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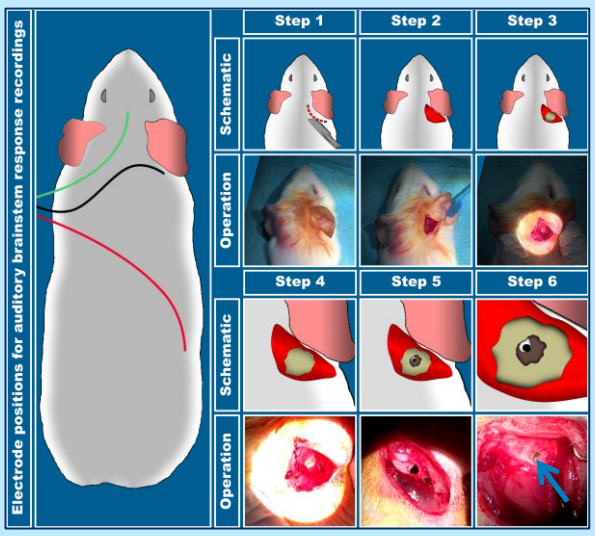
Does Ouabain Induce Selective Degeneration of Type-I Spiral Ganglion Neurons in the Guinea Pig Cochlea?

Introduction

Round window membrane application of the specific Na^+/K^+-ATPase inhibitor ouabain is known to selectively destroy type-I spiral ganglion neurons (SGNs) in the cochlea of several rodent species (for a review, see Lang *et al*., 2016). This protocol has been used in several cell-based regeneration studies – mostly in rats and Mongolian gerbils – to induce partial ablation of the cochlear nerve. Data on the morphological effects of cochlear application of ouabain in the guinea pig are limited and conflicting. Hamada and Kimura (1999) observed that ouabain results, in a dose-dependent way, in degeneration of type-I SGNs and outer hair cells (OHCs), loss of nerve endings at the base of the inner hair cells (IHCs) and vacuolation in the stria vascularis. In contrast, Cho *et al*. (2011) found that ouabain selectively destroys type-I SGNs without affecting the OHCs. Therefore, we have repeated the latter study and investigated if round window application of ouabain induces selective degeneration of SGNs in the guinea pig cochlea.

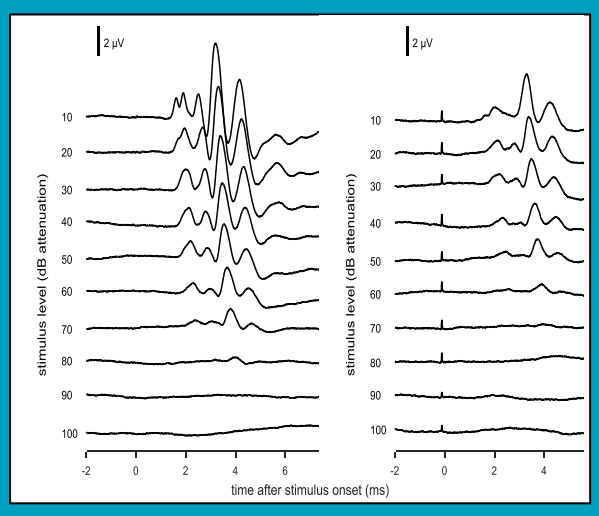
Experimental Design

The auditory bulla of the right ears of female albino guinea pigs (Dunkin Hartley strain; N=20) was opened via a retro-auricular surgical approach.
Small cubes (1 mm^3) of gelfoam (Willospon® Special) soaked in 10 µl of either ouabain solution (10 mM, 1 mM, 0.1 mM and 0.01 mM) or PBS were placed upon the round window membrane (Havenith *et al*., 2011). The hole in the bulla was fully closed with self-curing glass ionomer restorative dental cement (GC Fuji PLUS® II). The left cochleas served as controls.
Click-evoked auditory brainstem responses (ABRs) were recorded to determine pre-treatment (Day 0) and post-treatment hearing thresholds at 2, 4, and 7 days after gelfoam placement. Animals were euthanized at Day 4 or Day 7.
Immediately after the final ABR recordings, animals were euthanized and the cochleas were fixed by intralabyrinthine perfusion with a tri-aldehyde fixative (De Groot *et al*., 1987) and processed for histological examination.

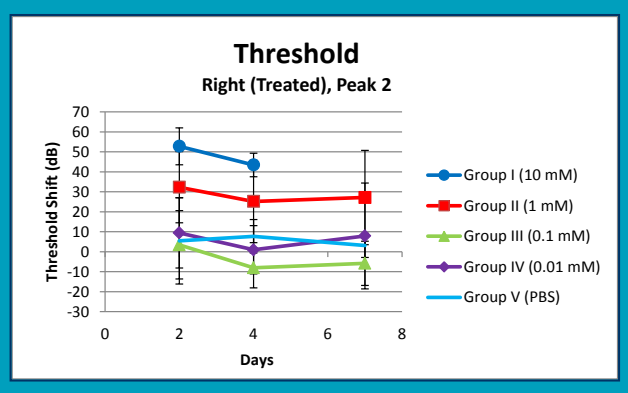


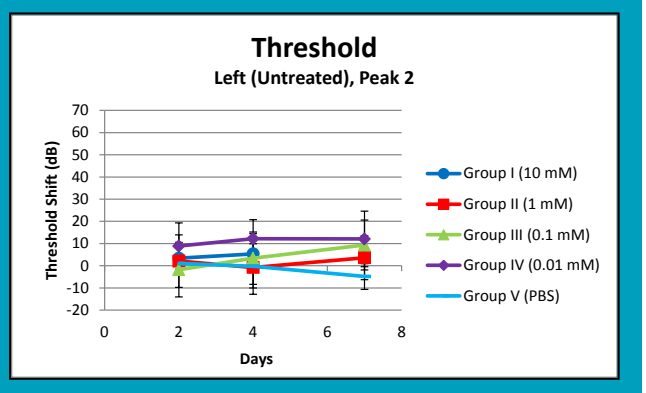
ABR Audiometry

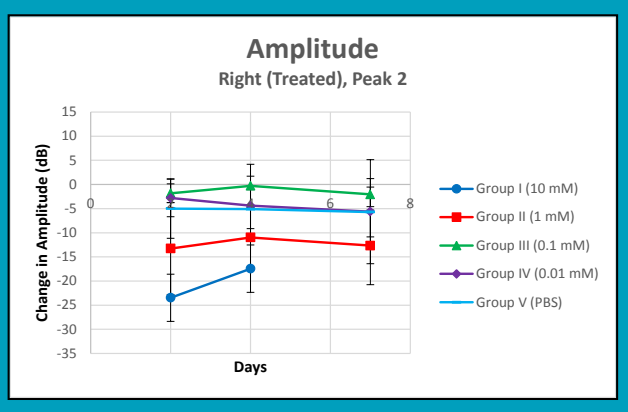
Animals treated with 1 mM ouabain (Group I) or 10 mM ouabain (Group II) showed a progressive shift in ABR thresholds and a significant change in the amplitude of ABR peak 2. After receiving lower doses of ouabain (Groups III and IV) or after treatment with PBS alone (Group V) animals did not show any changes in ABR thresholds and amplitudes.

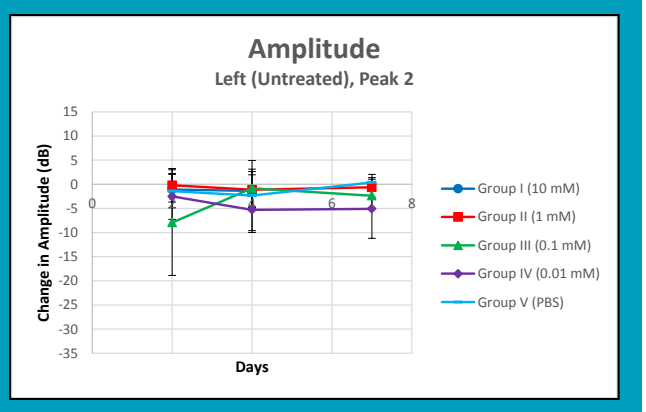


Line graphs depicting click-evoked auditory ABR recordings of the right ear from an animal treated with 1 mM ouabain. Shown are pre-treatment (Day 0; left panel) and post-treatment (Day 7; right panel) thresholds.









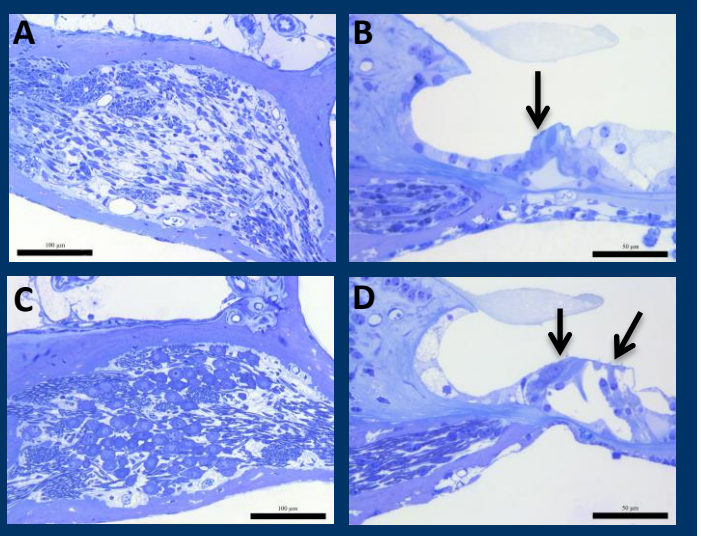
Histology

In animals treated with 10 mM ouabain (Group I) or with 1 mM ouabain (Group II) histological changes were observed in the spiral ganglion (Fig. A) and the organ of Corti (Fig. B), already within 4 days after gelfoam placement. The effects of ouabain varied considerably and can be divided into three groups:

- Complete loss of OHCs in the basal and middle turns (Fig. B) and partial loss in the apical turn, together with complete loss of SGNs in the basal and middle turns (Fig. A)
- Complete loss of OHCs in the basal and middle turns, without any SGN loss
- No loss of OHCs and SGNs

In none of the animals IHC loss (Fig. B) or histological changes in the stria vascularis could be observed.

Animals receiving 0.1 mM ouabain (Group III), 0.01 mM ouabain (Group IV) or PBS alone (Group V) expressed normal morphology of the spiral ganglion (Fig. C) and organ of Corti (Fig. D).



Conclusions

Our preliminary results show that round window membrane application of ouabain does not induce selective degeneration of SGNs in the guinea pig cochlea.
However, it does – in a dose-dependent way – result in both OHC and SGN loss, already within 4 days after round window membrane application.

References

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De Groot *et al.* (1987) *Acta Otolaryngologica* 104: 234-242
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Acknowledgements

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