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Beta Agonist Regulation of Sodium Transport in Fetal Lung Epithelium: Roles of Cell Volume, Cytosolic Chloride and Protein Tyrosine Kinase

Yoshinori Marunaka*, Naomi Niisato, Yasushi Ito

Department of Physiology*, Kyoto Prefectural University of Medicine, Kyoto, Japan
Department of Lung Biology, Hospital for Sick Children, University of Toronto, Toronto, Canada, Second Department of Internal Medicine, Nagoya University School of Medicine, Nagoya, Japan

1) A beta agonist stimulated Na^+ transport and decreased the intracellular Cl^- concentration ($[Cl^-]_c$) associated with cell shrinkage via an increase in cytosolic cAMP level by activating adenylate cyclase in rat fetal distal lung epithelial (FDLE) cells. 2) Lowering $[Cl^-]_c$ activated a 28-pS nonselective cation (NSC) channel by elongating the open time of the channel. 3) cAMP signals were converted to a protein tyrosine kinase (PTK)-mediated signal. 4) The PTK-mediated signal was involved in the cAMP-stimulated Na^+ transport in rat FDLE cells.

The fetal lung fluid secreted by lung epithelial cells plays an important role in development, differentiation and growth of the fetal lung (1-3). This fluid secretion depends on Cl^- secretion from the basolateral to the apical space (3). However, the fluid must be cleared from alveolar air space immediately at birth to allow normal gas exchange. Catecholamines, circulating levels of which increase during labor and delivery, have been suggested to induce clearance of the fluid by stimulating amiloride-sensitive Na^+ transport in lung epithelial cells via a beta-adrenergic receptor (1, 4).

A beta agonist, the intracellular second messenger of which is cAMP, stimulated a 28-pS amiloride-sensitive NSC channel, resulting in an increase of amiloride-sensitive Na^+ transport in rat FDLE cells (5-7) which were isolated from the fetuses of pregnant Wistar rats whose gestational ages were 20 days (term, 22 days) and cultured at 37℃ in 95% air and 5% CO_2 humidified incubator for 3 days. A beta-agonist, forskolin and cAMP caused cell shrinkage under isotonic conditions by stimulating KCl release in rat FDLE cells (8), decreasing $[Cl^-]_c$. Lowering $[Cl^-]_c$ activated the NSC channel by elongating the open time of the channel.

Recent reports (9, 10) have shown that the change in cell volume causes an increase in tyrosine phosphorylation which is involved in regulation of ion transport and gene transcription. These studies provide a possibility that in rat FDLE cells cAMP-induced cell shrinkage might also cause an increase in tyrosine phosphorylation, although cAMP-dependent signals are generally converted to cAMP-dependent protein kinase (PKA)-mediated signals. To study if in rat FDLE cells the cAMP-dependent signalings induces PKA activation, we measured PKA activity in unstimulated and forskolin-stimulated cells (11). Stimulation with forskolin increased PKA activity about 3-fold. To study whether forskolin stimulates Na^+ transport through a PKA-mediated signaling pathway, we next examined effects of PKA inhibitors on the forskolin action. KT5720, H8 and myristoylated PKA inhibitor peptide (myr-PKI14-22), PKA inhibitors (12-14), decreased the basal Isc, suggesting that the inhibitors applied in the present study were effective as PKA inhibitors in rat FDLE cells. However, even in rat FDLE cells treated with these PKA inhibitors forskolin still stimulated the amiloride-sensitive Isc, and the forskolin-induced amiloride-sensitive Isc (Isc) was not affected by these PKA inhibitors. These results strongly suggest that the forskolin-activated PKA may not be involved in the forskolin regulation of the amiloride-sensitive Na^+ transport.

Tyrphostin A23, a PTK inhibitor (15), decreased the basal Isc, and in the cell treated with 100 μM tyrphostin A23 for 30 min, forskolin failed to increase Isc. Tyrphostin A23 diminished the forskolin-stimulated amiloride-sensitive Isc dose-dependently. These results suggest that forskolin stimulates the Na^+ transport by increasing the 28-pS NSC channel activity via a pathway dependent on PTK (maybe non-receptor type). Forskolin also increased phosphorylation of tyrosine residues of ~70-80 kDa, ~97 and ~110-120 kDa proteins. A PTK inhibitor, tyrphostin A23, abolished the forskolin action on tyrosine

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Address for correspondence: Yoshinori Marunaka, M.D., Ph.D.
Department of Physiology, Kyoto Prefectural University of Medicine, Kyoto 602-0841, Japan, Tel: +81.75-251-5310, Fax: +81.75-251-0295,
E-mail: marunaka@phys.kpu-m.ac.jp

phosphorylation. These results suggest that forskolin would activate PTK, leading to tyrosine phosphorylation and supporting an idea that the activated PTK may be involved in the forskolin stimulation of Na^+ transport in rat FDLE cells.

NPPB (100 μM) abolished the forskolin action on cell volume and amiloride-sensitive Isc. The stimulatory action of forskolin on phosphotyrosine was also abolished by NPPB. These observations suggest that NPPB abolishes the forskolin action on the amiloride-sensitive Na^+ transport by preventing activation of PTK via blockade of cell shrinkage through its inhibitory action on Cl^- channels (conductances) which are required for cell shrinkage, indicating that physiological significance of cell volume change.

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References

1. Brown MJ, Olver RE, Ramsden CA, Strang LB, Walters DV. *Effects of adrenaline and of spontaneous labour on the secretion and absorption of lung liquid in the fetal lamb. J Physiol (London) 1983; 344: 137-52.*
2. Alcorn D, Adamson TM, Lambert TF, Maloney JE, Ritchie BC, Robinson PM. *Morphological effects of chronic tracheal ligation and drainage in the fetal lamb lung. J Anat 1977; 123: 649-60.*
3. O'Brodovich H. *Epithelial ion transport in the fetal and perinatal lung. Am J Physiol 1991; 261: C555-64.*
4. Olver RE, Schneeberger EE, Walters DV. *Epithelial solute permeability, ion transport and tight junction morphology in the developing lung of the fetal lamb. J Physiol (London) 1981; 315: 395-412.*
5. Tohda H, Foskett JK, O'Brodovich H, Marunaka Y. *Cl^- regulation of a Ca^{2+}-activated nonselective cation channel in β agonist treated fetal lung alveolar epithelium. Am J Physiol 1994; 266: C104-9.*
6. Ito Y, Niisato N, O'Brodovich H, Marunaka Y. *The effect of brefeldin A on terbutaline-induced sodium absorption in fetal rat distal lung epithelium. Pflugers Arch 1997; 434: 492-4.*
7. Marunaka Y, Niisato N, O'Brodovich H, Eaton DC. *Regulation of amiloride-sensitive Na^+-permeable channel by a β_2-adrenergic agonist, cytosolic Ca^{2+} and Cl^- in fetal rat alveolar epithelium. J Physiol (London) 1999; 515: 669-83.*
8. Nakahari T, Marunaka Y. *Regulation of cell volume by β_2-adrenergic stimulation in rat fetal distal lung epithelial cells. J Membr Biol 1996; 151: 91-100.*
9. Sadoshima J, Qiu Z, Morgan JP, Izumo S. *Tyrosine kinase activation is an immediate and essential step in hypotonic cell swelling-induced ERK activation and c-fos gene expression in cardiac myocytes. EMBO J 1996; 15: 5535-46.*
10. O'Donnell ME, Martinez A, Sun DD. *Endothelial Na-K-Cl cotransport regulation by tonicity and hormones: phosphorylation of cotransport protein. Am J Physiol 1995; 269: C1513-23.*
11. Niisato N, Ito Y, Marunaka Y. *cAMP stimulates Na^+ transport in rat fetal lung epithelium: involvement of a PTK- but not a PKA dependent pathway. Am J Physiol 1999; 277: L727-36.*
12. Hidaka H, Watanabe M, Kobayashi R. *Properties and use of H-series compounds as protein kinase inhibitors. Methods Enzymol 1991; 201: 328-39.*
13. Gadbois DM, Crissman HA, Tobey RA, Bradbury EM. *Multiple kinase arrest points in the G1 phase of nontransformed mammalian cells are absent in transformed cells. Proc Natl Acad Sci USA 1992; 89: 8626-30.*
14. Harris TE, Persaud SJ, Jones PM. *Pseudosubstrate inhibition of cyclic AMP-dependent protein kinase in intact pancreatic islets: effects on cyclic AMP-dependent and glucose-dependent insulin secretion. Biochem Biophys Res Commun 1997; 232: 648-51.*
15. Kovalenko M, Gazit A, Bohmer A, Rorsman C, Ronnstrand L, Heldin CH, Bohmer FD, Levitzki A. *Selective platelet-derived growth factor receptor kinase blockers reverse sis-transformation. Cancer Res 1994; 54: 6106-14.*