ERK mediates inhibition of Na\(^{+}/\)H\(^{+}\) exchange and HCO\(_{3}^{-}\) absorption by nerve growth factor in MTAL

BRUNS A. WATTS III AND DAVID W. GOOD

Departments of Medicine and Physiology and Biophysics, University of Texas Medical Branch, Galveston, Texas 77555

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Watts, Bruns A. III, and David W. Good. ERK mediates inhibition of Na\(^{+}/\)H\(^{+}\) exchange and HCO\(_{3}^{-}\) absorption by nerve growth factor in MTAL. Am J Physiol Renal Physiol 282: F1056–F1063, 2002; 10.1152/ajprenal.00133.2001.—Mitogen-activated protein (MAP) kinases mediate a variety of critical cellular events, but their role in the regulation of epithelial transport is largely undefined. Recently, we demonstrated that activation of the ERK signaling pathway in the rat medullary thick ascending limb (MTAL) through an unusual mechanism: 1) NGF inhibits basolateral membrane Na\(^{+}/\)H\(^{+}\) exchange activity, an effect opposite to the stimulation of Na\(^{+}/\)H\(^{+}\) exchange by growth factors in other cells; and 2) inhibition of basolateral Na\(^{+}/\)H\(^{+}\) exchange results secondarily in inhibition of apical Na\(^{+}/\)H\(^{+}\) exchange, thereby inhibiting HCO\(_{3}^{-}\) absorption. In this study, we examined the role of MAP kinases in mediating inhibition by NGF. In tissue strips from the inner stripe of the outer medulla and in microdissected MTALs, NGF increased extracellular signal-regulated kinase (ERK) activity twofold but had no effect on c-Jun NH\(_{2}\)-terminal kinase (JNK) or p38 MAP kinase activity. The selective MAP kinase inhibitor U0126 and PD-98059 abolished the NGF-induced ERK activation and largely eliminated (≥60%) the effects of NGF to inhibit basolateral Na\(^{+}/\)H\(^{+}\) exchange activity and transcellular HCO\(_{3}^{-}\) absorption in perfused MTALs. The MEK1/2 inhibitors did not affect inhibition of HCO\(_{3}^{-}\) absorption by bath ethylisopropyl amiloride, indicating that ERK activation is not involved in mediating interaction between the basolateral and apical Na\(^{+}/\)H\(^{+}\) exchangers. These results demonstrate that NGF inhibits basolateral Na\(^{+}/\)H\(^{+}\) exchange activity and HCO\(_{3}^{-}\) absorption in the MTAL through activation of the ERK signaling pathway. These findings identify a novel action of ERK to inhibit Na\(^{+}/\)H\(^{+}\) exchange activity and establish a role for MAP kinase pathways in the acute regulation of Na\(^{+}/\)H\(^{+}\) exchange activity and transcellular acid secretion in renal tubules.

mitogen-activated protein kinase; epithelial transport; kidney; extracellular signal-regulated kinase; medullary thick ascending limb

PLASMA MEMBRANE Na\(^{+}/\)H\(^{+}\) EXCHANGERS are involved in mediating a variety of cellular processes, including intracellular (pH\(_{i}\)) and cell volume regulation, epithelial Na\(^{+}\) absorption, and cell growth (19, 38, 46). At least six NHE isoforms (NHE1–6) have been identified in mammalian cells (12). These differ in their tissue distribution, inhibitor sensitivity, and responses to regulatory stimuli (12, 38, 46). A prominent feature of Na\(^{+}/\)H\(^{+}\) exchange is its stimulation by growth factors. This stimulation is rapid, occurs in a wide variety of cell types, and is observed with virtually all mitogens (12, 19, 38, 46). In addition, Na\(^{+}/\)H\(^{+}\) exchangers activated by growth factors include NHE1, the ubiquitously expressed exchanger isoform present in nonpolar cells and in the basolateral membrane of epithelia, and NHE2 and NHE3, isoforms that are localized selectively in the apical membrane of certain epithelial cells in the kidney and gastrointestinal tract (38, 46). The mechanisms involved in growth factor stimulation of Na\(^{+}/\)H\(^{+}\) exchange have been a subject of active investigation because of the close association of increased exchange activity with cell proliferation, transformation, hypertrophy, and adhesion (19, 21, 24, 30, 37, 38).

The medullary thick ascending limb (MTAL) of the mammalian kidney participates in acid-base regulation by reabsorbing a sizable fraction of the HCO\(_{3}^{-}\) filtered at the glomerulus (15). The proton secretion necessary for this HCO\(_{3}^{-}\) absorption is mediated by the apical membrane Na\(^{+}/\)H\(^{+}\) exchanger NHE3 (3, 8, 16, 25, 42). The MTAL also expresses Na\(^{+}/\)H\(^{+}\) exchange activity in the basolateral membrane (18, 40). Recently, we demonstrated that nerve growth factor (NGF) inhibits HCO\(_{3}^{-}\) absorption in the MTAL through a unique mechanism: 1) NGF primarily inhibits basolateral membrane Na\(^{+}/\)H\(^{+}\) exchange activity, an effect opposite to the virtually universal stimulation of Na\(^{+}/\)H\(^{+}\) exchange by growth factors in other cells; 2) inhibition of basolateral Na\(^{+}/\)H\(^{+}\) exchange activity results secondarily in inhibition of apical Na\(^{+}/\)H\(^{+}\) exchange activity, due to an undefined interaction between the exchangers; and 3) inhibition of apical Na\(^{+}/\)H\(^{+}\) exchange activity reduces H\(^{+}\) secretion and therefore HCO\(_{3}^{-}\) absorption (40). These studies provided the first evidence for inhibition of Na\(^{+}/\)H\(^{+}\) exchange by a growth factor and established a role for basolateral Na\(^{+}/\)H\(^{+}\) exchange in the regulation of renal tubule HCO\(_{3}^{-}\) absorption. The signaling mechanisms by which NGF inhibits Na\(^{+}/\)H\(^{+}\) exchange activity in the MTAL are unknown.

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Address for reprint requests and other correspondence: D. W. Good, 4.200 John Sealy Annex 0562, Univ. of Texas Medical Branch, 301 Univ. Blvd., Galveston, TX 77555-0562 (E-mail dgood@UTMB.edu).
Mitogen-activated protein (MAP) kinases are essential intermediates in signaling pathways activated by multiple stimuli, including growth factors, hormones, neurotransmitters, cytokines, and environmental stress (32, 43). MAP kinase subgroups in mammalian cells include extracellular signal-regulated kinases (ERKs), c-Jun NH2-terminal kinases (JNKs), and p38 MAP kinases. These kinases are regulated through distinct signaling cascades, leading to the activation of a MAP kinase kinase (MEK or MKK) that activates MAP kinase through direct phosphorylation on threonine and tyrosine (43). Recent work supports a role for MAP kinase pathways in the regulation of Na+/H+ exchange activity (6, 20, 24, 27, 36). In particular, ERK has been demonstrated to play a key role in mediating stimulation of NHE1 by growth factors in a number of nonepitheal systems (6, 12, 20, 24, 27, 36). At present, however, it is unknown whether MAP kinase pathways are relevant for the regulation of Na+/H+ exchange activity and its related functions in renal tubules or other epithelia.

The purpose of the present study was to investigate the role of MAP kinase pathways in the regulation of Na+/H+ exchange by NGF in the MTAL. We found that the novel inhibitory actions of NGF on basolateral Na+/H+ exchange activity and HCO3 absorption are mediated through activation of the ERK signaling pathway.

METHODS

Tubule perfusion and measurement of net HCO3 absorption. MTALs from male Sprague-Dawley rats (50–90 g; Taconic) were isolated and perfused in vitro at 37°C as previously described (16, 17). For HCO3 transport experiments, the tubules were perfused and bathed in control solution that contained (in mM) 146 Na+, 4 K+, 122 Cl−, 25 HCO3, 2.0 Ca2+, 1.5 Mg2+, 2.0 phosphate, 1.2 SO42−, 1.0 citrate, 2.0 lactate, and 5.5 glucose (equilibrated with 95% O2:5% CO2, pH 7.45 at 37°C). Experimental agents were added to the bath solution as described in RESULTS. For all experiments involving PD-98059 and U0126, an equal concentration of a MAP kinase pathway inhibitor was added to the control solution (39). The protocol for study of HCO3 absorption was as described previously (16, 17). The tubules were equilibrated for 20–30 min at 37°C in the initial perfusion and bath solutions, and the luminal flow rate (normalized per unit tubule length) was adjusted to 1.5–2.0 nl·min⁻¹·mm⁻¹. One to three 10-min tubule fluid samples were then collected for each period (initial, experimental, and recovery). The tubules were allowed to reequilibrate for 5–10 min after a change in the composition of the bath solution. The absolute rate of HCO3 absorption (pmol·min⁻¹·mm⁻¹) was calculated from the luminal flow rate and the difference between total CO2 concentrations measured in perfused and collected fluids (16, 17). An average HCO3 absorption rate was calculated for each period studied in a given tubule. When repeat measurements were made at the beginning and end of an experiment (initial and recovery periods), the values were averaged. Single tubule values are presented in the figures. Mean values ± SE (n = number of tubules) are reported in the text.

Measurement of intracellular pH and Na+/H+ exchange activity. pHi was measured in isolated and perfused MTALs with the pH-sensitive dye 2′,7′-bis(carboxyethyl)-5(6)-carboxyfluorescein as described previously (18, 40, 41). For pHi experiments, tubules were perfused and bathed in Na+-free HEPES-buffered solution that contained (in mM) 145 N-methyl-D-glucammonium, 4 K+, 147 Cl−, 2.0 Ca2+, 1.5 Mg2+, 1.0 phosphate, 1.0 SO42−, 1.0 citrate, 2.0 lactate, 5.5 glucose, and 5 HEPES (equilibrated with 100% O2, titrated to pH 7.4). Basolateral membrane Na+/H+ exchange activity was determined by measurement of the initial rate of pHi increase after addition of 145 mM Na+ to the bath solution (Na+ replaced 145 mM N-methyl-D-glucammonium), the intracellular buffering power, and cell volume, as previously described (40). In this approach, the pHi recovery induced by bath Na+ addition is interrupted at various points along the recovery curve, which unmasks background acid loading (40, 41). At the point of interruption, the Na+/H+ exchange rate is determined by correcting the net pHi recovery rate for the background acid loading rate (40, 41). NGF has no effect on background acid loading rate in the MTAL. The Na+-dependent pHi recovery rate was inhibited ≥90% by bath ethylisopropyl amiloride (EIPA; 50 μM) under all experimental conditions.

Tissue preparations and protein kinase assays. MAP kinase activities were studied by using two previously described preparations: 1) strips of tissue dissected from the inner stripe of the outer medulla (the region of the kidney highly enriched in MTALs), and 2) microdissected MTALs (4, 39). Use of the two preparations in combination permits comprehensive study of MAP kinase activities under multiple experimental conditions plus direct confirmation of key observations in dissected MTALs (4, 39). The tissue strips or tubules were incubated in vitro at 37°C in the control solution used for HCO3 transport experiments. The tissue was equilibrated for 15 min in control solution before the start of kinase experiments. The specific protocols used for incubations are given in RESULTS. After incubation, the tissue was lysed, and protein kinases were immunoprecipitated with polyclonal antibodies bound to protein A-agarose, as previously described (39). ERK1/2, JNK, and p38 MAP kinase activities were measured in immune complex kinase assays that used [γ-32P]ATP and myelin basic protein (ERK1/2), glutathione S-transferase-c-Jun (JNK), or glutathione S-transferase-activating transcription factor 2 (p38) as substrates (29, 39, 44). Phosphorylated substrates were isolated by SDS-PAGE, visualized by autoradiography, and quantified by densitometry. Equal amounts of protein kinases in immunoprecipitates were verified within experiments by immunoblotting with the same antibodies used for immunoprecipitation, as previously described (39). Anti-ERK1/2 antibody (anti-rat MAPK R2) was purchased from Upstate Biotechnology. Antibodies against JNK and p38 MAP kinase were kindly provided by R. J. Davis and have been described previously (29, 33, 39, 44).

Statistical analysis. Results are presented as means ± SE. Differences between means were evaluated by using Student’s t-test for paired or unpaired data or ANOVA with the Newman-Keuls multiple range test, as appropriate. P < 0.05 was considered statistically significant.

RESULTS

NGF increases ERK activity. Strips of tissue from the inner stripe of the outer medulla were incubated in vitro for 15 min in the absence (control) and presence of 0.7 nM NGF, and then MAP kinase activities were measured as described (39). NGF increased ERK activity 1.9-fold but had no effect on JNK or p38 MAP kinase activity (Fig. 1).
ERK-MEDIATED INHIBITION OF Na\textsuperscript{+}/H\textsuperscript{+} EXCHANGE

Fig. 1. Nerve growth factor (NGF) increases extracellular signal-regulated kinase (ERK) activity. A: inner stripe tissue was incubated in the absence (Cont) and presence of 0.7 nM NGF for 15 min at 37°C, and then mitogen-activated protein kinase activities were measured in immune complex assays that used myelin basic protein (MBP; ERK), glutathione S-transferase-c-Jun (JNK), and glutathione S-transferase-activating transcription factor 2 (p38) as substrates (39). Phosphorylated substrates were detected by autoradiography after SDS-PAGE. Autoradiograms are of representative experiments. B: substrate phosphorylation was quantified by densitometry, and kinase activities are presented as a percentage of control activity measured in the same experiment. Data are means ± SE for 4 experiments with ERK and 3 experiments each with JNK and p38. *P < 0.005 vs. control.

Although MTALs constitute the majority of the tissue mass of the inner stripe (4, 22), this region contains other nephron segments that may contribute to MAP kinase activity. To confirm that ERK activation occurs in the MTAL, ERK activity was examined in microdissected MTALs incubated in the absence and presence of NGF for 15 min. NGF increased ERK activity 2.0-fold (Fig. 2). These results establish that ERK is activated by NGF in the MTAL and that changes observed in the inner stripe reflect changes in the MTAL.

The time course of ERK activation was investigated by using inner stripe tissue. The tissue was incubated in the absence and presence of 0.7 nM NGF for different times, and ERK activity was measured as described (39). ERK activity increased within 5 min of exposure to NGF and remained elevated for at least 30–60 min (Fig. 3). ERK activity was increased 1.7 ± 0.1-fold at 5 min (n = 4, P < 0.05) and 1.8 ± 0.2-fold at 30 min (n = 6, P < 0.05) relative to control tissue not treated with NGF. These data demonstrate that NGF induces a rapid and sustained increase in ERK activity. The results are temporally consistent with a role for ERK in mediating inhibition of HCO\textsubscript{3}\textsuperscript{-} absorption, which occurs within 15 min of treatment with NGF and is sustained for at least 60 min (17).

**ERK activation is blocked by inhibitors of MEK.** To investigate the role of the ERK pathway in mediating NGF-induced inhibition of HCO\textsubscript{3}\textsuperscript{-} absorption, it was necessary to establish a method for blocking ERK activation. We therefore examined the effects of U0126 and PD-98059, two selective inhibitors of MEK1/2, which directly activates ERK (1, 13, 43). Pretreatment of inner stripe tissue for 30 min with either 15 μM U0126 or PD-98059 completely blocked the activation of ERK by NGF (Fig. 4). These data indicate that the activation of ERK by NGF is mediated through MEK1/2. Basal ERK activity was slightly lower (~20%) in the presence of the MEK1/2 inhibitors, but the difference did not achieve statistical significance.\(^1\)

**Inhibitors of ERK activation reduce inhibition of HCO\textsubscript{3}\textsuperscript{-} absorption by NGF.** To test whether activation of ERK plays a role in mediating inhibition of HCO\textsubscript{3}\textsuperscript{-} absorption by NGF, we examined the effects of U0126 and PD-98059 on NGF action. The MEK1/2 inhibitors were studied at the same concentration (15 μM) that blocked ERK activation (Fig. 4). In the absence of the inhibitors, NGF decreased HCO\textsubscript{3}\textsuperscript{-} absorption by 37%, from 12.5 ± 0.9 to 7.9 ± 0.7 pmol·min\textsuperscript{-1}·mm\textsuperscript{-1} (n = 5, P < 0.001; Fig. 5A). In MTAL bathed with U0126 or PD-98059, NGF decreased HCO\textsubscript{3}\textsuperscript{-} absorption by only 16%, from 11.2 ± 0.5 to 9.4 ± 0.5 pmol·min\textsuperscript{-1}·mm\textsuperscript{-1} (n = 9, P < 0.001; Fig. 5B). The net decrease in HCO\textsubscript{3}\textsuperscript{-} absorption induced by NGF was reduced 60% by the MEK1/2 inhibitors \(\Delta[HCO\textsubscript{3}\textsuperscript{-}] = 4.6 ± 0.4\) pmol·min\textsuperscript{-1}·mm\textsuperscript{-1} without inhibitors (Fig. 5A) vs.\(^2\)

\(^1\)In some experiments, ERK activity with inhibitor + NGF was less than activity measured with inhibitor alone (illustrated by the U0126 experiment in Fig. 4A). This may reflect NGF activation of a MAP kinase phosphatase (11), which results in a decrease in MAP kinase activity when the normally opposing stimulatory action of MEK is eliminated by pretreatment with the MEK inhibitors. The reduced ERK activity with inhibitor + NGF was not a consistent finding in other experiments and overall was not statistically significant (Fig. 4B).

\(^2\)
In separate, paired experiments, bath addition of U0126 or PD-98059 caused a small (−10%) decrease in HCO₃⁻ absorption that was of marginal significance and could not explain the difference in the response to NGF.

1.8 ± 0.2 pmol·min⁻¹·mm⁻¹ with inhibitors (Fig. 5B); P < 0.001). The basal rate of HCO₃⁻ absorption did not differ in the two series of experiments. Thus agents that prevent ERK activation markedly reduce the inhibition of HCO₃⁻ absorption by NGF. These results support an important role for the ERK pathway in mediating NGF-induced inhibition of HCO₃⁻ absorption. The HCO₃⁻ absorption rate returns to its control value within 15 min after removal of NGF from the bath solution.

Inhibitors of ERK activation reduce inhibition of basolateral Na⁺/H⁺ exchange activity by NGF. NGF inhibits HCO₃⁻ absorption primarily through inhibition of basolateral membrane Na⁺/H⁺ exchange (40). To determine whether ERK is involved in the inhibition of basolateral Na⁺/H⁺ exchange activity, we examined the effect of NGF in the absence and presence of U0126. Basolateral Na⁺/H⁺ exchange activity was determined by measurement of the initial rate of pHₐ increase in response to the addition of bath Na⁺, as described previously (40). Under basal conditions, NGF decreased basolateral Na⁺/H⁺ exchange activity at all pHₐ values studied (control vs. NGF; Fig. 6A). In contrast, in MTAL bathed with 15 µM U0126, the effect of NGF to decrease Na⁺/H⁺ exchange activity was largely eliminated (Fig. 6A). Overall, NGF decreased basolateral Na⁺/H⁺ exchange activity by 67% in the absence and 26% in the presence of U0126 (P < 0.05; Fig. 6B). U0126 alone did not affect basolateral Na⁺/H⁺ exchange activity (Fig. 6B). These results indicate that NGF inhibits basolateral Na⁺/H⁺ exchange activity through an ERK-dependent pathway.

Inhibitors of ERK activation do not reduce inhibition of HCO₃⁻ absorption by bath EIPA. NGF inhibits HCO₃⁻ absorption by inhibiting basolateral Na⁺/H⁺ exchange activity, which results secondarily in inhibition of apical Na⁺/H⁺ exchange activity due to an undefined interaction between the exchangers (40). The preceding experiments show that ERK plays a major role in mediating the primary inhibition of basolateral Na⁺/H⁺ exchange. Further experiments were performed to determine whether ERK also is involved in mediating interaction between the basolateral and apical Na⁺/H⁺ exchangers. To test this possibility, we took advantage of our previous finding that the interaction between the exchangers, and the resulting inhibition of HCO₃⁻ absorption, can be induced directly by inhibiting basolateral Na⁺/H⁺ exchange activity with EIPA (18, 40). In control solution, addition of 50 µM EIPA to the bath decreased HCO₃⁻ absorption by 54%, from 11.7 ± 0.9 to 5.4 ± 0.3 pmol·min⁻¹·mm⁻¹ (n = 4, P < 0.025). In tubules bathed with 15 µM U0126, addition of EIPA to the bath decreased HCO₃⁻ absorption by 65%, from 10.0 ± 0.4 to 3.6 ± 0.7 pmol·min⁻¹·mm⁻¹ (n = 4, P < 0.005). These results suggest

Fig. 5. Effect of NGF (0.7 nM added to the bath) on HCO₃⁻ absorption in the absence (A) and presence (B) of U0126 and PD-98059. B: 15 µM U0126 (●) or PD-98059 (○) was present in the bath throughout the experiments. Data points are average values for single tubules. Lines connect paired measurements made in the same tubule. Mean values are given in RESULTS. J_HCO3, absolute rate of HCO₃⁻ absorption. P values are for paired Student’s t-test.
Fig. 6. Effect of NGF on basolateral membrane Na+/H+ exchange activity (JNa/H+) in the absence and presence of U0126. A: MTAL were studied under control conditions and with 0.7 nM NGF or 0.7 nM NGF + 15 μM U0126 in the bath solution. JNa/H+ was determined at various intracellular pH values as the product of the initial rate of Na+/H+ exchange (NHE1) activity early in activation of the small guanine nucleotide-binding protein Ras, followed by the sequential phosphorylation and activation of Raf, MEK, and ERK (31). We believe that a similar cascade likely mediates the NGF-induced activation of ERK in our experiments; however, the identity of the NGF receptor and the precise upstream signaling components leading to MEK/ERK activation in the MTAL remain to be determined. Whether NGF acts on other nephron segments is unknown.

Stimulation of Na+/H+ exchange by growth factors has been a virtually universal finding (12, 19, 38, 46). Increases in Na+/H+ exchange activity (NHE1) activity early in the mitogenic response may increase pH2 and induce cell signals that are obligatory or permissive for proliferation, differentiation, or hypertrophy (19, 24, 37, 38). In contrast, we found that NGF uniquely inhibits basolateral Na+/H+ exchange activity in the MTAL, which results in inhibition of transepithelial HCO3 absorption (40). The present study demonstrates that activation of ERK is a critical component of the signaling pathway through which NGF inhibits Na+/H+ exchange activity. This conclusion is supported by several observations: 1) both the inhibition of basolateral Na+/H+ exchange activity and the resulting inhibition of HCO3 absorption are largely reduced by U0126 and PD-98059, two chemically unrelated MEK inhibitors with different mechanisms of action (1, 13); 2) NGF increased ERK activity in the MTAL under conditions that the interaction between basolateral and apical Na+/H+ exchangers does not involve ERK activation. Taken together, our findings support the conclusion that the MEKI/2 inhibitors decrease NGF-induced inhibition of HCO3 absorption by preventing ERK-dependent inhibition of basolateral Na+/H+ exchange activity.

**DISCUSSION**

MAP kinase pathways mediate a variety of critical cellular events, including growth, differentiation, survival and repair, inflammation, and responses to toxins and physical stress (14, 32, 43). In contrast, the role of MAP kinase pathways in the regulation of epithelial transport is largely undefined. Recently, we demonstrated that NGF inhibits transepithelial HCO3 absorption in the MTAL through a unique action to inhibit basolateral membrane Na+/H+ exchange (40). In the present study, we demonstrate that NGF inhibits basolateral Na+/H+ exchange activity and HCO3 absorption through activation of the ERK signaling pathway. These results identify a novel inhibitory action of ERK on Na+/H+ exchange activity and establish a role for MAP kinase pathways in the acute regulation of Na+/H+ exchange activity and transepithelial acid secretion in renal tubules.

NGF regulates the ERK, JNK, and p38 MAP kinase pathways in nerve cells, and the integrated action of these signaling pathways plays a crucial role in mediating NGF-induced control of neuronal cell proliferation, differentiation, and survival (26, 31, 44, 45). In the MTAL, we found that exposure to NGF caused selective activation of ERK, with no effect on JNK or p38 MAP kinase. The activation of ERK is rapid and sustained, consistent with a direct and continuous role in mediating NGF-induced regulation of HCO3 absorption (see below). The sustained activation of ERK by NGF is in contrast to the response of the MTAL to hyperosmotic stress; hyperosmolality causes transient ERK activation, and ERK is not involved in mediating hyperosmotic inhibition of HCO3 absorption (39). In the pheochromocytoma cell line PC-12, prolonged activation of ERK by NGF is required to induce neuronal differentiation, whereas agents that cause transient ERK activation, such as EGF, do not cause differentiation, indicating that the duration of ERK activation may be a critical factor in determining cellular responses (26). It is possible, therefore, that the regulation of Na+/H+ exchange activity and HCO3 absorption by NGF may depend on prolonged ERK activation.

Recently, we demonstrated that NGF inhibits basolateral Na+/H+ exchange activity (40). In the present study, we found that exposure to 0.7 nM NGF increased ERK activity in the MTAL under conditions shown in A, plus additional data from 4 tubules studied with U0126 alone. #P < 0.05 vs. control or U0126; *P < 0.05 vs. NGF (ANOVA).
similar to those used in HCO$_3$ transport experiments; 3) U0126 and PD-98059 completely blocked NGF-induced ERK activation; and 4) both the rapidity (≤5 min) and the duration (at least 60 min) of ERK activation correlate temporally with NGF-induced inhibition of HCO$_3$ absorption. At the concentrations used, U0126 and PD-98059 are selective inhibitors of ERK activation, with no significant effects against a wide range of other kinases (1, 13). In addition, U0126 and PD-98059 do not affect inhibition of HCO$_3$ absorption by hyperosmolality, vasopressin, or angiotensin II (Ref. 39; Good DW, unpublished observations), indicating that their action is not due to nonspecific cytotoxic or metabolic effects or to effects on the apical Na$^+$/H$^+$ exchanger (NHE3) that prevent its physiological regulation. Taken together, these data indicate that the novel action of NGF to inhibit basolateral Na$^+$/H$^+$ exchange activity in the MTAL is mediated through activation of the ERK signaling pathway. These studies also provide the first evidence for a role for ERK in the regulation of Na$^+$/H$^+$ exchange activity and transcellular acid transport in epithelial cells.

In contrast to its role in mediating inhibition of basolateral Na$^+$/H$^+$ exchange in the MTAL, ERK has been demonstrated to play an important role in activation of Na$^+$/H$^+$ exchange (NHE1) by growth factors in other cells (6, 20, 24, 27, 36). Several mechanisms have been identified that may contribute to ERK-dependent stimulation of NHE1 in these systems: 1) direct phosphorylation of NHE1 by ERK (24, 27); 2) direct phosphorylation of NHE1 by a downstream target of ERK [recent studies have shown that phosphorylation of NHE1 on serine-703 by p90RSK, a direct substrate of ERK, is important for growth factor-induced exchanger activation (36)]; and 3) ERK-dependent phosphorylation of accessory proteins that interact with and regulate NHE1 (6). The relative roles of these mechanisms in mediating ERK-dependent activation of NHE1 have not been defined; however, they provide an important framework for future analysis of how ERK uniquely inhibits Na$^+$/H$^+$ exchange activity in the MTAL. For example, it is possible that ERK activation leads to inhibition of Na$^+$/H$^+$ exchange in the MTAL due to activation of downstream targets or interaction with accessory proteins that differ from those in other cell types. Immunolocalization of protein expression (5, 7), localization of mRNA expression (9, 23), and functional studies of inhibitor kinetics (18) indicate that basolateral Na$^+$/H$^+$ exchange activity in the MTAL is mediated by NHE1. However, our studies do not rule out the possibility that NGF may act via ERK to inhibit an as yet unidentified exchanger that functions in parallel with and has an amiloride sensitivity similar to NHE1 in the basolateral membrane.

Although ERK plays a predominant role in mediating the inhibition of basolateral Na$^+$/H$^+$ exchange by NGF, significant inhibition of Na$^+$/H$^+$ exchange activity and HCO$_3$ absorption persists when ERK activation is abolished (Figs. 4–6). This indicates that an additional signaling pathway(s) induced by NGF functions in parallel with ERK to mediate inhibition of basolateral Na$^+$/H$^+$ exchange activity. We have previously shown that the inhibition of HCO$_3$ absorption by NGF does not involve cAMP or protein kinase C (17). Other signaling components that may influence Na$^+$/H$^+$ exchange activity and that are activated by NGF receptor binding in other systems include c-src, phosphatidylinositol 3-kinase, and Ca$^{2+}$/calmodulin (10, 31). The role of these pathways in mediating the ERK-independent inhibition of Na$^+$/H$^+$ exchange by NGF is presently under investigation.

NGF inhibits transepithelial HCO$_3$ absorption in the MTAL through an unusual mechanism whereby primary inhibition of basolateral Na$^+$/H$^+$ exchange activity results secondarily in inhibition of apical Na$^+$/H$^+$ exchange activity (40). The mechanism of interaction between the exchangers has not been defined. However, it cannot be explained by a change in the net driving force for the apical Na$^+$/H$^+$ exchanger and thus appears to be mediated via a signal transduction mechanism (18). The interaction between basolateral and apical Na$^+$/H$^+$ exchangers and the resulting inhibition of HCO$_3$ absorption can be induced directly in the absence of NGF by inhibiting the basolateral exchanger with EIPA (18, 40). We found that inhibitors of ERK activation did not reduce inhibition of HCO$_3$ absorption by bath EIPA, indicating that the ERK pathway is unlikely to be involved in mediating interaction between the exchangers. Thus ERK is essential for inhibition of HCO$_3$ absorption by NGF because it mediates NGF-induced inhibition of basolateral Na$^+$/H$^+$ exchange activity. NGF has no direct effect on apical Na$^+$/H$^+$ exchange activity (40), indicating that under the conditions of our experiments the ERK pathway is not coupled directly to regulation of NHE3. NGF does inhibit apical Na$^+$/H$^+$ exchange activity through ERK activation; however, this inhibition occurs secondarily to inhibition of basolateral Na$^+$/H$^+$ exchange activity (40).

Although NGF and its receptors are expressed in the kidney, their role in the regulation of kidney function remains to be determined (17, 40). The magnitude of the change in HCO$_3$ absorption induced by NGF in the MTAL is comparable to that observed with other regulatory factors such as angiotensin II, chronic metabolic acidosis and alkalosis, and vasopressin, consistent with a significant role for NGF in acid-base regulation (15, 17, 40). However, information is lacking on several important issues, including sites of NGF expression in the renal medulla, factors that influence NGF levels in the kidney, and the identity of the MTAL NGF receptor(s) (17, 40). In many systems, activation of the Ras/ERK pathway is a major signaling event triggered by membrane receptors to induce cell proliferation, hypertrophy, or differentiation (14, 26, 31, 32, 43). Our studies with NGF provide new evidence that the ERK pathway is involved in the regulation of Na$^+$/H$^+$ exchange activity and transepithelial acid secretion in renal tubules. In the kidney, there is a close association between altered ion transport and cell growth in a variety of pathophysiological conditions,
including reduction in renal mass, compensatory hypertrophy, diabetic nephropathy, potassium deficiency, and cyst formation (2, 28, 34, 35). Our finding that ERK participates directly in the regulation of ion transport in the MTAL suggests that this signaling pathway could play a general role in mediating the integrated control of growth and ion transport in renal epithelial cells.

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