THE EFFECTS OF SALICYLATE ON AUDITORY EVOKED POTENTIAL AMPLITUDE FROM THE AUDITORY CORTEX AND AUDITORY BRAINSTEM

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Abstract

Tinnitus has often been studied using salicylate in animal models as they are capable of inducing temporary hearing loss and tinnitus. Studies have recently observed enhancement of auditory evoked responses of the auditory cortex (AC) post salicylate treatment which is also shown to be related to tinnitus like behavior in rats. The aim of this study was to observe if enhancements of the AC post salicylate treatment are also present at structures in the brainstem. Four male Sprague Dawley rats with AC implanted electrodes were tested for both AC and auditory brainstem response (ABR) recordings pre and post 250 mg/kg intraperitoneal injections of salicylate. The responses were recorded as the peak to trough amplitudes of P1-N1 (AC), ABR wave V, and ABR wave II. AC responses resulted in statistically significant enhancement of amplitude at 2 hours post salicylate with 90 dB stimuli tone bursts of 4, 8, 12, and 20 kHz. Wave V of ABR responses at 90 dB resulted in a statistically significant reduction of amplitude 2 hours post salicylate and a mean decrease of amplitude of 31% for 16 kHz. Wave II amplitudes at 2 hours post treatment were significantly reduced for 4, 12, and 20 kHz stimuli at 90 dB SPL. Our results suggest that the enhancement changes of the AC related to salicylate induced tinnitus are generated superior to the level of the inferior colliculus and may originate in the AC.

Keywords: Salicylate; Tinnitus; Auditory brainstem response

Introduction

Subjective tinnitus is the sensation of sound in the absence of an acoustic stimulus. Research suggests that tinnitus affects between 15-19% of the population \cite{1-3} and can be induced by noise exposure \cite{4,5} or ototoxic drugs \cite{6,7} (Huang & Schacht, 1989; Day et al., 1989) and is usually associated with a cochlear damage \cite{3,5}. Salicylate has long been known for inducing temporary hearing loss and tinnitus in humans \cite{8,9}. Observations of tinnitus-like behaviors suggest that animals also experience tinnitus with treatment of salicylate \cite{10,13}. Consequently, salicylates have often been used in research to study the neurophysiologic mechanisms of tinnitus. Although tinnitus is most commonly associated with cochlear damage, there is evidence that it may originate in the central nervous system. The following background will discuss previous observations regarding tinnitus and the questions they provoke regarding the structure of origin.

Yang et al. (2007) has shown enhancement of auditory evoked cortical electrophysiological response to high intensity tone burst stimuli in awake rats after administering 150 mg/kg of salicylate. They measured the P1-N1 amplitude, presumably the response from the auditory cortex (AC), pre-salicylate treatment. The measurement was repeated again at 2 hours, 1, 2 and 3 days post treatment. Yang et al. observed the cortical enhancement was most prominent in response to 16 and 20 kHz tone bursts 2 hours post salicylate treatment. These frequencies and time intervals closely correlated to tinnitus-like behavior in another group of rats treated similarly with salicylate \cite{13}. The authors suggested that the amplitude enhancement of P1-N1 observed with super threshold tone bursts in rats treated with salicylate may be associated with tinnitus and hyperacusis.

Several studies have observed a similar auditory evok-
ed electrophysiological enhancement lower in the brainstem post noise exposure. Salvi et al. (2000) reviewed changes of auditory evoked potentials in chinchillas from the cochlea, the cochlear nucleus, and the inferior colliculus (IC) pre and post noise exposure or carboplatin in treatment. Post noise exposure, poorer thresholds were observed for soft auditory stimuli at all three structures, but enhancement of amplitude with high intensity stimuli was observed at the level of the IC and AC. As the auditory enhancement was not seen at the cochlea or cochlear nucleus, they concluded that this enhancement originated at or just before the IC. Liu et al., (2003) observed increases in extracellular levels of dailysate glucose and lactate in both the AC and the IC after administration of salicylate. Basta and Ernst (2004) found an overall increase in spontaneous firing rates in mouse IC brain slices post salicylate superfusion. Bauer et al. (2000) observed altered GABA(A) receptor binding and GAD expression with chronic exposure to salicylate in the IC of rats. Taken together, these studies all suggest that neurophysiologic changes related to tinnitus occur at the level of the IC.

Kaltenbach and associates (2002) evaluated the relationship between near field recordings of spontaneous discharge rates from the dorsal cochlear nucleus and the number of missing inner and outer hair cell loss post cisplatin-induced cochlear damage in hamsters. They observed hyperactivity in spontaneous discharge rate in the dorsal cochlear nucleus when the cochlea sustained significantly more outer hair cell damage than inner hair cell damage. It was suggested that hyperactivity in the dorsal cochlear nucleus may be a cause of tinnitus. It may be reasonable to consider that Salvi et al. did not observe this change at the level of the dorsal cochlear nucleus due to electrode placement at the IC or because of differences in neurological changes with noise exposure or carboplatin induced hearing loss compared to Kaltenbach’s use of cisplatin.

Prior to these studies, it has been shown that salicylate in guinea pigs causes reduced amplitude of the compound action potential of the cochlea and eighth cranial nerve for low intensity stimuli, but no change with higher intensity stimuli. Another study that suggests that the cochlea is not the place of origin of changes related to tinnitus was performed by Müller et al. They used local electrodes placed near the round window to record the compound action potential, and micropipettes inserted into the cochlear nerve to record spontaneous activity in anesthetized gerbils. After systemic application of salicylate, the compound action potential thresholds were elevated, single fiber thresholds were elevated, and spontaneous discharge rates were significantly reduced. They asserted that changes associated with increased activity in the central auditory system post salicylate are not likely caused by hyperactivity of the cochlear nerve.

Collectively, these results suggest that hearing loss occurs at the periphery and changes of increased spontaneous discharge rates, metabolic changes, and enhanced response to high intensity stimuli originate in the cochlear nucleus, IC, or AC. These changes are hypothesized to be related to the perception of tinnitus. The purpose of this study is to observe if the auditory evoked potential super threshold enhancement previously observed at the cortical level post salicylate treatment in rats will also be present in the brainstem evoked waveform. Previous studies using noise exposure, carboplatin or cisplatin suggest that enhancement may be observed at the level of the IC and the cochlear nucleus.

Previous studies suggest that salicylate causes an amplitude enhancement of the auditory evoked potential waveform of the AC. It is not clear if the enhancement originates in the AC, the IC, or lower levels of the auditory pathway. To address this question, we recorded AC and brainstem evoked potential waveforms from four implanted animals both pre and post salicylate. The brainstem waveform was analyzed at specific latencies to attempt to recognize the source of these changes.

**Experimental procedures**

**Experimental animals and restraint procedures:** Four male Sprague Dawley rats, implanted approximately 2-3 months old, were tested for auditory cortical and brainstem evoked electrophysiological testing at approximately 6-7 months of age. The implant consists of two implanted electrodes and a threaded holding bar for head restraint, all fixed in place to the surface of the skull with stainless steel screws and dental cement. One of the electrodes was made from A-M Systems Inc. silver Teflon wire (.008 inches) melted into a ball-end electrode placed on the right AC. The second electrode was made from tungsten Teflon wire (.008 inches) and affixed to the surface of the skull to serve as the reference.

The AC electrode position was identified from the suture on the skull and the blood vessel on the surface of the cortex. The implant procedure has been previously described in detail. During testing, the animals were restrained inside a modified rat restrainer (Braintree Scientific, G-4) to provide unrestricted acoustic access to both ears. Moderately firm head restraint was used that restricts head movement to small twisting motions by fixing a bar to the implanted threaded holding bar that was held with a World Precision Instruments M11 holding device. The animal was trained to the procedure until it could remain relaxed throughout the restraint process. This procedure has been extensively used within our
and saved in Microsoft Excel for later statistical analysis. P1 to N1 amplitude as a function of stimulus intensity was recorded as middle latency recording peak to peak later comparison to post salicylate recordings. The data, were recorded and averaged for two to four baselines, depending on stability and consistency. Recording duration was 300 ms with no onset delay. Significant changes in amplitude and latency have been observed in wave V, and even wave V of some animals, after ablation of the IC. These results, combined with the understanding of latency of the lateral lemniscus and IC, suggest that waves IV and V of the ABR are interactions of the impulses from these structures. For this study, the data was analyzed as wave II peak to trough amplitude as representing the cochlear nucleus and wave V peak to trough amplitude as representing the response from the IC.

**ABR recording:** The data for auditory brainstem response (ABR) was recorded with the same software set up as AC recording with the exception of the following changes: high pass filter of 100 Hz and a low pass filter of 3000 Hz, a click rate of 19/second, averaged 512 sweeps with duration of 10 ms for each average. Recording intensity schedules were performed as described above for AC recording. As previously explained, the transducer was placed close to the subject’s right ear, and the electrode placement was channel 1 at the base of the skull, and the negative and common at the AC. Although atypical for recording ABR, this electrode placement provides far field recordings for the auditory pathway ipsilateral to the stimulus ear. The positive peaks in the far field ABR occur around 1.3 (I), 2.1 (II), 2.9 (III), 3.7 (IV), and 4.6 (V) [Shaw, 1995]. The responses in far field ABR recordings are a complex interaction of electrical impulses due to dipole orientations of different structures of the auditory pathway resulting in fast and slow waves [21]. Peak II has been shown to be closely associated with the cochlear nucleus in rats [31]. Local field potentials and electrical vector fields show the latency of electrical potentials in the IC to be approximately 5.6 ms and the lateral lemniscus to be approximately 3-3.5 ms. Although the IC response is approximately at 5.6 ms, significant changes in amplitude and latency have been observed in wave V, and even wave V of some animals, after ablation of the IC. These results, combined with the understanding of latency of the lateral lemniscus and IC, suggest that waves IV and V of the ABR are interactions of the impulses from these structures. For this study, the data was analyzed as wave II peak to trough amplitude as representing the cochlear nucleus and wave V peak to trough amplitude as representing the response from the IC.

**Salicylate administration:** The administration of salicylate was 250 mg/kg intraperitoneal injection mixed with saline solution in a concentration of 25 mg/ml. AC and ABR recordings were taken at 2 hours, 1 day, and 3 days post salicylate and compared to the averaged pretreatment baseline.

**Statistical analysis:** Our testing hypothesis was for the presence of amplitude enhancement of auditory evoked potentials with high intensity stimuli in the brainstem response for wave II or wave V representing the cochlear nucleus and IC respectively. The data was analyzed comparing the average responses of all animals at 90 dB stimuli for each stimulus frequency using a paired t test with an alpha value of .05. The analysis was performed with Microsoft Excel 2003 software and graphed as pre and post treatment line graphs. Examples of input/output functions are displayed for a qualitative description of da-
**Results**

**AC recordings:** To verify the presence of enhancement of evoked responses at the AC, which were previously observed in other studies, the amplitude of the peak to trough P1-N1 was recorded pre and post salicylate treatment (Figure 1A). Figure 1B plots an input/output function of one stimulus frequency recording from one animal for all recordings. The input/output data illustrates the lower amplitude 2 hour post recordings for low intensity stimuli and enhancement with high intensity stimuli. As expected, the AC amplitude results to 90 dB SPL stimuli were significantly enhanced at 2 hours post salicylate treatment compared to the pretreatment baseline (P < 0.05, paired t-test). The enhancement was significant with all tested stimulus frequencies with the exception of 16 kHz (P = .0685). Figure 1A illustrates a typical waveform amplitude enhancement of P1-N1 recorded between 12 and 35 ms post stimulus. Example of an input/output function for amplitudes recorded from the AC all time intervals from one animal in response to 8 kHz tone bursts was shown in Figure 1B.

Figure 1 (A) Example of a comparison AC waveforms pre and 2 hours post salicylate treatment in response to 90 dB stimuli. (B) Example of an input/output function for amplitudes recorded from the AC all time intervals from one animal in response to 8 kHz tone bursts.

Figure 2A displays the comparison of the AC amplitudes of pre and post salicylate at 4, 8, 12, 16 and 20 kHz. It was noted that the overall AC amplitudes averaged across animals did not resolve completely at the 1 day or the 3 day recordings. One animal (092707) has no data to calculate into the 1 and 3 day averages as it was necessary for the animal to be euthanized prior to completing the testing. Figure 2B illustrates the differences from baseline and enhanced response at 2 hours post treatment (n = 4) compared to the level of resolve at 1 and 3 days after treatment (n = 3).

**IC response:** In contrast to the AC response enhancements, the wave V amplitude of the ABR was decreased overall at two hours post salicylate treatment compared pretreatment baseline averages.

One animal (100107) had extinguished wave V responses for 8 and 20 kHz at 2 hours post salicylate that resumed at 1 day and 3 day recordings. Three of the four animals (102607, 092807, and 100107) had missing responses for 4 kHz stimuli for both pre and 2 hours post recordings. Consequently, statistical analysis was only appropriate for 12 and 16 kHz stimuli recordings due to the missing data. Analysis of the two complete data sets resulted in a statistically significant reduction in amplitude (P < 0.05, paired t-test) for 12 kHz stimuli and a non-statistically significant reduction of 31% for 16 kHz responses (P = .0613). Figure 3B plots the data used for statistical analysis (n = 4). Due to the small amplitudes of the ABR recordings and large standard deviations, attempts to plot error bars in the negative direction results in a calculation error due to the first standard deviation value being below zero micro volts. Further, adding plots for the 1 and 3 day recordings (n = 3) as in figure 3B results in values much more reduced than the 2 hours post salicylate values. These problems with the initial view of the data are explained if the relative amplitudes for each animal are plotted separately to display the intra subject variability as in figure 4A. Animal 092707, which was euthanized prior to recording 1 and 3 days post treatment, has wave V amplitudes grossly larger than the other three. For this remaining sample, statistical analysis would provide poor reliability due to its size (n = 3).
However, the qualitative display of the data in figure 9 illustrates a reliable pattern of decrease in overall amplitudes 2 hours post salicylate treatment and resolve to near baseline values at 1 and 3 days.

Cochlear nucleus response: Similar to wave V of the ABR, wave II amplitudes in response to 90 dB SPL stimuli were overall reduced in amplitude post salicylate treatment. Responses to 4, 16 and 20 kHz stimuli were reduced without reaching the level of statistical significance (P = .323; P = .480).

Discussion

The results of the AC amplitude enhancement with 90 dB SPL stimuli post salicylate treatment are consistent with findings in Yang et al. (2007) who showed a relationship between these findings and tinnitus-like behaviors [13]. Although the findings are similar, Yang et al.’s data had a statistically significant increase in amplitude of AC responses for 16 and 20 kHz stimuli. Our observations resulted in statistically significant increases in AC responses with 4, 8, 12 and 20 kHz stimuli, and not with 16 kHz. Although not statistically significant, a mean increase in amplitude to 135% of the baseline was present 2 hours post salicylate with 16 kHz. The exact physiological mechanism for the differences in results between the results in this study and that observed by Yang is uncertain, but the increased dosage of salicylate from 150 mg/kg used by Yang compared to our dose of 250 mg/kg is the most likely factor. It was also noted that the amplitudes of the 1 and 3 day AC recordings did not completely resolve to baseline amplitudes which is also likely to be dose related. It must also be considered that the 1 and 3 day post treatment data were only based on three of the four original subjects as previously described.

The results of pre and post salicylate treatment for wave II and V of the ABR resulted in overall decreases in amplitude or extinction of the response for some stimulus frequencies. These results suggest that the physiological mechanism related to the perception of tinnitus occurs at the AC, or at least superior to the level of the IC. A noted weakness of this study is poor signal to noise ratio when recording small amplitude ABR responses from animals while awake using far field electrode recordings. However, these results are supported by a recent study performed by Sun et al. since the completion of data collection for the present study. Their results confirm the absence of IC response enhancement with high level stimuli with locally implanted electrodes [22]. Although the results of these two studies are in agreement, the findings contrast with previously described studies that recorded increased spontaneous neural firing rates at the level of the cochlear nucleus post treatment with cisplatin [18] and increased amplitudes for high level stimuli recorded locally in the IC post noise exposure or carboplatin [14]. These differences in results suggest different physiological mechanisms of auditory system changes related to tinnitus between salicylate from that of cisplatin, carboplatin, or noise exposure pathology. Another limitation of this current study is a small sampling size. This may also affect the results reaching to a statistic significance.

References

[1] Axelsson A, Ringdahl A. Tinnitus--a study of its prevalence