, MA). The animal was placed in a custom head-holder, the right bulla was exposed and a small fistula made to access the cochlear round window. A Teflon-coated gold wire (76.2 µm in diameter, Cat#75810, A-M System Inc.) ring-electrode was placed on the round window and a silver chloride reference electrode was inserted into the neck muscles. The tone bursts (2, 4, 6, 8, 12, 16, 20, 24, 30, 35, & 40 kHz) were generated using a Tucker Davis Technologies (TDT) real-time processor (RP2.1, System 3, TDT; Alachua, FL). The signal (duration: 20ms; rise/fall time: 1ms) was presented via a high frequency transducer crafted from an ACO ½" microphone. Stimulation intensities decreased in 10 dB steps from 80 dB SPL using a TDT PA5 programmable attenuator. The cochlear response within a 20 ms time window was amplified (1000x) with a Grass AC preamplifier (Model P15; West Warwick, RI, USA) and filtered online (0.1–50 kHz) using a custom MATLAB program. The CAP component was isolated off-line by low-pass filtering 1 kHz below the stimulation frequency. The vertical distance between N1 and P1 was measured and used to represent the CAP amplitude. Data were plotted and analyzed with

GraphPad software. CAP threshold was defined as the stimulus intensity that elicited a 4 μ V N1 response. Responses were discarded if a reduction in stimulation level did not result in reduced amplitude measurement and demonstrated poor waveform morphology suggestive of electrical noise. Changes in CAP amplitude I/O functions following treatment were tested for significance using two-way ANOVA, and changes in CAP thresholds were tested for significance using one-way ANOVA (Prism GraphPad v5).

1.3 Distortion Product Otoacoustic Emissions

Rats were anesthetized with a ketamine (60 mg/kg) and xylazine (6 mg/kg) injection (i.p.). Using a commercial instrument (Intelligent Hearing Systems; Miami, FL), DPOAEs were obtained with two primary tones (f1 and f2) presented at an f2/f1 ratio of 1.2. The intensity of f1 (L1) was presented 10 dB higher than the intensity of f2 (L2). I/O functions for distortion products 2f1-f2 were plotted for f2 frequencies of 4, 6, 8.6, 13.2, 16, 24, 30 and 35 kHz using L1 intensities of 25-80 dB SPL (5 dB steps). I/O functions were analyzed for significance using two-way ANOVA (Prism GraphPad v5).

1.4 Experimental Conditions

1.4.1 Experiment 1: Vehicles (DMSO & Saline)

Saline was used to dissolve SS and DMSO (Sigma-Aldrich, St. Louis, MO) was used to dissolve Maxipost and RTG. One vehicle group was treated with saline alone and the other vehicle group was treated with DMSO alone. CAPs and DPOAEs were acquired three hours later.

1.4.2 Experiment 2: SS

SS (Sigma-Aldrich) was dissolved in saline (50 mg/ml), and administered via i.p injection (200 mg/kg). This dose reliably induces tinnitus and hearing loss (Chen et al., 2013; Lobarinas et al., 2004; Sheppard et al., 2014; Stolzberg et al., 2011). For DPOAEs, a within-subject experiment, measurements

were made at baseline and three hours post SS. For CAPs, a between-group experiment, recordings were performed three hours post SS.

1.4.3 Experiment 3: Maxipost or RTG Alone

Maxipost (Axon Medchem, 9713 GZ Groningen, The Netherlands) was dissolved in DMSO (25 mg/ml) and administered via i.p. injection (10 mg/kg). Previous reports indicate this dose eliminated behavioral evidence of SS-induced tinnitus in rats (Lobarinas et al., 2011). RTG (Alomone Labs, Har Hotzvim Hi-Tech Park, P.O.Box 4287 Jerusalem 9104201, Israel) was dissolved in DMSO (50 mg/ml) and administered via i.p. injection (10 mg/kg). Previous reports indicate this dose prevents noise-induced tinnitus in mice (Li et al., 2013). DPOAEs were obtained at baseline and again 2 hours post Maxipost or RTG. CAPs were obtained approximately three hours post drug administration.

1.4.4 Experiment 4: Maxipost or RTG +SS

Maxipost or RTG (10 mg/kg i.p.) was administered ten minutes prior to SS (200 mg/kg i.p.). DPOAEs or CAPs were obtained three hours later.

1.5 Compliance

The research project was performed in accordance with regulations approved by the Institutional Animal Care and Use Committee (IACUC, HER05080Y)) at the University of Buffalo and was carried out in accordance with National Institute of Health guidelines.

2. Results

2.1 Vehicles

Figure 1A illustrates CAP amplitudes as a function of tone bursts presented at 80 dB SPL for control, saline, and DMSO groups. Figure 1B illustrates DPOAEs as function of f2 frequencies with L1 intensity set at 70 dB SPL for control, saline, and DMSO groups. There is no significant difference between control

and vehicle (saline and DMSO) treated animals. This indicates that the vehicles used had no significant impact on peripheral hearing.

2.2 SS

It is well-established that SS is ototoxic (Chen et al., 2010; Chen et al., 2013; Stolzberg, Salvi, & Allman, 2012). Figure 2A illustrates that mean ($\pm$ -SEM) CAP thresholds were significantly higher than saline vehicles by approximately 30 dB SPL across the tested frequency range (two-way ANOVA, $p=0.0001$, $F_{1,10}=205$). Figure 2B illustrates mean ($\pm$ -SEM) CAP I/O functions at 6, 20 and 35 kHz. The average CAP amplitudes decreased significantly at all frequencies three hours post SS (two-way ANOVA, 4 kHz $p<0.001$, $F_{1,2}=13.17$; 6-40 kHz $p<0.0001$, $F_{1,4}=13-37$). Figure 2C illustrates mean ($\pm$ -SEM) DPOAE I/O functions 3 hours post SS. DPOAEs were significantly reduced at all tested frequencies (two-way ANOVA, 4-24 kHz $p<0.0001$, $F_{1,11}=18-40$; 30 kHz $p<0.02$, $F_{1,7}=5.948$; 35 kHz $p<0.04$, $F_{1,11}=4.212$). These results indicate that SS induced a mild-to-moderate CAP threshold shift, moderately decreased CAP amplitudes, and mildly reduced DPOAEs, indicative of OHC dysfunction.

2.3 Maxipost and RTG Alone

Figure 3A illustrates CAPs at 80 dB SPL as a function of tone bursts and figure 3B illustrates DPOAEs at $L1=70$ dB SPL as a function of $f2$ frequencies for rats treated with Maxipost, RTG, or the DMSO vehicle. CAPs and DPOAEs for Maxipost or RTG alone were not significantly different from the DMSO vehicle. Similar results were obtained at all other measured intensities. These results indicate that Maxipost or RTG alone did not have a significant effect on peripheral hearing.

2.4 Maxipost and SS

Figure 5A illustrates mean ($\pm$ -SEM) CAP I/O functions at a low (6 kHz), mid (16 kHz), and high (35 kHz) frequency for Maxipost + SS and SS alone groups. For reference, data are shown for saline and DMSO vehicles. Compared to SS, Maxipost + SS group had significantly enhanced mean CAPs at high

frequencies ≥ 20 kHz (two-way ANOVA, 20 kHz: $p=0.024$, $F_{1,4}=5.408$; 24 kHz: $p=0.019$, $F_{1,5}=5.870$; 30 kHz: $p=0.0015$, $F_{1,2}=12.15$; 35 kHz: $p=0.0001$, $F_{1,4}=17.64$; 40 kHz: $p=0.025$, $F_{1,3}=5.354$), but not low or mid frequencies ≤ 16 kHz. Figure 4A illustrates CAP thresholds for Maxipost + SS and SS alone. Maxipost + SS was not significantly different from SS alone (one-way ANOVA, $p>.05$). Figure 5B illustrates mean DPOAE I/O functions for Maxipost + SS and SS alone. There was no significant difference between Maxipost + SS and SS alone (two-way ANOVA, $p>.05$). Since Maxipost reversed SS-reduced CAP amplitudes, but not DPOAEs, these data suggest that Maxipost inhibits SS-induced K⁺ channel blockage of IHCs in the basal high-frequency region of the cochlea.

2.5 RTG and SS

Figure 6A illustrates mean ($\pm$ SEM) CAP I/O functions at a low (6 kHz), mid (16 kHz), and high (35 kHz) frequency for RTG + SS and SS alone groups. For reference, data are shown for saline and DMSO vehicles. Compared to SS alone, RTG + SS significantly enhanced mean CAPs in low frequencies ≤ 8 kHz (two-way ANOVA, 2 kHz: $p<0.05$, $F_{1,1}=6.215$; 4 kHz: $p<0.05$, $F_{1,2}=4.84$; 6 kHz: $p<0.0001$, $F_{1,4}=18.92$; 8 kHz: $p<0.01$, $F_{1,3}=9.796$), but not in mid or high frequencies ≥ 12 kHz. Figure 4B illustrates that CAP thresholds in the low frequencies were significantly reduced in RTG + SS compared to SS alone (one-way ANOVA, 2 kHz: $p<0.01$, $F_{3,4}=8.899$; 4 kHz: $p<0.05$, $F_{3,4}=5.462$; 6 kHz: $p<0.05$, $F_{3,4}=10.42$; 8 kHz: $p<0.01$, $F_{3,4}=20.22$). Additionally, CAP thresholds were not significantly different between saline and RTG + SS (two-way ANOVA, $p>.05$: 2 kHz, 4 kHz, 6 kHz, & 8 kHz), indicating that RTG completely reversed the SS-induced threshold shift in low frequencies. Figure 6B illustrates mean DPOAE I/O functions for RTG + SS and SS alone. Results indicate no significant difference between RTG + SS and SS alone in all tested frequencies (two-way ANOVA, $p>.05$). Since RTG reversed SS-reduced CAP amplitudes, but not DPOAEs, these data suggest that RTG inhibits SS K⁺ channel blockage in the apical low-frequency region of the cochlea.

3. Discussion

3.1 Salicylate Pathophysiology of Kv7.4 and BK K⁺ channels

Previous research using knockout mouse models have determined that BK and Kv7.4 channel function is essential for normal hearing (Kharkovets et al., 2006; Ruttiger et al., 2004), although their direct mechanisms are not well understood. It is speculated that Kv7.4 modulates the resting potential, and therefore excitation of sensory cells through removal of intracellular K⁺ at the basal pole of OHCs (Kubisch et al., 1999). This theory is supported by the basal pole location of Kv7.4 on OHCs (Kharkovets et al., 2000). Salicylate blocks Kv7.4, reduces K⁺ conductance, and subsequently depolarizes guinea-pig OHCs (Wu et al., 2010). This depolarization could reduce the net driving force for K⁺ influx, and subsequently reduce electromotility of OHCs (Wu et al., 2010), resulting in increased thresholds. BK channel function in the mammalian cochlea is unclear but they may play a role in modulating neurotransmitter release (Skinner et al., 2003). This theory is supported by the observation that specific BK channel blockers, charybdotoxin (ChTX) and iberiotoxin (IbTX), significantly reduce sound-evoked cochlear CAPs in guinea pigs (Skinner et al., 2003). Salicylate blocks the IHC BK sensitive K⁺ current $I_{K,f}$ (Kimitsuki et al., 2011), which plays a role in cellular high frequency transduction by accelerating membrane repolarization (Kros & Crawford, 1990). This suggests that salicylate-induced high-frequency hearing loss is due to blocking BK channels, causing impaired IHC repolarization and therefore reduced transmitter release.

3.2 High Frequency Maxipost Protection at BK channels

Our results suggest that Maxipost protection from SS results from an opening action on BK channels in the basal region of the cochlea. BK channels are likely the primary contributor since they are blocked by salicylate (Kimitsuki et al., 2011), opened by Maxipost, but not RTG (Jensen, 2002; Korsgaard et al., 2005; Tatulian, Delmas, Abogadie, & Brown, 2001), and are primarily expressed in the high frequency region of the cochlea (Kimitsuki et al., 2003; Wersinger et al., 2010). Previous studies demonstrate BK α ^{-/-} mice develop a progressive high-frequency hearing loss (Ruttiger et al., 2004). SS-

induced BK channel blockage could impose a similar hearing loss and be reversed by treatment with a BK channel opener, such as Maxipost. Figure 7A speculates the antagonistic influences that SS and Maxipost have on BK channels in the basal region of cochlea and illustrates mean CAP waveforms obtained from saline, SS alone, and Maxipost + SS to a high frequency stimuli (35 kHz).

3.3 Low Frequency Retigabine Protection at Kv7.4 Channels

Our results suggest that RTG protection from SS results from an opening action on Kv7.4 channels in the apical region of the cochlea. Both Maxipost and RTG open Kv7.4 channels (Schroder et al., 2001); however, RTG has a higher affinity for Kv7.4 than Maxipost (Jensen, 2002; Korsgaard et al., 2005; Schroder et al., 2001). Salicylate blocks OHC Kv7.4 currents (Wu et al., 2010), which are primarily expressed on OHCs in the apical region of the cochlea (Beisel et al., 2000). Previous studies indicate that Kv7.4^{-/-} mice develop hearing loss with elevated ABR thresholds and reduced DPOAEs (Kharkovets et al., 2006), suggestive of OHC dysfunction. Therefore, SS-induced Kv7.4 blockage could impose a similar hearing loss and be reversed by treatment with a Kv7.4 channel opener, such as RTG. In contradiction, in the present study, RTG failed to reverse SS-reduced DPOAEs, suggesting that RTG protection was not mediated at the level of OHCs. Additional studies using more sensitive measures such as whole-cell patch clamping are needed for further clarification. Figure 7C speculates the antagonistic influence that SS and RTG have on Kv7.4 channels in the apical region of the cochlea and demonstrates mean CAP waveforms obtained from saline, SS alone, and RTG + SS to a low frequency stimuli (6 kHz).

3.4 Tinnitus and potassium channel openers

While chronic tinnitus is generated, at least partially, within the CNS (House & Brackmann, 1981), the peripheral auditory system plays a role in precipitating tinnitus onset (Bartels, Staal, & Albers, 2007; Salvi, Wang, & Ding, 2000). Therefore, considering the results of the current study, Maxipost or RTG might prevent tinnitus generation by reducing peripheral damage. RTG would suppress low-frequency tinnitus, while Maxipost, in contrast, would suppress high frequency tinnitus. Since SS is

believed to induce a high pitched tinnitus (Jastreboff & Sasaki, 1994; Lobarinas et al., 2004; Yang et al., 2007), these results explain why Maxipost was effective at preventing SS-induced tinnitus (Lobarinas et al., 2011).

Maxipost and RTG also influence the CNS (Brenner et al., 2005; Gribkoff, Starrett, Dworetzky, et al., 2001; Li et al., 2013). Maxipost has cortical neuroprotective properties (Gribkoff, Starrett, & Dworetzky, 2001; Jensen, 2002) and regulates neurotransmitters important for excitation (Jensen, 2002). RTG suppresses noise-induced spontaneous hyperactivity in high frequency fusiform cells of the dorsal cochlear nucleus *in vitro* (Li et al., 2013). Therefore, more detailed research is needed to characterize the effects that Maxipost and RTG have on central auditory structures involved with tinnitus perception.

4. Summary

Consistent with previous studies, our results indicate that CAP amplitudes are suppressed and thresholds increased three hours after a high dose of SS (Chen et al., 2010; Stolzberg et al., 2011; Sun et al., 2009). Recent studies suggest that this observation results in part by SS blockage of BK and Kv7.4 K⁺ channels (Kimitsuki et al., 2011; Wu et al., 2010). Combined treatment of Maxipost or RTG with SS partially or completely reversed SS-reduced CAP amplitudes and thresholds. Maxipost protection was observed in the basal high frequency region (≥ 20 kHz), and RTG protection was observed in the apical low frequency region (≤ 8 kHz). These results are likely due to (1) the tonotopic dependent expression of K⁺ channels in the cochlea, and (2) the antagonistic influence of SS (blocking K⁺ channels) and Maxipost or RTG (opening K⁺ channels).

Conflict of Interest

The authors declare that there is no conflict of interests regarding the publication of this article.

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Figure Legends

Fig.1 – Vehicles had no impact on peripheral hearing. (A) CAP amplitudes obtained with 80 dB SPL tone bursts (6, 8, 12, 16, 20, 24, 30, 35, 40 kHz). (B) DPOAEs measured with L1=70 dB SPL ($f_2 = 5.3, 6, 12, 16, 20, 24, 30, 35$ kHz). Presented data includes control animals (n=6), and vehicles animals, saline (n=6) or DMSO (n=6), (ns= not significant)

Fig.2 – SS significantly reduces peripheral hearing. (A) CAP thresholds (smallest detectable response above $4\mu V$) three hours after saline and SS. CAP thresholds are significantly increased in the SS group across the frequency spectrum. (B) CAP amplitudes at a low- mid- and high- frequency. All amplitudes were significantly decreased in the SS group. (C) DPOAEs at a low- mid- and high-frequency. All DPOAEs were significantly decreased in the SS group. Circles represent saline group, X's represent SS group, and dotted lines in DPOAEs (C) represent noise floors. (***=p<0.001, **p<0.01, *=p<0.05)

Fig.3 – Maxipost or RTG alone have no impact on CAPs or DPOAEs. (A) CAP amplitudes for DMSO vehicle, Maxipost, and RTG alone groups acquired at 80 dB SPL (6, 8, 12, 16, 20, 24, 30, 35, 40 kHz). (B) DPOAEs obtained with L1= 70 dB SPL ($f_2 = 5.3, 6, 12, 16, 20, 24, 30, 35$ kHz), (ns= not significant).

Fig. 4- RTG but not Maxipost significantly reduces SS-enhanced thresholds. (A) Illustrates CAP threshold shift (DMSO thresholds = 0) for Maxipost + SS and SS. Maxipost did not significantly reduce SS enhanced thresholds. (B) Illustrates CAP threshold shift (DMSO thresholds = 0) for RTG + SS and SS. RTG + SS thresholds were significantly reduced in the low frequencies (2, 4, 6, 8 kHz) when compared to the SS group (one-way ANOVA, 2 kHz: p<.01, 4 kHz: p<.05, 6 kHz: p<.05, 8 kHz: p<.01) and were not significantly different from vehicle CAP thresholds (two-way ANOVA, p>.05, 2 kHz, 6 kHz, and 8 kHz)

Fig.5- Maxipost protects against SS-induced high-frequency amplitude reductions in CAPs, but not DPOAEs. (A) CAP amplitudes of Maxipost + SS and SS at a low- mid- and high-frequency. Maxipost +

SS significantly protected against SS-reduced CAP amplitudes in high frequencies (two-way ANOVA, $***=p<0.001$, ns=not significant). (B) DPOAE amplitudes from Maxipost + SS and SS at a low- mid- and high frequency. There was no significant difference in DPAOs (two-way ANOVA, $p>.05$, ns=not significant). Dashed lines represent saline vehicle and dotted lines represent DMSO vehicle for reference.

Fig. 6- RTG protects against SS-induced low-frequency amplitude reduction in CAPs, but not DPOAEs. (A) CAP amplitudes from RTG + SS and SS at a low- mid- and high-frequency. RTG + SS significantly protected against SS-reduced CAP amplitudes in low-frequencies (two-way ANOVA, $***=p<0.001$, ns=not significant). (B) DPOAEs from RTG + SS and SS at a low- mid- and high-frequency. There was no significant difference in DPOAE amplitudes between RTG + SS and SS across the frequency spectrum (two-way ANOVA, $p>.05$, ns=not significant). Dashed lines represent saline vehicle and dotted lines represent DMSO vehicle for reference.

Fig. 7- SS, Maxipost, and RTGs interaction on tonotopic specific K⁺ channels, and resulting normalized mean CAP waveforms demonstrating SS protection. (A) BK channels are primarily expressed in the basal high frequency region of IHCs, SS blocks BK channels, and Maxipost opens BK channels. (B) Saline (n=4), SS (n=4), and Maxipost + SS (n=4) normalized mean CAP waveforms (35 kHz, 60 dB SPL). SS alone reduces amplitudes while Maxipost + SS partially restore amplitudes. (C) Mild expression of BK and Kv7.4 channels in the mid frequency region of the cochlea. (D) Saline (n=4), SS (n=4), Maxipost + SS (n=4), and RTG + SS (n=4) normalized mean CAP waveforms (16 kHz, 60 dB SPL). SS reduced amplitudes, but Maxipost + SS nor RTG + SS restored amplitudes. (E) Kv7.4 channels are primarily expressed in the apical low frequency region of OHCs, SS blocks Kv7.4 channels, and RTG opens Kv7.4 channels. (F) Saline (n=4), SS (n=4), and RTG + SS (n=4) normalized mean CAP waveforms (6 kHz, 60 dB SPL). SS alone reduces amplitudes while RTG + SS fully restore amplitudes. Bar densities represent higher channel expression. Waveforms: Dashed black=saline, red=SS, blue=Maxipost + SS, green= RTG + SS.

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Figure 1

Figure 2

A

B

C

Figure 3

Figure 4

A

Maxipost CAP Threshold Shift

B

Retigabine CAP Threshold Shift

Figure 5

A

CAP

B

DPOAE

Figure 7