Biometals (2008) 21:571–580
DOI 10.1007/s10534-008-9143-x

Iron accumulation in bronchial epithelial cells is dependent on concurrent sodium transport

Jennifer L. Turi · Claude A. Piantadosi · Jackie D. Stonehuerner · Andrew J. Ghio

Received: 23 October 2007 / Accepted: 23 April 2008 / Published online: 16 May 2008

Abstract Airway epithelial cells prevent damaging effects of extracellular iron by taking up the metal and sequestering it within intracellular ferritin. Epithelial iron transport is associated with transcellular movement of other cations including changes in the expression or activity of Na, K-ATPase and epithelial Na^+ channel (ENaC). Given this relationship between iron and Na^+, we hypothesized that iron uptake by airway epithelial cells requires concurrent Na^+ transport. In preliminary studies, we found that Na^+-free buffer blocked iron uptake by human airway epithelial cell. Na^+ channels inhibitors, including furosemide, bumetanide, and ethylisopropyl amiloride (EIPA) significantly decreased epithelial cell concentrations of non-heme iron suggesting that Na^+-dependent iron accumulation involves generalized Na^+ flux into the cells rather than participation of one or more specific Na^+ channels. In addition, efflux of K^+ was detected during iron uptake, as was the influx of phosphate to balance the inward movement of cations. Together, these data demonstrate that intracellular iron accumulation by airway epithelium requires concurrent Na^+/K^+exchange.

Keywords Iron · Sodium · Cation exchange

J. L. Turi (✉)
Department of Pediatrics, Duke University Medical Center, P.O. Box 3046, Durham, NC 27710, USA
e-mail: turi0002@mc.duke.edu

C. A. Piantadosi
Department of Medicine, Duke University Medical Center, Durham, NC 27710, USA

J. D. Stonehuerner · A. J. Ghio
National Health and Environmental Effects Research Laboratory, Environmental Protection Agency, Research Triangle Park, NC 27711, USA

Introduction

While iron's oxidation–reduction (redox) potential is necessary for its biological functions, it can catalyze reactive oxygen species (ROS) production and participate in cell and tissue injury. As a result, iron acquisition and distribution are tightly regulated in vivo. This is particularly important in the lower respiratory tract, which is exposed to iron in airborne particulates and that mobilized by inhaled microbes (Schroeder et al. 1987). The toxicity of free iron can be limited by storage within ferritin, which packages the metal in a less chemically reactive form inside the cell (Balla et al. 1992, Olakanmi et al. 1993). Iron sequestration by ferritin requires that extracelluar iron be transported across the cell membrane either by a number of transport proteins such as transferrin (Tf) and lactoferrin (Lf) (Aisen and Listowsky 1980, Thorstensen and Romslo 1990) or as non-transferrin bound iron (NTBI) (Turi et al. 2004).

Epithelial transport of iron and certain other metals into the cell appears to be associated with transcellular

movement of other ions, particularly sodium. For instance, copper uptake across epithelia in fish gills, frog skin, and vertebrate intestines appears to be dependent on the presence of Na^+ either via a common pathway, or a Cu-specific pathway modulated by Na^+ (Handy et al. 2002). This transport is significantly decreased in the presence of amiloride suggesting the involvement of the epithelial sodium channel (ENaC). In addition, Murr1, a protein implicated in copper transport (van De Sluis et al. 2002) has been demonstrated to interact with the C-terminus of the δENaC subunit to inhibit amiloride sensitive Na^+ transport (Biasio et al. 2004). Like copper, iron can induce ENaC expression and amiloride sensitive Na^+ transport in fetal lung epithelial cells (Rafii et al. 2000). In addition, regulation of Na, K-ATPase expression requires the presence of transferrin and can impact iron transport (Yin et al. 2003). These investigations suggest that iron accumulation in airway epithelial cells may also be linked to the movement of Na^+ across the plasma membrane. Therefore, we tested the hypothesis that iron accumulation in human airway epithelial cells depends on concurrent Na^+ transport.

Materials and methods

Materials

All reagents were obtained from Sigma Chemical Co. (St. Louis, MO) unless otherwise specified.

Cell harvest and culture

Primary human bronchial epithelial (NHBE) cells were studied after harvesting them from healthy, nonsmoking adult volunteers by cytologic brushing during bronchoscopy on a protocol approved by the University of North Carolina Institutional Review Board. They were expanded to passage 2–4 in bronchial epithelial growth medium (BEGM; Cambrex, Walkersville, MD). The cells were then grown to 90–100% confluence on uncoated plastic twelve-well plates in 1.0 ml BEGM medium supplemented with 0.5 ng/ml epidermal growth factor (EGF), 5 mg/ml insulin, 0.5 ng/ml triiodothyronine, and 0.1 ng/ml retinoic acid. Fresh medium was provided every 48 h.

Measurement of total cellular non-heme iron concentrations

After cells were grown to confluence on plastic twelve-well plates, medium was removed, the cells washed with 1.0 ml Hank's Balanced Salt Solution (HBSS), and 1.0 ml HBSS added to the well. Cells were incubated with ferric ammonium citrate (25 μg/ml FAC in HBSS) for 15, 30, 60, and 90 min or with FAC (0, 12.5, 25, or 125 μg/ml) for 4 h to establish time and concentration dependent effects, respectively. While citrate was present in this compound to maintain the iron in solution, the amount of citrate available potentially allowed some degree of hydration of the ferric iron (Aisen et al. 1978). However, given the short time duration of cell exposure, this was unlikely to impact our investigation. Following exposure, HBSS containing the metal was removed, the cells were washed with 1.0 ml of HBSS, and 1.0 ml of 3 N HCl and 10% trichloroacetic acid was added. The cells were scraped into the acid, hydrolyzed at 70°C for 18 h, and centrifuged at $600g \times 10$ min. Iron concentrations were determined using inductively coupled plasma atomic emission spectroscopy (ICPAES) at a wavelength of 238.204 (Model P300; Perkin Elmer, Norwalk, CT). Single element standards were used to calibrate the instrument (Fisher, Pittsburgh, PA). The limit of detection was approximately 10 ppb. The use of this methodology to measure the concentration of non-heme iron in airway epithelial cells has been demonstrated previously to accurately reflect the concentration of metal imported into the cell (Wang et al. 2002).

A second set of cells was exposed to FAC (25 μg/ml) in HEPES buffer (50 mM; pH = 7.3) to assess whether extracellular Na^+ or K^+ are required for iron uptake. Calcium chloride, magnesium chloride, and glucose were added to the buffer to concentrations present in keratinocyte medium (KGM, Cambrex; 0.15, 0.6, and 6 mM, respectively). Cells were exposed either in (1) HEPES buffer adjusted to the ionic strength of physiological saline, 0.9% by the addition sodium chloride (50 mM), (2) HEPES buffer adjusted to the same ionic strength by the addition of 1.15% potassium chloride (50 mM), or (3) HEPES buffer containing both NaCl and KCl in order to distinguish the effects of the presence of Na^+ versus the absence of K^+. Additional cells were exposed to varying concentrations of FAC in the presence or absence of calcium

(1 mM) or phosphate (1 mM) to determine the involvement of these ions in iron accumulation. Four hours after exposure to FAC, the cells were washed and cellular iron was measured by ICPAES as above.

Human bronchial epithelial cells were pre-treated with the Na^+/K^+-ATPase inhibitor ouabain (0–100 μM), or sodium channel inhibitors, furosemide (0–100 μM), bumetanide (0–100 μM), ethylisopropyl amiloride (EIPA, 0–10 μM), or triamterene (0–100 μM), or the carbonic anhydrase inhibitor acetazolamide (0–100 μM). Other studies were conducted with K^+ channel inhibitor, glybenclamide (0–100 μM) or calcium channel inhibitors, verapamil and nifedipine (0–100 μM). The inhibitors were applied for 15 min followed by exposure to FAC (25 μg/ml). After 4 h, the metal was removed, the cells washed, and total cellular iron measured by ICPAES as described above.

Measurement of potassium, calcium, and phosphate concentration

Cells were grown to 90–100% confluence on plastic twelve-well plates in 1.0 ml BEGM medium. Medium was removed, the cells washed with 1.0 ml HBSS, and 1.0 ml HBSS added to the well. Cells were incubated with FAC (12.5, 25, or 125 μg/ml) in HBSS for 4 h or with FAC (25 μg/ml) for 15, 30, 60, and 90 min to establish time and concentration dependent effects of iron uptake on extracellular potassium transport. The potassium concentration in the supernatant was determined using ICPAES at a wavelength of 766.490. In addition, cell calcium and phosphorous concentrations were determined in the presence of exogenous iron using ICPAES at wavelengths of 317.933 and 214.914 nm, respectively. Measurements were performed on a PerkinElmer 4300DV ICPAES (Fisher, Pittsburgh, PA). Standards were prepared by gravimetric dilution of a multielement standard from Spex Certiprep and concentrations checked by using QC standards prepared by gravimetric dilution of a multielement standard from VHG Labs.

Statistical analysis

Data are expressed as mean ± standard error. A minimum of three separate experiments were performed for each measurement. Data were compared using one-way analysis of variance followed by the Fisher's protected least square difference test. Significance was assumed at $P \leq 0.05$.

Results

Iron accumulation is augmented by extracellular Na^+

We established the time course and concentration effects of iron uptake in human airway epithelial cells by exposing NHBE cells to varying concentrations of Fe^{3+} as ferric ammonium citrate (FAC) and measuring changes in cellular non-heme iron (Fig. 1a, c). In addition, we assessed cation flux with concurrent iron accumulation by measuring K^+ concentration in the cell buffer (HBSS), because the lower extracellular K^+ baseline allowed more sensitive detection of changes in concentration. We attempted to measure changes in extracellular Na^+ concentration associated with iron accumulation, but found that these values were relatively small compared to the high concentration of extracellular Na^+ at baseline. Our data showed that extracellular K^+ in the buffer increased with the cellular uptake of non-heme iron (Fig. 1b, d). The increase in extracellular K^+ correlated with iron uptake and increased with the duration and concentration of exposure.

We next investigated whether either extracellular Na^+ or K^+ was required for the uptake of iron into the cell by assessing cellular iron levels after an iron challenge in cells maintained in HEPES buffer in which K^+ or Na^+ were independently removed from the extracellular buffer. We found that minimal iron was taken into the cell in the presence HEPES buffer containing K^+ alone, while the cells maintained in HEPES containing Na^+ or Na^+ together with K^+ showed a significant increase in cellular iron accumulation (Fig. 2). This suggests that increased iron uptake was due to the presence extracellular Na^+ rather than an absence of extracellular K^+.

Inhibitors of Na^+ channels block iron accumulation

To more clearly elucidate the role that Na^+ may play in iron accumulation and determine whether a specific Na^+ channel could be implicated in cation-dependent iron accumulation, we chemically

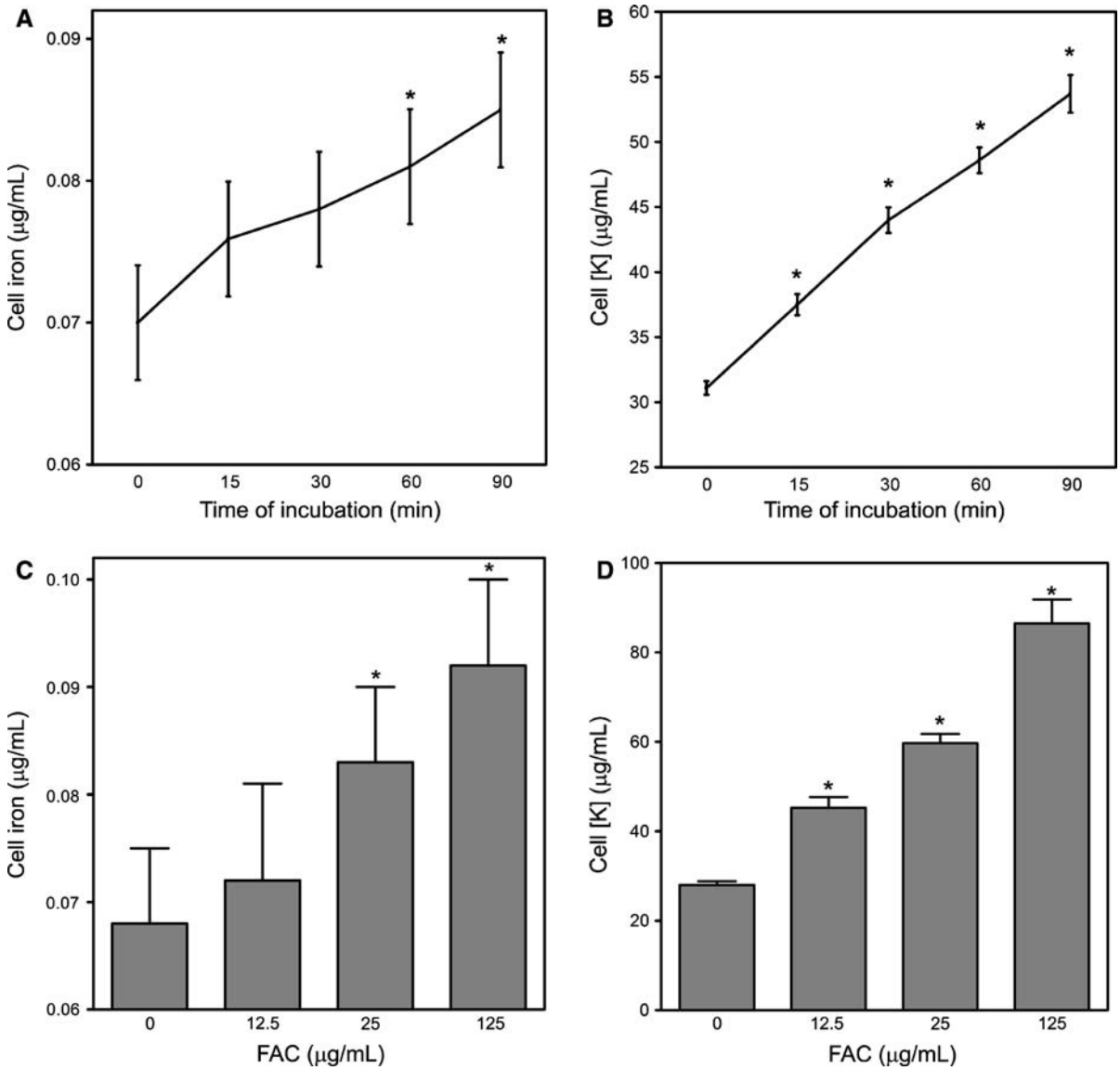

Fig. 1 Cellular iron accumulation and extracellular potassium increased in a dose and time dependent manner. NHBE cells were grown submerged in BEGM medium. The medium was removed and the cells were exposed to ferric ammonium citrate in HBSS (25 μg/ml) for 0, 15, 30, 60, and 90 min (**a**, **b**). Additional cells were incubated with FAC at varying concentrations for 60 min (**c**, **d**). The metal was removed and the cells washed, hydrolyzed in 3 N HCl and 10% trichloroacetic acid for 18 h, and centrifuged. Cellular non-heme iron concentration was measured using ICPAES ($\lambda = 238.204$; **a**, **c**). Supernatant potassium concentration was measured using ICPAES ($\lambda = 766.490$; **b**, **d**). $^*P < 0.05$ when compared to HBSS control

inhibited several types of Na^+ channels and measured the impact on iron uptake by NHBE cells. We found that cellular iron content was significantly decreased in cells pretreated with the ouabain, a relatively specific inhibitor of Na, K-ATPase (Fig. 3a). Similarly, cells pretreated with furosemide or bumetanide to inhibit the NKCC symporter, demonstrated a decrease in cellular uptake of iron after exposure to FAC (Fig. 3b). EIPA amiloride, which inhibits the ENaC channel and $Na^{+/}H^+$ exchanger (Fig. 3c) and acetazolamide or triamterene, K^+ sparing Na^+ inhibitors (Fig. 3d), also decreased cellular uptake of iron at the tested concentrations. This suggests that iron accumulation requires a generalized Na^+ flux to

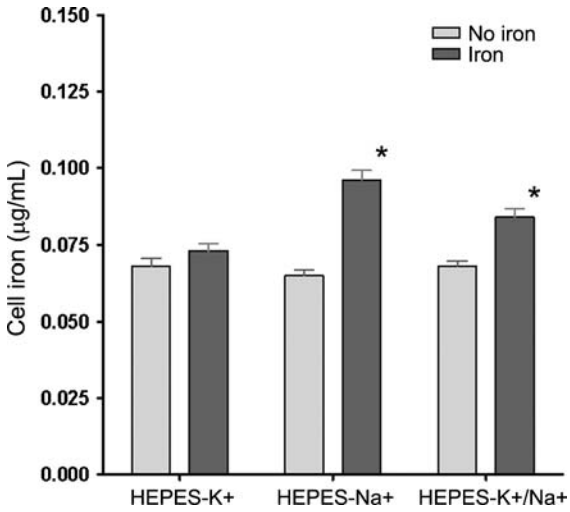

Fig. 2 Extracellular sodium augments iron accumulation. NHBE cells were grown submerged in BEGM medium. The medium was replaced with HEPES buffer containing either potassium (1.15% KCl), sodium (0.9% NaCl), or both NaCl and KCl. Cells were exposed to FAC in HBSS (25 μg/ml) or to HEPES buffer as negative control for 4 h. Iron uptake was measured using ICPAES ($\lambda = 766.490$). $^*P < 0.05$ when compared to buffer control

establish a gradient into the cells rather than the participation of a specific Na^+ channel.

Iron accumulation is augmented by extracellular calcium

To evaluate whether iron accumulation is dependent on Na^+ specifically or involves other cation channels, we measured changes in intracellular Ca^{2+} after treatment with Fe^{3+} and found a significant increase in cellular Ca^{2+} concentration following treatment of airway epithelial cells with iron (Fig. 4a). Further, the uptake of iron was significantly increased when the cells were exposed to iron in the presence of a Ca^{2+} containing buffer (Fig. 4b). Treatment of cells with Ca^{2+} channel inhibitors verapamil and nifedipine, however, did not alter the accumulation of iron in airway epithelial cells (data not shown). This may represent nonspecific passage of Ca^{2+} through the iron transporter or a direct interaction of Ca^{2+} on iron channels but suggests that a particular Ca^{2+} channels is not required.

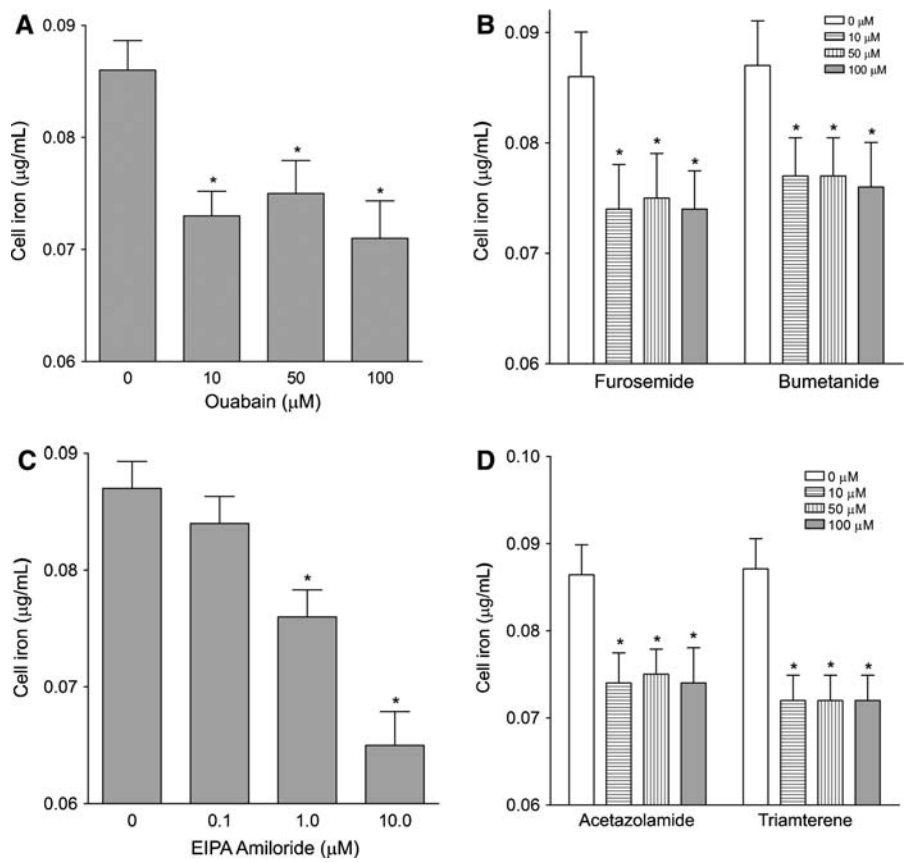

Fig. 3 Sodium channel inhibitors decrease intracellular iron accumulation. NHBE cells grown submerged in BEGM medium, were pre-treated with the Na, K-ATPase inhibitor, ouabain (**a**), Na/K/2Cl symporter inhibitors, furosemide or bumetanide (**b**), EIPA amiloride (**c**), or acetazolamide and triamterene (**d**) and then exposed to FAC (25 μg/ml) for 4 h. HBSS containing the metal was removed and the cells washed, hydrolyzed in 3 N HCl and 10% trichloroacetic acid for 18 h, and centrifuged. The iron content was measured using ICPAES ($\lambda = 238.204$). $^*P < 0.05$ when compared to HBSS control

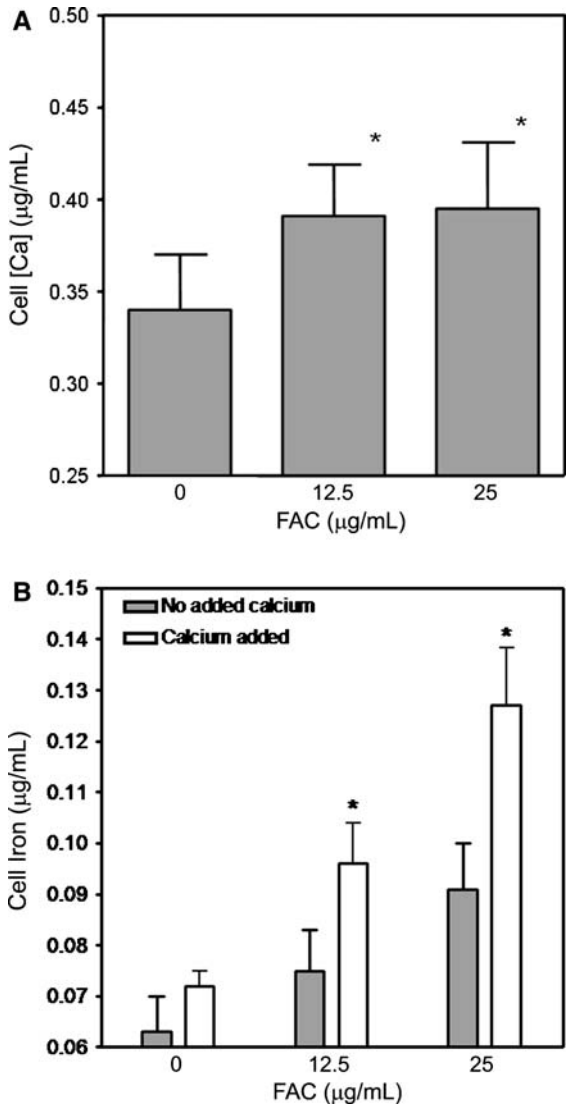

Fig. 4 Iron accumulation is linked to calcium uptake. NHBE cells were treated with varying concentrations of FAC in HBSS. After 4 h, the metal was removed and cellular Ca^{2+} was measured by ICPAES ($\lambda = 317.933$) (**a**). Additional cells were exposed to FAC in the presence or absence of a Ca^{2+}-containing buffer. Fours hours after exposure the metal was removed and cells washed, hydrolyzed in 3 N HCl and 10% trichloroacetic acid for 18 h, and centrifuged. The iron content was measured using ICPAES ($\lambda = 238.204$) (**b**). $^*P < 0.05$ when compared to medium control

Balance of charge is maintained by potassium and phosphate movement

The outward flow of K^+ with the uptake of Fe^{2+} suggests that a balance of charge is being maintained to balance the intracellular movement of Fe^{2+}, Na^+ and Ca^{2+}. To evaluate the extent of this, we measured changes in cellular phosphorus levels after exposure of NHBE cells to iron and found a significant increase in cellular phosphorus concentration with increasing exposure to exogenous iron (Fig. 5a). In addition, cells exposed to iron in a buffer containing phosphate demonstrated significantly increased concentrations of cellular iron (Fig. 5b). This suggests that the presence of extracellular phosphate may augment intracellular iron accumulation by providing a source of anions to maintain a balance of charge.

To determine if interference with the movement of K^+ out of the cell can alter the accumulation of iron, we pretreated NHBE cells with the K^+ channel inhibitor, glybenclamide, and measured cellular iron concentration after treatment with exogenous iron. As expected from the efflux of K^+ detected during iron uptake, treatment of cells with the K^+ channel blocker caused a significant decrease in the uptake of iron (Fig. 6).

Discussion

Respiratory epithelial cells serve to regulate pH, osmotic balance, and metal homeostasis at the air–liquid interface. This investigation proposes that some of the functions of airway epithelium may be interrelated, such that iron homeostasis requires intracellular Na^+ transport. We demonstrate that intracellular iron accumulation is augmented in the presence of increased extracellular Na^+ and that intracellular uptake of iron is associated with a time and concentration dependent efflux of K^+ from the cell. Further, uptake of iron into the cell is abrogated by inhibiting a number of different types of Na^+ channels. These data suggest that iron uptake is dependent on developing a transcellular Na^+ gradient, and is not linked to the function of a specific Na^+ channel.

Previous reports demonstrate that divalent and trivalent metal ions affect the activity of ion channels in a number of tissues (Assaf and Chung 1984; Gorman and Hermann 1979; Leinders et al. 1992; Vijverberg et al. 1994). In particular, iron can impact cation flux through single channels and at the whole cell level. Single channel recordings have demonstrated that Fe^{2+} can block single Ca^{2+} channels (Winegar et al. 1991) while activating an outward

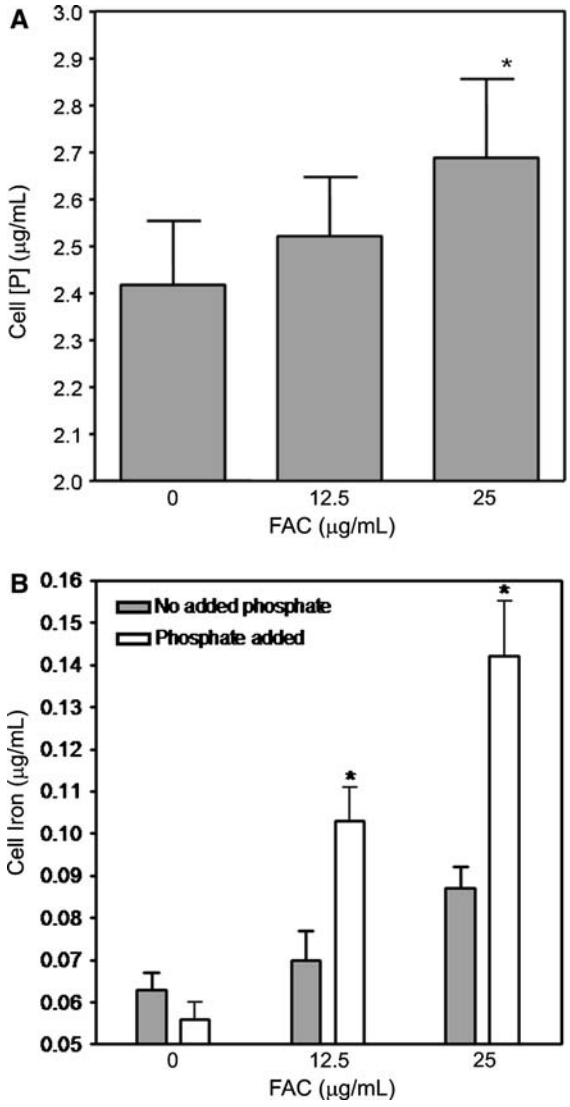

Fig. 5 Iron accumulation is balanced by increased cellular phosphate concentration. NHBE cells, grown submerged in BEGM medium, were treated with varying concentrations of FAC. After 4 h, the metal was removed and cell phosphorous concentration was measured by ICPAES ($\lambda = 214.914$) (**a**). Additional cells were exposed to FAC in the presence or absence of a phosphate-containing buffer. Fours hours after exposure the metal was removed and cells washed, hydrolyzed in 3 N HCl and 10% trichloroacetic acid for 18 h, and centrifuged. The iron content was measured using ICPAES ($\lambda = 238.204$) (**b**). $^*P < 0.05$ when compared to medium control

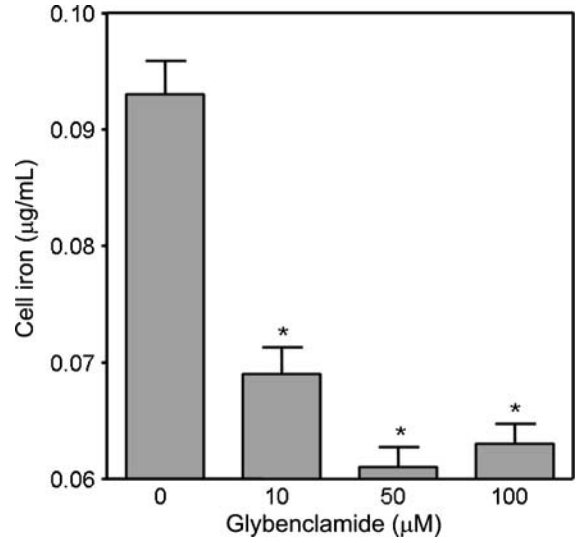

Fig. 6 K channel inhibitor decreases intracellular iron accumulation. NHBE cells were grown submerged in BEGM medium. The medium was removed and the cells pretreated with glybenclamide, then exposed to FAC (25 μg/ml) for 4 h. HBSS containing the metal was removed and the cells washed, hydrolyzed in 3 N HCl and 10% trichloroacetic acid for 18 h, and centrifuged. The iron content was measured using ICPAES ($\lambda = 238.204$). $^*P < 0.05$ when compared to medium control

current carried primarily by K^+ (Gorman and Hermann 1979). In the neuron, Fe^{2+} can enhance transient outward K^+ channels and Na^+ channels in a voltage and charge dependent manner (Ge et al. 2001), most likely due to an interaction between the gating charges and the ion bound at a non-specific site near the channel pore (Ge et al. 2001). The ability of iron to alter cation gradients across the cell surface suggests an integral link between the cation gradient created and iron-handling by the cell (Fig. 7).

To determine whether the Na^+-dependence of iron uptake into airway epithelial cells involves a specific Na^+ channel, we assessed iron accumulation in the presence of numerous Na^+ channel inhibitors. The lack of specificity of iron accumulation for Na^+ channel blockers suggests that this process may be due to vectorial Na^+ transport that is established by generating electrochemical gradients across the cell. To balance the movement of charge associated with intracellular uptake of Na^+ and Fe^{2+}, and in response to the gradient established by Na^+ K^+-ATPase, K^+ then exits the cell. The transcellular sodium gradient is established primarily via the action of the Na, K-ATPase in the basolateral membrane. However, inhibition with ouabain does not create a proportionally greater effect on iron accumulation than the other Na^+ channel blockers. This suggests that Na^+ electrodiffusion through the apical membrane, permitted

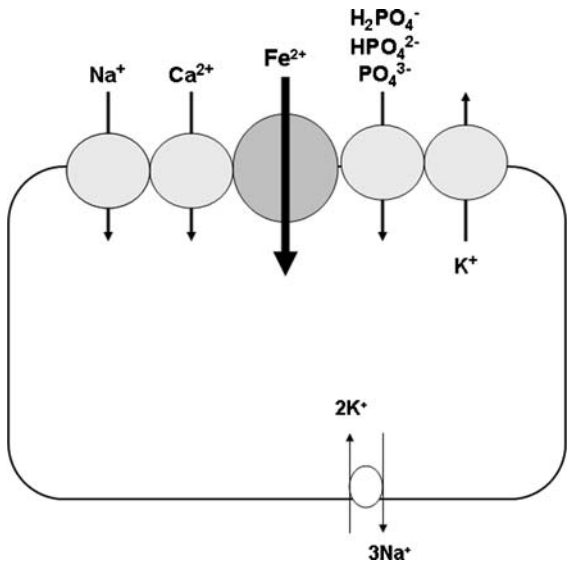

Fig. 7 Hypothetical Schema. Our data suggest the hypothesis that iron accumulation in airway epithelial cells requires the concurrent inflow of Na^+. This most likely represents vectorial Na^+ transport established by generating an electrochemical gradient across the cell via the activation of Na, K-ATPase. Movement of Na^+ and Fe^{2+} into the cell is also accompanied by inward movement of Ca^{2+}, though it remains unclear whether this represents nonspecific movement of Ca^{2+} through the iron transporter, DMT1 or actual involvement of a Ca^{2+} channel. Movement of these cations is balanced by inward movement of phosphate as well as outward flow of K^+

by the formation of a Na^+ gradient, is required for concurrent iron accumulation.

The creation and maintenance of the inward Na^+ and outward K^+ gradient across the plasma membrane is essential for numerous functions including nutrient uptake, apoptosis, and regulation of intracellular Ca^{2+} concentration. Na, K-ATPase levels and activity are regulated by the requirement for these gradients, such that a decrease in the K^+ or Na^+ gradient will increase the capacity of the Na^+ pump (Pressley et al. 1986). Low K^+ has been demonstrated in multiple cell types to increase the activity and expression of Na, K-ATPase. This effect in large part appears to require the presence of transferrin, and moreover, the cellular transport of ^{59}Fe in MDCK cells was increased in the presence of low K^+, but only in the presence of serum or transferrin (Yin et al. 2003). The effect was abrogated in the presence of deferoxamine and superoxide dismutase suggesting that the effect of low K^+ on Na,K-ATPase occurs via increased iron transport and reactive oxygen species activity, which may act as second messengers in the signaling cascade (Zhou et al. 2003).

Our data demonstrate that NHBE cells maintained in a buffer containing Na^+ but not K^+ had significantly increased iron uptake when compared to cells maintained in a K^+-containing buffer without Na^+. This supports the idea that the presence of Na^+ is required for the uptake of iron across the cell membrane. While low extracellular K^+ concentrations has been demonstrated to stimulate the function of Na, K-ATPase (Zhou et al. 2003), our data show that iron accumulation occurred to a significant extent in the presence of Na^+ and K^+ as well as with Na^+ alone. This suggests that the impact of the sodium presence on iron accumulation is not due to the decreased level of K^+ alone.

An interdependence of iron and Na^+ transport has also been demonstrated for the functional epithelial Na^+ channel (ENaC). Oxygen-induced expression of ENaC in fetal lung cells, which is responsible for increased fluid reabsorption at birth, requires the presence of adequate iron (Rafii et al. 2000) in that activation of amiloride sensitive short-circuit current as well as α- and β-ENaC mRNA expression is decreased by the presence of deferoxamine. This suggests that the interaction between iron and oxygen participates in altering Na^+ transport required to stimulate fluid removal from the lungs at birth.

It was not surprising that the movement of Ca^{2+} followed the uptake of iron in these cells and that uptake was augmented in the presence of increased extracellular Ca^{2+}. Transport of Fe^{2+} through the divalent metal transporter (DMT)-1, is associated with the transport of other divalent cations, including Ca^{2+} (Gunshin et al. 1997, Oudit et al. 2006). Further, Fe^{2+} can more directly impact Ca^{2+} transport by prolonging the activation of L-type Ca^{2+} channels in the myocardium to augment Fe^{2+} uptake. The link between Ca^{2+} flux and iron transport in airway epithelial cells, however, remains unclear. In the myocardium, iron transport can be blocked by Ca^{2+} channel blockers, nifedipine and verapamil (Tsushima et al. 1999; Oudit et al. 2003). We were not able to alter iron accumulation in our cells in the presence of Ca^{2+} channel blockers, suggesting that specific Ca^{2+} channels may not be involved in this process in the lung.

Transport of iron across the cell membrane requires that iron be reduced to Fe^{2+} prior to transport across the cell membrane. We used a ferric compound to test

our hypothesis because Fe^{3+} is far more available in an O_2-rich environment. Our previous studies utilizing Fe^{3+}-citrate and Fe^{2+}-citrate have demonstrated disparities in the rate of their uptake by airway epithelial cells in the first 10 min, but metal import is equivalent after this time using ICPAES methodology (Wang et al. 2002). Therefore, while ferri-reduction can be a limiting step in iron uptake by airway epithelial cells, this process is short lived and likely does not impact the results of our investigation. Following ferri-reduction, iron is transported in airway epithelial cells by DMT1 (Wang et al. 2002). Further investigation is warranted to determine the role of DMT1 in Na^+ dependent iron accumulation.

The presence of excessive iron on the surface of the airway epithelium presents a significant source of oxidative stress and injury as well as provides an important nutrient for microorganisms. The lung has mechanisms to detoxify this iron by taking it into the cell to sequester it in a chemically less reactive form within ferritin. Despite this, numerous inflammatory diseases such as cystic fibrosis (CF), emphysema, and acute respiratory distress syndrome are associated with markedly elevated levels of iron in the airway (Mateos et al. 1998). These elevated levels of iron are associated with increased lung injury. Significant iron present in the airways in CF, a disorder of Cl^- and Na^+ transport, raises the possibility that altered Na^+ transport in this disease may impact the ability of the airway epithelium to take up and sequester excess iron. The inability to metabolize excess iron may explain, in part, the significant oxidant injury and inflammation seen in these patients.

In conclusion, cellular uptake of iron by airway epithelial cells requires the presence of extracellular Na^+, and movement of Na^+ into the cell augments the uptake of iron. This suggests that altered iron cycling in the airways may be exacerbated in the presence of altered cation balance. Specific ionic mechanisms, as well as the mechanism by which iron enters the cell during Na^+ transport will require further investigation.

Acknowledgement This work was supported by National Institutes of Health Grant 5K08HL081269–02 (J. L. Turi).

References

Aisen P, Listowsky I (1980) Iron transport and storage proteins. Annu Rev Biochem 49:357–393

Aisen P, Leibman A, Zweier J (1978) Stoichiometric and site characteristics of the binding of iron to human transferrin. J Biol Chem 253:1930–1937

Assaf SY, Chung SH (1984) Release of endogenous Zn2+ from brain tissue during activity. Nature 308: 734–736

Balla G, Jacob HS, Balla J, Rosenberg M, Nath K, Apple F, Eaton JW, Vercellotti GM (1992) Ferritin: a cytoprotective antioxidant strategem of endothelium. J Biol Chem 267:18148–18153

Biasio W, Chang T, McIntosh CJ, McDonald FJ (2004) Identification of Murr1 as a regulator of the human delta epithelial sodium channel. J Biol Chem 279:5429–5434

Ge SY, Ruan DY, Yu K, Chen JT, Wang M, Zhong GS (2001) Effects of Fe(2+) on ion channels: Na(+) channel, delayed rectified and transient outward K(+) channels. Food Chem Toxicol 39:1271–1278

Gorman AL, Hermann A (1979) Internal effects of divalent cations on potassium permeability in molluscan neurones. J Physiol 296:393–410

Gunshin H, Mackenzie B, Berger UV, Gunshin Y, Romero MF, Boron WF, Nussberger S, Gollan JL, Hediger MA (1997) Cloning and characterization of a mammalian proton-coupled metal-ion transporter. Nature 388:482–488

Handy RD, Eddy FB, Baines H (2002) Sodium-dependent copper uptake across epithelia: a review of rationale with experimental evidence from gill and intestine. Biochim Biophys Acta 1566:104–115

Leinders T, van Kleef RG, Vijverberg HP (1992) Distinct metal ion binding sites on Ca(2+)-activated K + channels in inside-out patches of human erythrocytes. Biochim Biophys Acta 1112:75–82

Mateos F, Brock JH, Perez-Arellano JL (1998) Iron metabolism in the lower respiratory tract. Thorax 53:594–600

Olakanmi O, McGowan SE, Hayek MB, Britigan BE (1993) Iron sequestration by macrophages decreases the potential for extracellular hydroxyl radical formation. J Clin Invest 91:889–899

Oudit GY, Sun H, Trivieri MG, Koch SE, Dawood F, Ackerley C, Yazdanpanah M, Wilson GJ, Schwartz A, Liu PP, Backx PH (2003) L-type $Ca2^+$ channels provide a major pathway for iron entry into cardiomyocytes in iron-overload cardiomyopathy. Nat Med 9:1187–1194

Oudit GY, Trivieri MG, Khaper N, Liu PP, Backx PH (2006) Role of L-type Ca^{2+} channels in iron transport and iron-overload cardiomyopathy. J Mol Med 84:349–364

Pressley TA, Haber RS, Loeb JN, Edelman IS, Ismail-Beigi F (1986) Stimulation of Na, K-activated adenosine triphosphatase and active transport by low external K^+ in a rat liver cell line. J Gen Physiol 87:591–606

Rafii B, Coutinho C, Otulakowski G, O'Brodovich H (2000) Oxygen induction of epithelial Na(+) transport requires heme proteins. Am J Physiol Lung Cell Mol Physiol 278:L399–L406

Schroeder WH, Dobson M, Kane DM, Johnson ND (1987) Toxic trace elements associated with airborne particulate matter: a review. Japca 37:1267–1285

Thorstensen K, Romslo I (1990) The role of transferrin in the mechanism of cellular iron uptake. Biochem J 271:1–9

Tsushima RG, Wickenden AD, Bouchard RA, Oudit GY, Liu PP, Backx PH (1999) Modulation of iron uptake in heart

by L-type Ca^{2+} channel modifiers: possible implications in iron overload. Circ Res 84:1302–1309

Turi JL, Yang F, Garrick MD, Piantadosi CA, Ghio AJ (2004) The iron cycle and oxidative stress in the lung. Free Radic Biol Med 36:850–857

van De Sluis B, Rothuizen J, Pearson PL, van Oost BA, Wijmenga C (2002) Identification of a new copper metabolism gene by positional cloning in a purebred dog population. Hum Mol Genet 11:165–173

Vijverberg HP, Leinders-Zufall T, van Kleef RG (1994) Differential effects of heavy metal ions on Ca(2+)-dependent K^+ channels. Cell Mol Neurobiol 14:841–857

Wang X, Ghio AJ, Yang F, Dolan KG, Garrick MD, Piantadosi CA (2002) Iron uptake and Nramp2/DMT1/DCT1 in human bronchial epithelial cells. Am J Physiol Lung Cell Mol Physiol 282:L987–L995

Winegar BD, Kelly R, Lansman JB (1991) Block of current through single calcium channels by Fe, Co, and Ni. Location of the transition metal binding site in the pore. J Gen Physiol 97:351–367

Yin W, Jiang G, Takeyasu K, Zhou X (2003) Stimulation of Na, K-ATPase by low potassium is dependent on transferrin. J Membr Biol 193:177–184

Zhou X, Yin W, Doi SQ, Robinson SW, Takeyasu K, Fan X (2003) Stimulation of Na, K-ATPase by low potassium requires reactive oxygen species. Am J Physiol Cell Physiol 285:C319–C326