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Regulation of paracellular Na^+ and Cl^- conductances by hydrostatic pressure

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Abstract

The effect of hydrostatic pressure on the paracellular ion conductance (Gp) composed of the Na^+ conductance (G_{Na}) and the Cl^- conductance (G_{Cl}) has been Investigated. Gp, G_{Na} and G_{Cl} were time-dependently increased after applying an osmotic gradient generated by NaCl with basolateral hypotonicity. Hydrostatic pressure (1−4 cm H_2O) applied from the basolateral side enhanced the osmotic gradient-induced increase in Gp, G_{Na} and G_{Cl} in a magnitude-dependent manner, while the hydrostatic pressure applied from the apical side diminished the osmotic gradient-induced increase in Gp, G_{Na} and G_{Cl}. How the hydrostatic pressure influences Gp, G_{Na} and G_{Cl} under an isosmotic condition was also investigated. Gp, G_{Na} and G_{Cl} were stably constant under a condition with basolateral application of sucrose canceling the NaCl-generated osmotic gradient (an isotonic condition). Even under this stable condition, the basolaterally applied hydrostatic pressure drastically elevated Gp, G_{Na} and G_{Cl}, while apically applied hydrostatic pressure had little effect on Gp, G_{Na} or G_{Cl}. Taken together, these observations suggest that certain factors controlled by the basolateral osmolality and the basolaterally applied hydrostatic pressure mainly regulate the Gp, G_{Na} and G_{Cl}.

Keywords: Epithelial cells; Ion conductance; Osmotic pressure; Hydrostatic pressure; A6 cells

1. Introduction

Transepithelial ion transport in distal tubules of the kidney plays an essential role in the homeostasis of blood pressure and ionic environments of the body. Transepithelial ion transport is composed of 2 pathways; transcellular and paracellular pathways. Transcellular ion transport in distal tubules of the kidney participates in an important role in the homeostasis; e.g., ENaC (epithelial Na^+ channel) located in the apical membrane of epithelial cells of the cortical collecting duct plays as a sodium entry pathway in the transcellular Na^+ reabsorption to keep sodium in the body (Lang et al., 2005; Schafer, 2002; Schild, 2004). In addition to transcellular ion transport pathway, the paracellular pathway also contributes to transepithelial ion transport (Blikslager et al., 2007; Kahle et al., 2004; Landau, 2006). However, we unfortunately have little information on regulation of the paracellular ion transport. As already reported (Tokuda et al., 2007, 2008), the paracellular pathway is regulated by the osmotic gradient, which is one of the most important factors providing the driving force for water movements, leading us to speculate that water movements regulate paracellular ion conductance. Therefore, the effect of hydrostatic pressure, which generates water movement, on the paracellular ion conductance in renal epithelial cells has been studied.

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2. Materials and methods

2.1. Cell culture

Renal epithelial A6 cells derived from *Xenopus laevis* were purchased from American Type Culture Collection were used at passages 76–79 cultured on plastic flasks in an NCTC-109 culture medium modified for amphibian cells containing 10% fetal bovine serum, streptomycin, and penicillin in a humidified incubator at 27 °C with 1.0% CO_2 in air similar to previous studies (Miyazaki et al., 2007; Taruno et al., 2007; Tokuda et al., 2002). Monolayers of cells were subcultured on tissue culture-treated transwell filter cups to measure paracellular ion conductance, (Costar, Cambridge, MA, USA) for 12–14 days.

2.2. Materials

Benzamil, 5-nitro-2-(3-phenylpropylamino)benzoate (NPPB) and $BaCl_2$ were purchased from Sigma (St. Louis, MO, USA), and NCTC-109 medium and fetal bovine serum from Gibco (Grand Island, NY, USA)., The compositions of solutions A, B and C are shown in Table 1, their pH being adjusted to 7.4 with NaOH or HCl.

2.3. Measurement of short-circuit current (Isc) and transepithelial conductance (Gt)

We rinsed A6 cells subcultured on filter cups were with the solution A and transferred the cells to a modified Ussing chamber (Jim's Instrument, Iowa City, IA, USA) designed to hold the filter cup (Niisato et al., 2007b). Solution A in the apical and basolateral chambers was stirred with 21% O_2/79% N_2. Under an open-circuit condition, the transepithelial potential (Vt) was continuously measured with a high-impedance millivoltmeter functioning as a voltage clamp with automatic fluid resistance compensation (VCC-600, Physiologic Instrument, San Diego, CA, USA), and with a pair of calomel electrodes that were immersed in a saturated KCl solution and bridged to the modified Ussing chamber by a pair of polyethylene tubes filled with a solution of 2% (weight/volume (w/v)) agarose in a 2 M KCl solution (Niisato et al., 2007b; Taruno et al., 2007). Under short-circuit conditions, the current (short-circuit current: Isc) was measured with an amplifier, VCC-600, using a pair of silver-silver chloride electrodes immersed in a 2 M NaCl solution and bridged to the modified Ussing chamber by a pair of polyethylene tubes filled with 2% (w/v) agarose in a 2 M NaCl solution. For measurement of the transepithelial conductance (Gt), a pulse of 1 μA constant current with 1 s duration under an open-circuit condition was applied to the A6 cell monolayer every 10 s, calculating the Gt from the change in the Vt (ΔVt) using Ohm's law (Gt (mS) = 1 (μA)/ΔVt (mV)) (Hasegawa et al., 2006; Niisato et al., 2007a, 2007c; Taruno et al., 2007). A positive current represented a net flow of cation from the apical to the basolateral side or a net flow of anion form the basolateral to the apical side. We also estimated the Isc by calculating the product of Gt and Vt (Isc = Gt × Vt) using the measured Gt and Vt.

Table 1
Compositions of solutions used.

Solution	NaCl (mM)	Sucrose (mM)
A	120	0
B	60	0
C	60	110
D	30	165
E	15	192.5

These solutions contained 3.5 mM KCl, 1 mM $CaCl_2$, 1 mM $MgCl_2$, 5 mM glucose, and 10 mM HEPES in addition to NaCl and/or sucrose.

2.4. Measurement of paracellular ion conductance

To measure the paracellular ion conductance, the method previously reported (Tokuda et al., 2007) was used. Monolayers of cells subcultured were rinsed on tissue culture-treated Transwell filter cups with solution A, which were transferred to a modified Ussing chamber (Jim's Instrument, Iowa City, IA, USA) designed to hold the filter cup. Solution A was put in the chambers as the apical and basolateral solutions were stirred with 21% O_2/79% N_2. Transepithelial conductance (Gt) was done by the previously reported method (Niisato et al., 2004a, 2007a,b; Yasuda et al., 2007a,b) of continuously measuring the transepithelial potential (Vt) with a high-impedance millivoltmeter that could function as a voltage clamp with automatic fluid resistance compensation (VCC-600, Physiologic Instrument, San Diego, CA, USA) (Fujimoto et al., 2005; Hasegawa et al., 2006; Niisato et al., 2007c; Taruno et al., 2008; Ueda-Nishimura et al., 2005). Gp, G_{Na} (Na^+-selective Gp) and G_{Cl} (Cl^--selective Gp) were measured and calculated in the presence of 10 μM benzamil, a specific blocker of ENaC (Eaton and Marunaka, 1990; Kleyman and Cragoe, 1990), 300 μM NPPB, a blocker of Cl^- channel (Wangemann et al., 1986; Yasuda et al., 2007a) and 1 mM $BaCl_2$, a blocker of the K^+ channel (Hanrahan et al., 1986) in the apical solution. Application of these agents to the apical solution abolished the Isc from 0.82 ± 0.0 to 0.00 ± 0.0 mA/cm^2 (mean ± S.E., $p < 0.00001$, $n = 169$), and diminished the Gt from 91.4 ± 1.36 to 72.6 ± 1.3 mS/cm^2 (mean ± S.E., $p < 0.00001$, $n = 169$; Tokuda et al., 2007). The apical and/or basolateral solutions were replaced with solutions C, D or E containing the channel blockers (benzamil, NPPB and $BaCl_2$) 40 min after the application of these channel blockers into the solution A abolishing the Isc. Under conditions where the Isc (i.e., the transcellular transport) is abolished, transepithelial conductance (Gt) would represent the paracellular conductance (Gp), which is composed of G_{Na}, G_{Cl} and the conductance of other charged substances (Go) via the paracellular pathway. As the paracellular ion conductance is dependent on the strength of ions existing in the bathing solutions, the Go would be negligibly small compared with G_{Na} and G_{Cl} (Tokuda et al., 2007). Therefore, bbGp is represented as follows (equation (1)).