Nerve growth factor regulates \( \text{HCO}_3^- \) absorption in thick ascending limb: modifying effects of vasopressin

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Good, David W. Nerve growth factor regulates \( \text{HCO}_3^- \) absorption in thick ascending limb: modifying effects of vasopressin. Am. J. Physiol. 274 (Cell Physiol. 43): C931–C939, 1998.—Growth factors stimulate \( \text{Na}^+/\text{H}^+ \) exchange activity in many cell types but their effects on acid secretion via this mechanism in renal tubules are poorly understood. We examined the regulation of \( \text{HCO}_3^- \) absorption by nerve growth factor (NGF) in the rat medullary thick ascending limb (MTAL), which absorbs \( \text{HCO}_3^- \) via apical membrane \( \text{Na}^+/\text{H}^+ \) exchange. MTAL were perfused in vitro with 25 mM \( \text{HCO}_3^- \) solutions (pH 7.4; 290 mosmol/kg H\(_2\)O). Addition of 0.7 nM NGF to the bath decreased \( \text{HCO}_3^- \) absorption from 13.1 ± 1.1 to 9.6 ± 0.8 pmol·min\(^{-1} \)·mm\(^{-1} \) (\( P < 0.001 \)). In contrast, with 10\(^{-9}\) M arginine vasopressin (AVP) in the bath, addition of NGF to the bath increased \( \text{HCO}_3^- \) absorption from 8.0 ± 1.6 to 12.5 ± 1.3 pmol·min\(^{-1} \)·mm\(^{-1} \) (\( P < 0.001 \)). Both effects of NGF were blocked by genistein, consistent with the involvement of tyrosine kinase pathways. However, the AVP-dependent stimulation required activation of protein kinase C (PKC), whereas the inhibition was PKC independent, indicating that the NGF-induced signaling pathways leading to inhibition and stimulation of \( \text{HCO}_3^- \) absorption are distinct. Hypertonicity blocked the inhibition but not the AVP-dependent stimulation, suggesting that hypertonicity and NGF may inhibit \( \text{HCO}_3^- \) absorption via a common mechanism. These data demonstrate that NGF inhibits \( \text{HCO}_3^- \) absorption in the MTAL under basal conditions but stimulates \( \text{HCO}_3^- \) absorption in the presence of AVP, effects that are mediated through distinct signal transduction pathways. They also show that AVP is a critical determinant of the response of the MTAL to growth factor stimulation and suggest that NGF can either inhibit or stimulate apical \( \text{Na}^+/\text{H}^+ \) exchange activity depending on its interactions with other regulatory factors. Locally produced growth factors such as NGF may play a role in regulating renal tubule \( \text{HCO}_3^- \) absorption.

GROWTH FACTORS INFLUENCE A variety of renal processes, including cellular proliferation, development and differentiation, hypertrophy, matrix production, and repair following injury (29, 30, 44, 50). Growth factors that are produced by the kidney include insulin-like growth factor, platelet-derived growth factor, nerve growth factor (NGF), hepatocyte growth factor, transforming growth factor-\( \beta \), and epidermal growth factor (30). Much recent work has focused on the involvement of these factors in the pathophysiological control of glomerular function (1, 30, 50) and in recovery from acute renal failure (8, 29, 30, 36). In contrast, with the exception of epidermal growth factor (11, 28, 48), little is known about the effects of locally produced growth factors on the function of renal tubules, particularly with respect to the regulation of ion transport and signal transduction pathways.

The medullary thick ascending limb (MTAL) of the rat participates in the renal regulation of acid-base balance by reabsorbing a sizable fraction of the \( \text{HCO}_3^- \) filtered at the glomerulus (21). The \( \text{H}^+ \) secretion required for this \( \text{HCO}_3^- \) reabsorption is mediated virtually completely by apical membrane \( \text{Na}^+/\text{H}^+ \) exchange (26). Furthermore, the regulation of \( \text{HCO}_3^- \) absorption is mediated through regulation of this apical exchanger (19, 21, 26, 52, 55). Because of their potent stimulation of \( \text{Na}^+/\text{H}^+ \) exchange activity in other systems, we investigated whether growth factors might stimulate \( \text{HCO}_3^- \) absorption in the MTAL. Our results demonstrate that NGF regulates \( \text{HCO}_3^- \) absorption in the MTAL in a complex manner: it inhibits \( \text{HCO}_3^- \) absorption under basal conditions but stimulates \( \text{HCO}_3^- \) absorption in the presence of arginine vasopressin (AVP). In addition, we show that these regulatory effects are mediated through signal transduction pathways. These findings indicate that AVP is a critical determinant of the response of MTAL cells to NGF stimulation and suggest that locally produced growth factors such as NGF may play a role in the regulation of renal tubule acid excretion.

METHODS

The methods for in vitro microperfusion of MTAL from Sprague-Dawley rats (50–100 g body wt; Taconic, German-
Effects of NGF on HCO₃⁻ Absorption

NGF inhibits HCO₃⁻ absorption. Based on the virtually universal finding that growth factors stimulate Na⁺/H⁺ exchange (27, 54), it was anticipated that NGF would stimulate HCO₃⁻ absorption. Instead, addition of 0.7 nM NGF to the bath decreased HCO₃⁻ absorption from 13.1 ± 1.1 to 9.6 ± 0.8 pmol·min⁻¹·mm⁻¹ (n = 6; P < 0.001; Fig. 1). The inhibition by NGF was reversible, was observed within 15 min after addition of NGF to the bath, and was stable for up to 2 h.

Hypertonicity prevents inhibition by NGF. In other systems, stimulation of Na⁺/H⁺ exchange by hypertonicity precludes subsequent stimulation by growth factors (16). In the MTAL, hypertonicity inhibits HCO₃⁻ absorption (20, 22). Therefore, we tested for possible interactions between hypertonicity and NGF. Hypertonicity was produced by adding 75 mM NaCl to the bath, and was stable for up to 2 h. Hypertonicity prevented inhibition by NGF (Fig. 2).

Fig. 1. Effect of nerve growth factor (NGF; 0.7 nM added to bath) on HCO₃⁻ absorption in MTAL (n = 6). Data points are average values from single tubules. Lines connect paired measurements made in the same tubule. P value is for paired t-test. Mean values are in text. Cont, control.

Fig. 2. Effect of NGF (0.7 nM added to bath) on HCO₃⁻ absorption in hypertonic solutions. Hypertonicity (Hyper) was produced by adding 75 mM NaCl to perfusate and bath. Data points are average values for single tubules. Lines connect paired measurements made in the same tubule. P value is for paired t-test. Mean values are in text. NS, not significant.
NGF stimulates HCO$_3^-$ absorption in the presence of AVP. AVP inhibits HCO$_3^-$ absorption in the MTAL, and the inhibitory effects of AVP and hypertonicity are additive (19, 20). We therefore tested whether inhibition by NGF also is additive to inhibition by AVP. Surprisingly, in MTAL bathed with 10$^{-10}$ M AVP, addition of 0.7 nM NGF to the bath increased HCO$_3^-$ absorption from 8.0 ± 1.6 to 12.5 ± 1.3 pmol·min$^{-1}$·mm$^{-1}$ (n = 4; P < 0.01; Fig. 3A). NGF also increased HCO$_3^-$ absorption in the presence of AVP in hypertonic solutions (2.9 ± 0.2 pmol·min$^{-1}$·mm$^{-1}$ for AVP + hypertonic vs. 5.7 ± 0.5 pmol·min$^{-1}$·mm$^{-1}$ for AVP + hypertonic + NGF; n = 3; P < 0.05; Fig. 3B). Thus NGF inhibits HCO$_3^-$ absorption under basal conditions but stimulates HCO$_3^-$ absorption in the presence of AVP. These results show that AVP is a critical determinant of the response of MTAL cells to NGF stimulation and suggest that NGF may regulate HCO$_3^-$ absorption through at least two distinct signal transduction pathways.

Effects of NGF-β. To define further the regulatory action of NGF, we examined the effects of NGF-β (or 2.55 NGF), the biologically active subunit of the 7S NGF complex (53). In MTAL perfused and bathed in control solution, addition of 0.7 nM NGF-β to the bath decreased HCO$_3^-$ absorption from 11.4 ± 0.6 to 7.2 ± 0.8 pmol·min$^{-1}$·mm$^{-1}$ (n = 3; P < 0.05). In tubules bathed with 10$^{-10}$ M AVP, addition of 0.7 nM NGF-β to the bath increased HCO$_3^-$ absorption from 6.5 ± 0.7 to 8.3 ± 0.7 pmol·min$^{-1}$·mm$^{-1}$ (n = 3; P < 0.001). Thus results with NGF-β were similar to those obtained with 7S NGF.$^1$

Signaling Pathways Involved in Regulation by NGF

Previously, we demonstrated that cAMP, protein kinase C (PKC), and protein-tyrosine kinase pathways play key roles in the regulation of MTAL HCO$_3^-$ absorption (19, 22, 23). We therefore examined the importance of these signaling pathways for regulation by NGF.

Inhibition by NGF does not involve cAMP or PKC. cAMP inhibits HCO$_3^-$ absorption in the MTAL (19). We therefore tested whether this pathway is involved in inhibition by NGF. MTAL were bathed with forskolin or 8-Br-cAMP, agents that induce maximal cAMP-dependent inhibition of HCO$_3^-$ absorption (19, 24). The results in Fig. 4 show that, in the presence of 10$^{-6}$ M forskolin or 10$^{-4}$ M 8-Br-cAMP, addition of 0.7 nM NGF to the bath decreased HCO$_3^-$ absorption from 7.2 ± 1.1 to 3.8 ± 1.0 pmol·min$^{-1}$·mm$^{-1}$ (n = 4; P < 0.001). Thus the inhibition by NGF is not mediated by an increase in cell cAMP.

Many of the cellular actions of growth factors are mediated through activation of PKC (41, 57). The role of PKC in the inhibition of HCO$_3^-$ absorption by NGF was investigated using staurosporine and chelerythrine chloride, inhibitors of PKC that selectively abolish PKC-dependent regulation of HCO$_3^-$ absorption in the MTAL (22, 23). In tubules bathed with 10$^{-7}$ M staurosporine or 10$^{-8}$ M chelerythrine, addition of 0.7 nM NGF to the bath decreased HCO$_3^-$ absorption from 9.1 ± 0.4 to 5.4 ± 0.5 pmol·min$^{-1}$·mm$^{-1}$ (n = 4; P < 0.005; Fig. 5A). In MTAL studied with 0.7 nM NGF in the bathing solution, addition of staurosporine or chelerythrine to the bath had no effect on HCO$_3^-$ absorption (7.2 ± 1.2 pmol·min$^{-1}$·mm$^{-1}$ for NGF vs. 7.2 ± 1.1 pmol·min$^{-1}$·mm$^{-1}$ for NGF + inhibitor; n = 4; NS; Fig. 5B). Thus the inhibition of HCO$_3^-$ absorption by NGF does not involve PKC.

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$^1$The effect of NGF-β to inhibit HCO$_3^-$ absorption was not fully reversible. The explanation for this difference from 7S NGF was not investigated but may relate to differences in the extent of proteolysis of the β-subunit when it is isolated from the 7S complex (53).
Stimulation by NGF is mediated by PKC. In the MTAL, prostaglandin E2 (PGE2) stimulates HCO3 absorption in the presence of AVP through activation of PKC (23). We therefore tested the hypothesis that PKC mediates the AVP-dependent stimulation by NGF. In MTAL bathed with AVP plus 10^{-7} M staurosporine or 10^{-8} M chelerythrine, addition of 0.7 nM NGF to the bath had no effect on HCO3 absorption (5.7 ± 0.5 pmol·min^{-1}·mm^{-1} for AVP + inhibitor vs. 5.8 ± 0.6 pmol·min^{-1}·mm^{-1} for AVP + inhibitor + NGF; n = 4; NS; Fig. 6A). Thus pretreatment with inhibitors of PKC abolished the stimulation of HCO3 absorption by NGF.

In tubules studied with AVP and NGF in the bath solution, addition of staurosporine or chelerythrine to the bath decreased HCO3 absorption from 7.7 ± 0.5 to 4.4 ± 0.4 pmol·min^{-1}·mm^{-1} (n = 4; P < 0.01; Fig. 6B). In contrast, addition of these agents had no effect on HCO3 absorption in the presence of NGF (Fig. 5B) or AVP (23) alone. Thus the PKC inhibitors targeted specifically the interaction between NGF and AVP, reversing the effect of NGF to stimulate HCO3 absorption when AVP is present. Together, these results establish that PKC mediates the AVP-dependent stimulation of HCO3 absorption by NGF.

Both NGF and PGE2 stimulate HCO3 absorption in the presence of AVP through activation of PKC. We therefore examined the interaction between NGF and PGE2. In MTAL bathed with AVP plus 10^{-6} M PGE2, addition of 0.7 nM NGF to the bath had no effect on HCO3 absorption (13.1 ± 1.3 pmol·min^{-1}·mm^{-1} for AVP + PGE2 vs. 13.0 ± 1.2 pmol·min^{-1}·mm^{-1} for AVP + PGE2 + NGF; n = 3; NS). Thus the stimulatory effects of NGF and PGE2 were not additive, suggesting that these factors may act via a common mechanism (see DISCUSSION).

Inhibition and stimulation by NGF are blocked by genistein. Tyrosine kinases are important intermediates in the signal transduction pathways activated by growth factors (17, 33) and play a key role in mediating inhibition of HCO3 absorption by hypertonicity in the MTAL (22). We therefore examined whether tyrosine kinase pathways are involved in the regulation of HCO3 absorption by NGF. MTAL were bathed with genistein, which selectively blocks tyrosine kinase-dependent regulation of HCO3 absorption in the MTAL (22). Under basal conditions, the inhibition of HCO3 absorption by NGF was eliminated nearly completely by 7 µM genistein (15.9 ± 0.4 pmol·min^{-1}·mm^{-1} for genistein vs. 14.6 ± 0.6 pmol·min^{-1}·mm^{-1} for genistein + NGF; n = 4; P < 0.05) and was abolished by 70 µM genistein (11.6 ± 0.8 pmol·min^{-1}·mm^{-1} for genistein + NGF vs. 11.5 ± 0.9 pmol·min^{-1}·mm^{-1} for genistein + NGF).
NGF; n = 4; NS; Fig. 7A). The stimulation of HCO$_3^-$ absorption by NGF in the presence of AVP was abolished by either 7 or 70 µM genistein (6.5 ± 1.4 pmol·min$^{-1}$·mm$^{-1}$ for AVP + genistein vs. 6.4 ± 1.3 pmol·min$^{-1}$·mm$^{-1}$ for AVP + genistein + NGF; n = 4; NS; Fig. 7B). Thus tyrosine kinase pathways appear to be involved in both the inhibition and the AVP-dependent stimulation of HCO$_3^-$ absorption by NGF.

DISCUSSION

Regulation of HCO$_3^-$ absorption in the MTAL by a variety of physiological factors, including vasopressin, changes in osmolality, and chronic metabolic acidosis, is achieved through regulation of apical membrane Na$^+$/H$^+$ exchange activity (19, 26, 52, 55). Because growth factors are potent activators of Na$^+$/H$^+$ exchange in many other systems, we tested the hypothesis that they would stimulate HCO$_3^-$ absorption in the isolated, perfused MTAL. Our results show that NGF regulates HCO$_3^-$ absorption in the MTAL but that its effects are complex: NGF inhibits HCO$_3^-$ absorption under basal conditions but stimulates HCO$_3^-$ absorption in the presence of AVP. Furthermore, hypertonicity blocks the inhibition by NGF but has no effect on the AVP-dependent stimulation. We also found that activation of PKC plays a key role in the AVP-dependent stimulation of HCO$_3^-$ absorption but is not involved in HCO$_3^-$ transport inhibition, indicating that the NGF-induced signaling pathways leading to inhibition and stimulation of HCO$_3^-$ absorption are distinct. Together, these results identify a pattern of growth factor regulation of acid-base transport unlike that described in

Fig. 6. Effects of staurosporine (10$^{-7}$ M) and chelerythrine chloride (10$^{-8}$ M) on regulation of HCO$_3^-$ absorption by NGF in presence of AVP. A: MTAL were bathed with AVP (10$^{-10}$ M) + inhibitor, and then NGF (0.7 nM) was added to bath solution. PKC inhibitors blocked stimulation of HCO$_3^-$ absorption by NGF. B: MTAL were bathed with AVP (10$^{-10}$ M) + NGF (0.7 nM), and then inhibitor was added to bath solution. Inhibitors reversed NGF stimulation of HCO$_3^-$ absorption. Data points are average values for single tubules. Lines connect paired measurements made in same tubule. P values are for paired t-test. Mean values are in text.

Fig. 7. Effects of genistein on regulation of HCO$_3^-$ absorption by NGF. Tubules were studied in absence (A) and presence (B) of AVP (10$^{-10}$ M in bath). Genistein (Gen) was present in bath solution at 7 µM (●) or 70 µM (○) throughout experiments. Genistein blocked effects of NGF to inhibit HCO$_3^-$ absorption under basal conditions and to stimulate HCO$_3^-$ absorption in presence of AVP. Data points are average values for single tubules. Lines connect paired measurements made in same tubule. P values are for paired t-test. Mean values are in text.
other cell types and establish an important role for AVP in determining the response of MTAL cells to growth factor stimulation. As discussed below, our findings suggest that NGF can either inhibit or stimulate apical membrane Na\(^+\)/H\(^+\) exchange activity depending on its interactions with other regulatory factors.

Effects of NGF on HCO\(_3^-\) Absorption

Inhibition of basal HCO\(_3^-\) absorption. Under a wide variety of experimental conditions, apical membrane Na\(^+\)/H\(^+\) exchange mediates virtually all of the H\(^+\) secretion necessary for HCO\(_3^-\) absorption in the MTAL (26). Hence, the rate of HCO\(_3^-\) absorption serves as a measure of apical Na\(^+\)/H\(^+\) exchange activity under steady-state transporting conditions. In the present study, we found that NGF inhibits HCO\(_3^-\) absorption, which suggests strongly that NGF inhibits apical membrane Na\(^+\)/H\(^+\) exchange. Mitogens have been shown to stimulate Na\(^+\)/H\(^+\) exchange activity in many systems; however, to our knowledge, inhibition of Na\(^+\)/H\(^+\) exchange by growth factors has not been reported. The predominant Na\(^+\)/H\(^+\) exchanger isoform in the apical membrane of the MTAL is NHE3, which is stimulated by growth factors when expressed in exchange-deficient cell lines (35). Thus inhibition by growth factors may be a novel functional property of the apical Na\(^+\)/H\(^+\) exchanger in the MTAL. At this point, we do not know whether the NGF pathway is coupled directly to inhibition of the apical exchanger or whether NGF may act indirectly to reduce the driving force for the exchanger through effects on other transporters such as the Na\(^+\)-K\(^+\)-ATPase. Relevant to this question, however, we found that the effects of NGF and hypertonicity to inhibit HCO\(_3^-\) absorption are not additive, suggesting that these factors may act via a common mechanism. Hypertonicity inhibits the apical exchanger (NHE3) directly by decreasing its sensitivity to intracellular H\(^+\) (55). Together, these data suggest that NGF may activate signals that couple directly to inhibition of the apical membrane Na\(^+\)/H\(^+\) exchanger. Study of the effects of NGF on the transport properties of this exchanger, independent of effects on other transporters, will be required to test this hypothesis.

Stimulation of HCO\(_3^-\) absorption in the presence of AVP. In contrast to its inhibitory effect under basal conditions, NGF stimulates HCO\(_3^-\) absorption in MTAL exposed to AVP. As discussed below, this stimulation likely occurs as the result of NGF inhibiting AVP-stimulated cAMP production, which results in an increase in apical membrane Na\(^+\)/H\(^+\) exchange activity (19, 23). Thus NGF apparently is capable of inducing the classical growth factor stimulation of Na\(^+\)/H\(^+\) exchange activity in the MTAL; however, this stimulation requires the interaction of NGF with AVP. These studies identify a potentially important physiological function of AVP in the kidney, namely, to modify the response of tubule epithelial cells to growth factor stimulation. Our data establish that stimulation of luminal acidification by NGF in the MTAL is dependent on AVP. In many systems, stimulation of Na\(^+\)/H\(^+\) exchange activity by growth factors is an early event that may be permissive for cell proliferation (18, 27, 34, 43). Based on our results suggesting that NGF stimulates apical Na\(^+\)/H\(^+\) exchange only when AVP is present, we speculate that AVP could be a key cofactor in determining the actions of growth factors to regulate other cellular processes in the MTAL such as survival, growth, and repair.

Signal Transduction by NGF

NGF activates at least two distinct signaling pathways in the MTAL: one that is PKC independent and leads to inhibition of HCO\(_3^-\) absorption and another that is PKC dependent and leads to stimulation of HCO\(_3^-\) absorption. The inhibitory pathway predominates under basal conditions, whereas stimulation is observed in tubules exposed to AVP. Hence, functional expression of the signaling pathway coupled to HCO\(_3^-\) transport stimulation requires the interaction of NGF with AVP. Hypertonicity blocks the inhibition but not the stimulation of HCO\(_3^-\) absorption, providing further evidence that these pathways are distinct. Both transport effects are blocked by genistein, suggesting an important role for tyrosine kinases in mediating NGF action. These findings are discussed below in the context of current knowledge of signal transduction in the MTAL.

NGF receptors. NGF and other neurotrophins induce intracellular signals through two classes of cell-surface receptors: the Trk family of receptor tyrosine kinases (6, 33) and p75, a member of the tumor necrosis factor receptor superfamily (6, 10). TrkA is a high-affinity NGF receptor [dissociation constant (Kd) of 10\(^{-11}\) to 10\(^{-10}\) M] that is tyrosine phosphorylated on NGF exposure and is coupled to the downstream activation of multiple signaling proteins, including phosphatidylinositol 3-kinase, mitogen-activated protein kinase, and phospholipase C-γ (pathways that may lead to activation of PKC) (6, 33). In contrast, the p75 receptor binds NGF with low affinity (Kd of 10\(^{-9}\) to 10\(^{-8}\) M), lacks intrinsic tyrosine kinase activity, and couples to the activation of the transcription factor NF-κB, ceramide production, and c-jun NH\(_2\)-terminal kinase (JNK), signals thought to participate in neuronal cell death (10, 12, 13). The receptor(s) that mediates NGF action in the MTAL is unknown; however, both the p75 and TrkA receptors are expressed in the kidney (3, 8, 49).

We found that NGF altered HCO\(_3^-\) absorption in the MTAL at 7 × 10\(^{-10}\) M (Figs. 1–5) but had no effect at 7 × 10\(^{-11}\) M (data not shown), suggesting that the responses to NGF may be mediated via the low-affinity receptor. On the other hand, our results show that NGF signaling involves both tyrosine kinases and PKC pathways more consistent with signaling via high-affinity TrkA receptors. Evidence in other systems indicates that the p75 and TrkA receptors may coexist and interact functionally, with activation of one receptor altering the affinity or signaling efficiency of the other receptor (6, 10). Assessment of whether this type of interaction may explain our observations will require the molecular identification of the receptor(s) that mediates NGF signal transduction in the MTAL.

Role of PKC in regulation by NGF. NGF stimulates HCO\(_3^-\) absorption in the presence of AVP through
activation of PKC. This conclusion is based on the observations that pretreatment with inhibitors of PKC prevented the stimulation of $\text{HCO}_3^-$ absorption and that the stimulation was reversed when the inhibitors were added in the presence of NGF (Fig. 6). Virtually identical results were obtained with staurosporine and chelerythrine chloride, two chemically different PKC inhibitors with different kinetic properties (23). Furthermore, these inhibitors have no effect on $\text{HCO}_3^-$ absorption under basal conditions or in the presence of AVP alone (23) and do not prevent inhibition of $\text{HCO}_3^-$ absorption by NGF alone (Fig. 5A). Thus the PKC inhibitors blocked specifically the signaling pathway through which NGF interacts with AVP. Together, these data establish that the stimulation of $\text{HCO}_3^-$ absorption occurs through a PKC-dependent mechanism, whereas the inhibition of $\text{HCO}_3^-$ absorption occurs independently of PKC. This difference indicates that the NGF-induced signaling pathways leading to stimulation and inhibition of $\text{HCO}_3^-$ absorption are distinct.

Insight into the mechanism of the PKC-dependent stimulation of $\text{HCO}_3^-$ absorption by NGF can be obtained from our previous studies of the interactions between AVP and PGE$_2$. PGE$_2$, like NGF, acts via PKC to stimulate $\text{HCO}_3^-$ absorption in the presence but not in the absence of AVP (23, 24). In the MTAL, AVP inhibits $\text{HCO}_3^-$ absorption by increasing cAMP, in turn inhibiting apical membrane Na$^+$/H$^+$ exchange activity (9, 19, 52). PGE$_2$ reverses the inhibition by AVP by activating PKC, which inhibits AVP-stimulated cAMP production and thereby reverses the cAMP-dependent inhibition of Na$^+$/H$^+$ exchange (23). We propose that NGF stimulates $\text{HCO}_3^-$ absorption by a similar mechanism, that is, that activation of PKC by NGF leads to decreased AVP-dependent cAMP production, thereby reversing AVP inhibition of apical Na$^+$/H$^+$ exchange activity and increasing $\text{HCO}_3^-$ absorption. Thus, similar to PGE$_2$, NGF likely stimulates apical Na$^+$/H$^+$ exchange activity indirectly through modulation of the adenyl cyclase system. A test of this hypothesis will require study of the effects of NGF on cell cAMP levels and direct analysis of the effects of NGF on apical Na$^+$/H$^+$ exchange activity. Our finding that the stimulatory effects of NGF and PGE$_2$ are not additive supports the view that these factors act via a common mechanism. In the MTAL, PGE$_2$ appears to reverse AVP action through the selective activation of PKC by NGF has been reported to activate PKC, -6, -e, and -z (57), all of which are expressed in the MTAL (5). Thus an additional goal for future studies will be to determine whether NGF and PGE$_2$ reverse AVP action in the MTAL through activation of the same or different PKC isoforms. Whether NGF may stimulate $\text{HCO}_3^-$ absorption through effects on basolateral membrane transport pathways in addition to apical membrane Na$^+$/H$^+$ exchange activity also remains to be determined.

Role of tyrosine kinase pathways in regulation by NGF. Binding of NGF to its high-affinity receptor stimulates receptor-tyrosine kinase activity and induces the tyrosine phosphorylation and activation of a number of intracellular target molecules, including phospholipase C-$\gamma$ (33). We found that both the inhibition and the stimulation of $\text{HCO}_3^-$ absorption by NGF were blocked by genistein, which selectively inhibits tyrosine kinase-dependent regulation of $\text{HCO}_3^-$ absorption in the MTAL (22) and prevents NGF-stimulated tyrosine phosphorylation in PC-12 cells (45). Thus tyrosine kinases appear to be components of the NGF signal transduction pathways that lead to inhibition and PKC-dependent stimulation of $\text{HCO}_3^-$ absorption. Of interest, genistein did not block the PKC-dependent stimulation of $\text{HCO}_3^-$ absorption by PGE$_2$ (22). This suggests that the genistein-sensitive step in the NGF stimulatory pathway is upstream of PKC activation. At this point, we do not know whether NGF activates two separate genistein-sensitive pathways or activates a common pathway that diverges to regulate distinct pathways coupled to $\text{HCO}_3^-$ transport inhibition and stimulation. A simple explanation for our results is that genistein inhibits the tyrosine kinase activity of the NGF receptor, thereby blocking both downstream signaling pathways. However, studies in PC-12 cells show that genistein concentrations in excess of 100 $\mu$M are needed to inhibit NGF-stimulated tyrosine phosphorylation via the high-affinity (TrkA) receptor (45). In our study, complete or nearly complete inhibition of NGF action was observed using only 7 $\mu$M genistein (Fig. 7). Thus the effects of genistein in the MTAL may be mediated through inhibition of NGF-stimulated tyrosine kinase(s) distinct from the NGF receptor. Alternatively, it is possible that the NGF receptor in the MTAL is much more sensitive to genistein than the receptor in PC-12 cells. Resolution of these issues will require molecular identification of the signaling proteins that undergo tyrosine phosphorylation in response to NGF stimulation.

The signaling pathway by which NGF inhibits basal $\text{HCO}_3^-$ absorption is unknown, although our results indicate that CAMP and PKC do not appear to be involved (Figs. 4 and 5). We found that hypertonicity prevents inhibition by NGF, suggesting that these factors may inhibit $\text{HCO}_3^-$ absorption via a common pathway. Hypertonicity inhibits $\text{HCO}_3^-$ absorption via a tyrosine kinase-dependent mechanism (22), and we have recently identified focal adhesion kinase (FAK) as a signaling protein activated by tyrosine phosphorylation in response to hypertonic stress in the renal outer medulla (56). Growth factors have been shown to activate FAK and related components of integrin signaling complexes in other systems (15, 39). Thus FAK may be a point of convergence of the hypertonic and NGF signaling pathways that lead to inhibition of $\text{HCO}_3^-$ absorption in the MTAL. This hypothesis remains to be tested but is consistent with the notion presented above that genistein may block NGF action through inhibition of nonreceptor tyrosine kinases.

Physiological Implications

The kidney produces a number of growth factors that are known to influence the activities of acid-base transporters and intracellular pH in many cell types (18, 27, 32, 35, 42, 54). Despite this, growth factors generally have not been considered to play a role in the regulation
of renal tubule acid excretion. Recent studies indicate, however, that levels of growth factors within the kidney are altered in a variety of pathophysiological conditions that are associated with changes in renal tubule acid-base transport, including reduction of renal mass, ureteral obstruction, K+ depletion, and diabetes (14, 28, 30, 31, 37, 44, 51). The results of the present study demonstrating regulation of HCO3− absorption by NGF in the MTAL raise the possibility that changes in the levels of growth factors could participate in the local control of urinary net acid excretion in these conditions. NGF and its receptors are expressed in the kidney, but their functions are unknown. Our results establish directly that NGF can influence the function of renal tubules and identify a possible role for NGF in the control of urinary acidification. In view of the close association between effects of mitogens on acid-base transporters and cell proliferation (18, 27, 34, 43) and the important role of NGF in determining the survival and differentiation of nerve cells (33, 53), it is also possible that the signaling pathways and acid-base transport effects we identified may be indicative of a role for NGF in controlling the growth and survival of MTAL cells. At present, information is lacking on many critical issues, including sources of NGF in the renal medulla, factors that regulate NGF levels in the kidney, and effects of NGF on renal function. Further work on these and other basic issues is needed before the physiological relevance of the effects of NGF on signal transduction and HCO3− transport in the MTAL can be defined. An additional goal will be to examine the effects of other growth factors in order to determine whether the unusual pattern of HCO3− transport regulation that we observe is specific for NGF or represents a general response of MTAL cells to growth factor stimulation.

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