Endolymphatic sac is involved in the regulation of hydrostatic pressure of cochlear endolymph

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Running head: Catecholamines elevate cochlear endolymph pressure

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ABSTRACT

To clarify the role of the endolymphatic sac (ES) in the regulation of endolymphatic pressure, the effects of isoproterenol, a β-adrenergic receptor agonist, and acetazolamide, a potent carbonic anhydrase inhibitor, both of which decrease ES direct current potential (ESP), on cochlear hydrostatic pressure were examined in guinea pigs. When isoproterenol was applied intravenously, hydrostatic pressures of cochlear endolymph and perilymph were significantly increased with no change in endocochlear potential (EP) or the hydrostatic pressure of cerebrospinal fluid. Acetazolamide produced no marked change in the hydrostatic pressure of cochlear endolymph. In ears with an obstructed ES, the action of isoproterenol on the hydrostatic pressure of cochlear endolymph and perilymph was suppressed. These results suggest that the ES may regulate the hydrostatic pressure of the endolymphatic system via the action of the agents such as catecholamines on the ES.

Key words: endolymphatic sac; direct current potential; cochlear endolymph; hydrostatic pressure; isoproterenol; acetazolamide
INTRODUCTION

The endolymphatic sac (ES), which is a part of the inner ear, is believed to absorb endolymph, since surgical blockage of the ES and the endolymphatic duct causes accumulation of endolymph in the cochlea and vestibule, as so-called endolymphatic hydrops(11), a characteristic pathological finding in Meniere’s disease. Endolymphatic hydrops in the cochlea causes deafness, while endolymphatic hydrops in the vestibule causes vertigo(22, 31). Endolymph regulation is thus important for hearing and the sense of equilibrium(7, 29). Although the ES is generally accepted to contain active ion transport systems and may absorb endolymph(11), no mechanisms of endolymph regulation by the ES have actually been established.

ES direct current potential (ESP) and endocochlear direct current potential (EP) are known to be present in the ES and the cochlea, respectively(3, 12, 15). ESP and EP are generated by active ion transports in the ES and cochlea, respectively(3, 15, 21). ESP and EP can thus be used as indices of function for the ES and cochlea, respectively(15, 21). Catecholamines reportedly depress ESP through  β-adrenergic receptors(19). The finding that isoproterenol, a β-adrenergic receptor agonist, at a dose of 12.5 μg/kg decreases ESP without changing EP(16) suggests that isoproterenol would suppress active ion transport in the ES without inhibiting active ion transport in the cochlea. Acetazolamide, a potent carbonic anhydrase inhibitor, reportedly depresses ESP more sensitively than EP (32, 33). The result that co-treatment with isoproterenol and acetazolamide at doses producing
near-maximum reduction of ESP depresses almost all parts of the ESP, suggesting that isoproterenol and acetazolamide may depress ESP via different mechanisms, and that ESP may be composed of isoproterenol- and acetazolamide-sensitive parts(17).

The ES has been hypothesized to regulate endolymphatic hydrostatic pressure(4, 9). However, no reports have described hydrostatic pressure regulation by the ES, as direct measurement of endolymphatic hydrostatic pressure in the ES is very difficult due to the proximity of the sigmoid sinus and dura mater(24). We initially tried to measure endolymphatic hydrostatic pressure in the ES, but were able to achieve stable measurements in only 2 of 205 experiments. We therefore examined changes in endolymphatic hydrostatic pressure in the cochlea with administration of isoproterenol and acetazolamide to verify the regulation of endolymphatic hydrostatic pressure by the ES.

**MATERIALS AND METHODS**

**Animal preparation and recording technique**

Albino guinea pigs with a positive Preyer’s reflex (weight, 300 - 400 g) were used. Protocols for animal care and use were approved by the Experimental Animal Committee of the Faculty of Medicine at Kagawa University (Protocol #31) in accordance with the principles of the Declaration of Helsinki. During all experiments, the animals were deeply anesthetized with ketamine (Daiichi Sankyo, Tokyo, Japan) (50 mg/kg body weight, intramuscularly) and xylazine (Sigma-Aldrich, Tokyo,
Japan) (5 mg/kg body weight, intramuscularly) and allowed to breathe spontaneously. Electrocardiography and heart rate were monitored in all animals throughout the experiments. Body temperature was maintained at 36 - 38 °C using a heating pad. Left jugular vein cannulation was performed for the administration of the agents.

The head of the animal was fixed in a prone position using a head holder (SH-15; Narishige, Tokyo, Japan). The bulla was opened using a retroauricular approach to expose the round window. Recording pipettes were single-barreled, beveled to a diameter 5 - 10 μm, and filled with 2 M KCl(28). With the aid of an operating microscope and micromanipulator, the tip of a micropipette was inserted through the round window membrane into the scala tympani, then through the basilar membrane into the scala media, in which the hydrostatic pressure of cochlear endolymph was measured. EP was simultaneously measured to verify the position of the pipette tip(4, 5, 27). To measure the hydrostatic pressure of cochlear perilymph, the tip of a micropipette was inserted through the round window membrane into the scala tympani(28). The tip of a micropipette was inserted into the cisterna magna to measure the hydrostatic pressure of cerebrospinal fluid (CSF).

The pipette was connected to a servo-null system (900A Micropressure System; World Precision Instruments, Sarasota, FL, USA). The system was calibrated by lowering the micropipette 0 and 1 cm into an electrically grounded beaker of normal saline(4).

ESP was recorded as described previously(19). Briefly, the ES was exposed extradurally using a
posterior occipital approach. The sigmoid sinus was detached medially from the medial surface of the temporal bone. The ES was identified just behind the sigmoid sinus. A glass microelectrode (diameter, 2 - 5 μm) filled with 154 mM NaCl was inserted into the intermediate portion of the ES to measure ESP. Connection of the microelectrode for the measurement of ESP was made via Ag-AgCl to an amplifier with high-input impedance (FD223; World Precision Instruments).

An Ag-AgCl reference electrode was placed on the neck muscles. All experiments were performed in an electrically shielded booth.

**Drug administration**

As isoproterenol has the strongest potency of any catecholamines for action on the ESP, this substance was used as one of the catecholamines(16, 19). Isoproterenol (l-isoproterenol hydrochloride; Kowa, Tokyo, Japan) was infused at a concentration of 6.25 μg/kg/min, which suppresses the ESP to near the lowest level(17), through a catheter inserted into the jugular vein using an infusion pump (STC-521; Terumo, Tokyo, Japan).

A dose of 10 mg/kg acetazolamide (acetazolamide sodium; Sanwa Kagaku Kenkyusyo, Nagoya, Japan) dissolved in 2 ml of saline was infused for 1 min through a catheter inserted into the jugular vein using an infusion pump (STC-521; Terumo)(17). The dose of acetazolamide producing the maximum reduction in ESP is 10 mg/kg(33).
**Data analysis**

Data from the experiment with pre-infusion EP values of less than 70 mV were excluded. Values are presented as mean ± standard error of the mean (SEM). Student’s paired and unpaired \( t \) tests were used to determine statistical differences.

**RESULTS**

Figure 1 shows the response of the hydrostatic pressure of cochlear endolymph and EP with the ESP response to intravenous application of isoproterenol at 6.25 \( \mu \)g/kg/min for 5 min. The initial values of hydrostatic pressure for cochlear endolymph, EP and ESP were 2.6 ± 0.2 mmHg (n=14), 80.6 ± 1.4 mV (n = 14) and 19.4 ± 2.1 mV (n = 5), respectively. Isoproterenol reversibly elevated the hydrostatic pressure of cochlear endolymph with no change in EP. Changes in the hydrostatic pressure of cochlear endolymph produced by isoproterenol corresponded to ESP changes.

Figure 2 shows the response of the hydrostatic pressure of cochlear endolymph and EP with ESP response to intravenous application of acetazolamide at 10 mg/kg/min for 1 min. Initial values of the hydrostatic pressure of cochlear endolymph, EP and ESP were 2.4 ± 0.5 mmHg (n =5), 89.0 ± 2.6 mV (n = 5) and 18.2 ± 0.6 mV (n = 5), respectively. Acetazolamide at the dose producing the maximal change in ESP produced no significant change in the hydrostatic pressure of cochlear endolymph or
Figure 3 shows changes in the hydrostatic pressure of cochlear perilymph and CSF when isoproterenol was applied intravenously. The initial values for hydrostatic pressure of cochlear perilymph and CSF were $2.6 \pm 0.2$ mmHg (n = 10) and $4.1 \pm 0.4$ mmHg (n = 5), respectively. Isoproterenol elevated the hydrostatic pressure of cochlear perilymph with no significant changes in hydrostatic pressure of the CSF.

To verify the role of the ES in the change in hydrostatic pressure of cochlear endolymph and perilymph by isoproterenol, the influence of ES obstruction on the responses of hydrostatic pressure of cochlear endolymph and perilymph to isoproterenol was examined. EP and the hydrostatic pressure of cochlear endolymph and perilymph were recorded immediately after the ES was obstructed extradurally using autologous bone putty in the same manner as described previously(8). Figure 4 shows the responses of EP and the hydrostatic pressure of cochlear endolymph and perilymph to isoproterenol in ears with an obstructed ES. Initial values of the EP and hydrostatic pressure of cochlear endolymph and perilymph were $86.1 \pm 1.9$ mV (n = 10), $3.3 \pm 0.1$ mmHg (n = 10) and $2.5 \pm 0.3$ mmHg (n = 5), respectively. In ears with an obstructed ES, the elevation of cochlear hydrostatic pressure by isoproterenol was suppressed. Table 1 indicates the influence of ES obstruction on the response of hydrostatic pressure of cochlear endolymph to isoproterenol. Although the initial values for the hydrostatic pressure of cochlear endolymph tended to be higher in ears with an obstructed ES.
than in ears with an intact ES, the difference was statistically insignificant \((p > 0.01)\). The maximum change in hydrostatic pressure by isoproterenol was significantly smaller in ears with an obstructed ES than in ears with an intact ES \((p < 0.0001)\).

**DISCUSSION**

Two agents decreasing ESP, isoproterenol and acetazolamide, showed different actions on the hydrostatic pressure of cochlear endolymph. Isoproterenol increased hydrostatic pressure of the cochlea, whereas acetazolamide produced no significant change. The result that the increase in hydrostatic pressure of the cochlea produced by isoproterenol was suppressed in ears with an obstructed ES suggests that isoproterenol may increase cochlear hydrostatic pressure via actions on the ES.

Longitudinal flow of endolymph in the cochlea is thought to be relatively slow\((23)\). In this study, ESP reached the lowest potential value around 2 min after injection of isoproterenol and changes in endolymphatic pressure of the cochlea seem to have started simultaneously with decreases in ESP. These results suggest that the changes in endolymphatic pressure might not be due to endolymph volume change or longitudinal flow change in the endolymph. Conceivably, according to Pascal’s law, the change in cochlear endolymphatic pressure may reflect the ES endolymphatic pressure change caused by changes in ion and water transport in the ES at almost the same time.
Ion transporters of sodium and/or chloride from the ES lumen to the outside are suggested to be present in ES epithelial cells (3, 14, 15, 20). Several Na⁺ and/or Cl⁻ transporters (1, 2, 14, 20) and pH-regulating proteins, H⁺ ATPase, Cl⁻/HCO₃⁻ exchanger and Na⁺-H⁺ exchanger (25, 38), are reportedly localized in ES epithelial cells. Acetazolamide reportedly increases sodium and chloride concentrations in ES endolymph with decreased HCO₃⁻ and unchanged potassium concentrations (18, 32). Bafilomycin, a vacuolar type H⁺-ATPase inhibitor, depressed ESP, suggesting that H⁺-ATPase may be involved in ESP generation (6). Recent results indicate that acetazolamide affects acid-base balance in the ES via H⁺ ATPase, Cl⁻/HCO₃⁻ exchanger and Na⁺-H⁺ exchanger (6, 25). The present finding that acetazolamide failed to affect endolymphatic pressure suggests that ion transporters in the ES other than H⁺ ATPase, Cl⁻/HCO₃⁻ exchanger and Na⁺-H⁺ exchanger are involved in the regulation of endolymphatic pressure.

Catecholamines have also been reported to increase potassium secretion in marginal cells of the stria vascularis via β₁ adrenergic actions (36). If isoproterenol changes endolymphatic pressure by increasing potassium secretion in marginal cells, EP would be increased with no influence of ES obstruction on the response of cochlear hydrostatic pressure to isoproterenol.

A previous study demonstrated that no pressure difference exists between perilymph and endolymph, suggesting that Reissner’s membrane separating perilymph and endolymph has very high plasticity and negligible elasticity (28). The high compliance of Reissner’s membrane may transmit
endolymphatic pressure to the perilymphatic pressure immediately. Increased endolymphatic pressure may thus cause enlargement of the endolymphatic space as endolymphatic hydrops, resulting in dysfunctions in hearing and equilibrium\(^{34, 35}\). Endolymphatic hydrops is a characteristic finding in Meniere’s disease, which is associated with recurrent symptoms of vertigo, hearing loss and tinnitus\(^{10}\). The present study shows that the ES may be involved in endolymphatic pressure changes in the cochlea, suggesting that the ES may play an important role in the regulation of endolymph pressure. Endolymph pressure regulation by the ES is considered important for homeostasis in the endolymphatic system.

Previous studies have reported that endolymphatic pressure is not directly influenced by CSF pressure, as permeability of the cochlear aqueduct is not constant\(^{13, 37}\), and that a CSF pressure increase of more than 10 mmHg is needed to increase endolymphatic pressure\(^{26, 30}\). The present study detected no endolymphatic pressure change synchronized with heartbeat and no significant change in CSF pressure by isoproterenol, indicating that the increase in endolymphatic pressure by isoproterenol is unaffected by CSF changes.

**Perspectives and Significance**

The present study demonstrated that the ES directly affects endolymphatic pressure in the cochlea, and suggests that the ES may regulate hydrostatic pressure of the endolymphatic system via the action
of agents such as catecholamines on the ES. The control of ion and fluid transport in the ES may thus improve endolymphatic hydrops such as in Meniere’s disease. Further studies are necessary to elucidate the mechanisms underlying the action of catecholamines on ESP and endolymphatic pressure.

**GRANTS**

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### Table 1

<table>
<thead>
<tr>
<th>Treatment of ES</th>
<th>n</th>
<th>Hydrostatic pressure of cochlear endolymph (mmHg)</th>
<th>Isoproterenol (6.25 μg/kg/min for 5 min)</th>
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<tr>
<td></td>
<td></td>
<td>Pre-treatment</td>
<td>Maximum post-treatment change</td>
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<td>Intact ES</td>
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<td>2.6 ± 0.2</td>
<td>1.6 ± 0.1</td>
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<tr>
<td>Obstructed ES</td>
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<td>3.3 ± 0.1</td>
<td>0.2 ± 0.1**</td>
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</tbody>
</table>

**P < 0.0001
TABLE LEGENDS

Table 1: Effect of isoproterenol on hydrostatic pressure of cochlear endolymph in ears with intact endolymphatic sac (ES) and with obstructed ES
FIGURE LEGENDS

Figure 1: a) Response of hydrostatic pressure of cochlear endolymph to intravenous application of isoproterenol (6.25 μg/kg/min) (mean ± SEM, n = 14). b) Response of endocochlear potential to intravenous application of isoproterenol (6.25 μg/kg/min) (mean ± SEM, n = 14). c) Response of endolymphatic sac DC potential to intravenous application of isoproterenol (6.25 μg/kg/min) (mean ± SEM, n = 5).

Figure 2: a) Response of hydrostatic pressure of cochlear endolymph to intravenous application of acetazolamide (10 mg/kg/min) (mean ± SEM, n = 5). b) Response of endocochlear potential to intravenous application of acetazolamide (10 mg/kg/min) (mean ± SEM, n = 5). c) Response of endolymphatic sac DC potential to intravenous application of acetazolamide (10 mg/kg/min) (mean ± SEM, n = 5).

Figure 3: a) Response of hydrostatic pressure of cochlear perilymph to intravenous application of isoproterenol (6.25 μg/kg/min) (mean ± SEM, n = 10). b) Response to hydrostatic pressure of CSF to intravenous application of isoproterenol (6.25 μg/kg/min) (mean ± SEM, n = 5).

Figure 4: a) Response of endocochlear potential to intravenous application of isoproterenol (6.25
µg/kg/min) in ears with obstructed endolymphatic sac (mean ± SEM, n = 10). b) Response of hydrostatic pressure of cochlear endolymph to intravenous application of isoproterenol (6.25 µg/kg/min) in ears with obstructed endolymphatic sac (mean ± SEM, n = 10). c) Response of hydrostatic pressure of cochlear perilymph to intravenous application of isoproterenol (6.25 µg/kg/min) in ears with obstructed endolymphatic sac (mean ± SEM, n = 5).
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Fig. 1a

Endymphatic pressure (mmHg)

Time (min)
Fig. 1b

Endocochlear potential (mV)

Time (min)

Isoproterenol
Fig. 1c

Isoproterenol

Endolymphatic sac DC potential (mV)

Time (min)
Fig. 2a

Endolymphatic pressure (mmHg)

Time (min)

Acetazolamide
Fig. 2b

Endocochlear potential (mV)

Time (min)

Acetazolamide
Fig. 2c

Endolymphatic sac DC potential (mV)

0 2 4 6 8 10 12 14 16 18
Time (min)

Acetazolamide
Fig. 3a

- **Isoproterenol**

- Perilymphatic pressure (mmHg)

- Time (min)

- Figure shows the effect of Isoproterenol on perilymphatic pressure over time. The pressure increases and then decreases after the Isoproterenol treatment.
Fig. 4a

Endocochlear potential (mV)

Time (min)

Isoproterenol
Fig. 4b

Endolymphatic pressure (mmHg)

Time (min)

Isoproterenol

0 2 4 6 8 10 12 14 16 18

0.5 1 1.5 2 2.5 3 3.5 4
Fig. 4c

Perilymphatic pressure (mmHg)

Time (min)

Isoproterenol