73. **Effects of Neomycin on the Lateral-Line Organ of the Mudpuppy**

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It is known that the lateral-line organs of aquatic animals are homologous to the mammalian inner ear, especially in the receptive mechanism of the hair cells. The initial step of the sensory transduction of mechanical stimulation in hair cells probably takes place at the apical cell surface,\(^1\) but detailed mechanisms of mechanoreception are still unknown. Sand\(^2\) described the effect of different ionic compositions of the external water medium on the mechano-sensitivity of the lateral-line organ. He reported that the mechano-sensitivity was a function of the \(\text{Ca}^{2+}\) concentration in the external medium. Further information is needed regarding the \(\text{Ca}^{2+}\) effect on the sensory transduction in hair cells.

Recently a model was proposed for the mechanism of neomycin ototoxicity. In this model the basic groups of the antibiotic bind directly to polyphosphoinositides, which are thought to be the sites of calcium binding in the membrane.\(^3\) We therefore investigated the effects of neomycin and calcium on the mechano-sensitivity of the lateral-line organ.

**Materials and methods.** Experiments were performed on the neuromasts of the epidermal lateral-line organs in the tail of the mudpuppy (*Necturus maculosus*). The neuromasts on the tail are grouped in so-called “stitches”, with two to six neuromasts per stitch. Each neuromast in a single stitch is innervated by two afferent neurons. A single neuromast contains eight to ten hair cells.

The animal was immobilized by a gallamine triethiodide injection of 1 mg per 100 g body weight. The tail of the animal was fixed to a wooden board in the experimental chamber, while the head, gills, and trunk were kept in a separate bath filled with aerated water. The blood flow was monitored locally by examining capillaries near the neuromasts.

The afferent spikes (the lateral-line nerve discharges) were recorded by a suction electrode enclosing one of the neuromasts of a stitch. 8 Hz sine waves of mechanical vibration were employed as stimuli, and were applied to another neuromast within the same stitch.
by a glass sphere of about 50 μm in diameter. Each spike triggered a square pulse of 5 ms duration, and these pulses were averaged by a signal processor (SANEI-7T07). The processor was triggered by the sine wave generator that controlled the vibrator. The averaged value for a certain stimulus phase gives the probability of an action potential occurring within 5 ms. Therefore, the mechano-sensitivity was expressed as an increment of the synchrony between the stimulus and action potentials. Usually 100 sweeps were averaged and the intensity of the stimulus was kept constant throughout the experiment.

To investigate the effect of neomycin on the mechano-sensitivity, solutions of neomycin in distilled water with concentrations from 1:500,000 to 1:10,000 were applied to the lateral-line organ. In order to test the effects of neomycin on the chemoreception, solutions of 30 mM NaCl, 50 mM NaCl, or 1 mM CaCl₂ dissolved in 50 mM NaCl solution were applied before and after the neomycin treatment. Chemical responses were recorded by means of a ratemeter as described in the other paper.

Results. Synchronization between 8 Hz sine waves of mechanical vibration and afferent nerve discharges were examined in distilled water and in neomycin solution. When a solution of neomycin with a concentration of 1:10,000 was applied to the neuromasts for 5 min, the afferent synchronization was completely suppressed (Fig. 1A).

Fig. 1. Effects on afferent synchronization when neomycin is applied to the lateral-line organ of the mudpuppy. A: Responses averaged by signal processor. DW: Distilled water. 8 Hz stimulus indicated by lowest trace. B: Applications of three different concentrations of neomycin, and recovery in DW. Bar at bottom indicates application time of neomycin. Ordinate: Normalized synchronization rate. Standard level is synchronization in DW. Abscissa: Time in minutes.
In order to study whether this effect of neomycin was reversible or not, the organ was rinsed in distilled water. Recovery of the afferent synchronization was gradual and complete recovery was obtained after 80 min. Effects of lower concentrations of neomycin were also studied. At a neomycin concentration of 1:100,000 the afferent synchronization decreased to 30% of the original value obtained in distilled water and recovery to the original level required 50 min. At a neomycin concentration of 1:500,000 no suppressive effect could be recognized (Fig. 1B).

It has been reported that increasing Ca\(^{2+}\) concentration in the external medium enhanced the mechano-sensitivity of the lateral-line organs.\(^2\) The same Ca\(^{2+}\) effect was observed in the present study. Afferent synchronization was improved remarkably by application of 20 mM CaCl\(_2\) solution. The rate of synchronization in 20 mM CaCl\(_2\) solution was 5 times greater than in distilled water. Next, the influence of Ca\(^{2+}\) on the neomycin effects was tested. Neomycin solution

![Graph](image_url)

**Fig. 2.** Influence of CaCl\(_2\) or KCl on effects of neomycin. The solid line at bottom indicates application time of neomycin solution. The dotted line at bottom indicates application time of a mixed solution of neomycin and CaCl\(_2\), or neomycin and KCl. Neomycin concentration is unchanged in all cases. Filled circle at top right indicates synchronization in 20 mM CaCl\(_2\) without neomycin. Ordinate and abscissa: As in Fig. 1.
at a concentration of 1:20,000 was applied to the neuromasts for 5 min. Then the external medium was changed to a mixed solution of neomycin and CaCl₂. Three different concentrations (5 mM, 10 mM, and 20 mM) of CaCl₂ were used. Even after 3 min afferent synchronization in the mixed solutions improved markedly. After 20 min rates of synchronization in the mixed solutions containing CaCl₂ at 5 mM, 10 mM, and 20 mM were 2, 2.6 and 3.6 respectively as shown in Fig. 2.

K⁺ and Na⁺ have been found to enhance the mechano-sensitivity of the lateral-line organs, but the effect of these ions is much less for Ca²⁺. Open squares in Fig. 2 show the results after substituting CaCl₂ for KCl. No recovery of mechano-sensitivity was obtained in KCl.

Chemical responses of the lateral-line organ to mono- and divalent cations have been described in various aquatic animals by Katsuki and his co-workers. Effects of neomycin on the chemoreception was tested. Neomycin solution caused no effect on the spontaneous discharges. As described above application of neomycin with a concentration of 1:20,000 for 5 min caused complete suppression of afferent synchronization to mechanical stimulation. However, the chemical response to 30 mM NaCl solution was unchanged before and after application of neomycin. We also tested whether the suppressive effect of Ca²⁺ on the stimulating effect of NaCl was influenced by treatment with neomycin. When 1 mM CaCl₂ was added to 50 mM NaCl solution, the response to NaCl was suppressed markedly, but this suppressive effect was unchanged by neomycin. Therefore, chemical responses to mono- and divalent cations were not affected by neomycin.

Discussion. The morphological and electrophysiological changes accompanying the ototoxic actions of aminoglycosidic antibiotics have been frequently and extensively described. Wersäll et al. reported that streptomycin reversibly reduced the microphonic output from the lateral-line organ and suggested that this antibiotic primarily affected the surface membrane of the receptor cells. The suppressive effect of neomycin on the mechano-sensitivity in our experiments was also reversible, and this supports their hypothesis.

EGTA has been found to decrease the mechano-sensitivity of the lateral-line organ by chelating Ca²⁺ in the external medium. The same mechanism might be suggested for the effect of neomycin in eliminating the mechano-sensitivity. However, the suppressive effect of Ca²⁺ on the chemical stimulation of NaCl was unchanged by neomycin when complete suppression of afferent synchronization was observed. In other words, the mechanical suppression was caused by neomycin even in Ca²⁺ containing medium. It is therefore suggested
that the mechanisms of mechanical suppression by neomycin and
EGTA might be different.

Schacht demonstrated a high rate of polyphosphoinositide meta-
bolism in the inner ear and interference of neomycin with this meta-
bolism.\textsuperscript{3,4} He proposed, as a hypothesis for the mechanism of neomycin
ototoxicity, that the basic groups of the antibiotic bound directly to
the polyphosphoinositides in the membrane and interfered with the
control of selective membrane permeability. Furthermore, this drug
binding would impose conformational changes on the membrane. Such
an action on the membrane may explain the suppression of the
mechano-sensitivity of the lateral-line organ by neomycin. The sup-
pressive effects of neomycin were reversed by application of excessive
calcium. This fact suggests competitive occupation of sites on the
membrane by neomycin and calcium.

It has been reported that the mechano-sensitivity of the receptor
cells of the lateral-line organ is enhanced by potassium ions in the
external medium.\textsuperscript{5} The irreversible suppression of mechano-sensi-
tivity in KCl containing medium in our study indicates different
actions of K\textsuperscript+ and Ca\textsuperscript{2+} on the mechanoreception. Sand also suggested
that Na\textsuperscript+ and K\textsuperscript+ might influence the mechano-sensitivity indirectly
by influencing the Ca\textsuperscript{2+} effect.\textsuperscript{2,3}

After application of neomycin the mechano-sensitivity was sup-
pressed, but the chemical responses to cations were unchanged. This
phenomenon may be explained by differing receptive mechanisms in
mechano- and chemoreception. From the results of experiments on
the lateral-line organ of the tadpole, Yoshioka \textit{et al.} proposed the
existence of chemical adsorption of ions on the receptor cell mem-
brane.\textsuperscript{10,11} They explained the chemical responses with the aid of a
site-binding chemical adsorption model and suggested that these sites
were in the protein at the membrane surface. From observing the
drug action of neomycin, we conclude that the action site of calcium
for enhancement of mechano-sensitivity is related to membrane lipids.
However, since calcium actions on the cell membrane and intracellular
structures are complicated, we admit the need for further studies.

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\textbf{References}