Published in final edited form as:

J Neurosci Methods. 2005 August 30; 147(1): 55–64.

Simultaneous measurement of electrocochleography and cochlear blood flow during cochlear hypoxia in rabbits

Erdem Yavuz^{a,*}, Krzysztof Morawski^b, Fred F. Telischi^{a,c}, Özcan Özdamar^{a,c}, Rafael E. Delgado^{a,d}, Fabrice Manns^{a,e}, and Jean-Marie Parel^a

^a Department of Biomedical Engineering, University of Miami, PO Box 248294, Coral Gables, FL 33124, USA

^b Department of Otolaryngology, Medical University of Warsaw, Warsaw Poland

^c Department of Otolaryngology, University of Miami, Miami, FL, USA

^d Intelligent Hearing Systems Inc., Miami, FL, USA

^e Bascom Palmer Eye Institute, University of Miami, Miami, FL, USA

Abstract

In this study, a new monitoring system is developed to measure cochlear blood flow (CBF) and electrocochleography (ECoChG) during transient ischemic episodes of the cochlea. A newly designed otic probe was used for the simultaneous recordings of laser-Doppler CBF and ECoChG directly from the round window (RW). The probe enabled the recording of high amplitude compound action potentials (CAP) and cochlear microphonics (CM) with few averages. Experiments were conducted on rabbits to generate episodes of cochlear ischemia by using timed compressions of the internal auditory artery (IAA). The computer monitoring system extracted and measured CAP and CM components from ECoChG in real-time. Results indicate that CM and CAP generally followed CBF during compressions and releases of IAA. Both CBF values and CAP amplitudes showed an overshoot following the reperfusion. CAP amplitude measures were found to be very sensitive to ischemia showing very rapid amplitude, latency and morphological changes. CM amplitude decreased more slowly than the CAP and CBF. Simultaneous recordings of CBF and ECoChG using the otic probe provide a valuable neuromonitoring tool to investigate the dynamic behavior of the cochlea during ischemia.

Keywords

Electrocochleography; Compound action potential; Cochlear blood flow; Neuromonitoring; Hypoxia; Ischemia

1. Introduction

Impairment of the cochlear blood flow (CBF) and damage to the acoustic nerve has been implicated as one of the causes of post-operative sensorineural hearing loss (SNHL) that may occur after cerebellopontine angle (CPA) region surgical manipulations (Millen et al., 1982). Space-occupying lesions of CPA such as acoustic neuromas (AN) and meningiomas are tumors that often require surgical removal. These neoplasms may also cause hearing loss due to the compression of the cochlear nerve and internal auditory artery (IAA) (Colletti et al., 1997).

* Corresponding author. Tel.: +1 305 284 5272; fax: +1 305 284 6494. E-mail address: eyavuz@umsis.miami.edu (E. Yavuz)..

During surgical excision with attempted hearing conservation, unrecognized compressions of IAA may result in cochlear ischemia producing unwanted outcomes.

A monitoring system that can detect the changes in CBF and cochlear function would be useful in preserving hearing, not only for intraoperative applications, but to detect the progression of cochlear impairments in other clinical settings as well. Simultaneous acquisition of CBF with different electrophysiological parameters was proposed and tested on different animal models as the result of increasing interest in cochlear monitoring.

Auditory brain stem response (ABR) is the most commonly used intraoperative auditory function monitoring method due to its ease of acquisition and resistance to anesthesia. ABRs, however, lack acquisition speed because of their poor signal to noise ratio (SNR), resulting in delayed detection of responses and events (Telischi et al., 1999). Direct near field cochlear nerve action potential (CNAP) recording provides better SNR and has been successfully used for intraoperative monitoring (Colletti et al., 1997). CNAP recording, however, has its limitations due to its invasive nature, as CNAP requires the placement of an electrode directly on the eighth cranial nerve. Distortion-product otoacoustic emissions (DPOAE) were reported to be a rapid responding method in correlation with CBF changes that can be used as a cochlear monitoring tool, but were difficult to acquire intraoperatively due to their high sensitivity to acoustic noise (Telischi et al., 1996; Mom et al., 1999). Endocochlear potentials (EPs) were also used in animals to monitor cochlear function, but such measurements are highly invasive prohibiting clinical usage (Tabuchi et al., 2001).

Electrocochleography (ECochG) is an electrophysiological recording technique, which is minimally invasive, using easily applied transtympanic needle electrodes or wick electrodes. ECochG contains both eighth nerve compound action potentials (CAP) and cochlear microphonics (CM) thus providing information from two different parts of the auditory periphery. CM reflects primarily the outer hair cell function, while CAPs represent synchronous eighth nerve activity originating from the inner hair cells. Although the promontory is traditionally used as the site for ECochG recordings, electrode placement in the RW niche may increase the signal level. Positioning the recording electrode at or near the RW generates a signal with a relatively high SNR decreasing the number of required sweeps and, therefore, acquisition time.

1.1. Laser-Doppler measurement technique

Laser-Doppler (LD) blood flow measurement technique is based on the Doppler effect created by moving particles, which are irradiated with a laser beam. LD measurement systems generally use a low power, semiconductor laser diode as the coherent light source. The wavelength is usually selected in the near infrared range. In clinical systems, the laser source is usually coupled to an optical fiber that is placed in contact with the vascularized tissue of interest. Light that penetrates into the tissue is scattered by both the tissues and static and dynamic components of the moving red blood cells (RBCs). Light interacting with the moving RBCs undergoes a frequency shift related to the velocity of RBCs following the Doppler principle. Due to several factors, such as the number of times a photon is scattered, differences between RBC velocities and the angle between RBCs and the incident light, a spectrum of Doppler shifts is generated (Levine et al., 1993). A portion of the scattered light returns to a collecting fiber which delivers the detected Doppler shifted signal to a photodetector connected to a signal processing unit. Received signals are processed to obtain a mean difference frequency (i.e., mean Doppler frequency) that defines the mean RBC velocity, which is mostly measured with the unit of mm/s. RBC volume fraction is calculated by the comparison of static and fluctuating portions of the signal and shown as the fraction of tissue volume that is occupied by the moving RBC. Flow is calculated as the product of velocity and volume with the unit of

ml/(min 100 g of tissue) to measure the speed of RBCs moving in random directions (Vasamedics Inc., 1993).

Optical properties of the tissue, laser wavelength and characteristics of the optical fibers define the volume probed by the laser (Weiss et al., 1989). With small animals, like guinea pigs and gerbils, the laser beam can be delivered to the capillary bed of stria vascularis through the promontory bone of cochlea (Miller et al., 1983; Asami et al., 1995). In humans and larger animals, such as rabbits, however, where the cochlea is surrounded by dense otic capsule bone, the penetration depth of the laser is greatly attenuated, decreasing the measurement capability and resulting with unreliable CBF values (Miller et al., 1991; Mom et al., 1999). Methods used for having a better penetration depth, like thinning of the bone, not only increase the invasiveness of the procedure but also increase the risk of damage to fine structures of the cochlea as well. Placing the LD probe on the bone also increases the occurrences of artifacts created by non-blood flow originated movement of the probe due to stabilization problems. The round window niche may provide better location for monitoring CBF, providing better stabilization of the measurement probe with close access to the intracochlear vasculature.

1.2. Background

Animal models were used long before the introduction of LD systems for CBF measurement. In guinea pigs, Perlman et al. (1959) made microscopic observation of blood flow through individual vessels of stria vascularis and described a decrease in CBF following vascular compression. Development of LD technique and its successful use in other tissues enabled researchers to measure CBF with accuracy and minimal invasiveness (Stern et al., 1977). LD rapidly became popular for measuring CBF and other physiological parameters in animal models such as guinea pig, rat, gerbil, and rabbit (Miller et al., 1983; Scheibe et al., 1990; Asami et al., 1995; Ren et al., 1995). Generally, the LD probe was placed on the cochlear promontory bone throughout these experiments, resulting in attenuation of measurement penetration depth due to dense bone tissue. Placement of the LD probe on the RW niche was performed later and reported to overcome these problems (Mom et al., 1999; Telischi et al., 1999). In this approach, the laser beam is directed medially towards to the spiral modiolar vasculature. The RW placement is likely to solve the artifact problem caused by the unwanted movement of the probe, by better stabilization of the probe at the RW niche. Appropriate selection of the probe's tip size may increase the stabilization.

The RW may be a suitable location to obtain better measurements of ECoChG and CBF. Recording both parameters simultaneously from the same location was not possible until recently. A newly developed otic-probe was designed to overcome previous problems for LD measurements (Telischi et al., 2003). The microsurgical otic probe combines a modified commercial laser-Doppler probe (Vasamedics, St. Paul, MN), a thin ECoChG electrode to measure auditory electro physiological responses, an irrigation/aspiration (I/A) system for removal of excess fluid. The laser-Doppler probe and ECoChG electrode were contained within a 0.5 mm diameter stainless steel tube with a bent tip that was shaped to facilitate insertion through the ear canal for placement in contact with the round window. The final probe shape was selected by performing experiments on a fixed cadaver head to ensure feasibility of round window placement in a clinically relevant setting. The irrigation/suction system consisted of a sleeve made of a thin walled hollow silastic tube into which the stainless steel probe was inserted. A single tube was used for both irrigation and suction. The sleeve was made disposable to avoid contamination. Successive larger diameter silastic tubes were used as mediators to connect the silastic tube at the tip to the probe handle. The outer diameter of the irrigation tube at the tip was 1.19 mm. The total length from the handle to the tip of the bent probe was approximately 10 cm. The size of the tip of the probe was designed to fit easily in the RW niche.

This study describes the methods and the results of simultaneous monitoring of CBF and ECoChG recorded before, during and after experimentally initiated ischemic episodes of cochlea. These two signals were recorded from the RW with the otic-probe to determine their usefulness as a neuromonitoring tool.

2. Methods

2.1. Animals and surgical procedure

Seven young albino rabbits, weighing 3–3.5 kg, were used as the experimental subjects. The protocol for the care and use of the rabbits was reviewed, approved and monitored by the Institutional Animal Care and Use Committee of the University of Miami (UM) School Of Medicine.

A mixture of ketamine hydrochloride (50 mg/kg) and xylazine hydrochloride (10 mg/kg) was used intramuscularly to provide an anesthesia of surgical level, preserving the spontaneous respiration. To ensure that the experiments were performed under adequate anesthesia during surgical procedure and recordings, intramuscular (IM) injections were repeated as needed, monitoring the corneal reflex and the vibrissae twitching.

Body hair around the neck and forehead were shaved by an electrical hair trimmer. The surgery and recordings were carried out in a double-walled, walk-in, sound-treated, chamber that was also electromagnetically shielded (Industrial Acoustics Corp., Bronx, NY). Subjects were immobilized by a surgically implanted head holder device so that the head could be firmly oriented at any desired angle. Surgical approaches established earlier by the Ear Institute of UM were then carried out to expose both cochleae and internal auditory canals (IAC) (Widick et al., 1994; Mom et al., 1999; Telischi et al., 1999). Under the operating microscope, a post-auricular cochlear approach to the middle ear through the auditory bulla was made after dissecting the necessary neck muscles. An electrical drill with a diamond burr was used to enter the middle ear cavity below the horizontal border between the bulla's thick and thin posterior walls. The upper section of the wall was delicately enlarged using the diamond burr and curette until a wide, clear exposure to RW was achieved. The entrance was covered by several pieces of surgical gauze during the preceding surgical maneuvers. The same process was repeated to the second ear. After the middle ears were reached, both IACs were bilaterally exposed by sub-occipital craniotomy to be able to occlude the blood flow to the inner ears. Dorsal neck muscles were sectioned and resected in the mid-line to have clear access to the base of the skull. A wide entrance was achieved by removing the posterior calvarium.

2.2. Instrumentation

After completing the surgical process, the otic probe was used to measure CBF and ECoChG simultaneously from the RW. After inserting and stabilizing in the RW niche using a micromanipulator, the probe was connected to the LD system (LaserFlo BMP², Vasamedics, St. Paul, MN) and a commercially available evoked potential measurement system (SmartEP, Intelligent Hearing Systems, Miami, FL). This system was able to record ECoChG and CBF measures continuously. LD data were sampled at 2–3 s⁻¹, while the EP data were acquired at a rate of 16 sweeps every 2–3 s. Probe placement and instrumental setup are shown in Fig. 1.

The delivered laser beam passes through the round window membrane and travels inside the cochlea. Although the exact trajectory of the delivered light is not certain, the placement of the otic probe at the round window niche (positioned at an angle that pointed the laser beam slightly upward and in medial direction that was approximately 45° from the vertical plane) directs the laser beam towards to spiral modiolar artery. The main cochlear artery, which is a branch of the IAA, supplies the upper three quarters of the cochlea including the modiolus, while the

cochlear ramus supplies the basal one-quarter of the cochlear and its adjacent modiolus. What we are measuring with the LD probe at the round window can be better described as the result of the circulation of a summation of vessels around the basal turn of the cochlea and modiolus.

The ECochG electrode from the probe was connected to the negative port of the amplifier. A positive needle electrode was placed in the midline on dorsal neck muscles. The needle electrode used for ground was stabilized at the lumbar region on the midline. Sampling period of the system was set to 25 μ s. An amplification of 30,000–100,000 was used, depending on the acquired response level ensuring the elimination of a saturation of the digital-to-analog converter of the system. The band pass filter was set between 300 and 1500 Hz (6 dB/oct). Auditory stimulation was presented using a 10B+ probe with ER2 insert earphones (Etymotic Research Inc., IL).

2.3. Experimental procedures

A sequence of three groups of click stimuli were presented at a rate of 19.3 s⁻¹. The first group consisted of 16 rarefaction clicks at 82 dB SPL. The second group consisted of 16 condensation clicks at 82 dB SPL. The last group contained 16 rarefaction clicks at 60 dB SPL. The results from the 60 dB SPL recordings were not used in this study since CAP and CM cannot be separated in this group. Each group was averaged in order to further increase SNR yield.

Compressions of the eighth nerve complex at the porus of the IAC were conducted using the tip of a custom made micro glass pipette with a spherical end, under a surgical microscope. Although it would be possible to visualize the IAA, this procedure would be very difficult and time consuming, significantly extending the preparatory phase of the experiment. Additionally, by compressing the eighth nerve complex, the cochlear blood deficit was achieved without directly touching the artery so that vasospasm of the artery was mostly avoided. Vasospasm of the artery creates unpredictable, prolonged durations of blood flow deficits, which results in early termination of the experiment.

Baseline recordings were made before each compression for several minutes and recording continued during and after the compressions. Experiments were conducted by two researchers. As the first researcher started each compression, the second researcher enabled a separate timer in the recording system to keep track of the compression time. The timer data was also saved as a separate text file for offline analysis. CBF recordings from the RW were used as the objective compression indicator. An example of a set of simultaneous ECochG and CBF recordings evoked by consecutive rarefaction and condensation clicks during an 1-min compression (OMC) acquired from one of the rabbits is shown in Fig. 2.

The change in the ECochG waveform was monitored constantly by the second researcher. CBF and ECochG were used to monitor unsuccessful compressions. If a compression was unsuccessful or incomplete, the event was terminated as quickly as possible. In these cases, an additional 5 min delay was added to the onset of the next compression attempt. Three OMCs and one 3-min compression (TMC) were made for each ear. CBF and ECochG were continuously recorded for 1–2 min as baseline before compressions. Recordings were continued during and after the compressions. After the release of the compressions, additional recordings of 10 min were done for each OMC. Recording time after release for each TMC was 15 min. After recording from both ears, the rabbits were terminated by the overdose injection of anesthetics.

2.4. Signal and data processing

Along with the data acquisition system, two custom Matlab programs were used to analyze the data. Compound action potential amplitudes were extracted by the summation of rarefaction

and condensation responses resulting in the cancellation of the CM component of the waveforms. Subtracting condensation from rarefaction responses resulted in cancellation of the CAP component to yield the CM component. This process is shown in Fig. 3. The CAP N_1 amplitude was measured between N_1 and P_2 . The P_1 peak was not used in this measurement since P_1 and N_2 peaks were not easily identifiable following the compressions. The CM amplitude was measured between the extreme peaks of the waveform.

3. Results

In this study, data from seven ears are reported with all of the four compression recordings. Data from other ears were discarded because of either missing CBF data due to instrumentation or operational failure, vasospasm of the artery or early spontaneous death of the animal before collection of the data from four compressions. CAP recordings varied in amplitude from 50 μ V to 450 μ V due to probe placement differences from animal to animal. It was also found that as the duration of surgical manipulation (e.g. drilling) increased, the amplitude of the recorded signals decreased, possibly due to mechanical trauma.

All of the seven ears showed a similar rapid CBF change occurring after the each compression onset, which was used to confirm a successful compression. Mathematically separated CM, CAP and the raw recordings, which were acquired during an OMC, are shown in Fig. 2. During the compression, CBF reached a minimal level and then made a quick recovery followed by an overshoot, after the release of the compression. In the data following the onset of the compression, the CAP portion of the ECoG waveform changed dramatically as the amplitude decreased and the waveform broadened with a slight delay in the latency of the peak. The N_2 peak component disappeared in the broadened waveform. As the ischemia progressed, the CAP disappeared. Following the release of the compression, N_1 amplitude increased, reaching and exceeding the baseline level, with an overshoot similar to the CBF data. The latency of the N_1 peak also recovered rapidly after the reperfusion.

Changes in the CAP and CM responses are shown in Fig. 4. Each waveform is shown before compression, after compression (at the maximum overshoot point) and just prior to the second compression. The time location of these waveforms during the compression sequence is marked and shown in Fig. 5. This figure shows the relation between CBF, CAP and CM that was recorded continuously from rabbit 13. CBF and CAP were found to drop very quickly with the onset of ischemia. CBF and CAP presented similar recovery patterns followed by an overshoot. The CM results were quite different. Although CM rapidly dropped with the onset of the compression, a stable minima was only reached during the TMC and no overshoot was seen after the reperfusion.

Each compression group was averaged and the resulting graphs are shown in Figs. 6 and 7. For objective descriptions of changes in CAP, CM and CBF, several critical points are defined and shown graphically in Fig. 8.

General observation of averaged results showed that with the onset of the compression, CBF values decreased dramatically and stabilized at a minimum level until the release of the compression. Onset end point was reached between 2 and 3 data points (14–17 s) after each compression. Following the release of the compressions, the CBF levels quickly rose to pre-compression levels within the next 2–3 data points (14–17 s). Regardless of compression duration, overshoots of between 130% and 148% were observed.

A closer look at Figs. 6 and 7 shows several differences between CAP amplitude and the CBF behavior. CAP amplitude decreased very rapidly just after the onset of IAA compression. Onset start delay of CAP was found to be about one measurement cycle (7–8.5 s). The slope of the decrease of CAP was very similar to that of CBF. CAP amplitude measurements continued to

drop even after CBF reached its minimum level. Offset start delay was also one measurement cycle (7–8.5 s), similar to CBF behavior. Although we observed the same delay time for offset end delay for the first two OMCs, further compressions resulted in longer delay times for CAP compared to CBF. A delayed, but higher, overshoot was also observed for CAP in each compression group. CAP amplitudes were differing between 158% and 171% of baseline whereas CBF measures were between 130% and 148%. CAP overshoot was found to take place later than CBF by 1–4 measurements (28–36 s) for OMCs. In the TMCs CAP overshoot levels and delay times were significantly higher. CAP amplitudes gradually decreased after the overshoot. During the recorded time, it was not possible to observe the recovery to the baseline value for the first two compressions. CAP measurements dropped below the baseline values after the latter two compression release episodes.

A slower drop was observed for CM after the onset of the compressions. Both onset start and onset end delay times were longer for CM, which caused a longer time to reach to a stable drop level. After the offset, a rapid recovery with a short offset start delay was observed. Unlike CAP and CBF, CM did not show a significant overshoot. Although in the first OMC a slightly higher value compared to baseline was seen, in the rest of the compressions CM recovered close to the baseline and did not make an overshoot.

4. Discussions

The newly designed otic probe was found to be capable of conducting stable and fast measurements of CBF, CAP and CM at the RW very close to their generation points, thus overcoming the problems that occur in the measurements made through the bone. The onset and offset characteristics of these parameters reflect the physiological properties of the generators. The CAP amplitude measurements followed the changes in the CBF very rapidly, which shows that CAP is a good predictor of the changes in the CBF. Although CAP followed the changes in CBF, CAP showed alterations in the waveform where no change in CBF was observed, demonstrating the necessity of simultaneously monitoring these two parameters.

During the monitored time, CBF slowly decreased to the baseline level in two out of three OMCs. CBF in the first OMC did not reach baseline value during the monitored duration. Also the TMC showed a similar decrease down to the baseline but in a comparatively longer time, nearly twice as long than OMCs. The findings described above about CBF behavior were similar to previous studies (Miller et al., 1995; Mom et al., 1999, 2000). In studies where CBF measurements were made through the bone, the finer details of the responses such as the overshoot was either not recognizable or not described most likely to the previously mentioned problems (Levine et al., 1993).

The overshoot of CBF following the reperfusion has been observed by other authors (Ren et al., 1995; Nakashima et al., 2001). This may be the result of the formation and the effects of the local vasodilator chemicals (Miller et al., 1995). Increase in CO₂, H⁺, adenosine K⁺, NO and low pH are known to cause vasodilatation and, thus, increase the cerebral blood flow (Clarke and Sokoloff, 1999). Sudden release of blood pressure built behind the compression point might also have a facilitating effect on the increase of the CBF. Ren et al. (1995) reported that the longer ischemia episodes result in delayed overshoot onset and longer recovery time. We observed similar responses, as TMCs resulted in delayed CBF overshoot onset compared to OMCs (about 64 ms versus 96 ms in average). Although the first OMC never did recover to baseline, the second and third OMCs recovered faster.

Overshoot in the CAP followed the release of IAA compression similar to the CBF but with a longer delay (64 ms versus 88 ms for 1-min and 96 ms versus 200 ms for 3-min compressions). The CAP overshoot is likely to be caused by the excitatory neurotransmitter glutamate.

Glutamate is thought to be the primary amino acid at the synapses of cochlear hair cells and spiral ganglion neurons (Puel, 1995; Matsuda et al., 2000). Hypoxic–ischemic stress is known to cause excessive release and decreased uptake of glutamate at the synaptic cleft in the neural tissue. Decreased O₂ and ATP levels disrupt the function of active ion pumps, such as Na/K-ATPase, which are essential for action potential generation and conduction. The resulting membrane depolarization is rapidly followed by the depolarization induced entry of Ca²⁺ via voltage-sensitive Ca²⁺ channels which stimulate the release of vesicular neurotransmitters, including the excitatory amino acid glutamate. Ionic pump insufficiency also decreases the reuptake of glutamate from the synaptic cleft due to altered ionic concentrations of Na⁺ and K⁺ which are essential for glutamate uptake (Clarke and Sokoloff, 1999). Either decreased or total interruption of ionic transport by the blood beyond the occlusion point might also have an additional effect on the diminishing and disappearing of the CAP during the compression. Release of the compression rapidly increases the O₂ presence and reestablishes the ionic normal compartmentalization, which results in rapid recovery of the CAP waveform. Based on the earlier observations, we expect that excitatory effect of glutamate showed itself by the overshoot following a short delay from the release of the compression (Katsura et al., 1994; Kristian and Siesjö, 1997). The delay was likely related to the time necessary for re-compartmentalization of the ions.

Excitotoxicity of glutamate seems to be important in the recovery and post-overshoot recordings. Additional effects of free radical formation, cytotoxic changes and related deterioration of cellular mechanism might also contribute to the observed results. Ischemia was also shown to cause rapid changes on the recorded EP (Levine et al., 1993). After the ischemia onset, a decrease in the EP amplitude to negative values was seen. Reperfusion was followed by the recovery of the EP with an initial transient overshoot. This observation shows the speed of compartmental ionic concentration changes likely to have an influential contribution to our results. EP is commonly accepted to be dependent on the functioning of the Na/K-ATPase pump, which actively transports potassium from marginal cells of stria vascularis into the scala media and maintain high K⁺ and low Na⁺ concentration in the endolymph. EP is highly dependent on O₂ and blood supply (Hu and Jiang, 1995). The question of whether the overshoot in the CBF might contribute to the overshoot of CAP by the increased ionic transport is yet to be answered.

The duration of our recordings was not sufficiently long enough to observe the chronic changes in the CAP. Longer durations of post-ischemic recording might allow time for further changes in the CAP waveform shape and amplitude to be observed.

In each compression, the N₂ peak disappeared with accompanied broadening of the CAP waveform. The reason for this behavior was not clear but collateral damage to the eighth nerve during the blockage of the IAA might be responsible due to the edema that occurred after the mechanical stress applied. Delayed conduction of the action potentials around the edema line might cause the broadening effect. The exact reason for the N₂ disappearance remains unknown.

CM data distinguished itself by both a general lack of overshoot and a slower decrease in the amplitude. Unlike CAP, CM amplitudes decreased slowly and only in the 3-min compressions a lower plateau value was reached. This may be due to two reasons: either CM generation needs less energy or generation is done using a reserve, which can sustain itself for about 1 min before depletion. CM is well known to be recorded even after death, thus giving support to second hypothesis. Although the recording amplitude dramatically decreases, CM still follows the envelope of the stimulus and can be traced even for hours. High ionic concentrations in the scala media, most likely act as the reservoir. Hypoxia can alter the ATP levels in the neural tissue, which resulted with failure of ATP dependent ion compartmentalization. An early,

slowly increasing K^+ efflux towards to the extracellular compartment is followed by an extremely fast efflux phase (occurring between 60 s and 90 s of ischemia). Other ions, like Ca^{2+} , Na^+ and Cl^- , similarly show an early phase of slow extracellular increase followed by an extreme rapid ex-tracellular decrease (Katsura et al., 1994; Kristian and Siesjö, 1997). These studies suggest the possibility of a reservoir that slowly becomes depleted but still can be sustained for approximately 1 min.

After the first OMC, a small transient overshoot was observed in CM. After the other compressions, CM recovered either totally or very close to the baseline. Our previous studies showed that DPOAEs can be effectively used for monitoring cochlear hypoxia (Telischi et al., 1999; Mom et al., 2000). Our CM results are similar to the DPOAE findings observed in these previous studies. DPOAEs were shown to recover to the baseline without an overshoot after the release of the compressions. Such similar behavior of CM and DPOAE would be expected since both are believed to take their origins from the outer hair cell activity. Further studies in which simultaneous recording of DPOAEs and CM can give a better understanding of these two parameters and their behavior under hypoxia–ischemia.

The experimental set up was based on consecutive compressions of eight nerve complex of the same cochlea under repetitive ischemic episodes, which were likely to create cumulative effects in the cochlear function. This is similar to situations during the course of a real surgery in which multiple blockages of the blood supply may occur. The cumulative effects of these blockages, however, remain to be explored. Likely the current experimental setup was not designed to isolate the effects of the efferent system. Future experiments should be conducted to isolate the possible effects of the efferents, which were likely to reactivate after the release of the nerve.

Variability among recordings was measured by calculating standard deviations. The standard deviations were not included in the graphs for clear visualization of the data behavior. The CM and CBF variability was fairly low and showed similar behavior for the same phases of the compression (maximum of 20% for 1-min, 30% for 3-min compression). The CAP however showed the largest variability among all the parameters recorded. The moderate variability (between 14% and 33%) in the pre and during compression phase, drastically increased during the overshoot phase (31–67% for 1-min compressions and 80% for the 3-min compression), that later decreased with the recovery. This increase could be due to the compression technique used. Variability in the nerve injury, efferent blockage and complex effects of the ischemia are likely to act together increasing the range of measured CAP variability which has to be explored further to solve the underlying complicating factors.

In this study, measurements were made every 7–8.5 s. With further technical improvements, the measurement interval will be reduced to 1–2 s, thus resulting in better event resolution for interactive monitoring during surgery. The addition of OAE measurement may be helpful in further identification of the roles and behaviors of different levels of cochlear function.

In summary, this study shows that simultaneous recordings of CBF and CAP using the otic probe placed at the RW niche are a valuable neuromonitoring tool, with the capability of reflecting the behaviors of different levels of cochlear function. Hypoxia alters ECoG signals with different effects on CM and CAP portions. CBF is a fast, reliable measurement to show the ischemia at the level of cochlea. Reperfusion yields an easily observable overshoot in the CBF measurements. CAP closely follows the changes of CBF, reflecting the affected cochlear function. A similar overshoot with a persistent delay follows the reperfusion. CM data distinguishes itself by the absence of an overshoot generally and a slower decrease in the amplitude. This difference from CAP relates to the different generation points of these two measurements.

Acknowledgements

This study was partially supported by NIH SBIR grants (1 R44 DC005720-01 and 1R43 DC05720-01) to Intelligent Hearing Systems and University of Miami. The authors kindly acknowledge the help of Jorge Bohorquez, Ph.D., Lidet Wlgiorgise Abiy, M.S., Tatiana Pereira DaCunha, BS and Jennifer Smullen, M.D. for their assistance.

References

- Asami K, Nakashima T, Morisaki H, Akanabe K, Kuno K, Yanagita N. Effects of hypercapnia on cochlear and cerebral blood flow in rabbits. *ORL J Otorhinolaryngol Relat Spec* 1995;57:239–44. [PubMed: 8587774]
- Clarke, DD.; Sokoloff, L. Circulation and energy metabolism of the brain. In: Siegel, GJ.; Agranoff, BW.; Albers, RW.; Fisher, SK.; Uhler, MD., editors. *Basic neurochemistry*. Philadelphia, PA: Lippincott–Raven; 1999. p. 637–69.
- Colletti V, Fiorino FG, Carner M, Tonoli G. Mechanisms of auditory impairment during acoustic neuroma surgery. *Otolaryngol Head Neck Surg* 1997;117:596–605. [PubMed: 9419085]
- Hu HB, Jiang SG. Effect of focal cochlear vascular lesion on endocochlear potential in guinea pigs. *Hear Res* 1995;89:69–75. [PubMed: 8600133]
- Katsura K, Kristian T, Siesjo BK. Energy metabolism, ion homeostasis, and cell damage in the brain. *Biochem Soc Trans* 1994;22:991–6. [PubMed: 7698500]
- Kristian T, Siesjo K. Changes in ionic fluxes during cerebral ischemia. *Int Rev Neurobiol* 1997;40:27–45. [PubMed: 8989615]
- Nakashima T, Suzuki T, Iwagaki T, Hibi T. Effects of anterior inferior cerebellar artery occlusion on cochlear blood flow—a comparison between laser-Doppler and microsphere methods. *Hear Res* 2001;162:85–90. [PubMed: 11707355]
- Matsuda K, Komune S, Tono T, Yamasaki M, Haruta A, Kato E. A role of glutamate in drug induced ototoxicity: in vivo microdialysis study combined with on-line enzyme fluorometric detection of glutamate in the guinea pig cochlea. *Brain Res* 2000;852:492–5. [PubMed: 10678781]
- Levine RA, Bu-Saba N, Brown MC. Laser-Doppler measurement and electrocochleography during ischemia of the guinea pig cochlea: impacts for hearing preservation in acoustic neuroma surgery. *Ann Otol Rhinol Laryngol* 1993;102:127–36.
- Millen SJ, Toohill RJ, Lehman RH. Sudden sensorineural hearing loss: operative complication in nonotological surgery. *Laryngoscope* 1982;92:913–7. [PubMed: 7098739]
- Miller JM, Marks NJ, Goodwin PC. Laser-Doppler measurements of cochlear blood flow. *Hear Res* 1983;11:385–94. [PubMed: 6226636]
- Miller JM, Bredberg G, Snopaa J, Liuolstrom B, Didier A. Measurement of human cochlear blood flow. *Ann Otol Rhinol Laryngol* 1991;100:44–53. [PubMed: 1824672]
- Miller JM, Ren T, Nuttall AF. Studies of inner ear blood flow in animals and human beings. *Otolaryngol Head Neck Surg* 1995;112:101–13. [PubMed: 7816443]
- Mom T, Telischi FF, Martin GK, Lonsbury-Martin BL. Measuring the cochlear blood flow and distortion-product otoacoustic emissions during reversible cochlear ischemia: a rabbit model. *Hear Res* 1999;133:40–52. [PubMed: 10416863]
- Mom T, Telischi FF, Martin GK, Stagner BB, Lonsbury-Martin BL. Vasospasm of the internal auditory artery: significance in cerebellopontine angle surgery. *Am J Otol* 2000;21:735–42. [PubMed: 10993468]
- Perlman HB, Kimura R, Fernandez C. Experiments on temporary obstruction of the internal auditory artery. *Laryngoscope* 1959;69:591–613. [PubMed: 13673604]
- Puel JL. Chemical synaptical transmission in the cochlea. *Prog Neurobiol* 1995;47:449–76. [PubMed: 8787031]
- Ren T, Brown NJ, Zhang M, Nuttall AL, Miller JM. A reversible ischemia model in gerbil cochlea. *Hear Res* 1995;92:30–7. [PubMed: 8647743]
- Scheibe F, Haupt H, Berndt H, Magnus S, Weymar P. Laser light transmission and laser-Doppler flow measurement on the human, rat and guinea pig cochlea. *Eur Arch Otorhinolaryngol* 1990;247:20–3. [PubMed: 2310544]

- Stern MD, Lappe DL, Bowen PD, Chimosky JE, Holloway GA, Keiser HR, et al. Continuous measurement of tissue blood flow by laser-Doppler spectroscopy. *Am J Physiol* 1977;232:441–8.
- Tabuchi K, Okuba H, Fujihira K, Tsuji S, Hara A, Kusakari J. Protection of outer hair cells from reperfusion injury by an iron chelator and anitric oxide synthas inhibitor in the guinea pig cochlea. *Neurosci Lett* 2001;307:29–32. [PubMed: 11516567]
- Telisch FF, Mom T, Agrama M, Stagner BB, Özdamar Ö, Bustillo A, et al. Comparison of the auditory-evoked brainstem response wave I to distortion-product otoacoustic emissions resulting from changes to inner ear blood flow. *Laryngoscope* 1999;109:186–91. [PubMed: 10890763]
- Telisch FF, Widick MP, Lonsbury-Martin BL, McCoy MJ. Monitoring cochlear blood function intraoperatively using distortion product otoa-coustic emissions. *Am J Otolaryngol* 1996;16:597–608.
- Telisch, FF.; Ozdamar, O.; Parel, JM.; Manns, F. Otic probe for neurocochlear monitoring US Patent. #6,640,121. 2003.
- Vasamedics Inc. Laserflo BMP2 blood perfusion monitor operator manual. St. Paul; MN: 1993.
- Weiss G, Nossal R, Bonner R. Statistics of penetration depth of photons reemitted from irradiated tissue. *J Mod Opt* 1989;36:349–59.
- Widick MP, Telisch FF, Lonsbury-Martin BL, Stagner BB. Early effects of cerebellopontine angle compression on rabbit distortion-product otoacoustic emissions: a model for monitoring cochlear function during acoustic neuroma surgery. *Otolaryngol Head Neck Surg* 1994;111:407–16. [PubMed: 7936673]

Fig. 1.
Schematic representation of otic-probe placement and instrumental setup.

Fig. 2.

Continuous recordings ECoG evoked with subsequent rarefaction, condensation clicks displayed along with CBF measurements. CAP and CM after separation from ECoG are also added for a better interpretation. Arrows show the last recording before the onset and offset of the compression. An artifact in the waveforms was caused by the touching of neighboring tissue just before the onset of compression and is shown by “*”.

Fig. 3.

ECoG recordings to rarefaction and condensation clicks (top) and the separation of CAP and CM (bottom) from ECoG using the polarity reversal effect of CM. CAP is obtained by the addition of consecutive rarefaction and condensation recordings where CM is the result of the subtraction of the same waveforms. CAP and CM amplitudes are measured as shown in the bottom figures.

Fig. 4.

Change in the waveform of CAP and CM before the compression at the point of overshoot and just before the next compression are shown taken from left ear of rabbit 13. CM and CAP are scaled differently for better visualization of the changes. Numbers 1–3 are used to mark the time location of these waveforms in Fig. 5 where the continuous recordings of all three parameters are shown.

Fig. 5.

A typical continuous recording obtained from the same ear displayed in Fig. 2 is shown. Arrows indicates the location of the waveforms given in Fig. 4. When an incomplete compression such as the one marked with “*” was observed compression was stopped and a longer relaxation time was given to the system.

Fig. 6. Results of normalized and averaged data for the 1-min compressions. (a) Shows first, (b) second and (c) third subsequent compressions. Arrows indicate the last recording before the onset and offset of the compression.

Fig. 7.
Results of normalized and averaged data for the 3-min compression.

Fig. 8.

The diagram showing the general behavior of changes in CAP, CM, CBF. The ideal compression is shown in the top waveform and the typical physiological response is shown on the lower panel with significant markers labeled as shown. The gray regions displays the tolerance levels defined by $\pm 5\%$ of the baseline and 5% of the lowest drop level. These levels are used to determine the onset-start, offset-start (if the data point value is outside the tolerance level), onset-end, and the offset-end points (if the data value is inside the tolerance level).