Paradoxical enhancement of active cochlear mechanics in long-term administration of salicylate

Zhiwu Huang*, Yanyun Luo*, Zhanyuan Wu*, Zhezhang Tao*, Raleigh O. Jones#, Hong-Bo Zhao#

*Dept. of Otolaryngology, People’s Hospital, Faculty of Medicine, Wuhan University, Wuhan, China.
#Dept. of Surgery – Otolaryngology, University of Kentucky Medical Center, Lexington, KY 40536-0293.

Running title: Increase in DPOAEs in long-term salicylate treatment

Corresponding author:
Hong-Bo Zhao, Ph.D./M.D.
Assistant Professor
Dept. of Surgery – Otolaryngology
University of Kentucky Medical Center
800 Rose Street
Lexington, KY 40536 – 0293
Tel: 859-257-5097 x 82138
Fax: 859-257-5096
E-mail: hzhao2@uky.edu

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Abstract.

Aspirin (salicylate) is a common drug and frequently used long-term in the clinic. It has been well documented that salicylate can cause reversible hearing loss and tinnitus, and diminish outer hair cell (OHC) electromotility, which is capable of actively boosting the basilar membrane vibration and producing acoustic emission. However, aspirin’s ototoxic mechanisms still remain largely unclear. In this experiment, the effects of long-term salicylate administration on cochlear hearing functions were investigated by measuring distortion product otoacoustic emissions (DPOAEs) in awake guinea pigs. A single injection of sodium salicylate (200 mg/kg) could reduce the amplitude of the cubic distortion product of $2f_1-f_2$ within 2 hours. The reduction was significant at 20-50 dB SPL stimulus levels and recovered after 8 hours. However, following daily injections of sodium salicylate (200 mg/kg, b.i.d.), the distortion product of $2f_1-f_2$ progressively increased. After injection for 14 days, the distortion product increased about 2-3.5 dB SPL. The increase rate was about 0.2 dB SPL/day. The DP-I/O function remained nonlinear. The increase was greater at 40-70 dB SPL primary sound intensities and reversible. After cessation of salicylate treatment for 4 weeks, the increased distortion product returned to the initial normal levels. The rate of recovery was 0.1 dB SPL/day. In the control animals with saline injection, there was no change in DPOAEs. The data revealed that long-term administration of salicylate could paradoxically enhance active cochlear mechanics. The data also suggested that salicylate-induced tinnitus might be generated at the OHC level.
Introduction

Otoacoustic emissions are sounds emitted by the cochlea and can be acoustically recordable in the external auditory canal (Kemp 1978). These emissions provide evidence for an active mechanical amplification of sounds in the inner ear (Wilson 1980; Kim 1980; Rosowski et al. 1984; Norton 1992). Many experimental data (Probst 1990; Whitehead et al. 1992a, b; Fitzgerald et al. 1993; Frolenkov et al. 1998; Grosh et al. 2004), including genetic studies (Liberman et al. 2002; Cheatham et al. 2004a), indicate that these active cochlear mechanics mainly originate from outer hair cells (OHCs). In the mammalian cochlea, OHCs have electromotility that can actively enhance the basilar membrane vibration in response to acoustic stimulation (Brownell et al. 1985). Elimination of OHC electromotility can induce hearing loss and also result in elimination or decrease in otoacoustic emissions (Lonsbury-Martin and Martin 1990; Harris 1990; Liberman et al. 2002). Hence, otoacoustic emission measurement provides a reliable noninvasive test to examine OHC and cochlear functions in vivo.

Salicylate (aspirin) is a widely used drug in clinics. It has been known that salicylate can cause reversible tinnitus and hearing loss (Myers and Bernstein 1965; Matz 1990; Boettcher and Salvi 1991; Brien 1993; Cazals 2000). Electrophysiological and histological studies suggest that salicylate mainly acts on cochlear OHCs to influence hearing function. Salicylate can reversibly eliminate OHC electromotility. Outer hair cells perfused with salicylate solution show vesiculation of subsurface cisternae in their lateral walls, and a reversible reduction in the turgidity, axial stiffness, electromotility, and motility-associated nonlinear capacitance (Dieler et al. 1991; Shehata-Dieler et al. 1991; Tunstall et al. 1995; Kakehata and Satons-Sacchi 1996; Lue and Brownell 1997). A recent experiment further revealed that salicylate can competitively bind the motor protein prestin with its external voltage sense of Cl⁻ ions to inhibit OHC electromotility (Zheng et al. 2000; Oliver et al. 2001). In in vivo recording, salicylate reversibly eliminated spontaneous otoacoustic emissions
SOAEs) and reduced distortion product otoacoustic emissions (DPOAEs) acutely after administration (McFadden and Plattsimer 1984; Long and Tubis 1988; Martin et al. 1988; Wier et al. 1988; Kujawa et al. 1992; Fitzgerald et al. 1993; Janssen et al. 2000). These effects are consistent with hearing loss observed in the clinic after treatment with a high dose of salicylate.

However, it is difficult to reconcile these changes with salicylate-induced tinnitus generation. Tinnitus is a virtual auditory sense without a corresponding acoustic stimulus, demonstrating hypersensitivity in the auditory system. Many factors can act on central and peripheral auditory systems to induce tinnitus (Jastreboff 1990; Kaltenbach 2000; Salvi et al. 2000; Baguley 2002; Lockwood et al. 2002). In the clinic, repetitive administration of a relatively high dose of salicylate can uniformly cause tinnitus perception (see a review, Cazals 2000). Daily treatment with salicylate can also induce animals to develop tinnitus (Jastreboff and Sasaki 1986; Jastreboff et al. 1988a, b; Guitton et al. 2003). It has been reported that salicylate increases the spontaneous activity of the auditory nerves (Evans and Borerwe 1982; Stypulkowski 1990) and changes the average spectrum of cochleoneural activity (Schreiner and Snyder 1987; Martin et al. 1993; Cazals et al. 1998). Increased spontaneous neural activities also occurred in the inferior colliculus (Jastreboff and Sasaki 1986; Chen and Jastreboff 1995; Manabe et al. 1997) and the auditory contex (Ochi and Eggermont 1996). However, the precise mechanism still remains unclear. In this experiment, we adopted recording of DPOAEs in awake guinea pigs to examine the effect of salicylate on cochlear and OHC functions. The data showed that a single injection of salicylate could reduce the amplitude of distortion product of $2f_1-f_2$ in otoacoustic emissions, but long-term administration of salicylate progressively raised the distortion product. This suggested that the long-term administration of salicylate may paradoxically enhance OHC electromotility. The data also provides evidence that OHCs may play an important role in salicylate-induced tinnitus generation.
Methods

Animal preparation

Adult guinea pigs (250~300g) of either sex with normal pinna reflex and no middle ear infections were used in the experiments. During the DPOAE measurement, the guinea pig was placed in a box in a double-wall soundproof cabin without anesthesia and its head was gently fixed with a nose ring. Before measurement, animals were trained one or two times to accustom them to the testing environment. Most animals were quiet during the recording. The testing would be repeated if the recording was unstable.

Distortion product measurement

A cubic distortion component of \(2f_1-f_2\) in DPOAEs was measured using a CELESTA 503 Cochlear Emission Analyzer (Madsen, Denmark). Two plastic tubes with 5-cm long and 3-mm inner diameter were inserted into the external ear canal and sealed with an earplug. Two pure tones \(f_1\) and \(f_2\) were simultaneously delivered into the ear. The ratio of \(f_2\) versus \(f_1\) \((f_2/f_1)\) was 1.22. The test frequency was presented by a geometric mean of \(f_1\) and \(f_2\) \([f_0=(f_1\times f_2)^{1/2}]\) from \(f_0=0.75\) to 8 kHz. The intensity of \(f_1\) (L_1) was set at 5 dB SPL (0 dB SPL re. 20 \(\mu\)Pa) higher than that of \(f_2\) (L_2). The distortion product was recorded from the L_1/L_2 level of 15/10 dB SPL to 70/65 dB SPL in a step of 5 dB SPL. One hundred-fifty responses were averaged. The recording was set to be automatically stopped when the amplitude of the 2f_1-f_2 component was lower than 2 standard deviations of the noise floor level.

Salicylate administration and experiment procedure

Sodium salicylic acid was purchased from Sigma (St. Lois, MO, USA) and freshly dissolved in saline to a concentration of 200 mg/ml. In the acute effect study, a single dose of sodium salicylate
(200 mg/kg) was administered to 10 guinea pigs through intramuscular injection. The equivalent volume of saline was given to 5 guinea pigs at the same time as control. The distortion product of $2f_1-f_2$ was measured at 30 min prior to the injection and at 2, 4, and 8 hours after the injection. In the long-term administration experiment, sodium salicylate of 200mg/kg was intramuscularly injected into 8 guinea pigs at 9:00 am and 6:00 pm every day for 14 days. Three other guinea pigs were given injection of the equivalent volume of saline as a control group. The distortion product of $2f_1-f_2$ was measured at 30 min prior to the injection at 9:00 am. The day of beginning injection was referred to day 0. Distortion product otoacoustic emissions were measured at day –7 and –3 prior to the administration of salicylate, day 0, 3, 7, 10 and 14 in the injection period, and the 1st, 2nd, 3rd, and 4th week after cessation of the injection (see Fig. 3).

Data analysis

The statistical analyses were performed using a commercial software, SPSS v10.0 (SPSS Inc, Chicago, IL). A level of $P<0.01$ was accepted as a statistical significance.

This work was approved and carried out in conformity with all applicable regulations and institutional use rules for the use of animals in research.
Results

DPOAEs in normal awake guinea pigs

In this experiment, we measured DPOAEs in awake guinea pigs to study the effects of salicylate on the cochlear function. Fig. 1 shows the distortion product of $2f_1-f_2$ in the normal awake guinea pigs (35 ears). The distortion product of $2f_1-f_2$ shows an increase for the test frequency ($f_0$) from 0.75 kHz to 8 kHz (Fig. 1A). Inset in Fig. 1A is a recording spectrum showing that the amplitude of $2f_1-f_2$ was about 50 dB SPL at $f_0$ of 8 kHz and the primary sound pressure levels were 70/65 dB SPL. The recording also had a good repeatability. The distortion product audiograms (DP-gram) were almost identical in the repeated recordings at different days (Fig. 1A). The distortion product of $2f_1-f_2$ increased with stimulus sound intensity (Fig. 1B). There is a notch visible at 60/55 dB SPL in the I/O function. The recording noise levels were about –20 dB SPL and were flat in the test intensity range from $L_1/L_2$ of 20/15 dB to 70/65 dB SPL.

Fig. 1

Acute effect of single injection of salicylate on DPOAEs

A single salicylate injection could reduce distortion products in acoustic emissions. Fig. 2 shows the reduction in otoacoustic emissions after the single injection of salicylate. The reduction in the distortion product of $2f_1-f_2$ was significant at 2 hours after the injection of salicylate (Fig. 2). The acute reduction in the distortion product of otoacoustic emissions for salicylate injection was reversible. Eight hours after the injection, the distortion product level had almost recovered to the initial normal level. In the control group with saline injection, the distortion product had no alteration and remained stable during the whole test period (empty circles in Fig. 2).
In contrast with the acute effect of short-term application of salicylate, long-term application of salicylate induced an increase in the distortion product of $2f_1-f_2$ (Figs. 3-5). Fig. 3 shows the audiogram of $2f_1-f_2$ in long-term administration of salicylate measured from $f_0$ of 0.75 to 8 kHz. The distortion product of $2f_1-f_2$ progressively increased during the salicylate treatment at every test frequency (Figs. 3 and 4). After daily injection of salicylate for 14 days, the distortion product raised about 2-3.5 dB SPL, which was significantly different from the normal level ($p<0.01$, ANOVA). The increase was reversible. After cessation of the injection of salicylate, the raised distortion product level slowly reduced (Fig. 3). Four weeks after cessation of the injection, the distortion product returned to the normal control level and was completely recovered (Figs. 3 and 4A). In the control group with saline injection, there was no change in the distortion product of $2f_1-f_2$ (data not shown).

The increase and recovery in the distortion product of $2f_1-f_2$ during long-term administration of salicylate demonstrated linear changes for our experimental period (Fig. 5). The slope of increase in the distortion product of $2f_1-f_2$ was about 0.2 dB SPL/day at $f_0=1$ kHz to 3 kHz, and 0.15 dB SPL/day at low (0.75 kHz) and high (6-8 kHz) test frequencies (Fig. 5B). Compared with the increase rate in the injection period, the decrease in the distortion product during the recovery
period was slow; the slope of recovery change was about 0.1 dB SPL/day and showed the same recovery rate among the test frequencies (Fig. 5B).

Fig. 5

*Effects of salicylate on the I/O function of distortion products*

Salicylate also altered the I/O function of distortion product (DP-I/O function). The left column in Fig. 6 shows that the reduction in the acoustic emission for a single injection of salicylate was significant at low and middle sound pressure levels and shifted the DP-I/O function downward. The nonlinearity of the DP-I/O functions was retained. The notch was visible but its position was shifted to high intensities. Compared with suppression on the I/O function of $2f_1-f_2$ in acute response to single salicylate injection, long-term administration of salicylate raised the distortion product levels and shifted the I/O function upward (right column in Fig. 6). The increase was significant at high sound pressure levels. The notch was also visible but shifted upwards.

Fig. 6

Fig. 7 shows the difference in distortion products ($\Delta 2f_1-f_2$) for acute and long-term administration of salicylate. For a single salicylate injection (the left column in Fig. 7), the reduction in the distortion product decreased with sound pressure levels. The change was almost linear except there was a notch at 60/55 dB SPL. The slope of change was 0.2 - 0.35 dB SPL. In the control group with saline injection, no change was detectable (empty circles in Fig. 7). For long-term administration of salicylate (the right column in Fig. 7), the distortion product increased at low sound pressure levels, became saturated and then slightly decreased at high sound pressure levels.
There was also no significant change in the distortion product in the control group with the saline injection (empty circles in Fig. 7). This also demonstrated that the recording in long-term repeated measurements was stable.

Fig. 7
Discussion

Effects of salicylate on otoacoustic emissions and cochlear mechanics

It has been well documented that salicylate can reduce otoacoustic emissions. In the clinic, consumption of aspirin uniformly reduced the SOAEs to levels that were unmeasurable, or approaching the noise floor of the measurement system (Johnsen and Elberling 1982; McFadden and Plattsmier 1984; Long and Tubis 1988; Ueda et al. 1996). The aspirin also reduced the amplitude of DPOAEs, but did not abolish them. Intracochlear perfusion of salicylate reduced basilar membrane movement (Murugasu and Russell 1995; Grosh et al. 2004) and 2f₁-f₂ distortion products in the ear canal spectrum and in the cochlear microphonic (CM) responses (Kujawa et al. 1992; Fitzgerald et al. 1993; Frank and Kossi 1996). In in vitro recording, perfusion of salicylate also diminished the electronic distortion products evoked by two sinusoidal stimuli in isolated OHCs (Takahashi and Santos-Sacchi 1999; Zhao and Santos-Sacchi 1999).

In this study, single salicylate injection could rapidly reduce the amplitude of 2f₁-f₂ (Fig. 2); the decrease was significant at low intensity levels (Figs. 6 and 7). This is consistent with previous reports that the degree of amplitude reduction was greater at low primary sound levels (Wier et al. 1988). It has been reported that intraperitoneal injection of 460 mg/kg sodium salicylate can induce a serum concentration of salicylate at 600-700 mg/L and of about 200 mg/L in the cochlear perilymph within 2-4 hours in the guinea pig (Jastreboff et al. 1986). In this experiment, we used 200 mg/kg of sodium salicylate intramuscular injection. The salicylate concentration in the cochlear perilymph would be ~ 90 mg/L (Cazals, 2000). This concentration of salicylate could reversibly eliminate OHC electromotility in in vitro patch clamp recording (Dieler et al. 1991; Shehata-Dieler et al. 1991; Tunstall et al. 1995; Kakehata and Satons-Sacchi 1996).

However, long-term administration of salicylate could increase the DPOAE levels (Figs. 3-7). As evidenced by their high emission levels, OHCs are obviously functioning. Although there is a
debate on the origination and mechanisms of otoacoustic emissions, there is no doubt that the
otoacoustic emissions reflect the active cochlear mechanics. In the mammalian cochlea, OHCs have
an electromotility capable of actively enhancing basilar membrane vibration (Brownell et al. 1985),
and this electromotility was termed an active cochlear amplifier (Dallos 1992). Knockout of the
prestin gene can result in reduction in DPOAEs and hearing loss (Liberman et al. 2002; Cheatham
et al. 2004a). It has also been found that Cl⁻ ions work as an external voltage sensor of prestin to
trigger cell movement; salicylate can competitively bind prestin with Cl⁻ to eliminate OHC
electromotility (Oliver et al. 2001). However, long-term administration of salicylate increased the
distortion products (Figs. 3-7). Several mechanisms could be underlying this enhancement. For
example, long-term use of salicylate may induce prestin upregulation and/or relative increase in
affinity of prestin with anionic ions (external voltage sensors). It has been reported that prestin is
upregulated in prestin⁻/⁺ heterozygous mice (Cheatham et al., 2004b). Long-term use of salicylate
may also result in increase in the mechanical nonlinearity associated with stereociliary transduction.
Stereocilia mechanics can produce acoustic emissions (Hudspeth 1997; Liberman et al. 2004).
Finally, long-term administration of salicylate may also induce alteration in cyclooxygenase activity
to affect active cochlear mechanics. Salicylate can inhibit cyclooxygenase activity (Vane 1971;
Mitchell et al. 1993; Vane and Botting 1998). However, it has been reported that daily
intraperitoneal injections of mefenamate, a potent cyclooxygenase inhibitor, did not change the
tinnitus-like behavior in animals (Guitton et al. 2003). It has also been found that intracochlear
perfusion of mefenamate had no effect on cochlear function (Puel et al. 1990).

The possible mechanisms of salicylate-induced tinnitus generation

Salicylate is a well-known ototoxic drug that can cause reversible tinnitus and hearing loss.
Tinnitus often appears as the first or as an only subjective symptom (Mongan et al. 1973; Day et al.
1989). In the clinic, tinnitus often appears during administration of aspirin (salicylate) after several days to several weeks, becomes louder as treatment is continued, and sounds like a high-pitch noise (McCabe and Dey 1965; Myers and Bernstein 1965; Mongan et al. 1973; Day et al. 1989). Behavioral experiments with rats also give evidence of tinnitus occurring after 26 hours of treatment, increasing in loudness with duration of treatment, and having a high pitch (Jastreboff and Sasaki 1994). Tinnitus is thought to be associated with increased or enhanced auditory neuronal activities. It has been reported that salicylate could increase spontaneous activity of the inferior colliculus (Jastreboff and Sasaki 1986; Chen and Jastreboff 1995; Manable et al. 1997) and the auditory cortex (Ochi and Eggermont 1996; Eggermont and Kenmochi 1998). These changes may cause generation of tinnitus perception. However, the most substantial pharmacological and pathological effects of salicylate occur in the cochlea (Cazals 2000). At the auditory nerves, salicylate could increase spontaneous activity, broaden tuning curve, and reduce $Q_{10}$ values (Evans and Borerwe 1982). Cazals et al. (1998) monitored changes in the average spectrum of electrophysiological cochleoneural activity (ASECA) in long-term administration of salicylate. They found that the ASECA rose at 1 kHz during the treatment. The increase was reversible and returned to the normal level after cessation of the treatment. It has been hypothesized that imbalance between inner hair cell and outer hair cell activities can trigger tinnitus happening (Jastreboff, 1990). In this experiment, short-term application of salicylate reversibly reduced the distortion product and long-term administration of salicylate increased the amplitude of $2f_1-f_2$ (Figs. 2-7). Both changes could be associated with the OHC activity. This further indicates that OHCs may play an important role in salicylate-induced tinnitus generation.

It has been reported that DPOAEs could be measured in tinnitus affected ears despite the severe hearing loss (Janssen et al. 1998). Enhancement of otoacoustic emissions was also reported in temporary noise-induced tinnitus after exposure of a loud low-frequency tone or in acoustic trauma
ears with tinnitus (Attias et al. 1996). Computer modeling showed that the impedance alteration of cochlear micromechanics caused by OHC malfunction was responsible for the generation of inner ear standing waves (Zweig and Shera 1995), which can result in both spontaneous otoacoustic emissions and tinnitus. Recently, the distortion products have been demonstrated capable of directly generating from nonlinearity of voltage dependence of OHC electromotility (Takahashi and Santos-Sacchi 1999) and electro-mechanical coupling between OHCs (Zhao and Santos-Sacchi 1999). The generation of distortion products increased as the amplitude of stimulus voltage increased. In our in vivo recording, the increasing of the $2f_1-f_2$ component was greater at moderate-to-high sound intensities (Figs. 6 and 7). Apparently, OHCs would produce high mechanical distortion when stimulated by high-level stimuli. This can also induce improper excitation for the inner hair cells causing tinnitus.

In sum, we studied the effects of salicylate on cochlear function by measuring DPOAEs in awake guinea pigs in this experiment. We found that long-term administration of salicylate could paradoxically increase the cubic distortion product of $2f_1-f_2$. This finding implied that long-term administration of salicylate could enhance active cochlear mechanics. This also suggested that the salicylate-induced tinnitus might be generated at the OHC level.
Acknowledgments:

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Figure legends:

Fig. 1. Distortion products of acoustic emission (DPOAE) measured from awake guinea pigs. A. A cubic distortion product of 2f₁-f₂ in responses to two-tone stimuli with f₀ of 0.75 to 8 kHz at the sound pressure intensity of 70/65 dB SPL. The distortion product was repeatedly measured in the same animal group over 3 days. No significant difference (p > 0.05, ANOVA) is visible in the repeated measurements. Inset: The spectrum of response was recorded at f₀=8 kHz and 70/65 dB SPL. B. The distortion product of 2f₁-f₂ was measured with f₀ of 4, 6, and 8 kHz. L₁ and L₂ represent the intensities of f₁ and f₂, respectively. Symbol-lines represent the I/O functions of 2f₁-f₂ at f₀ of 4, 6, and 8 kHz from L₁/L₂ of 20/15 dB SPL to 70/65 dB SPL in a 5-dB step. Non-symbol lines represent the test noise floor levels. Error bars represent SD.

Fig. 2. Acute effect of single salicylate injection on the cubic distortion product of 2f₁-f₂. The time of salicylate injection is referred to zero, and indicated by a vertical dotted line. The distortion product of 2f₁-f₂ was measured at 30 min prior to the injection of salycilate or saline, and at 2, 4, and 8 hours after the injection. Solid circles and empty circles represent the distortion products measured from the salicylate injection group and the saline injection control group, respectively. Error bars represent SD. Stars indicate that the distortion product of 2f₁-f₂ in the salicylate injection group was significantly reduced at 2- and 4-hour points after the injection (p < 0.01, ANOVA). The distortion product almost completely recovered after 8 hours.

Fig. 3. Effect of long-term administration of salicylate on acoustic emission. The cubic distortion product of 2f₁-f₂ was measured before, during, and after the injection. A pair of vertical dotted lines represents the period of salicylate injection (14 days). The day of starting the salicylate injection is
defined as day 0. The distortion product of $2f_1-f_2$ was measured at $L_1/L_2=70/65$ dB SPL from $f_0=0.75$ to 8 kHz. Data were averaged from measurements in 14 ears. Error bars represent SD.

Fig. 4. Increases in DPOAEs for long-term administration of salicylate. *A.* Reversible increases in DP-gram. The DP-gram was measured from $f_0$ of 0.75 to 8 kHz at the intensity of 70/65 dB SPL. Solid circles, empty circles, and solid triangles represent the distortion product of $2f_1-f_2$ measured at 3 days prior to the injection of salicylate, at 14 days of injection, and after cessation of the injection for a month, respectively. The data were extracted from Fig. 3. Error bar represents SD. *B.* Histogram analysis of increase in distortion products for long-term administration of salicylate. The difference of $2f_1-f_2$ ($\Delta 2f_1-f_2$) was calculated from the distortion products at the injection day 14 subtracted by the distortion products measured at the day –3 prior to the injection in the same animals. Error bars represent SD.

Fig. 5. Quantitative analysis of changes in distortion products for long-term administration of salicylate. *A.* Changes in the distortion product of $2f_1-f_2$ measured at $f_0=4$ kHz and $L_1/L_2=70/65$ dB SPL. Long-dashed lines represent linear fitting of changes in distortion products during the salicylate injection and recovery after cessation of the salicylate treatment. $S_{\text{inj}}$ and $S_{\text{rec}}$ represent the fitting slopes at the injection and recovery period, respectively. The fitting equation is $y=0.2x + 42.1$ ($r=0.99$) for the injection period and $y=-0.1x + 45.8$ ($r=0.99$) for the recovery period. Stars indicate the significant changes in the distortion product compared with the pre-injection level ($p<0.01$, ANOVA). *B.* A plot of slope changes in $2f_1-f_2$ against $f_0$. Solid and empty circles represent the injection period slope and the recovery slope ($S_{\text{inj}}$ and $S_{\text{rec}}$), respectively. $S_{\text{inj}}$ is almost 2 times larger than $S_{\text{rec}}$. 
Fig. 6. Effects of salicylate on the I/O function of $2f_1-f_2$. Left column represents the effect of a single injection of salicylate on the DP-I/O function. Solid and empty circles represent the I/O functions of $2f_1-f_2$ measured before and at 2 hours after the salicylate injection, respectively. Data were averaged from 10 ears. Error bars represent SD. Right column represents the effect of long-term administration of salicylate on the I/O-function. Solid circles, empty circles, and solid triangles represent the I/O functions measured at day 3 before the injection, at day 14 during the injection, and at the 4th week after the cessation of salicylate injection, respectively. Data were averaged from 14 ears. Error bars represent SD.

Fig. 7. Difference plots of changes in the DP-I/O functions of the salicylate injection group and saline injection control group. Left and right columns represent effects of single injection and long-term injection of salicylate on the DP-I/O function, respectively. The DP-I/O functions were measured at the $f_0$ of 8, 6, and 4 kHz. Solid and empty circles represent differences in changes in the distortion product in the salicylate treated group and the saline control group, respectively. For acute effect, the difference between the distortion products of $2f_1-f_2$ ($\Delta 2f_1-f_2$) measured at 30 min prior to the injection and at 2 hrs after the injection in the same animal was calculated and then averaged (n=10 and 5 ears for salicylate and saline control groups, respectively). For long-term administration of salicylate, $\Delta 2f_1-f_2$ was calculated from the distortion product at the injection day 14 subtracted by the measurement at the day -3 prior to the injection in the same animal, then averaged (n=14 and 6 ears for salicylate treatment and saline control, respectively). Error bars represent SD. Dotted lines represent data linear fitting. In the right column for long-term effect, only the data points in the low intensity range (below 35/30, 45/40, and 50/45 dB SPL for $f_0=8$, 6, and 4 kHz, respectively) were used for fitting.
References


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Huang et al. Fig. 1
Huang et al., Fig. 2
2f₁ - f₂ (dB SPL) vs Time (day)

L₁/L₂ = 70/65 dB SPL

f₀:
- 8 kHz
- 6 kHz
- 4 kHz
- 3 kHz
- 2 kHz
- 1.5 kHz
- 1 kHz
- 0.75 kHz

n = 14

Huang et al. Fig. 3
Huang et al. Fig. 4
Huang et al. Fig. 5
Huang et al., Fig. 6
Huang et al., Fig. 7