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Dopamine increases Na^+ absorption in the Reissner's membrane of the gerbil cochlea

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ABSTRACT

Objective: The purpose of the present study was to investigate the effect of dopamine as a possible regulator of epithelial Na^+ channel (ENaC) in the Reissner's membrane (RM).
Methods: RM was freshly dissected from the gerbil cochlea, and short-circuit current (I_{sc}) was measured using the voltage-sensitive vibrating probe technique. The dopamine receptor expression was examined using immunohistochemistry.
Results: The results showed that dopamine induced activation of the amiloride-sensitive I_{sc}, but not after pre-treatment with amiloride. The D_1-like receptor antagonist SCH-23390, but not the D_2-like receptor antagonist sulpiride, decreased the stimulatory effect of dopamine on RM. The effect of dopamine on Na^+ transport via ENaC was still observed after blockade of the Na^+–K^+-ATPase by ouabain. D_1 receptor immunoreactivity was observed in RM, stria vascularis and spiral ganglion.
Conclusion: Na^+ transport in RM is activated by dopamine possibly via D_1-like receptors, and intracellular mechanisms other than cAMP-mediated pathway may be involved.

1. Introduction

Na^+ transport mechanisms in the cochlea have been much known in recent years. Epithelial cells of the Reissner's membrane (RM) [1–3], outer sulcus cells [4] and Claudius' cells [5] have been reported to contribute to endolymphatic homeostasis by active Na^+ transport.

RM forms the boundary between the scala media filled with K^+-rich endolymph and the scala vestibuli filled with Na^+-rich perilymph in the cochlea. RM consists of two cell layers (tight epithelia which face the endolymph, and mesothelia which face the perilymph) that are separated by a basement membrane and a thin layer of intercellular substance.

Recently, evidences that RM contributes to endolymphatic Na^+ homeostasis by Na^+ absorption via apical epithelial Na^+ channel (ENaC) have been reported. Localization of ENaC in the epithelial cells of RM has been demonstrated by immunohistochemistry or by in situ hybridization [6]. The α-subunit of the Na^+–K^+-ATPase, which provides the driving force for Na^+ transport via ENaC, was reported to be expressed at the basolateral membrane of RM epithelial cells [7]. Electrogenic transepithelial Na^+ transport has been demonstrated in freshly dissected gerbil RM by using the vibrating probe method [1]. The activity of ENaC is regulated by hormones, such as glucocorticoids, aldosterone, vasopressin, and local paracrine or autocrine factors including ATP. The transcripts for three subunits of ENaC are present in rat RM, and glucocorticoid upregulates the transcription of Na^+ transport gene. Purinergic receptors also regulate the function of ENaC [3]. Other types of currents, such as an inwardly rectifying chloride current, were identified in the epithelial cells of RM using patch clamp studies [8].

In the inner ear, diverse dopamine receptor subtypes have been identified in areas including spiral ganglion neurons, vestibular hair cells, strial marginal cells, and the contact between lateral efferent fibers and type I afferent neurons beneath the inner hair cells [9]. Dopamine released from lateral olivocochlear efferents is known to interact with dopamine receptors located postsynaptically on the afferent nerve fibers of inner hair cells, where it has been shown to modulate cochlear afferent neurotransmission [10].

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However, modulation of Na^+ transport through by dopaminergic signaling in the cochlea has not been demonstrated yet.

The present study was undertaken to investigate the effect of dopamine on ENaC function using the vibrating probe technique, and to use immunohistochemistry to measure the corresponding dopamine receptor expression in epithelial cells of gerbil RM.

2. Materials and methods

2.1. Tissue preparation

The gerbils (3–4 weeks old) were anesthetized with sodium pentobarbital (50–100 mg/kg; intraperitoneally) and killed to remove temporal bones. The methods used for dissecting RM have been previously described [1]. The stria vascularis was removed from the lateral wall of the apical cochlear turn and the attached portion of RM was folded over the suprastrial portion of the spiral ligament. The tissue was mounted in a perfusion chamber on the stage of an inverted microscope (Olympus IX70) and continuously perfused at 37 °C at an exchange rate of 3 times/min. All procedures conformed to the protocols approved by the Institutional Animal Care and Use Committee of Seoul National University.

2.2. Voltage-sensitive vibrating probe

The vibrating probe technique was used to measure the transepithelial currents under short-circuit conditions due to the small size of RM epithelium. The diameter of the vibrating probe tip was approximately 20 μm, which permits the detection of voltages in the low nanovolt range. The vibration between the 2 positions within the line of current flow yields voltages that correspond to current flow through the resistive physiological saline [11]. The vibrating probe technique used was identical to a previously described method [11]. Briefly, the short-circuit current (I_{sc}) was monitored by vibrating a platinum–iridium wire microelectrode insulated with parylene-C (Micro Electrodes, Gaithersburg, MD, USA), and coated with platinum-black on its exposed tip. The vibration was approximately 20 μm along the horizontal (X) and vertical (Z) axes. The X-axis was perpendicular to the face of the epithelium, and the probe was positioned 30 μm from the apical surface of the epithelium using computer-controlled, stepper-motor manipulators (Applicable Electronics, Forestdale, MA, USA) and a specialized probe software (ASET version 2.0, Science Wares, East Falmouth, MA, USA). The bath references were 26-gauge platinum-black electrodes. Calibration was performed in physiologic saline (see below) using a glass microelectrode (tip <1-μm outer diameter) filled with 3 mol/l KCl as a point source of current. The frequencies of the vibration used were in the 200–400-Hz range and were well separated for the 2 orthogonal directions. Signals from the oscillators driving the probe were also fed to a dual channel phase-sensitive detector. Asymmetry of probe design yielded different resonant frequencies for the two directions of vibration. X and Z detector signals were connected to a 16 bit analog-to-digital converter (CIO-DAS1602/16, ComputerBoards, Mansfield, MA, USA) in a Pentium IV computer. The sampling interval was 0.6 s, which was the minimum interval allowed by the software. The electrode was positioned where I_{sc} showed a maximum X value and minimum Z value. The data are expressed as the recorded X value and plotted using the Origin version 6.1 software (OriginLab Software, Northampton, MA, USA). The output from the vibrating probe depended not only on the specific short circuit current of the epithelium, but also on the position of the probe relative to the surface of the tissue and on the precise geometry of each tissue sample. The current density reported here refers to the flux at the probe position and represents only a fraction of the current crossing the epithelium. No changes in the relative position of the probe were observed owing to the swelling or shrinking of the tissues during the experimental treatments.

2.3. Immunohistochemistry

The gerbils at the age of 21 days were transcardially perfused with phosphate buffer solution (PBS) under deep anesthesia, and then with 4% paraformaldehyde in PBS. The cochleas were dissected out and postfixed by immersion in a fresh solution of 4% formaldehyde in PBS for 2 h. After postfixation, the tissues were washed with PBS and transferred to a decalcifying solution (0.12 M EDTA, pH 7.2) for 5 days at 4 °C. The EDTA solution was changed every 24 h. The cochlear tissues were dehydrated in a graded ethanol series and embedded in paraffin. Ten-μm sections of cochlea were obtained and sequentially incubated in 0.1% sodium borohydride (PBS plus 5 mM glycine), 3% hydrogen peroxide (for the avidin–biotin peroxidase method), and 2% normal serum (corresponding to the species in which the secondary antibody was made). The Vector ABC elite protocol was used with 3,3′-diaminobenzidine as the chromogen (Bio-Genex, San Ramon, CA, USA). The sections were incubated overnight at 4 °C with the primary antibody. The primary antibody used for D_1 receptor was the rabbit anti-dopamine D_1 receptor which was raised against a 13 amino-acid sequence (amino acids 403–415) from rat D_1A (AB1765P, Chemicon, Temecula, CA, USA) diluted at 1:100. The primary antibody used for D_2 receptor was the rabbit anti-dopamine D_2 receptor which was raised against a 28 amino-acid sequence from the human D_2 (AB5084P, Chemicon, Temecula, CA, USA) diluted at 1:100. The specificity of the immunohistochemical stain was controlled by the omission of the primary antibody and preincubation of the antiserum a peptide antigen.

2.4. Solutions and chemicals

The perfusate used as control solution was a perilymph-like physiologic saline of pH 7.4, containing (in mM) 150 NaCl, 3.6 KCl, 1 $MgCl_2$, 0.7 $CaCl_2$, 5 glucose, and 10 HEPES. Dopamine (Sigma H-8502) was directly dissolved in the control solution just before use. SCH-23390 (R(+)-7-chloro-8-hydroxy-3-methyl-1-phenyl-2,3,4,5-tetrahydro-1H-3-benzazepine hydrochloride, Sigma C-0206) and sulpiride (Sigma S-8010) were dissolved in ethanol at 0.01 M stock concentrations. Ethanol at this concentration had no effect on I_{sc}. Bromocriptine (Sigma B-2134) and 8-bromo-cyclic adenosine monophosphate (cAMP, Sigma B-5386) was dissolved in distilled water. Amiloride (Sigma A-7410) was predissolved in dimethyl sulfoxide (DMSO) and then diluted to 0.1% DMSO in the control solution before application. DMSO at this concentration had no effect on I_{sc}.

2.5. Data presentation and statistics

The tip of the probe was positioned approximately 20 μm from the apical surface of the RM, and a short-circuit current (I_{sc}) was recorded in the apical-to-basolateral direction. The baseline I_{sc} values in the control solution were obtained by averaging the data for 9 s just before solution change. For the analysis of the effect of each drug, the data were averaged for 9 s after reaching a steady-state. The increases or decreases in I_{sc} were considered significant at the $P < 0.05$ level. Statistical comparisons between two means were obtained with t-test (Mann–Whitney test, if $n < 5$). The data shown were expressed as mean ± SEM values (n = number of tissues) of the I_{sc}.