Original Article

Effects of glutamate on distortion–product otoacoustic emissions and auditory brainstem responses in guinea pigs

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Abstract Objectives To investigate changes in evoked potentials and structure of the guinea pig cochleae during whole cochlear perfusion with glutamate. **Methods** CM, CAP, DPOAE, and ABR were recorded as indicators of cochlear functions during whole cochlear perfusion. The morphology of the cochlea was studied via transmission electron microscopy. **Results** There were no significant changes in DPOAE amplitude before and after glutamate perfusion. CM I/O function remained nonlinear during perfusion. ABR latencies were delayed following glutamate perfusion. The average CAP threshold was elevated 35 dB SPL following glutamate perfusion.. The OHCs appeared normal, but the IHCs and afferent dendrites showed cytoplasmic blebs after glutamate perfusion. **Conclusions** While being a primary amino acid neurotransmitter at the synapses between hair cells and spiral ganglion neurons, excessive glutamate is neurotoxic and can destroy IHCs and spiral ganglion neurons. The technique used in this study can also be used to build an animal model of auditory neuropathy.

Key words glutamate; distortion product otoacoustic emission; auditory brainstem response; auditory neuropathy

Introduction

Glutamate is thought to be the primary amino acid neurotransmitter at the synapses formed by cochlea hair cells and spiral ganglion neurons. However, excessive glutamate shows excitotoxicity and can damage inner hair cells (IHCs) and afferent neurons. Studies have shown that the neurotoxic effect of glutamate analogs results in massive swelling and degeneration of IHCs and auditory afferent dendrites [1]. A report by Chinese researchers demonstrated that the input/output function of cochlear microphonics (CM) maintained a nonlinear pattern, while the compound action potential (CAP) threshold and amplitude changed, in response to glutamate instillation [2]. Few studies have simultaneously observed the effects of glutamate on both cochlear potentials and morphology. In this study, we investigated changes of distortion product otoacoustic emissions (DPOAEs) and auditory brainstem responses (ABRs) during whole cochlear perfusion with glutamate. Transmission electron microscopy (TEM) was used to examine the morphology of the cochleae. Understanding the nature of cochlear excitotoxicity can conceivably contribute to the prevention and management of certain types of hearing deficits.

Materials and methods

Thirty healthy guinea pigs of both sexes, weigh-

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ing 300~400g each and free of middle ear infection, were randomly selected into the following 3 groups with 10 in each group: 1) artificial perilymph (APL) group, with CM and CAP monitored; 2) 10mmol/L glutamate group, with DPOAEs and ABRs monitored; and 3) 10mmol/L glutamate group, with CM and CAP monitored.

The animal was anesthetized with sodium pentobarbital (40mg/kg, i.p.), intubated and maintained on a positive pressure ventilator. The external acoustic meatus was examined and cleaned. Whole cochlear perfusion was conducted as previously reported (Yu et al., 1999)[3]. The right cochlea was exposed via a dorsal approach. One window (0.2 mm in diameter) to the scala tympani and another to the scala vestibuli were carefully opened in the basal turn with a drill. A glass pipette filled with either APL or glutamate (Sigma, USA) and connected to a microinfusion pump previously filled with the same solution was advanced into the scala tympani using a micromanipulator.

DPOAEs were recorded using the ILO92 system (Otodynamics, UK) from the ear canals. The f2 was set at 1001, 2002, 4004 and 6006Hz, and presented at 65 dB SPL, while the level of f1 was set at 70 dB SPL with an f2/f1 ratio was of 1.22. The 2f1–f2 amplitudes were plotted as DPgrams.

ABRs were recorded via subdermal electrodes in a standard vertex to postaural configuration on a SmartEP2.22 system (Intelligent Hearing System, USA). Acoustic click stimuli were used and the evoked potentials were band–pass filtered (80 Hz to 3 kHz) and amplified in a conventional manner.

For CAP and CM recordings, an electrode was placed at the round window. The electrode for the reference was placed in the neck. CAP was evoked by click stimuli and CM by 4 kHz tone bursts (rise/fall 2 ms and plateau 20 ms). CAP thresholds and CM amplitude were determined before and after perfusion. The entire test procedure lasted about 2 hours.

Upon completion of recording, animals were sacrificed and the cochleae was removed and prepared for TEM examination. Small openings were made at the apical end of the cochlea and at the round window. The cochlea was fixed with perfusion of 2.5% glutaraldehyde and rinsed with 0.1mol/L phosphate buffer solution (PBS). This was followed by decalcification in 10% of EDTA. The specimen was then post–fixed with 1% osmium tetroxide and gradually dehydrated with ethyl alcohol followed with acetone. The specimen was subsequently embedded, dissected and dried.

Statistical analysis was performed using Strata 4.0. The rank sum test was used to compare DPOAEs before and after glutamate perfusion. Changes of ABRs were analyzed using paired t–test. One way analysis of variances was used to assess the statistical significance of CAP variation. The criterion for statistical significance was set at $P < 0.05$.

Results

DPOAEs

Little changes in of DPOAE amplitude were noticed after 10 mmol/L glutamate perfusion (Tab.1).

Changes in ABRs

Clicks at 100 dB SPL were used to elicit ABRs and latencies of waves and were measured. . ABR responses were lost in 2 ears following glutamate perfusion, Latencies of waves and were delayed in the remaining 8 ears after glutamate perfusion, with no significant changes in – intervals (Tab.2).

CM input/output (I/O) function

After artificial perilymph perfusion, the CM relative amplitude and I/O function were similar to

Table 1 DPOAE amplitude before and after glutamate perfusion ($\bar{x}$ ± SD, dB SPL)

	ears	1 kHz	2 kHz	4 kHz	6 kHz
Pre–perfusion	10	10.29 ± 6.70	15.23 ± 9.04	16.29 ± 3.37	20.78 ± 4.39
Post–perfusion	10	10.24 ± 5.72	17.1 ± 5.28	16.6 ± 3.34	21.05 ± 2.03

$P > 0.05$

Table 2 ABR latencies before and after glutamate perfusion ($\bar{x}\pm SD$, ms)

	ears	Latencies in wave	Latencies in wave	– intervals
Pre–perfusion	10	1.75±0.22	3.44±0.20	1.68±0.16
Post–perfusion	8	1.97±0.17*	3.81±0.34*	1.82±0.30

* $P<0.05$; $P>0.05$

pre–perfusion measures (Fig.1), The CM I/O function remained nonlinear after 10mmol/L glutamate perfusion, although the relative amplitude declined (Fig. 2).

Changes in CAP

The CAP threshold shift after 10mmol/L glutamate perfusion was higher than after APL perfusion [30.00±3.33(SD) 2.00 ± 2.58 dB SPL, $P<0.05$].

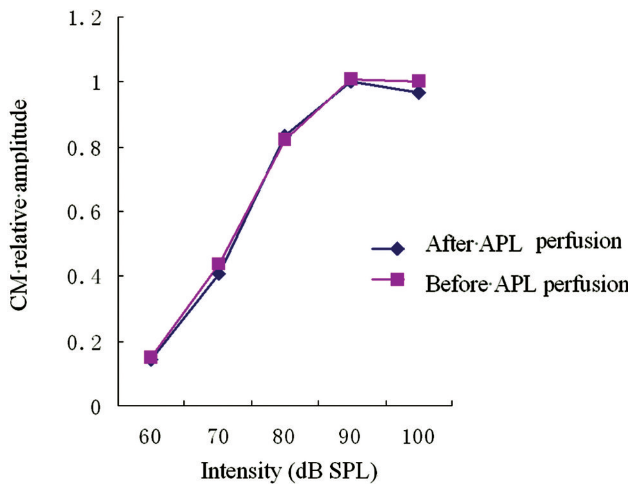

Figure 1 CM I/O functions before and after APL perfusion (n=10)

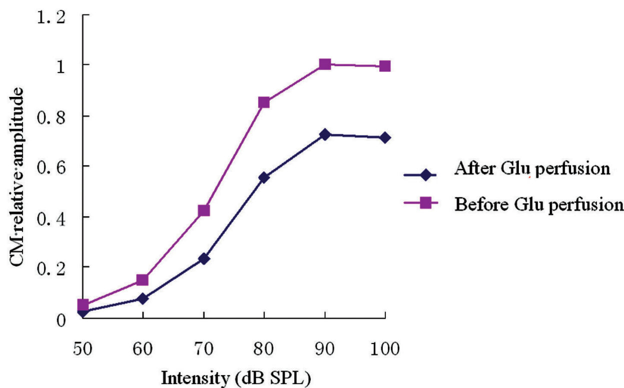

Figure 2 CM I/O functions before and after glutamate perfusion (n=10)

TEM

TEM showed normal IHCs and afferent nerve endings (Fig. 3) and intact OHCs (Fig. 4) after APL perfusion.

Cytoplasmic blebs were seen in IHCs and afferent dendrites (Fig.5), but not in OHCs (Fig.6) after 10mmol/L glutamate perfusion.

Discussion

Glutamate selectively damages the IHC and/or the afferent nerve system

Various electrophysiological measures have been used to study different aspects of cochlear function. CM mainly reflects OHC activities and CAP can be used to monitor IHC transduction and cochlear afferent neuronal activity [4, 5]. This study shows that the CM I/O function maintains its nonlinear pattern while the CAP threshold is elevated,

Figure 3 Normal–appearing IHC after APL perfusion

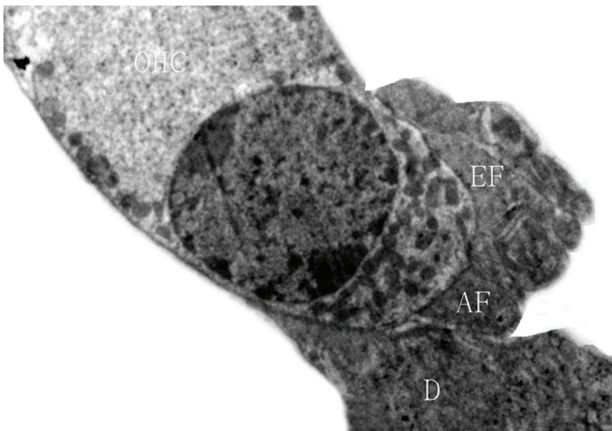

Figure 4 Normal–appearing OHC, afferent nerve fibers (AF), efferent nerve fibers (EF) and a Deiter cell (D) after APL perfusion

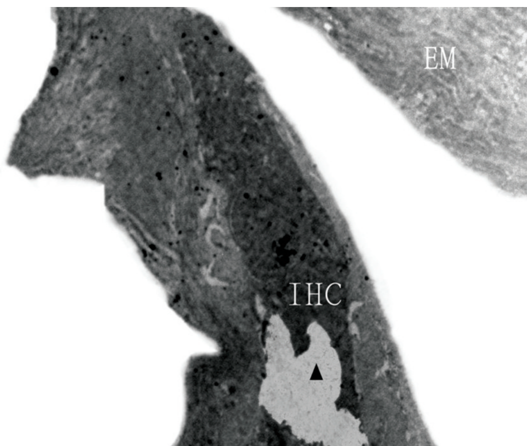

Figure 5 Vacuoles in the IHC and afferent nerve endings after glutamate perfusion(▲)

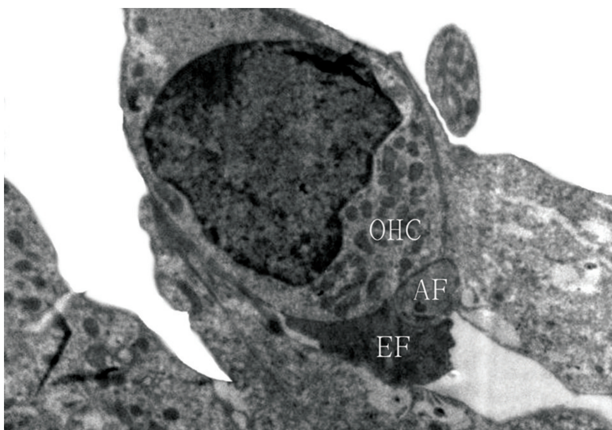

Figure 6 Intact OHC, AF, and EF after glutamate perfusion

indicating selective damage to IHC synapses and type I afferent dendrites by glutamate. DPOAEs are believed to reflect OHC functions. The wave I in ABRs, as well as CAP, come from auditory afferent nerve fibers[6]. Our study shows that DPOAE amplitudes show little change in response to glutamate perfusion, while ABRs are either lost or the wave I and III latencies are delayed. This observation confirms dysfunction of the IHC and/or cochlear nerve and preserved OHC function. Our TEM results are also consistent to the electrophysiological findings.

Glutamate is thought to be the neurotransmitter for the IHC–auditory nerve synapsis. Cochlear damage caused by noise, ischemia and hypoxia are very similar to those seen after exposure to glutamate receptor agonists. It has been suggested that noise–, ischemia– and hypoxia–induced hearing loss may be caused, in part, by glutamate excitotoxicity [7]. Tan et al [8]. reported lessened non–linearity in CM, elevated CAP threshold, and vacuolizations in the OHC and in the afferent nerve endings underneath the IHC after noise exposure, as well as significantly reduced immunogold particle densities in the IHC. They suspected an over–load of afferent neurotransmitter glutamate in IHCs noise exposure and the the excitotoxicity of excessive glutamate may be one of the injury mechanisms in noise induced hearing damage.

Mechanisms of cochlea damages by excessive glutamate

Glutamate is the neurotransmitter acting at IHC–primary auditory dendrite synapses. At least three pharmacologically distinct types of ionotropic glutamate receptors mediate the depolarizing effects of glutamate on the membrane potential of neurons. These ionotropic receptors can be divided into N–methyl–D–aspartate (NMDA), –amino–3–hydroxy–5–methyl–isoxazol–propionate(AMPA), and kainate subtypes. Exessive excitation of these postsynaptic receptors, referred to as excitotoxicity, can result in the destruction of the neuron. Acute postsynaptic overload causes an excessive influx of sodium into the auditory dendrites, and the resulting osmotic imbalance then provokes a massive water entry. In addition, it causes an increase in the intracellular calcium concentration, which leads to destruction of the primary auditory dendrites and later on to neuronal death [1]. The AMPA–induced damage to the afferent dendrites was protected by the administration

of glutamate antagonists 6, 7–dinitroquinoxaline–2, 3–dione (DNQX) prior to AMPA treatment [9]. We further speculate that the fundamental damaging effect on the IHC/cochlear afferents could be ultimately mediated by glutamate excitotoxicity.

Whole cochlear perfusion with glutamate for an animal model of auditory neuropathy (AN)

Starr et al.[10] identified a group of patients with hearing deficits who had normal otoacoustic emissions but absent or severely abnormal ABRs. They inferred that the disorder could be at the level of the IHC, between the IHC and VIII nerve fibers, the ganglion neurons, the nerve fibers, or any combination of the above. Miyamoto et al. [11] and Buss et al. reported that cochlear implantation was a feasible rehabilitation method for auditory neuropathy. Those clinical reports indicated the patient of AN might still have certain proportion of normal afferent nerve fibers. The pathological changes in AN are possibly in the IHC, synapses, and afferent nerve fibers.

After 10mmol/L glutamate perfusion for 2 hours, there were no significant changes in DPOAE amplitude. On the other hand, ABRs were lost in 2 and ABR latencies delayed in 8 of the 10 test ears. The differences in ABR changes in this study are possibly related to each animal's unique physiology as well as its sensitivity to glutamate. With glutamate perfusion, the conditions resulting in IHC /cochlear afferent system damage with relatively normal OHC functions mimic many of the characteristic symptoms seen in AN. The less severe changes of ABR than seen in clinical AN are possibly related to the glutamate concentration used in perfusion. Theoretically, an axonal neuropathy should not alter neural synchrony but only the number of conducting fibers [10]. We suggest that the failure to detect an averaged auditory brainstem potential in some of the animals reflects altered temporal synchrony of the auditory brainstem pathway activity due to the auditory nerve disorder. In these animals, the auditory nerve and brainstem discharges are not precisely time–locked to the acoustic signal so that the short duration components are cancelled in the averaging process, rendering them indistinguishable from the background electrical events. Excess extracellular glutamate may play a role in the degeneration of the organ of Corti in excitotoxic conditions.

In conclusion, we have demonstrated an animal model that shows many of the characteristics reported clinically in patients diagnosed with AN. The neural representation of sensory events depends upon neural synchrony, therefore a normal ABR is recorded only when multiple neurons fire synchronously at stimulus onset [13]. We therefore suggest that one possible underlying pathology for AN is a scattered loss of IHCs and cochlear afferent systems. We further propose that one of the causes of such pathology is related to glutamate excitotoxicity.

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