A Characteristic of Aspirin-Induced Hearing Loss in Auditory Brainstem Response of Conscious Rats

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ABSTRACT. The acute effects of aspirin on auditory functions were examined electrophysiologically in conscious rats with chronically implanted electrodes for auditory brainstem response (ABR) recording. A single intravenous injection of aspirin at a dose of 225 mg/kg caused a reduction in the amplitude of the ABR P1 wave evoked by a 2 kHz tone pip 1 and 24 hr after dosing at almost all sound intensity levels, while the P1 amplitude at 4 kHz was reduced mainly 1 hr after dosing, and the P1 amplitude at 8 kHz was not significantly affected at middle and high intensities even 1 hr after dosing. The audiogram obtained from the P1 amplitude showed a significant increase in the sound threshold 1 and 24 hr after dosing at 2 kHz, and 1 hr after dosing at 4 kHz, but not at 8 kHz. The peak latency of the P1 wave was also prolonged. Furthermore, reduction of the P2 and P4 wave amplitude and prolongation of the P1-P2 and P2-P4 interpeak latency were also observed at 2 kHz but not at 4 or 8 kHz. These results suggest that the rat auditory function for low frequency is vulnerable to the effects of aspirin. This paradigm, i.e., frequency selectivity, in rats may be useful to further assess the different outer hair cells along the cochlear duct and provide additional evidence for the mechanism(s) or site underlying aspirin ototoxicity. — KEY WORDS: ABR, aspirin, conscious rat, frequency selectivity.

The potential for temporary hearing loss following the administration of aspirin has long been known, and auditory impairment has been confirmed in experimental animals [18, 19, 30]. Human hearing loss affecting all frequencies of pure tones ranging from 10 to 45 dB, is often accompanied by tinnitus and is entirely reversible within 24 to 72 hr [8]. The extent of the hearing loss is also related to the plasma level of aspirin and the duration of the treatment [7, 23]. However, the mechanism or site underlying aspirin and/or salicylate ototoxicity remains poorly understood.

Generally, the mechanisms underlying the reversible hearing impairment produced by aspirin and salicylate may be considered to be as follows: in guinea pigs and/or rats, changes such as a decrease in prostaglandin (PG) synthesis by the lateral wall, a decrease in PG levels in perilymph changes such as a decrease in prostaglandin (PG) synthesis [17] occurs and is followed by morphological changes in the outer hair cells (OHCs) in the cochlea [11]. There have been numerous investigations of the electrical activity in the cochlea or auditory nerve on a salicylate effect, e.g., cochlear microphonic (CM) [4, 13, 24, 27, 28, 30], summating potential (SP) and endocochlear potential (EP) [27, 30], compound action potential (CAP) of the auditory nerve [27, 30, 31] and suppression of active cochlear processes [21]. Recent results suggest that the effects of salicylate may arise from alterations in the membrane capacitance of the OHC and consequent inhibition of OHC motility [32]. Therefore, the cochlea appears to be the primary site of ototoxicity and salicylate’s action within the cochlea may mainly involve the OHCs. However, the site and mechanism for treatment-related ototoxicity may not be the same in all the hair cell systems, and may include more than one site or mechanism.

The mammalian cochlea performs a frequency analysis of airborne sound by processing different frequencies at different portions along the cochlear duct: high frequencies at the base and low frequencies at the apex. It has been reported that the hearing impairment induced by aspirin and salicylates in humans does not depend on the sound frequency [25], but others have claimed that higher frequencies are more susceptible to the effects of these drugs in humans and/or guinea pigs [8, 10, 22]. Boettcher and Salvi have reported that the results of the effects of aspirin on frequency selectivity are conflicting [3]. Most reports do agree that hearing loss and/or tinnitus are caused by these drugs. In almost all studies, the ototoxicity of aspirin and/or salicylate has been assessed in guinea pigs [8, 11, 27], monkeys [25, 29] or cats [13, 14, 30], and little information is available on the effects of aspirin on the auditory function of rats. Recently it was indicated in rats that salicylate had a stronger effect on the otoacoustic emission distortion product for high and low frequencies with a clearly smaller effect for frequencies from the range of 6 to 8 kHz, and additionally, the loudness of salicylate-induced rat tinnitus was detected at moderate intensities corresponding to 62 dB SPL below 10 kHz by the behavioral response [18].

If aspirin has a different effect at different OHCs along the cochlear duct in rats, aspirin’s effect on the frequency selectivity may provide an additional approach to locating the mechanism or site of aspirin’s action within the cochlea. In the present study, we examined the effects of a single dose of aspirin on the ABR in conscious rats, paying special attention to frequency selectivity, to clarify the consecutive changes in the acoustic pathway.
MATERIAL AND METHODS

Animal: A total of 16 male Wistar rats (CLEA Japan Inc., Japan) weighing 350 to 400 g were used. At the start of treatment, the animals were 20 weeks old. Each animal was housed individually in a stainless steel cage in a room having a constant 12-h light-dark cycle. The room temperature was maintained at 23 ± 3°C with a relative humidity of 55 ± 15%. The animals were allowed free access to a laboratory diet (CE-2, CLEA Japan Inc., Japan) and tap water. Maintenance and experimental conditions conformed to the Guide for the Care and Use of Laboratory Animals of Takeda Chemical Industries, Ltd.

Drugs: Aspirin DL-lysine, a commercial product (Venopirin; Green Cross Pharmaceutical Co., Ltd.). Each vial, containing 497 mg of aspirin and 403 mg of DL-lysine, was dissolved in physiological saline at a concentration of 10% (w/v). The pH of the solutions was 5.6 to 6.0. A dose of 225 mg/kg was administered intravenously to 9 rats via a cannula implanted in the jugular vein at about 0.1 ml/min. The dosage level was selected on the basis of the results obtained from a preliminary ascending dose study in rats. Seven rats served as the control animals and were treated in the same manner with physiological saline.

Surgery and ABR recording: The techniques employed for surgery and the procedures for ABR recording are described in full elsewhere [34]. Briefly, the animals were anesthetized with ketamine hydrochloride (100 mg/kg, i.m.) and droperidol (1 mg/kg, i.m.). After anesthesia, a stainless steel screw was placed as the active electrode on the epidural surface 2 mm anterior and left lateral to the central portion of the lower margin of the parietal bone. Another screw, the reference electrode, was placed in the sinus of the frontal bone. In addition, a stainless steel wire was inserted into the right temporalis muscle and served as the ground. These electrodes were connected to a miniature socket (88-MF, Honda Communication MGF Co.) which was fastened to the skull with dental cement (POLISET, Yata Chemical Ind. Co., Ltd.). For intravenous administration, an indwelling siliconized rubber cannula was implanted in the jugular vein and passed subcutaneously to an exit on the animal’s back. A harness was put on the animal after surgery to protect the portion of the cannula outside of the body which was tipped with a stainless steel needle to prevent bleeding. The animals were allowed at least one week to recover from surgery. For ABR recording, a conscious rat was placed in a sound-attenuated box in an electrically shielded room. A slip ring (Electro-cannula slip ring. Airflyte Electronics Co.) was used to allow the animal to move in a relatively free manner without twisting the cables. The potentials were evoked by 2, 4 and 8 kHz tone pips of various intensities (60 to 90 dB SPL, in 5 dB steps) that had 0.25-millisecond exponential rise-fall times, a 10-millisecond duration and a 125-millisecond interstimulus interval. The tone pip was presented through a speaker located at the top of the box (50 cm in height). In the present study, rats could move freely in the sound-attenuated box, therefore, the sound intensity in the range of 60 to 90 dB SPL was calibrated by a sound level meter (MODEL NA-60, RION Co.) at the mid portion and the 4 corners of the bottom of the box. The sound intensities at these sites were confirmed to be within ±2 dB SPL of the target intensity at each frequency. The evoked potentials were then amplified and filtered with a bandpass between 320 Hz and 10 kHz. One hundred and twenty-eight responses to repetitive stimulation were averaged with an evoked response recording system (MEB-5100, Nihon Koden Co.). The ABR was recorded before dosing commenced (predrug values) and 1 and 24 hr after a single injection of aspirin or saline. ABR recording was begun after an adaptation period of 5 to 10 min, during which the animal became accustomed to the box. Body temperature measured before and after each ABR recording was 37.5 ± 0.2°C and was well maintained during the course of experiments.

The cochlear microphonic (CM) and 4 positive waves, P1, P2, P3 and P4 waves, could be recorded for ABR. The amplitude of CM was measured from peak to peak of the initial wave, and those of P1, P2, P3 and P4 from the baseline to the peak of each wave. The latency was measured as the time from stimulus onset to initial wave peak without correcting for stimulus transit time (about 0.7 ms). In addition, the sound threshold levels for each frequency were objectively estimated, by the input-output curve obtained from the change of P1 amplitudes, at the 5 dB steps, and at each frequency, the mean of P1 amplitudes was plotted against the corresponding sound intensities at the 5 dB steps. Linear and polynomial analyses were then performed to obtain the mathematical formulation which calculates the sound threshold at each frequency when zero is inserted into the y-axis of the regression line.

Statistical analysis: The data of the amplitude, peak latency and interpeak latency of ABR were averaged for the rats in the same experimental group in each session. Differences between predrug and postdrug values were evaluated using a paired t test.

RESULTS

Wave form: The typical wave form of the ABR recorded 50 dB SPL above threshold prior to aspirin administration using unrestrained rats, consisted of four positive waves labeled P1, P2, P3 and P4, and occurred within 5 ms poststimulus, following initial deflections corresponding to the cochlear microphonic (CM) (Fig. 1). In this paper, the amplitude and latency of all waves were assessed. However, in the various ABR components, the parameters of CM and of wave P3 were excluded from analysis, because these waves were not recorded reproducibly at the sound intensity levels used.

Effects of aspirin on the amplitude of P1, P2 and P4: In the control rats, P1 amplitude increased with increasing stimulus intensity at each frequency, though the degree of the increase at 8 kHz was smaller than that at 2 or 4 kHz. Throughout the intensity range of 60 to 90 dB SPL, the
The average increase in P1 amplitude was from $1.51 \pm 0.38 \mu V$ to $6.29 \pm 0.84 \mu V$ (n=7) at 2 kHz, from $2.76 \pm 0.61 \mu V$ to $9.06 \pm 0.65 \mu V$ (n=7) at 4 kHz and from $3.66 \pm 0.52 \mu V$ to $11.47 \pm 0.94 \mu V$ (n=7) at 8 kHz. The intensity-dependent increase in the amplitude of P2 and P4 at each frequency was much the same as that observed with P1 except for the degree of the changes. The values for each animal were reproducible during the experimental period, and there were no statistical differences between the predrug value and the values obtained at 1 and 24 hr after dosing with vehicle (data not shown).

Figure 2 shows the changes in the amplitude of P1 in the group (n=9) receiving 225 mg/kg of aspirin. The amplitude of P1 at 2 kHz was decreased over the intensity range of 60 to 90 dB SPL both 1 and 24 hr after dosing except in the cases of 65 and 90 dB SPL 1 hr after dosing, and 60 and 90 dB SPL 24 hr after dosing. At 4 kHz, P1 amplitude 1 hr after dosing was decreased at each intensity except 75 dB, while 24 hr after dosing a decrease in P1 amplitude was noted only at 70 dB. At 8 kHz, P1 amplitude was decreased only at 60 and 65 dB SPL 1 hr after dosing and 60 dB SPL 24 hr after dosing. A significant decrease in P2 and P4 amplitude was noted for most of the intensities at 2 kHz 1 hr after dosing. These changes also showed a tendency to disappear for all the intensities within 24 hr after dosing but remained at the middle and higher intensity levels (Fig. 3). No treatment related changes in P2 or P4 amplitude were observed at 4 or 8 kHz (data not shown).

Figure 4 illustrates the change in the threshold sound pressure level obtained from P1 amplitude at each frequency (control, n=7; 225 mg/kg aspirin, n=9). A significant hearing loss of $19.9 \pm 5.6$ and $15.6 \pm 4.9$ dB SPL was observed at 2 and 4 kHz, respectively, 1 hr after dosing. No hearing deficit at 4 kHz was observed 24 hr after dosing, but the loss at 2 kHz was still noted at that time.

**Effects of aspirin on the peak latency of P1:** In the control rats, intensity-dependent effects on P1 peak latency were characterized by decreased latency with increasing tone pip intensity or its tendency. Throughout the intensity range of 60 to 90 dB SPL, the average decrease in P1 peak latency was from $2.67 \pm 0.04$ ms to $2.40 \pm 0.04$ ms (n=7) at 2 kHz, from $2.62 \pm 0.05$ ms to $2.36 \pm 0.03$ ms (n=7) at 4 kHz and from $2.38 \pm 0.06$ ms to $2.23 \pm 0.03$ ms (n=7) at 8 kHz. The values for each animal were reproducible during the experimental period, and there were no statistical differences between the predrug value and the values 1 or 24 hr after the dosing in the saline group (data not shown).

Figure 5 shows the changes in peak latency of P1 at 2, 4 and 8 kHz in the group (n=9) receiving 225 mg/kg of aspirin. A significant increase in P1 peak latency was noted at each frequency over the intensity range of 60 to 90 dB SPL 1 hr after dosing except in the cases of 60 and 70 dB SPL at 2 kHz and 90 dB SPL at 8 kHz. Reversal of this change was not observed 24 hr after dosing except in the cases of 65 and 75 dB SPL at 2 kHz and 90 dB SPL at 8 kHz.

**Effects of aspirin on P1-P2 and P2-P4 interpeak latency:** In the control rats, the average increase in P2-P4 interpeak latency (IPL) over the intensity range of 60 to 90 dB SPL...
was from 1.16 ± 0.08 ms to 1.25 ± 0.15 ms (predrug value, n=7) at 2 kHz used. The P1-P2 IPL, on the other hand, tended to decrease with increasing stimulus intensity at 2 kHz. The intensity-dependent increase in P2-P4 IPL and decrease in P1-P2 IPL at 4 or 8 kHz were much the same as those observed at 2 kHz except for the degree of the changes. There were no significant differences in either parameter between the predrug values and the values 1 or 24 hr after dosing of saline (data not shown). In the group (n=9) receiving 225 mg/kg of aspirin, both parameters at 2 kHz were significantly prolonged 1 hr after dosing at all the intensity levels except in the cases of 85 dB SPL in P1-P2 IPL and 85 and 90 dB SPL in P2-P4 IPL. Twenty-four hours after dosing, these changes had almost disappeared for all the intensities except in the cases of 70 and 75 dB SPL in P1-P2 IPL and 80 dB SPL in P2-P4 IPL (Fig. 6). IPL was not significantly affected by aspirin at 4 or 8 kHz (data not shown).

DISCUSSION

Although more than one anatomical site may contribute to each wave [1, 2], the principal neural generators of the various ABR components in small laboratory animals are believed to be the auditory nerve (P1), the cochlear nucleus (P2) and the superior olivary complex/trapezoid body (P3). The focal generator of P4 has been variously attributed to neurons in the pontine tegmentum [33], the lateral lemniscus [6] and the inferior colliculus [15]. The CM potential is an electrical analogue of the sound stimulus and its origin is the electrical activity of the cochlear outer hair cells (OHCs), with a contribution from the inner hair cells (IHCs) [27].

The results presented here indicate that a single injection of aspirin causes an impairment of the auditory function in rats and that the function for low sound frequency is most vulnerable. The results also suggest that the auditory function remains unchanged at the best frequency in albino rat [20] except for a prolongation in P1 peak latency. The modification of P1 amplitude and peak latency seen in the present study using conscious rats is in agreement with
previous observations of the effect of aspirin on the cochlear nerve in anesthetized or conscious animals [8, 13, 26–28].

It has been reported that the hearing loss induced by aspirin and salicylates does not depend on the sound frequency in humans [25] and also that higher frequency is more susceptible in human and/or guinea pigs [8, 10, 22]. By the conditioned suppression technique, Kelly and Masterton [20] reported that best frequency for the normal rat was 8 kHz, although this point was closely rivaled (within 2 dB) by 38 kHz, and thresholds increased at a rate of several dB per octave except for the above frequencies. To restrict the variation normally found between sound threshold and frequencies and to cover the frequency spectrum of the tone pips, 8 kHz which was most sensitive for the normal rat was selected in the present study and also 2 and 4 kHz which showed an increase in the sound threshold. As described above, our results clearly demonstrate that low frequencies are vulnerable to aspirin in rats. Although we cannot completely explain the hearing threshold increase at low frequencies, it might be due to anatomical factors such as the number of neurons activated, the length and the geometry of the fibre tracts, the spatial alignment of neurons and conductivity of the brain tissue [9, 16]. It should be noted that salicylate had stronger effects on the otoacoustic emission distortion product for high and low frequencies with a clearly smaller effect for frequencies from the range of 6-8 kHz in rats [18]. Although the change at the high frequencies was not examined in our study, we obtained similar results concerning the stronger effect for low frequencies.

Among the documented adverse effects of aspirin and/or salicylates are tinnitus and hearing loss. Tinnitus in humans is described as a high-pitched ringing or hissing noise, commonly bilateral, associated with high doses of salicylates [23]. The loudness of salicylate-induced tinnitus has also been evaluated by the behavioral responses in rats and has
been detected at moderate intensities corresponding to 62 dB SPL below 10 kHz [18]. From these investigations and our results, we speculate that aspirin administration to rats seems to induce tinnitus, which is most likely to be at one frequency or centred at a very small number of frequencies, without hearing loss. In addition, a stronger effect concerning hearing loss at the low frequencies may be accompanied by the occurrence of tinnitus, while in its absence, only the hearing loss exists. Especially, the change at 8 kHz may indicate only tinnitus exists.

The mechanism(s) or underlying site(s) of aspirin and/or salicylate ototoxicity have not yet been resolved. When sodium salicylate was administered subcutaneously to a guinea pig, electron microscopy revealed vacuolization of the endoplasmic reticulum in OHCs and chronic salicylate administration caused bending of the OHCs stereocilia [11]. Brownell and Winston [5] reported that the turgor of OHCs (in vitro) decreased in the presence of salicylate. Furthermore, single unit recordings have shown that the auditory function changed with the membrane conductance of OHCs, in particular, an increase in K+ conductance of auditory function changed with the membrane conductance (in vitro) decreased in the presence of salicylate. 

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