Lysophosphatidic Acid Stimulates Brush Border Na+/H+ Exchanger 3 (NHE3) Activity by Increasing Its Exocytosis by an NHE3 Kinase A Regulatory Protein-dependent Mechanism*

Received for publication, January 17, 2003, and in revised form, February 12, 2003
Published, JBC Papers in Press, February 20, 2003, DOI 10.1074/jbc.M305302200

Whaseon Lee-Kwon‡, Kazuya Kawano‡, Jung Woong Choi‡, Jae Ho Kim‡¶§, and Mark Donowitz‡¶¶

From the §Departments of Physiology and Medicine, Gastrointestinal Division, The Johns Hopkins University School of Medicine, Baltimore, Maryland 21205 and ¶Department of Physiology, College of Medicine, Pusan National University, Pusan 602-739, Republic of Korea

Na+/H+ exchanger 3 (NHE3) kinase A regulatory protein (E3KARP) has been implicated in cAMP- and Ca2+-dependent inhibition of NHE3. In the current study, a new role of E3KARP is demonstrated in the stimulation of NHE3 activity. Lysophosphatidic acid (LPA) is a mediator of the restitution phase of inflammation but has not been studied for effects on sodium absorption. LPA has no effect on NHE3 activity in opossum kidney (OK) proximal tubule cells, which lack expression of endogenous E3KARP. However, in OK cells exogenously expressing E3KARP, LPA stimulated NHE3 activity. Consistent with the stimulatory effect on NHE3 activity, LPA treatment increased the surface NHE3 amount, which occurred by accelerating exocytic trafficking (endocytic recycling) to the apical plasma membrane. These LPA effects only occurred in OK cells transfected with E3KARP. The LPA-induced increases of NHE3 activity, surface NHE3 amounts, and exocytosis were completely inhibited by pretreatment with the PI 3-kinase inhibitor, LY294002. LPA stimulation of the phosphorylation of Akt was used as an assay for PI 3-kinase activity. LY294002 completely prevented the LPA-induced increase in Akt phosphorylation, which is consistent with the inhibitory effect of LY294002 on the LPA stimulation of NHE3 activity. The LPA-induced phosphorylation of Akt was the same in OK cells with and without E3KARP. These results show that LPA stimulates NHE3 in the apical surface of OK cells by a mechanism that is dependent on both E3KARP and PI 3-kinase. This is the first demonstration that rapid stimulation of NHE3 activity is dependent on an apical membrane PDZ domain protein.

Na+/H+ exchanger 3 (NHE3)1 plays an essential role in NaCl and NaHCO3 absorption in ileum, colon, gallbladder, and proximal tubule of kidney (1–3). NHE3 cycles between the plasma membrane and recycling endosomal compartment under basal conditions (4–9). NHE3 is both rapidly stimulated and inhibited by several growth factors, neurotransmitters, and hormones that are released as part of digestion (1). These act by altering the amount of the NHE3 in the plasma membrane, as well as by changes in the NHE3 turnover number (1). For instance, epidermal growth factor and clonidine stimulate ileal sodium absorption by increasing the percentage of total NHE3 in the ileal brush border (BB) (10). In PS120 fibroblasts stably transfected with NHE3, the surface NHE3 amounts are stimulated by treatment with fibroblast growth factor (4). In opossum kidney proximal tubule cells exogenously transfected with the endothelin β receptor, treatment with endothelin-1 increased the surface NHE3 amount and NHE3 activity (11, 12) by an increase in exocytosis. Thus modulation of surface NHE3 amounts is a major mechanism in the acute stimulation of NHE3 activity by growth factors and hormones.

Phosphatidylinositol (PI) 3-kinase has been implicated in stimulation of several plasma membrane transport processes, including those of GLUT4 and NHE3 (1, 4–9, 13–15). PI 3-kinase activation is associated with translocation of these transporters from intracellular storage sites to the plasma membrane. Activation of PI 3-kinase results in increased intracellular levels of 3'-phosphorylated inositol phospholipids and induction of signaling responses, including the activation of the Ser/Thr protein kinase Akt (6, 16). PI 3-kinase and Akt are intermediates in the insulin-injected GLUT4 translocation to plasma membranes, and a constitutively active Akt mutant stimulates GLUT4 translocation (13, 17). PI 3-kinase regulates the basal and stimulated level of plasma membrane NHE3 amount and NHE3 transport activity. In PS120 and OK cells, basal NHE3 activity and surface amount are decreased by 50% by the PI 3-kinase inhibitors, wortmannin and LY294002 (4, 7). Also, the epidermal growth factor- or fibroblast growth factor-induced increase of NHE3 activity in PS120 fibroblasts, Caco-2 cells, and ileal BB is inhibited by these PI 3-kinase inhibitors (4, 18). Consistent with these results, we reported recently (6) that constitutively active mutants of PI 3-kinase and Akt stimulated NHE3 activity in PS120 fibroblasts by increasing the NHE3 amount in the plasma membrane, and a peptide stimulator of PI 3-kinase had the same effect in OK cells (6). These results suggest that PI 3-kinase and Akt activation, which is stimulated by PI 3-kinase, play necessary and sufficient roles in the stimulation of exocytosis of NHE3 in PS120 fibroblasts.

NHERF and E3KARP, which are scaffolding proteins, possess two PDZ domains and an ERM-binding domain (19). Both have been implicated in cAMP-dependent inhibition of NHE3.
activity (19), whereas only E3KARP is involved in Ca\(^{2+}\)-dependent inhibition of NHE3 (20). These PDZ domain proteins interact with NHE3 through their C-terminally extended second PDZ domain, and via their C termini also associate with ezrin, which links to the actin cytoskeleton and also acts as an anchoring protein for protein kinase AII (21). Therefore, both NHERF and E3KARP physically link NHE3 to protein kinase AII for the acute inhibition of NHE3 activity through protein kinase A-dependent phosphorylation. The protein kinase A-dependent phosphorylation of NHE3 is a consequence of cAMP-stimulated endocytosis of NHE3 (22). It has not been studied whether these PDZ domain proteins are involved in rapid stimulation of NHE3 activity and in the endocytic recycling of NHE3 to the plasma membrane.

Lysophosphatidic acid (LPA) is an inflammatory mediator (23–26). It is a bioactive phospholipid released by activated platelets, fibroblasts, leukocytes, phagocytes, and endothelial cells. LPA is released in high concentrations at the site of injury in the gastrointestinal tract and proximal tubule. LPA enhances intestinal restitution and wound healing/remodeling (27–29). In opossum kidney proximal tubule cells, LPA also induces proliferation, although on a more prolonged time scale, through the activation of PI 3-kinase and the extracellular signal-regulated kinase (ERK) (30). In this study, we demonstrate the first evidence that LPA stimulates epithelial sodium absorption, acting by increasing NHE3 activity and the amount of NHE3 on the plasma membrane. This occurs by stimulation of exocytic trafficking by activation of PI 3-kinase. E3KARP but not NHERF is necessary for LPA-induced stimulation of NHE3 activity and increase of NHE3 in the plasma membrane. This is the initial demonstration of E3KARP being involved in rapid stimulation of sodium absorption.

EXPERIMENTAL PROCEDURES

Cell Culture and Generation of Stable Cell Lines—OK/E3V (NHE3 epitope tagged on the C terminus with the vesicular stomatitis virus G-protein (VSV-G epitope)) cells (generously provided by J. Noel, University of Montreal), as described (7, 31), were maintained in high glucose Dulbecco’s modified Eagle’s medium supplemented with 25 mM NaHCO\(_3\), 10 mM HEPES, 1 mM sodium pyruvate, 10% fetal bovine serum, 100 units/ml penicillin, and 50 µg/ml streptomycin in a 5% CO\(_2\)/95% O\(_2\) humidified incubator at 37 °C. Cells were maintained in growth medium containing 400 ng/ml streptomycin, 100 units/ml penicillin, and 50 µg/ml streptomycin, was then switched to Na\(^{+}\)/H\(^{+}\) exchange activity data was calculated as the product of NHE3-dependent changes in pH, times the buffering capacity at each pH, and were analyzed by nonlinear regression using Origin (Microcal Software) to estimate V\(_{\text{max}}\) and K\(_{\text{m}}\) (32).

In some cases, Na\(^+/\)H\(^+\) exchange activity was described as the rate of initial sodium-dependent alkalization over a minimum of 9 s, as determined by least square analysis.

Biotinylation and Immunoblotting—The amounts of NHE3 on the plasma membrane were measured by biotinylation of cell surface proteins as described (7, 32). OK cells as described above were serum-starved for 2 days and then exposed to LPA at 37 °C for the indicated times. All subsequent steps were performed at 4 °C. Cells were rinsed twice with ice-cold phosphate-buffered saline (150 mM NaCl, 20 mM Na\(_2\)HPO\(_4\), pH 7.4) and then in borate buffer (154 mM NaCl, 1.0 mM boric acid, 7.2 mM KCl, and 1.8 mM CaCl\(_2\), pH 9.0). Cells were then exposed to 0.5 mg/ml sulfo-NHS-SS-biotin in borate buffer for 40 min with gentle shaking. After labeling, cells were washed with quenching buffer (20 mM Tris-HCl, 120 mM NaCl, pH 7.4) to scavenge the unreacted biotin. Cells were washed three times with ice-cold phosphate-buffered saline and lysed in 1 ml of N\(^+\) buffer (60 mM HEPES-NaOH, pH 7.4, 150 mM NaCl, 3 mM KCl, 5 mM EDTA, 3 mM EGTA, and 1% Triton X-100) by sonication for 20 s. Insoluble cell debris was removed by centrifugation for 20 min at 12,000 × g. Supernatant representing the total NHE3 fraction was incubated with streptavidin-agarose for 2 h and then the resultant beads were washed four times in N\(^+\) buffer to remove nonspecifically bound proteins. The proteins bound to streptavidin-agarose beads, which represent surface fractions, were solubilized with Laemmli buffer. The total and surface fractions were resolved by SDS-PAGE and transferred to nitrocellulose membranes. After blocking with 5% nonfat milk, the blots were probed with a monoclonal anti-VSV-G antibody (PSD4) as the primary antibody and horseradish peroxidase-conjugated anti-mouse as the secondary antibody, and bands were visualized by enhanced chemiluminescence (33). The densities of NHE3 protein bands were quantitated by scanning densitometer and Imagequant software.

Endocytic Internalization—Endocytosis was measured by a protocol slightly modified from the reduced GSH-resistant endocytosis assay described previously by us (20). Cells were labeled with 1.5 mg/ml sulfo-NHS-SS-biotin for 40 min and quenched at 4 °C. Cells were then warmed to 37 °C, treated with LPA, dopamine (positive control) (34), or vehicle, for 50 min at 37 °C. LPA treatment was performed by incubating cells with ice-cold saline twice at 4 °C. Surface biotin was cleaved by washing with 50 mM Tris-HCl, 150 mM GSH, pH 8.8. The freshly endocytosed proteins bearing biotin were protected from cleavage with GSH. Cells were solubilized in N\(^+\) buffer, and biotinylated proteins were retrieved and assayed for endocytosed NHE3 as described above.

Exocytic Insertion (Endocytic Recycling) of NHE3 to the Apical Plasma Membrane—To measure exocytic insertion of NHE3, NHS reactive sites on the cell surface were first blocked by pretreatment with sulfo-NHS-acetate as described, with some slight modifications (12, 34). Cells were rinsed with PBS-Ca-Mg and then incubated with 50 mM LPA for 2 h. After quenching for 20 min, cells were rinsed with PBS at 37 °C and treated with LPA or vehicle for 30 min. Cells were then treated with 0.5 mg/ml sulfo-NHS-SS-biotin and lysed with N\(^+\) buffer. The biotinylated fraction, which represents newly inserted surface proteins, was precipitated by streptavidin agarose, and the precipitate was subjected to SDS-PAGE and Western blotting with anti-VSV-G antibody as described above.

RESULTS

Establishment of OK/E3V Cells Stably Expressing E3KARP—To address the question whether E3KARP is involved in stimulation of NHE3, we established an OK cell line stably expressing both NHE3V and E3KARP (OK/E3V/E3KARP cells) as described under “Experimental Procedures.” The expression levels of NHERF and E3KARP in the parental cell lines were determined. As shown in Fig. 1, E3KARP is expressed in OK/E3V/E3KARP cells but not in parental OK or OK/E3V cells. Endogenous NHERF is expressed in these OK cells in similar amounts. The level of E3KARP expression is less in OK/E3V/E3KARP cells than in the PS120/E3V/E3KARP cells we have described previously (20). Endogenous NHERF is highly expressed in OK/E3V/E3KARP cells, in contrast to the minimal expression of endogenous NHERF in PS120 cells (35).

LPA Stimulates NHE3 Activity in an E3KARP-dependent Fashion—Growth factors, including epidermal growth factor...
Increased up to 60 min. LPA activated NHE3 in a concentration-activity as early as 10 min, and the LPA-dependent activation, LPA significantly increased NHE3 activity. The dose and time dependence of the LPA-induced stimulation of NHE3 activity were determined. The net increase in surface NHE3 activity was not significantly affected by LPA treatment because of the increase of amount of surface NHE3.

Figure 1. Establishment of E3KARP expressing opossum kidney/E3V proximal tubule cells. 20 μg of lysates from OK (non-transfected), OK/E3V, OK/E3V/E3KARP, and PS120/E3V/E3KARP cells were separated by SDS-PAGE, and the presence of NHERF and E3KARP were probed with anti-NHERF (upper panel) and anti-E3KARP (lower panel) antibodies, respectively. Representative data from three similar experiments are shown.

and fibroblast growth factor, increase NHE3 activity in Caco-2 intestinal epithelial cells and in PS120 fibroblasts, respectively, and endothelin stimulates NHE3 in OK cells (4, 7, 11, 12). To determine whether LPA, as an example of the mediators of the restitution phase of inflammation, affects NHE3 activity in proximal tubule epithelial cells, we treated OK/E3V and OK/E3V/E3KARP cells with LPA for 30 min at 37 °C and then measured NHE3 activity. As shown in Fig. 2A, LPA treatment (100 μM, 30 min at 37 °C) caused a 50% increase in NHE3 V_{max} (1533 ± 63 μm/s, control versus 2285 ± 76 μm/s, LPA-treated cells, p < 0.01) in OK/E3V/E3KARP cells. In contrast, NHE3 activity was not significantly affected by LPA treatment in OK/E3V cells (Fig. 2A). The dose and time dependence of the LPA-induced stimulation of NHE3 activity were determined. As shown in Fig. 3A, LPA significantly increased NHE3 activity as early as 10 min, and the LPA-dependent activation increased up to 60 min. LPA activated NHE3 in a concentration-dependent manner from 10^{-6} M to 10^{-4} M (Fig. 3B), with no effect at 10^{-7} M. This dose dependence of LPA on NHE3 activity is similar to the concentration dependence of LPA on proliferation of OK cells (30). These results show that LPA stimulates NHE3 activity by an E3KARP-dependent mechanism.

LPA Increases the Surface Amount of NHE3 in E3KARP-containing Cells—To determine whether the LPA-induced increase of NHE3 activity was because of an increase of amount of plasma membrane NHE3 protein, we measured the surface NHE3 amounts by cell surface biotinylation. Plasma membrane proteins were biotinylated by reaction with sulfo-NHS-SS-biotin at 4 °C, and biotinylated proteins were isolated by precipitation with immobilized streptavidin-agarose. As shown in Fig. 4A, treatment with 10^{-4} M LPA for 60 min did not affect the surface abundance of NHE3 in OK/E3V cells (non-E3KARP expressing). In contrast, in OK/E3V/E3KARP cells, LPA treatment caused an increase in apical membrane NHE3 protein abundance in a time-dependent manner (Fig. 4B), consistent with the time-dependent stimulation of NHE3 activity by LPA treatment shown in Fig. 3B. 10 min of LPA exposure significantly increased the amount of surface NHE3. The LPA-induced increase in the surface NHE3 amount occurred without any change in total cellular NHE3 abundance. These results suggest that the LPA-induced activation of NHE3 is because of the increase of the amount of surface NHE3.

The LPA Increase in Surface NHE3 Amount Is Because of Increased Exocytic Insertion—The net increase in surface NHE3 amount could be because of a decrease in endocytic internalization and/or an increase in exocytic insertion of NHE3. To clarify which mechanism was involved in the increase of surface NHE3 amount, the rates of endocytosis and exocytosis of NHE3 in OK/E3V/E3KARP cells were measured. To quantitate the exocytic insertion, all plasma membrane reactive sites were blocked by pretreatment with sulfo-NHS-acetate (12). Cells were then treated with LPA or vehicle for 30 min, followed by biotinylation of cell surface proteins. Biotinylated NHE3 represents NHE3 that was initially located in intracellular compartments and then inserted on the plasma membrane after treatment with LPA. After treatment with LPA for 30 min, biotinylated amounts of NHE3 were increased in OK/E3V/E3KARP cells (Fig. 5A). LPA increased the NHE3 exocytosis by ~60% (Fig. 5A, inset on right). These results indicate that exocytic trafficking of NHE3 accounts for the LPA-induced increase of surface NHE3 amount with 30 min of LPA exposure.

To quantitate the endocytic internalization of NHE3, apical membrane proteins were labeled with sulfo-NHS-SS-biotin before treatment with LPA or vehicle. After biotinylation for 40 min at 4 °C, cells were exposed to reduced GSH, which removes biotin from surface proteins. With this protocol, biotinylated NHE3 represents the pool of NHE3 that was initially present on the apical membrane and then was endocytosed, thus protecting it from GSH. Consistent with a previous report (34), there was increased NHE3 internalized from plasma membrane after treatment with dopamine for 30 min (positive control). In contrast, LPA treatment for 30 min had no effect on the amount of endocytosed NHE3 (Fig. 5, B and inset on right). These results suggest that endocytosis of NHE3 is not involved in the LPA-induced (30 min) increase of surface NHE3 amount.

**LPA-induced NHE3 Stimulation Is PI 3-Kinase-dependent**—PI 3-kinase has been implicated in some basal and stimulated regulation of NHE3 activity in fibroblasts and Caco-2 cells and basal NHE3 activity in OK cells based on biochemical studies using specific inhibitors of PI 3-kinase, wortmannin and LY294002, and peptide stimulation of PI 3-kinase (4–7). We also reported recently (6) that constitutively active mutants of PI 3-kinase and Akt stimulated basal NHE3 activity by increasing surface NHE3 amounts in PS120 fibroblasts. To determine whether PI 3-kinase and Akt were involved in the LPA-induced activation of NHE3, we examined the effect of the PI 3-kinase inhibitor, LY294002, on the LPA-induced NHE3 activation in OK/E3V/E3KARP cells. As shown in Fig. 6, pretreatment with 50 μM LY294002 inhibited basal NHE3 activity by 50%, which is consistent with previous reports in OK cells (7). In addition, LY294002 pretreatment completely blocked the LPA-induced stimulation of NHE3 activity. These results indicate that PI 3-kinase is necessary for the LPA-induced stimulation of NHE3, as well as in basal NHE3 activity.

**PI 3-Kinase Is Necessary for the LPA-induced Increase of Plasma Membrane Amount and Exocytic Trafficking of NHE3**—LPA stimulates the NHE3 activity through increasing the amounts of NHE3 localized on the apical plasma membrane in OK/E3V/E3KARP cells (Fig. 4B). To determine whether the increase of surface NHE3 amounts requires the activation of PI 3-kinase, we next examined the effect of LY294002 on the surface NHE3 amounts in OK/E3V/E3KARP cells. As shown in Fig. 7A, the amounts of NHE3 localized on the plasma membrane were increased by LPA treatment, whereas LY294002 pretreatment completely blocked the LPA-induced increase of surface NHE3 amounts. LY294002 also caused a small but significant decrease in basal surface NHE3 amount. These changes in surface NHE3 parallel the inhibitory effect of LY294002 on the LPA-stimulated, as well as basal, NHE3 activity.
The LPA-induced increase of surface NHE3 amount is because of enhanced exocytic trafficking of NHE3 to the apical plasma membrane (Fig. 5, A and B). Therefore, we next measured the effect of LY294002 pretreatment on the LPA-induced increase of exocytic trafficking of NHE3. As shown in Fig. 7B, the LPA-induced increase of exocytic trafficking was completely prevented by pretreatment with LY294002. This shows that PI 3-kinase plays a necessary role in the LPA-dependent increase of NHE3 exocytosis to the apical plasma membrane. In addition, LY294002 pretreatment slightly reduced the basal rate of exocytic trafficking of NHE3 in the absence of LPA treatment.

The LPA-induced increase of surface NHE3 amount is because of enhanced exocytic trafficking of NHE3 to the apical plasma membrane (Fig. 5, A and B). Therefore, we next measured the effect of LY294002 pretreatment on the LPA-induced increase of exocytic trafficking of NHE3. As shown in Fig. 7B, the LPA-induced increase of exocytic trafficking was completely prevented by pretreatment with LY294002. This shows that PI 3-kinase plays a necessary role in the LPA-dependent increase of NHE3 exocytosis to the apical plasma membrane. In addition, LY294002 pretreatment slightly reduced the basal rate of exocytic trafficking of NHE3 in the absence of LPA treatment.

The E3KARP Dependence of LPA-stimulated NHE3 Activity Is Not Because of E3KARP-dependent Activation of PI 3-Kinase/Akt—The mechanism of the E3KARP dependence of LPA stimulation of NHE3 is not explained. The hypothesis was tested that E3KARP increased the LPA stimulation of PI 3-kinase as the mechanism by which E3KARP led to LPA stimulation of NHE3. The assay used for PI 3-kinase activity was activation of Akt as assessed as amount of phosphorylated Akt. We examined the phosphorylation of Akt after treatment with LPA in both OK/E3V and OK/E3V/E3KARP cells by using phospho-specific antibodies for Akt, which specifically recognize activated Akt, and anti-Akt antibodies for total Akt (Sigma). As shown in Fig. 8 in both OK/E3V and OK/E3V/E3KARP cells, Akt was phosphorylated by 1 min of LPA exposure, with maximal phosphorylation at 10 min. The Akt phosphorylation was sustained up to 30 min. Also, the magnitude of stimulation of Akt phosphorylation relative to basal Akt and the level of basal Akt phosphorylation were similar in OK/E3V and OK/E3V/E3KARP cells.

The LPA-induced phosphorylation of Akt was totally prevented by pretreatment with LY294002, consistent with the inhibitory effect of LY294002 on NHE3 activity and the exocytic trafficking of NHE3. Thus, although PI 3-kinase activity is required for the LPA-induced stimulation of NHE3 activity and exocytic trafficking of NHE3, these results show that PI 3-kinase/Akt activation do not explain why E3KARP is necessary for LPA stimulation of NHE3.

LPA Activates NHE3 Activity by an ERK-independent Manner—ERK has been implicated in growth factor-induced regulation of NHE1 and in acid-induced NHE3 activation (36, 37). In OK cells, LPA-dependent activation of ERK, as well as activation of PI 3-kinase, has been reported (30). To test
whether ERK is involved in the LPA-induced NHE3 activation, we examined the effect of PD98059, a specific inhibitor of ERK, on the LPA-dependent activation of NHE3 in OK/E3V/E3KARP cells. As shown in Fig. 9, basal NHE3 activity and the LPA-stimulated NHE3 activity were not affected by pretreatment with PD98059. We observed that the LPA-induced ERK phosphorylation is not altered by LY294002 treatment (data not shown). These results show that the LPA-induced ERK phosphorylation is mediated by a mechanism that is not PI 3-kinase dependent. Also LPA activation of ERK is not involved in LPA stimulation of NHE3. Thus LPA activates multiple signaling pathways in OK cells, only some of which are involved in NHE3 and PI 3-kinase stimulation.

**DISCUSSION**

In these studies, LPA was shown to stimulate NHE3 activity and to increase the percent of total NHE3 on the plasma membrane by increasing exocytic trafficking. These three related events were all dependent on the presence of E3KARP. Unlike some other reported regulation of NHE3 that could be mediated by either E3KARP or NHERF (cAMP) (19), the LPA stimulation of NHE3 activity only occurred in the presence of E3KARP and not NHERF. This specificity in an effect of E3KARP has only been reported previously (20) for Ca2+/H2O4101-dependent inhibition of NHE3, serum/glucocorticoid-inducible kinase 1-dependent stimulation of NHE3 (38), and activation of phospholipase C-β3 (39). The Ca2+/H2O4101-dependent inhibition of NHE3 was because of specific interaction of E3KARP but not NHERF with the cytoskeletal protein α-actinin-4. In addition, E3KARP directly interacts with SGK1 and phospholipase C-β3 to account for the E3KARP specificity. In contrast, NHERF and E3KARP both can reconstitute cAMP inhibition of NHE3, because they both bind ezrin, which links protein kinase AII to the PDZ domain protein NHE3 complex. Thus it is predicted...
that the specificity of E3KARP will be shown to be because of specific binding of a protein involved in LPA signal transduction that is necessary for NHE3 stimulation, although the reason for the E3KARP dependence of the LPA-induced NHE3 activation has not been identified.

These studies show that E3KARP is necessary to allow LPA-stimulated exocytic trafficking (also called endocytic recycling) of NHE3. A role for PDZ proteins in trafficking of transport proteins has been identified only recently. One type of involvement is via plasma membrane retention (40). In neurons, NMDA receptor and potassium channel Kv1.4 binding to PSD-95 increases plasma membrane retention (less endocytosis) and leads to a longer half-life (41). In Madin-Darby canine kidney cells, the PDZ domain binding C-terminal amino acids of CFTR and podocalyxin are necessary for their BB localization, whereas those of the potassium channel Kir 2.3 and
The mechanism by which E3KARP increases LPA-induced exocytic trafficking of NHE3 is not known. One possible explanation is that E3KARP increases or alters steps in LPA-induced signaling that are needed to stimulate exocytosis of NHE3 to the apical plasma membrane. LPA acts via multiple plasma membrane receptors and in other cells has been shown to affect at least Gq/phospholipase C activation, Gi/Ras activation and Gq/adenylate cyclase inhibition, and G12/13/Rho activation (23–25). At least in some cells, LPA rapidly activates PI 3-kinase (including in OK cells) and tyrosine kinases, including Src. It also elevates $[\text{Ca}^{2+}]_{\text{c}}$, in OK cells with a maximum effect at 100 nM LPA, IC$_{50}$ 2.5 $\times$ 10$^{-6}$ M and transactivates epidermal growth factor receptor, ERK (PI 3-kinase independent), Rho, and a Cl$^{-}$ channel (26, 30, 48, 49). Which of these are linked to LPA stimulation of NHE3 is not yet known beyond the involvement of PI 3-kinase. However, we have demonstrated that not all signal transduction initiated by LPA in OK cells is involved in stimulation of NHE3. In this study, we demonstrated that whereas LPA increased ERK activity and phosphorylation, blocking LPA activation of ERK with PD98059 did not alter LPA stimulation of NHE3, showing that ERK activation is not involved in NHE3 stimulation. Although it is still unknown which LPA-induced signaling steps are affected by E3KARP, the previously reported specific interactions of E3KARP with phospholipase C-β3, α-actinin-4, and serum/glucocorticoid-inducible protein kinase (20, 38, 50) suggests that E3KARP may be involved in LPA-induced NHE3 activation through direct interaction with a signaling molecule that is regulated by LPA.

Another explanation for the E3KARP dependence of the LPA-induced NHE3 activation is that E3KARP may regulate NHE3 by affecting a step in exocytic trafficking. von Zastrow and co-workers (52, 53) have reviewed several examples of receptors and transport proteins in which PDZ proteins appear...
to act as molecular switches determining whether these proteins move from the recycling compartment to the plasma membrane versus moving to lysosomes for degradation. Therefore, it is possible that E3KARP may act as a molecular switch by affecting components of the exocytic machinery with involvement of products of PI 3-K activation. Despite nearly all the LPA stimulation of NHE3 being prevented by LY294002, the fact that PI 3-K activation as judged by Akt activity was not affected by the presence of E3KARP suggests that the major effect of E3KARP on LPA stimulation of NHE3 was on the exocytic machinery at steps that are independent of Akt activation. Understanding LPA stimulation of NHE3 will require defining which additional steps in signal transduction and/or the components in the exocytic trafficking events initiated by LPA are necessary for LPA-induced NHE3 exocytosis.

Acknowledgments—We are grateful to Dr. Ming Tse and Dr. Pann-Ghill Suh (Pohang University of Science and Technology) for helpful discussions. We acknowledge the secretarial assistance of H. McCann.

REFERENCES
1. Donowitz, M., and Tse, M. (2000) in Gastrointestinal Transport Molecular Physiology: Current Topics in Membranes (Barrett, K. E. and Donowitz, M., eds) Vol. 50, pp. 437–498, Academic Press, San Diego
2. Brett, C. L., Wei, Y., Donowitz, M., and Rao, R. (2002) Am. J. Physiol. 282, C1031-C1041
3. Numata, M., and Orlowski, J. (2001) J. Biol. Chem. 276, 17387–17394
4. Janecki, A. J., Janecki, M., Akhter, S., and Donowitz, M. (2000) J. Biol. Chem. 275, 8135–8142
5. Donowitz, M., Janecki, A., Akhter, S., Cavet, M. E., Sanchez, F., Lamprecht, G., Khurana, S., and Yun, C. H. C. (2000) Ann. N. Y. Acad. Sci. 915, 30–42
6. Lee-Kwon, W., Johns, D. C. A., Cha, B., Cavet, M., Park, J., Tischis, P., and Donowitz, M. (2001) J. Biol. Chem. 276, 31296–31304
7. Akhter, S., Kovbasnjuk, O., Li, X., Cavet, M., Noël, J., Arpin, M., Hubbard, A., and Donowitz, M. (2002) Am. J. Physiol. 283, C927–C940
8. Kurashima, K., Szabo, E. Z., Lukacs, G., Orlowski, J., and Grinstein, S. (1998) J. Biol. Chem. 273, 3577–3582
9. Akhter, S., Kovbasnjuk, O., Li, X., Cavet, M., Noël, J., Arpin, M., Hubbard, A., and Donowitz, M. (2001) J. Biol. Chem. 276, 29397–29404
10. Li, X., Galli, T., Lo, S., Wied, E., Feinman, E. J., Leung, G., Cheng, A., Louvard, D., and Donowitz, M. (2001) J. Biol. Chem. 276, 537–552
11. Laghmani, K., Preissig, R. A., Moe, O. W., Yanagisawa, A. M., and Alpern, R. J. (2001) J. Clin. Invest. 107, 1563–1569
12. Peng, Y., Amemiya, M., Yong, X., Fan, L., Moe, O. W., Yin, H., Preissig, P. A., Yanagisawa, M., and Alpern, R. J. (2001) J. Biol. Chem. 276, F34–F42
13. Czech, M. P., and Corvera, S. (1999) J. Biol. Chem. 274, 1865–1868
14. Czech, M. P. (2002) Mol. Cell 9, 695–698
15. Calera, M. R., Martinez, C., Liu, H., Jack, A. K., Birnbaum, M. J., and Pilch, P. F. (1996) J. Biol. Chem. 271, 7293–7299
16. Cong, L. N., Chen, H., Li, Y., Zhuo, L., McGibbons, M. A., Taylor, S. L., and Qiao, M. J. (1997) Mol. Cell. Biol. 11, 1881–1890
17. Foster, L. J., Li, D., Randhawa, V. K., and Klip, A. (2001) J. Biol. Chem. 276, 44212–44221
18. Khurana, S., Nath, S. K., Levine, S. A., Bowser, J. M., Tse, C. M., Cohen, M. E., and Donowitz, M. (1996) J. Biol. Chem. 271, 9919–9927
19. Yun, C. H. C., Oh, S., Zizzak, M., Steplock, D., Tao, S., Tse, C. M., Weinman, E. J., and Donowitz, M. (1997) Proc. Natl. Acad. Sci. U. S. A. 94, 3010–3015
20. Kim, J. H., Lee-Kwon, W., Park, J. B., Roy, S. H., Yun, C., and Donowitz, M. (2002) J. Biol. Chem. 277, 23714–23724
21. Yun, C. H., Lamprecht, C., Forster, D. V., and Sidor, A. (1998) J. Biol. Chem. 273, 25858–25863
22. Zizzak, M., Lamprecht, G., Steplock, D., Tariq, N., Shenolikar, S., Donowitz, M., Yun, C. H. C., and Weinman, E. J. (1999) J. Biol. Chem. 274, 24753–24758
23. Xu, X., Gibbs, T. C., and Meier, K. R. (2000) Biochem. Biophys. Acta 1502, 270–281
24. Lynch, K. R., and Macdonald, T. L. (2002) Biochem. Biophys. Acta 1582, 273–279
25. Sardar, V. M., Bautista, D. L., Fischer, D. J., Yokoyama, K., Nusser, N., Virag, T., Baker, D. L., Tariq, N., and Tse, C. M. (2002) J. Biol. Chem. 277, 1881–1890
26. Moolenaar, W. H., Krump, E., Howard, C. M., Rubie, E. A., Tibbles, L. A., Woodgett, J. D., and Grinstein, S. (2001) J. Membr. Biol. 181, 205–214
27. Bianchini, L., L’Allemain, G., and Pouyssegur, J. (1997) J. Biol. Chem. 272, 271–279
28. Yuan, C., Chen, Y., and Lang, F. (2002) J. Biol. Chem. 277, 7676–7683
29. Suh, P. G., Hwang, J. I., Ryu, S. H., Donowitz, M., and Kim, J. H. (2001) J. Membr. Biochem. Biophys. Res. Commun. 288, 1–7
Lysophosphatidic Acid Stimulates Brush Border Na\(^+\)/H\(^+\) Exchanger 3 (NHE3) Activity by Increasing Its Exocytosis by an NHE3 Kinase A Regulatory Protein-dependent Mechanism

Whaseon Lee-Kwon, Kazuya Kawano, Jung Woong Choi, Jae Ho Kim and Mark Donowitz

*J. Biol. Chem.* 2003, 278:16494-16501.
doi: 10.1074/jbc.M300580200 originally published online February 20, 2003

Access the most updated version of this article at doi: 10.1074/jbc.M300580200

Alerts:
- When this article is cited
- When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

This article cites 52 references, 25 of which can be accessed free at [http://www.jbc.org/content/278/19/16494.full.html#ref-list-1](http://www.jbc.org/content/278/19/16494.full.html#ref-list-1)