J Korean Med Sci 2000; 15(Suppl): S31-3
ISSN 1011-8934

Control of Na^+ Channels in Salivary Duct Cells

Anuwat Dinudom, Permsak Komwatana, John Young, David Cook

Department of Physiology, University of Sydney, Sydney, Australia

The epithelial Na^+ channel (ENaC) is essential for transepithelial Na^+ transport in many epithelia, including the renal collecting duct, the descending colon, the salivary ducts and the respiratory epithelium (1, 2), and alterations in the activity of these channels lead to physiological significant disturbances in fluid and electrolyte homeostasis. Activating mutations in these channels, such as those that are found in the autosomal dominant form of hereditary hypertension known as Liddle's syndrome, produce hypertension (3, 4), whereas inactivating mutations lead to salt-sensitive hypotension and fluid accumulation in the respiratory tract (5-7).

Studies on humans with Liddle's syndome have indicated that this syndrome is due to mutations that cause to deletion or mutation of the PY motifs in the carboxyl termini of the β- or γ-subunits of the epithelial Na^+ channel (3, 8-11). These PY-motifs bind the WW domains of the ubiquitin-protein ligase, Nedd4 (12, 13), which then ubiquitinates the Na^+ channel (14-16) leading to its endocytosis (17) and degradation (16). As would be predicted by this model, in both renal epithelia (18) and in the *Xenopus* oocyte expression system (15, 19-21), mutations causing Liddle's syndrome lead to increased surface expression of Na^+ channels. Furthermore, increased expression of wild-type Nedd4 reduces Na^+ channel activity in *Xenopus* oocytes (15, 22), whereas expression of a dominant negative mutant of Nedd4 increases Na^+ channel activity (15, 22).

In both *Xenopus* oocytes and in mouse salivary duct cells, the interaction between Nedd4 and the epithelial Na^+ channel appears to be regulated by intracellular Na^+ in such a manner that increased intracellular Na^+ triggers the inactivation of Na^+ channels by Nedd4 (14, 20, 23, 24). In mouse mandibular duct cells, the evidence supporting a role for Nedd4 in Na^+-feedback inhibition of Na^+ channels is as follows: i) antibodies directed against murine Nedd4 block Na^+ feedback inhibition, ii) a mutant of murine Nedd4 containing the WW domains but no HECT (ubiquitin protein ligase) domain blocks Na^+ feedback inhibition, iii) a dominant negative mutant of ubiquitin blocks Na^+ feedback (14, 23), and iv) Nedd4 is expressed in mouse mandibular salivary duct cells (14). In salivary duct cells this control system involves sensing of cytosolic Na^+ by an intracellular receptor that can be blocked by amiloride and its analogs (25). This receptor in turn activates the G protein, G_o, (14, 26) which then activates Nedd4 by an unknown mechanism. In *Xenopus* oocytes, the effects of intracellular Na^+ on Na^+ channels and Nedd4 activity can be blocked by amiloride analogs (27), consistent with a role for an intracellular receptor for Na^+. Interestingly, however, a pertussis toxin-sensitive G protein does not appear to be involved (27). This postulated role of Nedd4 in Na^+ feedback control in *Xenopus* oocytes is supported by the failure of increased intracellular Na^+ to down-regulate Na^+ channels that contain Liddle's type mutations (20).

We have previously used whole-cell patch-clamp techniques in mouse mandibular salivary duct cells to show that WW1 of murine Nedd4 does not bind any of the subunits of the epithelial Na^+ channel (23) and this observation has recently been confirmed for WW1 of human Nedd4 (12). The question of whether the remaining two WW domains in murine Nedd4, WW2 and WW3, bind to all three subunits of the Na^+ channel indiscriminately, or whether they bind specifically to only two of them, has, however, not been answered.

In the present study, we have used the whole-cell patch-clamp technique to show that peptides and fusion proteins containing the PY motifs of β- and γ-ENaC prevent inhibition of the amiloride-sensitive Na^+ current by increased intracellular Na^+. Thus, given the essential role for Nedd4 in mediating the feedback control of Na^+ channels by cytosolic Na^+, we conclude that Nedd4 must interact with the β- and γ-subunits of ENaC.

On the other hand we find that the C-terminus of α-ENaC is unable to block Na^+ feedback regulation of the amiloride-sensitive Na^+ current. This fusion protein is thus unable to compete with the endogenous Na^+ channels for binding of Nedd4. Given that the α-subunits of rat, human and mouse ENaC have all been shown to bind Nedd4 in vitro (12, 13, 23), this indicates that in

Key Words: *Amiloride; Ubiquitin; Nedd4; ENaC*

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

vivo there is some constraint on the interaction of the PY motif of the α-subunit of the channel with Nedd4. One possible constraint could be that, unlike the carboxyl termini of the β- and γ-subunits, the α-subunit binds to α-spectrin (28).

Acknowledgements

This project was supported by the National Health and Medical Research Council of Australia and the National Heart Foundation of Australia. DIC is a Fellow of The Medical Foundation of the University of Sydney.

References

1. Garty H, Palmer LG. *Epithelial sodium channels: function, structure, and regulation. Physiol Rev 1997; 77: 359-96.*
2. Duc C, Farman N, Canessa CM, Bonvalet JP, Rossier BC. *Cell-specific expression of epithelial sodium channel alpha, beta, and gamma subunits in aldosterone-responsive epithelia from the rat: localization by in situ hybridization and immunochemistry. J Cell Biol 1994; 127: 1907-21.*
3. Shimkets RA, Warnock DG, Bositis CM, Nelson-Williams C, Hansson JH, Scambelan M, Gill JR, Ulick S, Milora RV, Finling JW, Canessa CM, Rossier BC, Lifton RP. *Liddle's syndrome: heritable human hypertension caused by mutations in the beta subnunit of the epithelial sodium channel. Cell 1994; 79: 407-14.*
4. Pradervand S, Wang Q, Burnier M, Beermann F, Horisberger JD, Hummler E, Rossier BC. *A mouse model for Liddle's syndrome. J Am Soc Nephrol 1999; 10: 2527-33.*
5. 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.*
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. Strautnieks SS, Thompson RJ, Gardner RM, Chung B. *A novel splice-site mutation in the gamma subunit of the epithelial sodium channel gene in three pseudohypoaldosteronism type 1 families. Nat Genet 1996; 13: 248-50.*
8. Tamura H, Schild L, Enomoto N, Matsui N, Marumo F, Rossier BC, Sasaki S. *Liddle disease caused by a missense mutation of β subunit of the epithelial sodium channel gene. J Clin Invest 1996; 97: 1780-4.*
9. Hansson JH, Nelson-Williams C, Suzuki H, Schild L, Shimkets R, Lu Y, Canessa C, Iwasaki T, Rossier B, Lifton RP. *Hypertension caused by a truncated epithelial sodium channel gamma subunit: genetic heterogeneity of Liddle syndrome. Nat Genet 1995; 11: 76-82.*
10. Hansson JH, Schild L, Lu Y, Wilson TA, Gautschi I, Shimkets RA, Nelson-Williams C, Rossier BC, Lifton RP. *A de novo missense mutation of the β subunit of the epithelial sodium channel causes hypertension and Liddle syndrome, identifying a proline-rich segment critical for regulation of channel activity. Proc Natl Acad Sci USA 1995; 92: 11495-9.*
11. Schild L, Lu Y, Gautschi I, Schneeberger E, Lifton RP, Rossier BC. *Identification of a PY motif in the epithelial Na^+ channel subunits as a target sequence for mutations causing channel activation found in Liddle syndrome. EMBO J 1996; 15: 2381-7.*
12. Farr TJ, Coddington-Lawson SJ, Snyder PM, McDonald FJ. *Human Nedd4 interacts with the human epithelial Na^+ channel: WW3 but WW1 binds to Na^+-channel subunits. Biochem J 2000; 345: 503-9.*
13. Staub O, Dho S, Henry PC, Correa J, Ishikawa T, McGlade J, Rotin D. *WW domains of Nedd4 bind to the proline-rich PY motifs in the epithelial Na^+ channel deleted in Liddle's syndrome. EMBO J 1996; 15: 2371-80.*
14. Dinudom A, Harvey KF, Komwatana P, Young JA, Kumar S, Cook DI. *Nedd4 mediates control of an epithelial Na^+ channel in salivary cells by cytosolic Na^+. Proc Natl Acad Sci USA 1998; 95: 7169-73.*
15. Goulet CC, Volk KA, Adams CM, Prince LS, Stokes JB, Snyder PM. *Inhibition of the epithelial Na^+ channel by interaction of Nedd4 with a PY motif deleted in Liddle's syndrome. J Biol Chem 1998; 273: 30012-7.*
16. Staub O, Gautschi I, Ishikawa T, Breitschopf K, Ciecanover A, Schild L, Rotin D. *Regulation of stability and function of the epithelial Na^+ channel (ENaC) by ubiquination. EMBO J 1997; 16: 6325-36.*
17. Shimkets RA, Lifton RP, Canessa CM. *The activity of the epithelial sodium channel is regulated by clathrin-mediated endocytosis. J Biol Chem 1997; 272: 25537-41.*
18. Snyder PM, Price MP, McDonald FJ, Adams CM, Volk KA, Zeiher BG, Stokes JB, Welsh MJ. *Mechanism by which Liddle's syndrome mutations increase activity of a human epithelial Na^+ channel. Cell 1995; 83: 969-78.*
19. Awayda MS, Tousson A, Benos DJ. *Regulation of a cloned epithelial Na^+ channel by its β- and γ-subunits. Am J Physiol 1997; 273: C1889-99.*
20. Kellenberger S, Gautschi I, Rossier BC, Schild L. *Mutations causing Liddle-syndrome reduce sodium-dependent down-regulation of the epithelial sodium channel in the Xenopus oocyte expression system. J Clin Invest 1998; 101: 2741-50.*
21. Firsov D, Schild L, Gautschi I, Merillat AM, Schneeberger E, Rossier BC. *Cell surface expression of the epithelial Na^+ channel and a mutant causing Liddle syndrome: a quantitative approach. Proc Natl Acad Sci USA 1996; 93: 15370-5.*
22. Abriel H, Loffing J, Rebhun JF, Pratt JH, Schild L, Horisberger JD, Rotin D, Staub O. *Defective regulation of the epithelial Na^+ channel by Nedd4 in Liddle's syndrome. J Clin Invest 1999; 103: 667-73.*
23. Harvey KF, Dinudom A, Komwatana P, Joliffe CN, Day ML,

Parasivam G, Cook DI, Kumar S. *All thee WW domains of murine Nedd4 are involved in the regulation of epithelial sodium channels by intracellular Na^+. J Biol Chem 1999; 274: 12525-30.*

24. Abriel H, Horisberger JD. *Feedback inhibition of rat amiloride-sensitive epithelial sodium channels expressed in Xenopus laevis oocytes. J Physiol (Lond) 1999; 516: 31-43.*
25. Komwatana P, Dinudom A, Young JA, Cook DI. *Activators of epithelial Na^+ channels inhibit cytosolic feedback controls. Evidence for the existence of a G protein coupled receptor for cytosolic Na^+. J Membr Biol 1998; 162: 225-32.*
26. Komwatana P, Dinudom A, Young JA, Cook DI. *Cytosolic Na^+ controls an epithelial Na^+ channel via the G_o guanine nucleotide-binding regulatory protein. Proc Natl Acad Sci USA 1996; 93: 8107-11.*
27. Huber M, Schreiber R, Boucherot A, Sanchez-Perez A, Poronnik P, Cook DI, Kunzelmann K. *Feedback inhibition of epithelial Na^+ channels in Xenopus oocytes does not require G_o or G_{i2} proteins. FEBS Lett 1999; 459: 443-7.*
28. Rotin D, Bar-Sagi D, O'Brodovich H, Merilainen J, Lehto PV, Canessa CM, Rossier BC, Downey GP. *A SH3 binding region in the epithelial Na^+ channel (ENaC) mediates its localization at the apical membrane. EMBO J 1994; 13: 4440-50.*