Fibroblast growth factor-10 upregulates Na,K-ATPase via the MAPK pathway

D. Upadhyay[a,b,*], E. Lecuona[a], A. Comellas[a], D.W. Kamp[a], J.I. Sznajder[a]

[a] *Division of Pulmonary and Critical Care Medicine, Northwestern University Medical School, Chicago, IL 60611, USA*
[b] *Pulmonary and Critical Care Medicine, Stanford University Medical Center, 300 Pasteur Drive, Stanford, CA 94305, USA*

Received 11 March 2003; revised 25 April 2003; accepted 5 May 2003

First published online 20 May 2003

Edited by Veli-Pekka Lehto

Abstract **We studied the effects of fibroblast growth factor (FGF-10) on alveolar epithelial cell (AEC) Na,K-ATPase regulation. Within 30 min FGF-10 increased Na,K-ATPase activity and α1 protein abundance by 2.5-fold at the AEC plasma membrane. Pretreatment of AEC with the mitogen-activated protein kinase (MAPK) inhibitor U0126, a Grb2-SOS inhibitor (SH3-b-p peptide), or a Ras inhibitor (farnesyl transferase inhibitor (FTI 277)), as well as N17-AEC that express a Ras dominant negative protein each prevented FGF-10-mediated Na,K-ATPase recruitment to the AEC plasma membrane. Accordingly, we provide first evidence that FGF-10 upregulates (short-term) the Na,K-ATPase activity in AEC via the Grb2-SOS/Ras/MAPK pathway.**

Key words: Fibroblast growth factor-10; Mitogen-activated protein kinase; Na,K-ATPase; Alveolar epithelial cell

1. Introduction

Lung edema clearance is effected by the osmotic gradient established by active vectorial Na^+ transport. Alveolar epithelial cell (AEC) Na,K-ATPase contributes to active sodium transport. Recent data indicate that upregulation of Na,K-ATPase is sufficient to increase alveolar fluid clearance [1–3]. Various agents such as dopamine, adrenergic agonists, mineralocorticoids and epidermal growth factor (EGF) increase fluid clearance by upregulating the alveolar epithelial Na,K-ATPase [4,5]. Na,K-ATPase activity is subjected to both short-term and long-term hormonal regulation (e.g. aldosterone, thyroid hormone) [4,5]. Short-term regulation of the Na,K-ATPase function can be achieved by increasing the number of molecules at the plasma membrane, changes in the catalytic property of enzymes already present at the plasma membrane, and by changes in enzyme affinity for sodium. Both keratinocyte growth factor (KGF) and EGF were reported to increase ion transport across AEC monolayers and lung edema clearance after 24–72 h exposure [6–9]. However, there is no information about the short-term effects (less than 60 min) of growth factors on Na,K-ATPase in the lungs.

Fibroblast growth factor-10 (FGF-10), a 19.3 kDa heparin binding protein structurally similar to KGF, is a potent AEC mitogen, promotes epithelial cell differentiation, migration, wound healing, prevents oxidant and cyclic stretch-induced DNA damage and is required for lung development [10–15].

Mitogen-activated protein kinase (MAPK) is a major signal transduction system by which cells transduce extracellular signals into intracellular responses. Several reports suggest an important role of the MAPK signal transduction pathway in the regulation of Na,K-ATPase. β-Adrenergic agonists as well as dopamine stimulate MAPK activation that results in (long-term, >12 h) upregulation of Na,K-ATPase [16,17]. MAPK pathway signaling typically occurs when growth factors, such as FGF-10, bind to their receptors resulting in downstream activation of MAPK family proteins that include extracellular signal-regulated kinases 1 and 2 (ERK-1/2) [14,15,18]. Because the Grb2-SOS, Ras and MAPK pathway plays a key role in mediating the protective effects of FGF-10 against cyclic stretch- and oxidant-induced AEC injury [14,15], we explored whether FGF-10 augments Na,K-ATPase activity by MAPK-dependent signal transduction pathways.

2. Materials and methods

2.1. Materials

FGF-10 was purchased from R&D systems (Minneapolis, MN, USA). U0126 was purchased from Promega (Madison, WI, USA). EZ-link NHS-SS-biotin and streptavidin beads were purchased from Pierce (Rockford, IL, USA). A monoclonal antibody specific to the Na,K-ATPase α1 subunit was obtained from Upstate Biotechnology, (Lake Placid, NY, USA). All other chemicals were purchased from Sigma (St. Louis, MO, USA).

2.2. Cell culture

A549 cells were obtained from the American Type Culture Collection (ATCC, Manassas, VA, USA) and maintained in Dulbecco's modified Eagle's medium supplemented with L-glutamine (0.3 μg/ml), non-essential amino acids, penicillin (100 U/ml), streptomycin (200 μg/ml), and 10% fetal bovine serum (Gibco, Grand Island, NY, USA). For each experiment, we used a seeding density of 3.0×10^5 cells/ml/well plated in six-well plates (Costar, Cambridge, MA, USA). The cells were grown to confluence over 24 h in a humidified 95% air–5% CO_2 incubator at 37°C.

2.3. Biotinylation of cell surface proteins

Cells were treated with different agonists/antagonists at 37°C, placed on ice, washed twice with ice-cold phosphate-buffered saline (PBS), and surface proteins were labeled for 1 h using 0.5 mg/ml EZ-link NHS-SS-biotin. After labeling, the cells were rinsed three times with PBS containing 50 mM glycine to quench unreacted biotin, and then lysed in modified RIPA buffer (50 mM Tris–HCl, pH 8, 150 mM NaCl, 1% NP-40 and 1% sodium deoxycholate, 1 μg/ml leupeptin, 100 μg/ml *N*-tosyl-L-phenylalanine chloromethyl ketone and 1 mM phen-

*Corresponding author. Fax: (1)-650-725 5489.
E-mail address: upadhyayd@pol.net (D. Upadhyay).

Abbreviations: AEC, alveolar epithelial cells; FGF-10, fibroblast growth factor-10; MAPK, mitogen-activated protein kinase

0014-5793/03/$22.00 © 2003 Published by Elsevier Science B.V. on behalf of the Federation of European Biochemical Societies.
doi:10.1016/S0014-5793(03)00527-1

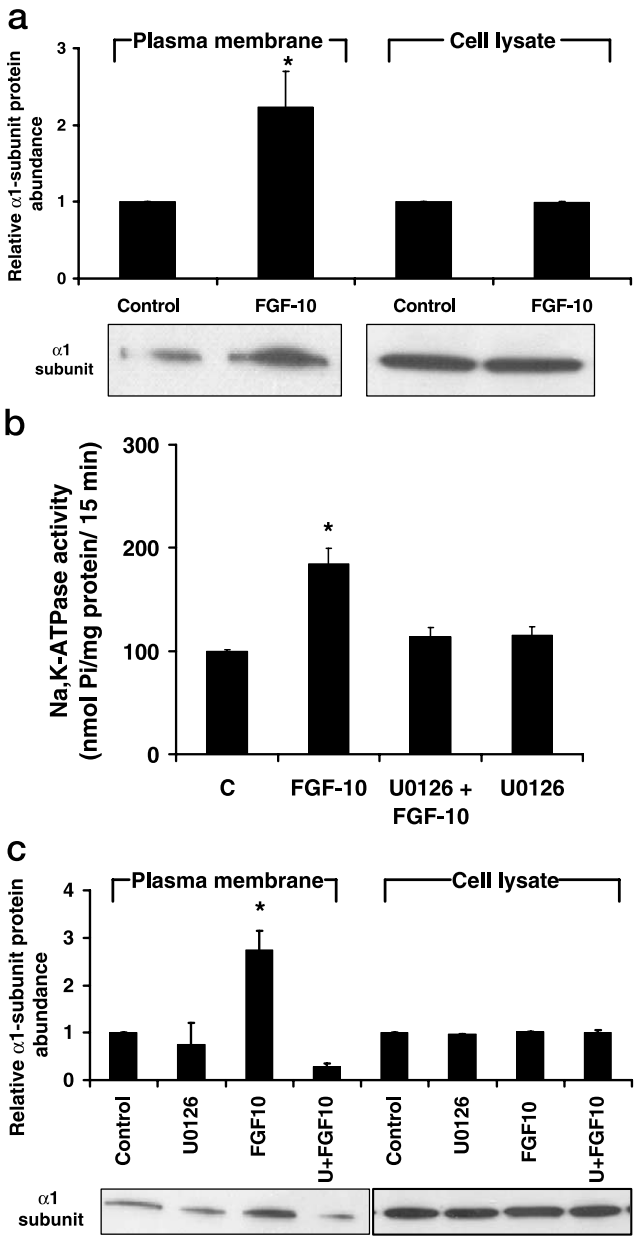

Fig. 1. a: FGF-10 upregulates (short-term) the AEC Na,K-ATPase. AEC were treated with FGF-10 (200 ng/ml) for 30 min. Na,K-ATPase α1 subunit protein abundance was assessed by biotinylation of cell surface proteins by Western blot. Compared to controls, FGF-10 increased Na,K-ATPase α1 subunit protein abundance. b: AEC were treated with FGF-10 (200 ng/ml) for 30 min. Na,K-ATPase activity was assessed by ^{32}P radiolabel assay. Compared to control, FGF-10 increased Na,K-ATPase activity by ~2-fold. Pretreatment of AEC with U0126 for 1.5 h blocked the increase in activity. c: AEC were pretreated with a specific MAPK inhibitor (U0126: 10 μM) for 1.5 h followed by treatment with FGF-10 (200 ng/ml) for 30 min. Na,K-ATPase α1 was assessed by biotinylation of cell surface proteins. U0126 blocked the FGF-10 effects. All data are expressed as means ± S.E.M., $n = 3$. *$P < .05$ vs. control.

ylmethylsulfonyl fluoride). 150–300 μg proteins were incubated overnight at 4°C with end-over-end shaking in the presence of streptavidin beads. Beads were thoroughly washed [19], resuspended in 30 μl of Laemmli's sample buffer solution [20] and analyzed by Western blot using a monoclonal antibody specific to the Na,K-ATPase α1 subunit.

2.4. Western blot analysis

Proteins were size fractionated by 1% sodium dodecyl sulfate/10% polyacrylamide gel electrophoresis and transferred to nitrocellulose membranes (Optitran, Schleicher and Schuell, Keene, NH, USA) using a semi-dry transfer apparatus (Bio-Rad, Hercules, CA, USA). Incubation of blots with a monoclonal antibody specific to the Na,K-ATPase α1 subunit (0.05 μg/ml) (Upstate Biotechnology) was performed overnight at 4°C. Blots were developed with an enhanced chemiluminescence detection kit (ECL$^+$, Amersham, Buckinghamshire, UK) used as recommended by the manufacturer. The bands were quantified by densitometric scan (Eagle Eye II, Stratagene, La Jolla, CA, USA).

2.5. Na,K-ATPase activity

Na,K-ATPase activity was determined as described before [21]. Briefly, after cells were incubated with the desired conditions, they were placed on ice and washed, and aliquots (~10 μg of protein) were transferred to the Na,K-ATPase assay medium, containing (in mM) 50 NaCl, 5 KCl, 10 $MgCl_2$, 1 EGTA, 50 Tris–HCl, and 7 Na_2ATP and [γ-^{32}P]ATP (specific activity 3000 Ci/mmol) in trace amounts (3.3 nCi/μl). Cells were transiently exposed to a thermic shock to render the membrane permeable to ATP. The samples were incubated at 37°C for 15 min and the reaction was terminated by the addition of 700 μl of a trichloroacetic acid:charcoal (5:10 w/v) suspension and rapid cooling to 4°C. After the charcoal phase containing the unhydrolyzed nucleotide was separated (12 000 × g for 5 min), the liberated ^{32}P was counted in an aliquot. The Na,K-ATPase activity was calculated as the difference between the samples assayed in the same medium but devoid of Na^+ and K^+ and in the presence of 2.5 mM ouabain (ouabain-insensitive ATPase activity).

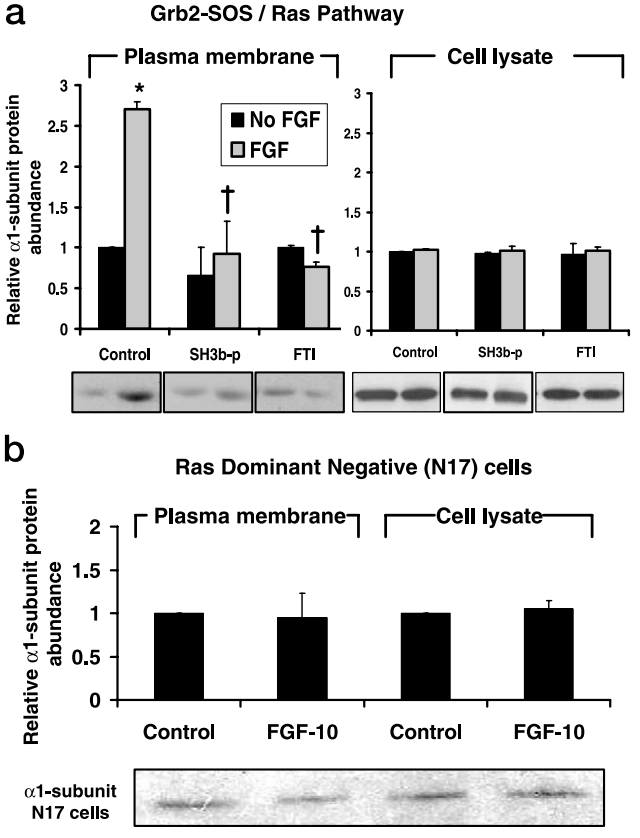

Fig. 2. FGF-10 regulation of AEC Na,K-ATPase is mediated by the Grb2-SOS/Ras/MAPK pathways. a: Pretreatment of AEC with SH3b-p for 2 h or the Ras inhibitor FTI 277 (10 μM) for 15 min prior to FGF-10 (200 ng/ml) blocked the FGF-10-mediated Na,K-ATPase upregulation. *$p < 0.05$ Control vs. FGF-10, $^{\dagger}p < 0.05$ FGF-10 vs SH3b-p+FGF-10, FTI+FGF-10. b: In N17-A549 cells expressing a Ras dominant negative protein treated with FGF-10 (200 ng/ml) did not increase the Na,K-ATPase. Data are presented as means ± S.E.M., $n = 3$, *$P < 0.05$

2.6. Statistical analysis

Data are reported as mean ± S.E.M. Statistical analysis was done by one-way analysis of variance and Tukey tests. Results were considered significant when $P < 0.05$.

3. Results and discussion

Growth factors are known to play a role in recovery from lung injury and to increase lung edema clearance [22–27]. EGF and KGF increased short-circuit current in AEC monolayers, upregulated Na,K-ATPase and increased lung liquid clearance [6,23–28] after ~24 h of exposure. Given the important role of growth factors regulating Na,K-ATPase in the lungs in the long term, we designed experiments to determine the short-term (< 60 min) effects of FGF-10, a potent AEC mitogen, on the Na,K-ATPase in AEC. We incubated AEC with FGF-10 (200 ng/ml) for 30 min and observed a 2.5-fold increase in α1 Na,K-ATPase protein abundance, assessed by biotinylation of AEC surface proteins (Fig. 1a). Na,K-ATPase translocation to the plasma membrane from intracellular compartments occurred without changing the Na,K-ATPase α1 subunit protein abundance in total cell lysates and resulted in an increase in Na,K-ATPase activity (Fig. 1b). Taken together, these data demonstrate short-term regulation (recruitment from intracellular pools) of Na,K-ATPase by FGF-10 in AEC.

The role of MAPK in the long-term (~24 h) regulation of Na,K-ATPase has been previously reported during dopaminergic type 2 receptor and β-agonist-mediated stimulation of Na,K-ATPase in AEC [16,17]. Given the role of MAPK in mediating the effects of FGF-10 as well as in regulation of Na,K-ATPase, we sought to determine the role of the MAPK pathway in the regulation of Na,K-ATPase by FGF-10. We treated AEC with a specific MAPK inhibitor (U0126: 10 μM) for 1.5 h followed by treatment with FGF-10 (200 ng/ml) for 30 min and as shown in Fig. 1b,c, U0126 blocked FGF-10-induced upregulation of AEC α1 Na,K-ATPase protein abundance at the plasma membrane as well as activity.

To investigate the role of upstream effectors of MAPK pathways, we incubated AEC with a SH3 binding domain inhibitor (SH3b-p; 10 μM) for 2 h [29,30] or a Ras inhibitor, farnesyl transferase inhibitor (FTI 277; 10 μM) for 30 min prior to treating the cells with FGF-10 (200 ng/ml). As shown in Fig. 2a, these inhibitors blocked FGF-10-mediated α1 Na,K-ATPase upregulation. Furthermore, we generated A549 cells expressing a Ras dominant negative protein (N17) which upon incubation with FGF-10 did not have an increase in Na,K-ATPase at the AEC plasma membrane (Fig. 2b) [30]. Taken together, these data suggest that FGF-10-induced (short-term) upregulation of AEC Na,K-ATPase occurs via the Grb2-SOS/Ras/MAPK pathways.

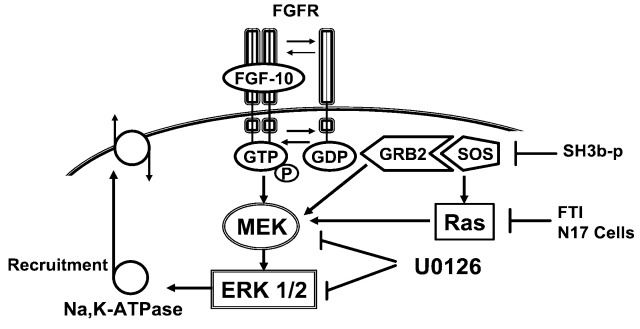

Fig. 3. Schematic diagram of FGF signaling pathways involved in regulation of AEC Na,K-ATPase. FGF-10 binds to activated FGF receptor which in turn facilitates activation of Grb2-SOS, Ras, ERK-1/2 pathways to recruit Na,K-ATPase to the cell plasma membrane.

In summary, we provide the first evidence that FGF-10-mediated increase in Na,K-ATPase activity and protein abundance at the plasma membrane of AEC function occurs within 30 min via the Grb2-SOS/Ras/MAPK pathways without increasing the whole cell Na,K-ATPase α1 protein expression (Fig. 3). We speculate that this novel mechanism of Na,K-ATPase regulation by FGF-10 may be of physiologic significance.

Acknowledgements: Supported in part by the NRSA (D.U.), and HL48129 (J.I.S.), Veterans Administration Merit Award (D.W.K.).

References

[1] Azzam, Z.S., Dumasius, V., Saldias, F.J., Adir, Y., Sznajder, J.I. and Factor, P. (2002) Circulation 105, 497–501.
[2] Factor, P., Dumasius, V., Brown, L.A. and Sznajder, J.I. (2000) Hum. Gene Ther. 11, 2231–2242.
[3] Factor, P., Saldias, F., Ridge, K., Dumasius, V., Zabner, J., Jaffe, H.A., Blanco, G., Barnard, M., Mercer, R., Perrin, R. and Sznajder, J.I. (1998) J. Clin. Invest. 102, 1421–1430.
[4] Berthiaume, Y., Staub, N.C. and Matthay, M.A. (1987) J. Clin. Invest. 79, 335–343.
[5] Panos, R., Bak, P., Simonet, W., Rubin, J. and Smith, L. (1995) J. Clin. Invest. 96, 2026–2033.
[6] Sznajder, J.I., Ridge, K.M., Yeates, D.B., Ilekis, J. and Olivera, W. (1998) Am. J. Physiol. Lung Cell. Mol. Physiol. 85, 1004–1010.
[7] Borok, Z., Spencer, I., Danto, L., Dimen, L., Zhang, X. and Lubman, L.L. (1998) Am. J. Physiol. Lung Cell. Mol. Physiol. 274, 149–158.
[8] Guery, B.P.H., Mason, C.M., Dobard, E.P., Beaucaire, G., Summer, W.R. and Nelson, S. (1997) Am. J. Respir. Crit. Care Med. 155, 1777–1784.
[9] Mason, C.M., Guery, B.P., Summer, W.R. and Nelson, S. (1996) Crit. Care Med. 24, 925–931.
[10] Igarashi, M., Finch, P.W. and Aaronson, S.A. (1998) J. Biol. Chem. 273, 13230–13235.
[11] Mason, J., Rampy, M.A., McCormack-Shannon, K., Edeen, K., Okoye, Z. and Shannon, J.M. (1999) Am. J. Respir. Crit. Care Med. 159, A172.
[12] Ware, L.B. and Matthay, M.A. (2002) Am. J. Physiol. Lung Cell. Mol. Physiol. 282, 924–940.
[13] Jimenez, P.A. and Rampy, M.A. (1999) J. Surg. Res. 81, 238–242.
[14] Upadhyay, D., Meyer, E.C., Sznajder, J.I. and Kamp, D.W. (2003) Am. J. Physiol. Lung Cell. Mol. Physiol. 284, 350–359.
[15] Upadhyay, D., Meyer, E.C., Pesce, L. and Kamp, D.W. (2001) Am. J. Respir. Crit. Care Med. 163, A793.
[16] Guerrero, C., Lecuona, E., Pesce, L., Ridge, K.M. and Sznajder, J.I. (2001) Am. J. Physiol. Lung Cell. Mol. Physiol. 281, 79–85.
[17] Pesce, L., Guerrero, C., Comellas, A., Ridge, K.M. and Sznajder, J.I. (2000) FEBS Lett. 486, 310–314.
[18] Szebenyi, G. and Fallon, J.F. (1999) Int. Rev. Cytol. 185, 45–105.
[19] Carranza, M.L., Rousselot, M., Chibalin, A.V., Bertorello, A.M., Favre, H. and Feraille, E. (1998) J. Physiol. 511, 235–243.
[20] Laemmli, U.K. (1970) Nature 227, 680–685.
[21] Bertorello, A.M., Ridge, K.M., Chibalin, A.V., Katz, A.I. and Sznajder, J.I. (1999) Am. J. Physiol. Lung Cell. Mol. Physiol. 276, 20–27.
[22] Kumar, R.K., O'Grady, R. and DiGirolamo, N. (1996) Lung 174, 171–179.
[23] Ridge, K.M., Dada, L., Lecuona, E., Bertorello, A., Katz, A.I., Mochly-Rosen, D. and Sznajder, J.I. (2002) Mol. Biol. Cell 3, 1381–1389.

[24] Sznajder, J.I., Factor, P. and Ingbar, D.H. (2002) J. Appl. Physiol. 93, 1860–1866.
[25] Bertorello, A., Komarova, Y., Efendiev, R., Pedemonte, C.H., Leibiger, I.B., Borisy, G. and Sznajder, J.I. (2003) Mol. Biol. Cell 14, 1149–1157.
[26] Ridge, K.M., Olivera, W., Saldias, F., Azzam, Z., Horowitz, S., Rutschman, D.H., Factor, P. and Sznajder, J.I. (2003) Circ. Res. 92, 453–460.
[27] Dada, L., Chandel, N., Pedemonte, C., Ridge, K.M., Bertorello, A.M. and Sznajder, J.I. (2003) J. Clin. Invest. 111, 1057–1064.
[28] Borok, Z., Hami, A., Danto, S.I., Lubman, R.L., Kim, K.J. and Crandall, E.D. (1996) Am. J. Physiol. Lung Cell. Mol. Physiol. 270, 559–565.
[29] Li, N., Batzer, A., Daly, R., Yajnik, V., Skolnik, E., Chardin, P., Bar-Sagi, D., Margolis, B. and Schlessinger, J. (1993) Nature 363, 85–88.
[30] Guerrero, C., Pesce, L., Lecuona, E., Ridge, K.M. and Sznajder, J.I. (2002) Am. J. Physiol. Lung Cell. Mol. Physiol. 282, 1099–1107.