J Korean Med Sci 2000; 15(Suppl): S59-60
ISSN 1011-8934

Copyright © The Korean Academy of Medical Sciences

Unexpected Effects of Pathogens on Epithelial Na^+ Channels

Karl Kunzelmann, Alex Beesley, Nicholas King*, Guna Karupiah*, John Young, David Cook

Departments of Physiology and Pathology*, University of Sydney, Sydney, Australia

The movement of fluid across the respiratory epithelium is controlled by a balance between the secretion of fluid and electrolytes mediated by apical Cl^- channels and the absorption of fluid and electrolytes mediated by amiloride-sensitive apical Na^+ channels (1, 2). Thus, the removal of fluid from the respiratory tract at birth is due to activation of the epithelial Na^+ channels (3). Conversely, pseudohypoaldosteronism type I, in which epithelial Na^+ channel function is defective, is accompanied by reduced lung fluid clearance (4-6). Disordered fluid balance across the respiratory mucosa is also a major feature of respiratory infections such as influenza (7-9).

It is well known that many bacteria causing diarrhea, including *V. cholerae* and *E. coli*, secrete enterotoxins that stimulate the secretion of fluid and electrolytes by the mucosa lining the gastrointestinal tract (10). Recently viral pathogens causing diarrhea have also been reported to produce diarrhea by stimulating fluid and electrolyte secretion by the gut mucosa (11-13). Thus, it occured to us that the fluid accumulation in the respiratory tract associated with respiratory infections may be due to altered epithelial transport.

In the present study we used micro-Ussing chamber methods (14) to investigate the acute effects of influenza virus on ion transport by mouse tracheal epithelium and found that it inhibits Na^+ transport by this epithelium. We found that exposure of the apical membrane of the epithelium to PR8 influenza virus (10^6 pfu/mL) for 1 hr inhibited the amiloride-sensitive Na^+ current by approximately 75%. Exposure to UV-inactivated PR8 virus or to virus-free allantoic fluid had no effect on the electrical properties of the epithelium.

The neurotropic influenza virus, WSN33 (10^6 pfu/mL), was also inhibitory, but the replication-deficient type-5 adenovirus, MX-17, was not (10^6 pfu/mL). PR8 virus had no significant effect on the time-course or the size of the responses of the tracheal epithelium to carbachol or forskolin. Other amiloride-sensitive Na^+ transporting epithelia were also sensitive to influenza virus: we found that PR8 virus inhibited amiloride-sensitive currents in both the M1 mouse collecting duct cell line and in mouse colonic epithelium.

We then used pharmacological blockers to define the step of the infection process which inhibits amiloride-sensitive Na^+ channel activity. We found that pre-treatment of the epithelium with neuraminidase, which blocks binding of the virus to its receptor on the apical surface of the epithelium, prevented the inhibition of the amiloride-sensitive current by PR8 virus. Inhibition of endocytosis (cytochalasin D) or uncoating (chloroquine, amantadine) of the viral particles, on the other hand, did not prevent inhibition of the Na^+ channels. Furthermore, a "split-virus" preparation, in which the virus had been disrupted with detergent to render it inactive but still capable of binding to the epithelium, was also inhibitory. This inhibition could be prevented by a monoclonal antibody directed against hemagglutinin. Thus the Na^+ channel inhibition produced by influenza virus seems to be due to hemagglutinin binding to a receptor in the apical membrane of the respiratory epithelium.

Finally, we examined the intracellular mechanisms of the inhibition produced by the influenza virus. The inhibitor of phospholipase Cβ, U-73122, blocked the response, as did a broad-spectrum inhibitor of protein kinase C, bisindolylmaleimide I (BIM), and the selective inhibitor of the α and β isoforms of protein kinase C, Go[[umlaut]] -6983. Staurosporine, a non-selective inhibitor of serine and threonine kinases, partially prevented the inhibitory effect of the virus, but also itself significantly reduced the amiloride-sensitive current. Since Na^+ channels in respiratory epithelium have not previously been shown to be regulated by protein kinase C, we checked, and confirmed, that the activator of protein kinase C, 1,2-dioctanoyl-*sn*-glycerol (DOG), inhibits the amiloride-sensitive current, whereas inhibition of protein kinase C with BIM stimulates it. Pertussis toxin (300 ng/mL for 3 hr) did not prevent the inhibitory effect of the virus, although, like staurosporine, it reduced the baseline amiloride-sensitive current.

Key Words: *Amiloride; Hemagglutinin; Influenza Virus; Protein Kinase C; ENaC; Phospholipase C*

Address for correspondence: Professor D.I. Cook
Department of Physiology (F-13), University of Sydney, NSW 2006, Australia
Tel: +61.2-9351-3477, Fax: +61.2-9351-9926, E-mail: davidc@physiol.usyd.edu.au

This is the first report of any viral or microbial pathogen that regulates amiloride-sensitive Na^+ channels. Given the role of amiloride-sensitive Na^+ channels in controlling the amount of fluid in the respiratory tract (4, 15), the present findings provide an explanation for the disturbances in respiratory tract fluid balance which accompany influenza infections (8, 16-19).

Acknowledgements

This project was supported by the National Health and Medical Research Council of Australia and the National Heart Foundation of Australia. DIC and GK are Fellows of The Medical Foundation of the University of Sydney.

References

1. Matalon S, O'Brodovich H. *Sodium channels in alveolar epithelial cells: molecular characterization, biophysical properties, and physiological significance. Annu Rev Physiol 1999; 61: 627-61.*
2. Boucher RC. *Molecular insights into the physiology of the 'thin film' of airway surface liquid. J Physiol (Lond) 1999; 516: 631-8.*
3. Olver RE, Ramsden CA, Strang LB, Walters DV. *The role of amiloride-blockable sodium transport in adrenaline-induced lung liquid reabsorption in the fetal lamb. J Physiol (Lond) 1986; 376: 321-40.*
4. Kerem E, Bistritzer T, Hanukoglu A, Hofmann T, Zhou Z, Bennett W, MacLaughlin E, Barker P, Nash M, Quittell L, Boucher R, Knowles MR. *Pulmonary epithelial sodium-channel dysfunction and excess airway liquid in pseudohypoaldosteronism. N Engl J Med 1999; 341: 156-62.*
5. Hummler E, Barker P, Gatzy J, Beermann F, Verdumo C, Schmidt A, Boucher R, Rossier BC. *Early death due to defective neonatal lung liquid clearance in alpha-ENaC-deficient mice. Nat Genet 1996; 12: 325-8.*
6. Barker PM, Nguyen MS, Gatzy JT, Grubb B, Norman H, Hummler E, Rossier B, Boucher RC, Koller B. *Role of gammaENaC subunit in lung clearance and electrolyte balance in newborn mice. Insights into perinatal adaptation and pseudohypoaldosteronism. J Clin Invest 1998; 102: 1634-40.*
7. Belshe RB, Mendelman PM, Treanor J, King J, Gruber WC, Piedra P, Bernstein DI, Hayden FG, Kotloff K, Zangwill K, Iacuzio D, Wolff M. *The efficacy of live attenuated, cold-adapted, trivalent, intranasal influenza virus vaccine in children. N Engl J Med 1998; 338: 1405-12.*
8. Doyle WJ, Skoner DP, Hayden F, Buchman CA, Seroky JT, Fireman P. *Nasal and otologic effects of experimental influenza A virus infection. Ann Otol Rhinol Laryngol 1994; 103: 59-69.*
9. Giebink GS, Ripley ML, Wright PF. *Eustachian tube histopathology during experimental influenza A virus infection in the chinchilla. Ann Otol Rhinol Laryngol 1987; 96: 199-206.*
10. Popoff MR. *Interactions between bacterial toxins and intestinal cells. Toxicon 1998; 36: 665-85.*
11. Ball JM, Tian P, Zeng CQ, Morris AP, Estes MK. *Age-dependent diarrhea induced by a rotaviral nonstructural glycoprotein. Science 1996; 272: 101-4.*
12. Dong Y, Zeng CQ, Ball JM, Estes MK, Morris AP. *The rotavirus enterotoxin NSP4 mobilizes intracellular calcium in human intestinal cells by stimulating phospholipase C-mediated inositol 1,4,5-trisphosphate production. Proc Natl Acad Sci USA 1997; 94: 3960-5.*
13. Zhang M, Zeng CQ, Dong Y, Ball JM, Saif LJ, Morris AP, Estes MK. *Mutations in rotavirus nonstructural glycoprotein NSP4 are associated with altered virus virulence. J Virol 1998; 72: 3666-72.*
14. Mall M, Bleich M, Greger R, Schreiber R, Kunzelmann K. *The amiloride-inhibitable Na^+ conductance is reduced by the cystic fibrosis transmenbrane conductance regulator in normal but not in cystic fibrosis airways. J Clin Invest 1998; 102: 15-21.*
15. Matthay MA, Wiener-Kronish JP. *Intact epithelial barrier function is critical for the resolution of alveolar edema in humans. Am Rev Respir Dis 1990; 142: 1250-7.*
16. Suarez DL, Perdue ML, Cox N, Rowe T, Bender C, Huang J, Swayne DE. *Comparisons of highly virulent H5N1 influenza A viruses isolated from humans and chickens from Hong Kong. J Virol 1998; 72: 6678-88.*
17. Winternitz MG, Wason IM, McNamara FP. *The Pathology of Influenza. New Haven: Yale University Press, 1920; 14-5.*
18. Kudlacz EM, Baugh LE, Porter WP, Kenny MT, Farrell AM. *A time-course study of airway hyperresponsiveness in concious parainfluenza virus type 3-infected guinea pigs. Lab Anim Sci 1993; 43: 445-53.*
19. Parusov VN, Zhunko VV. *Experimental toxic pulmonary edema in white mice with influenza. Biull Eksp Biol Med 1977; 83: 243-4.*