
Protecting the Inner Ear from Acoustic Trauma

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Abstract: Calcium activated proteases, or calpains, play an important role in neurodegeneration. In some cases, neural degeneration can be significantly reduced by leupeptin, a potent calpain inhibitor. To determine if leupeptin could protect against noise-induced hearing loss and hair cell loss, we infused leupeptin into scala tympani of one cochlea before, during and after a 14-day exposure to a 100 dB SPL, octave band noise centered at 4.0 kHz. Hearing loss, assessed with the auditory evoked response, was less in the leupeptin-treated ear than in the control ear during the early stage of recovery from acoustic trauma. In addition, hair cell loss in the leupeptin-treated ear was significantly less than in the control ear. These preliminary results suggest that leupeptin may protect against noise-induced hearing loss.

Keywords: leupeptin; calpain; neurodegeneration; acoustic trauma; hair cells

Over the past decade, considerable progress has been made in understanding the molecular and cellular mechanisms that promote the development and survival of various types of neurons [1–4]. Some factors exert their effects during specific periods of development while others act throughout the life span. While most neurons mature and survive, others undergo a carefully orchestrated process of programmed cell death or apoptosis. Progress has also been made in elucidating the biochemical signals that cause mature neurons to degenerate. With this increased knowledge, comes the possibility of developing drug intervention strategies that may delay or prevent many common neurodegenerative disorders such as stroke, Alzheimer's disease, hearing loss and tinnitus [5–8]. Hearing impairment affects roughly 7–8% of the population in the United States and for those over the age of 65, the prevalence of hearing loss exceeds 25% [9]. Sensorineural hearing loss is most often caused by exposure to high-level noise exposure, aging or some combination of the two [10]. Noise-induced hearing loss and presbycusis are typically associated with damage to the sensory hair

cells and spiral ganglion neurons. Thus, any drug that prevents the degeneration of the hair cells and spiral ganglion neurons could conceivably reduce the risk of hearing loss [11–15]. One approach to identifying potential otoprotective agents is to screen for their protective effects *in vitro*. However, acoustic overstimulation is impractical to apply to *in vitro*. In addition, the long-term consequences of these compounds must eventually be evaluated *in vivo* to determine their positive and negative side effects. In an earlier paper, one of the authors (Stracher) discussed the role of calpain in neural degeneration and the possibility of using calpain inhibitors as neuroprotective agents [16]. In this paper, we will discuss our preliminary studies with leupeptin, a calpain inhibitor that may protect against noise-induced hearing loss. We will briefly review some of the properties of calpains and the neuroprotective effects of calpain inhibitors.

Calpain

Calpains are a ubiquitous family of calcium-activated proteases. They are thought to play a role in the biochemical pathway leading to cell death in neurons and other cells [17–20]. When intracellular calcium levels increase under pathological conditions, calpain levels rise resulting in the proteolysis of cytoskeletal and membrane proteins, phosphatases, kinases and transcription factors. The two most common forms of calpain,

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calpain I (μ -calpain) and calpain II (m-calpain), are activated at micromolar and millimolar levels, respectively. Several calpain antagonists have been shown to be neuroprotective *in vitro* and *in vivo* during ischemia as well as brain, spinal cord and peripheral nerve injury [17,21–23]. However, the efficacy of calpain inhibitors varies with type of neuron and the mechanism of injury.

Previous work by Stracher has shown that oral administration of leupeptin, a potent calpain inhibitor, promoted the recovery muscles and neurons after median nerve transection and repair [19,22]. Furthermore, long-term administration of leupeptin did not cause any adverse side effects. These results suggest that leupeptin is safe and could conceivably be used to protect the hair cells and neurons in the inner ear from damage caused by acoustic overstimulation. A potential limitation is that it does not easily cross the blood brain barrier.

Local Drug Delivery

Because the cochlea is relatively easy to approach surgically, it is possible to deliver leupeptin directly to the inner ear using an osmotic pump [24]. The long-term effectiveness of the pump depends on the pumping duration and the degradation of leupeptin when maintained at body temperature in the carrier solution, Hanks Balanced Salt Solution (HBSS). In the present study, we used an osmotic pump (Alza, 0.5 μ L/hr, and 14-days duration) to deliver leupeptin into scala tympani of the chinchilla. The osmotic pump was implanted and secured under the skin in the back of the neck. The pump was connected to a microcatheter consisting of flexible polyethylene tubing terminated at its distal end by a short piece of metal tubing. A small ridge of silicone glue was placed near the end of the metal tubing to help to seal the tube in scala tympani and limit its insertion depth into scala tympani. The catheter was cemented to the bulla with dental cement as it exits the middle ear. The pump was filled with leupeptin (1 mg/ml) in Hank's Balanced Salt Solution (GIBCO).

Auditory Evoked Response

To determine if leupeptin, the carrier solution or the surgery had any negative effects on auditory function, we measured the auditory evoked response prior to and during infusion of leupeptin into scala tympani of the right cochlea. The auditory evoked response was measured from chronic electrodes implanted in the inferior colliculus according to procedures outlined in an earlier report [25]. The local field potential from the colliculus was recorded in response to tone bursts and the evoked response thresholds were determined using visual detection criteria. Figure 1 shows the shift in the evoked

response thresholds at four days post-implantation of the osmotic pump. The implantation of the pump and perfusion of leupeptin and carrier solution had no effect on auditory evoked response thresholds. This suggests that leupeptin has no short-term deleterious effect on auditory function.

Noise Exposure, Hearing Loss and Hair Cell Loss

To determine if leupeptin could protect the sensory cells in the cochlea from acoustic overstimulation, we used the osmotic pump to deliver leupeptin to the right cochlea. The left cochlea was untreated and served as a within subject control. After the pump had been implanted and operating for four days, the chinchillas were exposed for 48 hours to a 100 dB SPL, octave-band noise centered at 4.0 kHz. After the exposure, hearing loss was assessed at regular intervals. Approximately four weeks later, the animals were sacrificed and the left and right cochleae were removed, stained with succinate dehydrogenase histochemistry, fixed and dissected out as a flat surface preparation [26,27]. The percentage of missing inner hair cells (IHCs) and outer hair cells (OHCs) were determined as function of distance from the apex of the cochlea and the results plotted as a cytochleogram.

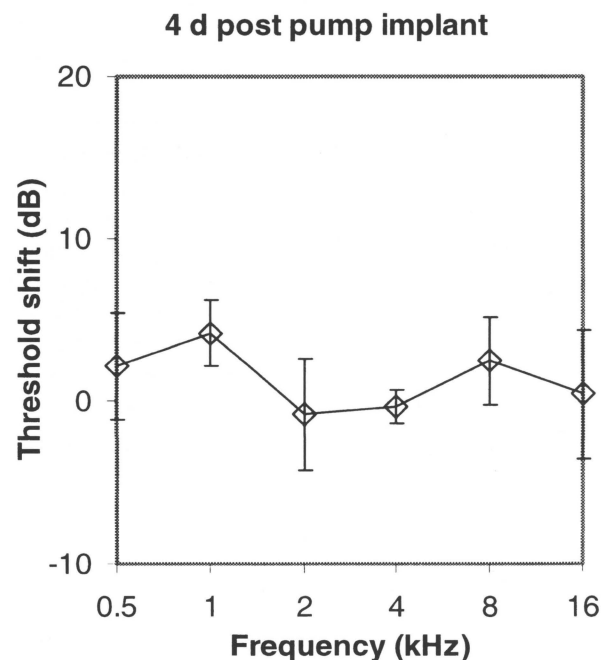


Figure 1. Change in average ($N = 4$, vertical bars ± 1 SD) change in evoked response thresholds measured four days following implantation of an osmotic pump in the basal turn of scala tympani of the chinchilla.

Figure 2 shows the threshold shift as a function of frequency 0-days and 7-days post-exposure. Immediately after the exposure, the threshold shifts at 4, 8 and 16 kHz were between 30 and 50 dB in the control ear; in the leupeptin-treated ear, the threshold shift was about 10 dB less than in the untreated ear. By 7-days post-exposure, the hearing loss had decreased significantly in both ears. However, a 30-dB threshold shift was still present in the control ear near the exposure frequency, 4 kHz. The threshold shifts in the leupeptin

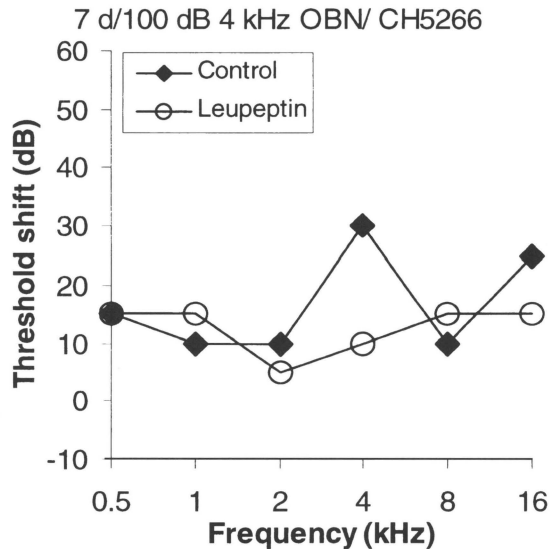
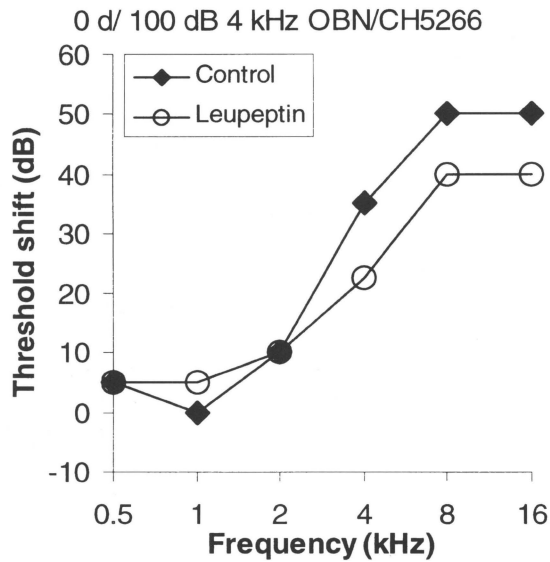


Figure 2. Threshold shift of the auditory evoked response as a function of stimulus frequency. Measurement obtained from the untreated control ear and the leupeptin treated ear.

treated ear were negligible. These results suggest that leupeptin may protect the ear from acoustic overstimulation.

Figure 3 shows the anatomical data obtained from the animal shown in Figure 2. Hair cell loss was evident in the untreated control ear near the 4 kHz region of the cochlea; however, there was little evidence of damage in the leupeptin-treated ear. Figure 3B shows a surface preparation view of the organ of Corti taken from the untreated, control ear in the region of maximum damage. Most of the OHCs were missing from all three rows; however, the IHCs were still present. Figure 3A shows the same region of the organ of Corti in the leupeptin-treated ear. The IHCs and nearly all of the OHCs were present in the leupeptin treated ear. Preliminary results similar to these have been obtained from other animals exposed to the same noise or a higher level of noise. While these preliminary results are encouraging, we have noted some deterioration in auditory thresholds when the osmotic pumps have been running for 14 days or more. This could be due to the depletion of the pump or more likely, the degradation of leupeptin when it is maintained in the pump at body temperature.

SUMMARY

Previous studies have shown that calpain inhibitors promote the survival and function of neurons in the peripheral and central auditory system [17,21-23]. Our preliminary studies suggest that leupeptin, a potent calpain inhibitor, may protect the hair cells in the inner ear from acoustic overstimulation. The histological re-

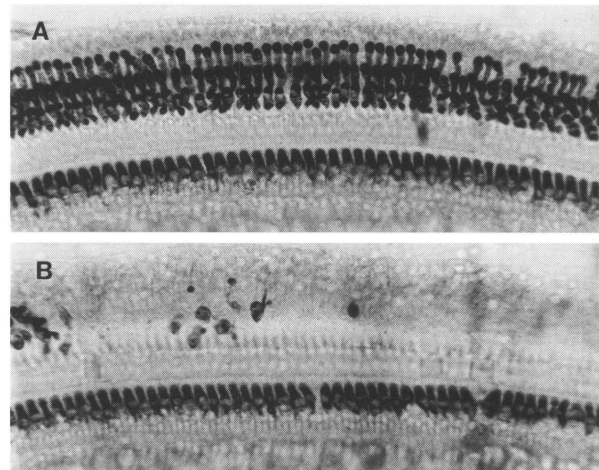


Figure 3. Surface preparation of the organ of Corti in the 4-kHz region of the cochlea. (A) Leupeptin-treated ear. (B) Normal control ear.

sults gathered to date suggest that leupeptin can significantly reduce the amount of hair cell loss from acoustic overstimulation. Our functional measures also show that leupeptin may reduce the amount of hearing loss during the early stage of recovery from acoustic overstimulation; however, its long-term effectiveness is not clear and need further investigation. While our preliminary results are encouraging, many other important issues need to be addressed. For example, what is the optimal dose of leupeptin? Because leupeptin was delivered into the basal turn of the cochlea, the concentration gradient is likely to vary along a base-to-apex gradient. In addition, leupeptin was delivered into a fairly, large fluid space. Therefore, we do not know its actual concentration in the fluid or various tissue compartments. In an attempt to optimize its effects, leupeptin was administered four days before the start of the exposure. Most individuals in the real world do not know in advance what type of noise they will be exposed to. Therefore, from a practical standpoint, it would be important to determine if leupeptin can prevent the degeneration of the hair cells if it is given after a traumatic sound exposure. If so, what are the time limits of the rescue effect? Obviously, this would have important implications for cases of sudden hearing loss. Acoustic damage to the inner ear is thought to involve at least two different mechanisms. Extremely high-level sound exposures (e.g., gun fire) are thought to damage the inner ear by direct mechanical destruction of the sensory cells and supporting cells [28]. By contrast, prolonged noise exposure at moderate intensities may damage the inner ear by mechanisms involving metabolic fatigue and production of lethal metabolites (e.g., free radicals) [14]. Clearly, it would be important to determine if leupeptin can protect against both short-duration, high-level exposures and moderate-level, long-duration exposure. Clearly, much further work is needed to fully address these important questions.

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