Aldosterone potentiates 1,25-dihydroxyvitamin D₃ action in renal thick ascending limb via a nongenomic, ERK-dependent pathway

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Good, David W., Thampi George, and Bruns A. Watts III. Aldosterone potentiates 1,25-dihydroxyvitamin D₃ action in renal thick ascending limb via a nongenomic, ERK-dependent pathway. *Am J Physiol Cell Physiol* 285: C1122–C1130, 2003. First published July 2, 2003; 10.1152/ajpcell.00125.2003.—Recently, we demonstrated that aldosterone inhibits HCO₃⁻ absorption in the rat medullary thick ascending limb (MTAL) via a nongenomic pathway blocked by inhibitors of extracellular signal-regulated kinase (ERK) activation. Here we examined the effects on the MTAL of 1,25-dihydroxyvitamin D₃ [1,25(OH)₂D₃], which regulates cell functions through nongenomic mechanisms in nonrenal systems. Addition of 1 nM 1,25(OH)₂D₃ to the bath decreased HCO₃⁻ absorption by 24%, from 15.0 ± 0.3 to 11.4 ± 0.5 pmol·min⁻¹·mm⁻¹ (P < 0.001). This inhibition was maximal within 60 min and was eliminated by pretreatment with actinomycin D, cycloheximide, or inhibitors of protein kinase C. In MTAL bathed with 1 nM aldosterone [added 15–20 min before 1,25(OH)₂D₃], the absolute (5.6 ± 0.3 vs. 3.6 ± 0.3 pmol·min⁻¹·mm⁻¹) and fractional (49 ± 2 vs. 24 ± 2%) decreases in HCO₃⁻ absorption induced by 1,25(OH)₂D₃ were significantly greater than those in the absence of aldosterone (P < 0.05). The effect of aldosterone to potentiate inhibition by 1,25(OH)₂D₃ was not affected by spironolactone but was eliminated by the MAPK kinase/ERK inhibitor U-0126. U-0126 did not affect inhibition of HCO₃⁻ absorption by 1,25(OH)₂D₃ alone. Aldosterone induced rapid activation of ERK via a transcription-independent pathway. We conclude that 1,25(OH)₂D₃ inhibits HCO₃⁻ absorption in the MTAL via a genomic pathway regulating protein kinase C, which may contribute to 1,25(OH)₂D₃-induced regulation of urinary net acid and/or Ca²⁺ excretion and 2) aldosterone potentiates inhibition by 1,25(OH)₂D₃ through an ERK-dependent, nongenomic pathway. These results identify a novel regulatory interaction whereby aldosterone acts via nongenomic mechanisms to enhance the genomic response to 1,25(OH)₂D₃. Aldosterone may influence a broad range of biological processes, including epithelial transport, by modifying the response of target tissues to 1,25(OH)₂D₃ stimulation.

1,25-dihydroxycholecalciferol; kidney; calcium homeostasis; bicarbonate absorption; acid-base transport

IN ADDITION TO THEIR CLASSICAL actions mediated through regulation of nuclear transcription and protein synthesis, steroid hormones have been recognized recently to induce rapid cellular responses that occur independently of transcription and translation (12). Nongenomic effects have been identified for all groups of steroids, including aldosterone, glucocorticoids, progesterone and estrogen, and vitamin D₃ (7–10, 12, 22, 33). The biological and clinical relevance of rapid steroid effects is not well understood. Nongenomic pathways have been implicated in a variety of important processes, including endothelial cell biology, cardiovascular function and blood pressure, fertility, neuronal excitability, and electrolyte homeostasis (7–10, 12, 22, 23, 33, 34). The rapid steroid effects likely are transmitted through cell-surface receptors (10, 12, 23, 36, 46), and there is increasing evidence that steroid hormones may control their own transcriptional regulation through the rapid activation of second messenger pathways (7–10, 12). However, the physiological and clinical relevance of rapid steroid effects is not well understood. Aldosterone potentiates 1,25-dihydroxyvitamin D₃ action in renal thick ascending limb through transcriptional regulation of electrolyte transport across renal tubule epithelia (1, 37, 43). Recently, we demonstrated that aldosterone inhibits transepithelial HCO₃⁻ absorption in the medullary thick ascending limb (MTAL) of the rat via a pathway that is highly selective, operates independently of transcription and translation, and is not mediated through the classical mineralocorticoid receptor (20). This nongenomic regulation is observed with physiological aldosterone concentrations and is blocked by inhibitors of the extracellular signal-regulated kinase (ERK) signaling pathway (19, 20). These studies provided the first evidence that nongenomic mechanisms mediate steroid-induced regulation of the transport function of renal tubules. Whether steroid hormones in addition to aldosterone influence renal tubule function through nongenomic actions has not been determined. How nongenomic and genomic steroid pathways may interact to regulate renal tubule transport also is unknown.

1α,25-Dihydroxyvitamin D₃ [1,25(OH)₂D₃], the major active metabolite of vitamin D₃, plays a crucial role

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in the regulation of Ca\(^{2+}\) and phosphate balance through its integrated actions on the intestine, kidney, bone, and parathyroid glands (7, 28, 42). Most of the biological actions of 1,25(OH)\(_2\)D\(_3\) are mediated through transcriptional regulation of target genes after the hormone binds to the nuclear vitamin D receptor (VDR) (7, 28). However, 1,25(OH)\(_2\)D\(_3\) also activates nongenomic signaling pathways in some systems (7, 12). 1,25(OH)\(_2\)D\(_3\) influences a variety of renal functions, including reabsorption of Ca\(^{2+}\) and phosphate and excretion of acid (7, 14, 25, 41, 42). It is unclear to what extent these effects are mediated through direct actions of 1,25(OH)\(_2\)D\(_3\) on the renal tubules or through indirect effects of the hormone on plasma Ca\(^{2+}\) concentration, parathyroid hormone (PTH) levels, or other confounding factors (7, 25, 42). The VDR is expressed in multiple nephron segments (26, 30), and 1,25(OH)\(_2\)D\(_3\) can influence Ca\(^{2+}\) transport in renal cell lines and isolated membrane preparations (5, 6, 15, 42). However, whether 1,25(OH)\(_2\)D\(_3\) regulates the transport function of renal tubules directly remains unclear. Whether 1,25(OH)\(_2\)D\(_3\) may influence renal tubule function through nongenomic mechanisms is unknown.

The purpose of the present study was to examine directly the effects of 1,25(OH)\(_2\)D\(_3\) on renal tubules or through indirect effects of the hormone on plasma Ca\(^{2+}\) concentration, parathyroid hormone (PTH) levels, or other confounding factors (7, 25, 42). The VDR is expressed in multiple nephron segments (26, 30), and 1,25(OH)\(_2\)D\(_3\) can influence Ca\(^{2+}\) transport in renal cell lines and isolated membrane preparations (5, 6, 15, 42). However, whether 1,25(OH)\(_2\)D\(_3\) regulates the transport function of renal tubules directly remains unclear. Whether 1,25(OH)\(_2\)D\(_3\) may influence renal tubule function through nongenomic mechanisms is unknown.

The protocol for study of transepithelial HCO\(_3\)\(^{-}\) absorption has been described elsewhere (18, 20, 49). In most experiments, 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 1.6–2.0 nl·min\(^{-1}\)·mm\(^{-1}\). One to five 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 an experimental agent was added to or removed from the bath solution. In some experiments, longer treatment periods were used. The absolute rate of HCO\(_3\)\(^{-}\) absorption was calculated from the luminal flow rate, and the difference between total CO\(_2\) concentrations measured in perfused and collected fluids (18, 20). An average HCO\(_3\)\(^{-}\) 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 Figs. 1–5. Values are means \(\pm\) SE; \(n\) is the number of tubules. The absolute decrease in HCO\(_3\)\(^{-}\) absorption was calculated for individual tubules as the difference between absorption rates measured in the absence and pres-
C from the inner thin strips of tissue were dissected at 4°C. Activity in the MTAL has been described (48, 50). Briefly, thin strips of tissue were dissected at 4°C from the inner stripe of the outer medulla, the region of the kidney highly enriched in MTAL (3, 48). The strips were divided into four stripe of the outer medulla, the region of the kidney highly enriched in MTAL (3, 48). The strips were divided into four

Effects of 1,25(OH)2D3 on HCO3− absorption. Addition of 1 nM 1,25(OH)2D3 to the bath for 60 min decreased HCO3− absorption by 24%, from 15.0 ± 0.3 to 11.4 ± 0.5 pmol·min−1·mm−1 (P < 0.001; Fig. 1A). The time course (Fig. 1B) shows that 1,25(OH)2D3 decreased HCO3− absorption within 40 min, with maximum inhibition at 60 min. The HCO3− absorption rate returned to its control value within 60 min after removal of 1,25(OH)2D3 from the bath solution (Fig. 1A). For subsequent experiments (Figs. 2–5), HCO3− absorption data show the maximum inhibition by 1 nM 1,25(OH)2D3. 1,25(OH)2D3 added to the bath at 0.1 nM decreased HCO3− absorption by 11%, from 15.3 ± 0.8 to 13.7 ± 0.9 pmol·min−1·mm−1 (n = 3, P < 0.005).

Effects of actinomycin D and cycloheximide. Inhibition of HCO3− absorption by aldosterone in the MTAL is not affected by inhibitors of transcription or translation (20). Similar protocols were used to assess the mechanism of inhibition by 1,25(OH)2D3. MTAL were bathed with the transcription inhibitor actinomycin D (12.5 µg/ml) for 100 min or the protein synthesis inhibitor cycloheximide (40 µg/ml) for 120 min before addition of 1 nM 1,25(OH)2D3. Actinomycin D or cycloheximide blocked completely the inhibition of HCO3− absorption by 1,25(OH)2D3 (Fig. 2). Thus, in contrast to aldosterone, inhibition by 1,25(OH)2D3 is dependent on gene transcription and protein synthesis. Actinomycin D and cycloheximide alone do not affect HCO3− absorption (data not shown).

Effects of protein kinase C inhibitors. Activation of protein kinase C (PKC) mediates certain cellular effects of 1,25(OH)2D3. The role of PKC in the inhibition of HCO3− absorption was examined using chelerythrine chloride and staurosporine, PKC inhibitors that selectively block PKC-dependent regulation of HCO3− absorption in the MTAL (3, 17–19). In tubules bathed with 10−7 M chelerythrine chloride or 10−7 M staurosporine, addition of 1 nM 1,25(OH)2D3 to the bath had

RESULTS

Effects of 1,25(OH)2D3 on HCO3− Absorption

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no effect on HCO$_3^-$ absorption: 11.6 ± 0.3 pmol·min$^{-1}$·mm$^{-1}$, inhibitors + 1,25(OH)$_2$D$_3$ (Fig. 3). These results suggest that 1,25(OH)$_2$D$_3$ inhibits HCO$_3^-$ absorption via a PKC-dependent pathway.

**Interaction of 1,25(OH)$_2$D$_3$ and Aldosterone**

1,25(OH)$_2$D$_3$ inhibits HCO$_3^-$ absorption in the MTAL through gene regulation, whereas inhibition by aldosterone is transcription and translation independent (20). Further experiments were carried out to investigate how these different pathways interact to regulate MTAL function.

**Aldosterone potentiates inhibition by 1,25(OH)$_2$D$_3$.**

In MTAL bathed with 1 nM aldosterone for 15–20 min, 1 nM 1,25(OH)$_2$D$_3$ decreased HCO$_3^-$ absorption by 48% (from 11.7 ± 0.3 to 6.1 ± 0.4 pmol·min$^{-1}$·mm$^{-1}$, $P < 0.001$) compared with a decrease of 24% observed under the same conditions in the absence of aldosterone (Fig. 4, A and B). The absolute and fractional decreases in HCO$_3^-$ absorption induced by 1,25(OH)$_2$D$_3$ were significantly greater in the presence than in the absence of aldosterone (Fig. 4, E and F). Thus aldosterone potentiates the inhibitory effect of 1,25(OH)$_2$D$_3$. Actinomycin D blocked inhibition of HCO$_3^-$ absorption by 1,25(OH)$_2$D$_3$ in the presence of aldosterone: 12.1 ± 0.8, aldosterone + actinomycin D vs. 11.3 ± 0.5 pmol·min$^{-1}$·mm$^{-1}$, aldosterone + actinomycin D + 1,25(OH)$_2$D$_3$ ($n = 4$, $P = $ not significant).

Further studies were designed to determine whether aldosterone influences 1,25(OH)$_2$D$_3$-induced regulation through nongenomic mechanisms. We previously identified two key features of the nongenomic pathway activated by aldosterone in the MTAL: 1) it is blocked by inhibitors of ERK activation, and 2) it is not affected by spironolactone, a competitive antagonist of the classical mineralocorticoid receptor (19, 20). We took advantage of these properties to test whether this non-

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**Fig. 4.** A and B: aldosterone (Aldo) potentiates inhibition of HCO$_3^-$ absorption by 1 nM 1,25(OH)$_2$D$_3$. Tubules were bathed for 15–20 min in the absence (A) or presence (B) of 1 nM aldosterone, and then 1,25(OH)$_2$D$_3$ was added to the bath solution. A: control data are repeated from Fig. 1A to facilitate comparison. Initial transport rates in B are less than those in A because of the previously described inhibitory effect of aldosterone (20). C and D: U-0126 blocks potentiation by aldosterone. Tubules were bathed with 15 μM U-0126 (C) or 15 μM U-0126 + 1 nM aldosterone (D), and then 1 nM 1,25(OH)$_2$D$_3$ was added to the bath solution. ∆HCO$_3^-$, lines, data points, and $P$ values are as described in Fig. 1A legend. E and F: summary of results in A–D. Data show absolute (E) and fractional (F) decreases in HCO$_3^-$ absorption induced by 1,25(OH)$_2$D$_3$. Values are means ± SE; $n$, number of tubules. Cont, control. *$P < 0.05$ vs. other 3 conditions.
genomic pathway mediates aldosterone-induced potentiation of 1,25(OH)2D3 action. If it does, then the potentiation by aldosterone should be blocked by ERK inhibitors, but not by spironolactone.

_**U-0126 blocks potentiation by aldosterone.**_ U-0126 is a selective inhibitor of the MAPK kinase MEK1/2, which directly activates ERK1/2 (13, 50). U-0126 blocks ERK activation in the MTAL and prevents the nongenomic inhibition of HCO3− absorption by aldosterone (19, 48, 50). We therefore tested whether U-0126 would influence the effect of aldosterone to potentiate inhibition by 1,25(OH)2D3. In MTAL bathed with 1 nM aldosterone + 15 μM U-0126, 1 nM 1,25(OH)2D3 decreased HCO3− absorption by 21% (from 14.5 ± 1.1 to 11.4 ± 1.1 pmol.min−1.mm−1, P < 0.001; Fig. 4D), an amount similar to that observed with 1,25(OH)2D3 alone (Fig. 4A). The effect of aldosterone to enhance the absolute and fractional inhibition of HCO3− absorption by 1,25(OH)2D3 was eliminated completely by U-0126 (Fig. 4, E and F). In contrast, U-0126 had no effect on the inhibition of HCO3− absorption by 1,25(OH)2D3 in the absence of aldosterone (Fig. 4, C, E, and F), indicating that ERK is not involved in mediating inhibition by 1,25(OH)2D3 and that the MEK/ERK inhibitor blocked specifically the regulatory action of aldosterone. These results indicate that aldosterone potentiates the inhibition by 1,25(OH)2D3 via an ERK-dependent mechanism and support the view that aldosterone acts via its nongenomic pathway.

_Spironolactone does not block potentiation by aldosterone._ In MTAL bathed with 1 nM aldosterone + 10 μM spironolactone, 1 nM 1,25(OH)2D3 decreased HCO3− absorption from 11.7 ± 0.5 to 5.5 ± 0.4 pmol.min−1.mm−1 (P < 0.001; Fig. 5). The absolute (6.2 ± 0.2 pmol.min−1.mm−1) and fractional (53 ± 2%) decreases in HCO3− absorption were similar to those induced by 1,25(OH)2D3 in the presence of aldosterone alone and were greater than the decreases observed with 1,25(OH)2D3 in the absence of aldosterone (Fig. 4, E and F). Thus the potentiation of 1,25(OH)2D3 action by aldosterone is not blocked by spironolactone and, therefore, not mediated through the classical mineralocorticoid receptor.

_Aldosterone activates ERK via a transcription-independent mechanism._ Results of the preceding experiments support the view that aldosterone acts through a nongenomic pathway involving ERK to potentiate inhibition by 1,25(OH)2D3. To confirm directly that aldosterone activates ERK via a nongenomic mechanism, inner stripe tissue was incubated in vitro in the absence and presence of aldosterone for 15 min, and then ERK activity was assessed by immune complex assay (48, 50). Incubation with 1 nM aldosterone increased ERK activity 1.6 ± 0.1-fold (n = 3, P < 0.05), and this increase was not prevented by pretreatment with actinomycin D (Fig. 6, A and B). These results demonstrate that aldosterone increases ERK activity via a transcription-independent pathway. The aldosterone-induced ERK activation was blocked completely by pretreatment with 15 μM U-0126 (Fig. 6C). Immunoblot analysis of inner stripe samples using antiphospho-ERK1/2 antibody showed that aldosterone increased ERK phosphorylation (results not shown).

**DISCUSSION**

The major findings of this study are that 1) 1,25(OH)2D3 inhibits HCO3− absorption in the MTAL via a genomic pathway likely involving PKC, and 2) aldosterone potentiates the inhibition by 1,25(OH)2D3 through a nongenomic pathway involving ERK activation. These results, to our knowledge, are the first in any system to identify a direct interaction between aldosterone and 1,25(OH)2D3 in the regulation of cell function and demonstrate that this interaction is the result of cross talk between nongenomic and genomic steroid hormone signaling pathways. Although nongenomic actions of aldosterone have been demonstrated in a variety of cell systems (12, 33), their physiological relevance to the regulation of epithelial transport has been unclear. Our results in the MTAL establish that nongenomic pathways activated by aldosterone not only regulate transepithelial transport directly (20) but also can modify the transport response to other, dissimilar steroid hormones.

**1,25(OH)2D3 Inhibits HCO3− Absorption in the MTAL**

1,25(OH)2D3 influences renal excretion of Ca2+, phosphate, SO42−, and net acid, but very little is known about the direct effects of this hormone on the transport function of individual nephron segments (7, 14, 25, 28, 41, 42). Our results are the first to identify the MTAL as a target for direct transport regulation by 1,25(OH)2D3. 1,25(OH)2D3 inhibited HCO3− absorption...
in the MTAL at 0.1 and 1.0 nM. These values encompass the range of circulating 1,25(OH)₂D₃ levels in serum (0.1–0.4 nM) (6, 16, 39, 47) and are similar to 1,25(OH)₂D₃ concentrations reported to stimulate Ca²⁺ transport in cultured distal tubule and collecting duct cells (5, 15). It is possible that intrarenal levels of 1,25(OH)₂D₃ may exceed concentrations in systemic plasma as the result of 1,25(OH)₂D₃ production by the proximal tubule. We found that 1,25(OH)₂D₃ inhibits HCO₃⁻ absorption in the MTAL at concentrations at or above systemic plasma levels, indicating that the transport effects are likely to represent physiologically relevant regulation.

1,25(OH)₂D₃ inhibits HCO₃⁻ absorption in the MTAL by an amount quantitatively similar to that observed with aldosterone; however, the mechanisms of action of the two steroids are different. Aldosterone inhibits HCO₃⁻ absorption via a nongenomic pathway that is blocked by MEK inhibitors, but not by PKC inhibitors (19, 20). In contrast, 1,25(OH)₂D₃ inhibits HCO₃⁻ absorption via a genomic pathway that is blocked by PKC inhibitors, but not by MEK inhibitors. Transport regulation by 1,25(OH)₂D₃ in the MTAL depends on gene transcription and protein synthesis, as evidenced by 1) its complete inhibition by actinomycin D or cycloheximide and 2) the time course, which demonstrates a lag period of up to 40 min for the 1,25(OH)₂D₃-induced inhibition. The MTAL expresses the VDR (26, 30), a member of the nuclear receptor superfamily that mediates 1,25(OH)₂D₃-induced transcriptional regulation (7, 28). Whether the VDR is involved in inhibition of HCO₃⁻ absorption in the MTAL remains to be determined.

The Na⁺/H⁺ exchanger isoform NHE3 is localized selectively in the apical membrane of certain renal and intestinal epithelial cells, where it mediates transepithelial reabsorption of NaCl and/or NaHCO₃ (35, 44). It is unknown whether the activity of this transporter is regulated by 1,25(OH)₂D₃. NHE3 mediates virtually all the luminal H⁺ secretion necessary for HCO₃⁻ absorption in the MTAL (2, 4, 21, 49). Thus it is highly likely that 1,25(OH)₂D₃ inhibits NHE3 in the MTAL. Further direct studies of the effect of 1,25(OH)₂D₃ on the apical Na⁺/H⁺ exchanger are needed to determine whether the 1,25(OH)₂D₃-induced signaling pathway is coupled directly to inhibition of the exchanger or whether 1,25(OH)₂D₃ may act indirectly to decrease exchanger activity through effects on another transporter, such as basolateral HCO₃⁻ efflux pathways or the Na⁺/K⁺-ATPase. It is noteworthy that inhibition of HCO₃⁻ absorption by 1,25(OH)₂D₃ likely involves PKC, which has been shown to inhibit HCO₃⁻ absorption in some systems (35, 44). However, in the MTAL, neither activation nor inhibition of PKC by itself leads to inhibition of HCO₃⁻ absorption (3, 18). Thus it is unlikely that PKC is a direct mediator of 1,25(OH)₂D₃-induced inhibition of apical Na⁺/H⁺ exchange activity.

Our results provide the first evidence for direct regulation of transepithelial H⁺ secretion by 1,25(OH)₂D₃ in a nephron segment. The inhibition of HCO₃⁻ absorption by 1,25(OH)₂D₃ in the MTAL may play a role in acid-base regulation. For example, metabolic acidosis can adversely affect the production of 1,25(OH)₂D₃ through several mechanisms: 1) decreasing the activity of the 1α-hydroxylase, which catalyzes the formation of 1,25(OH)₂D₃ from 25-hydroxyvitamin D₃ in the proximal tubule, 2) blunting the action of PTH to stimulate renal 1α-hydroxylase activity, and 3) increasing the activity of the renal 24-hydroxylase that catalyzes 1,25(OH)₂D₃ degradation (7, 29, 31, 41). On the basis of results of the present study, decreased 1,25(OH)₂D₃ levels in acidosis could lead to increased H⁺ secretion and HCO₃⁻ absorption by the MTAL, which would aid in limiting or correcting the acidemia. In addition, 1,25(OH)₂D₃ may directly affect other MTAL transport functions. Recent studies in rats show that chronic hypercalcemia induced by 1,25(OH)₂D₃ administration...
reduces the abundance of the apical Na\(^{+}\)-K\(^{+}\)-2Cl\(^{-}\) co-transporter (BSC-1 or NKCC2) and the apical K\(^{+}\) channel (ROMK), effects that would reduce MTAL NaCl absorption and contribute to the urinary concentrating defect associated with hypercalcemia (45). The adaptations in the two MTAL transporters were explained on the basis of renal and cellular responses to hypercalcemia (45). However, our results raise the additional possibility that the adaptations in BSC-1 and ROMK observed in these studies may involve direct receptor-mediated effects of 1,25(OH)\(_{2}\)D\(_{3}\) on the MTAL.

**Aldosterone Potentiates Inhibition by 1,25(OH)\(_{2}\)D\(_{3}\)**

In the presence of aldosterone, the inhibition of HCO\(_{3}\)\(^{-}\) absorption by 1,25(OH)\(_{2}\)D\(_{3}\) was markedly enhanced. The time course of inhibition by 1,25(OH)\(_{2}\)D\(_{3}\) was not affected by aldosterone, and actinomycin D eliminated the 1,25(OH)\(_{2}\)D\(_{3}\)-induced inhibition in the absence or presence of aldosterone, supporting the view that aldosterone upregulates the genomic pathway responsible for 1,25(OH)\(_{2}\)D\(_{3}\)-induced regulation. Several lines of evidence support the conclusion that aldosterone potentiates 1,25(OH)\(_{2}\)D\(_{3}\) action via a nongenomic, ERK-dependent pathway: 1) aldosterone activates ERK via a rapid, transcription-independent mechanism, 2) inhibitors of ERK activation prevent nongenomic inhibition of HCO\(_{3}\)\(^{-}\) absorption by aldosterone (19, 20), 3) inhibitors of ERK activation eliminate the potentiating effect of aldosterone on 1,25(OH)\(_{2}\)D\(_{3}\) but have no effect on the inhibition of HCO\(_{3}\)\(^{-}\) absorption by 1,25(OH)\(_{2}\)D\(_{3}\) alone, 4) 1,25(OH)\(_{2}\)D\(_{3}\) action is potentiated by short-term (15 min) aldosterone treatment, and 5) potentiation by aldosterone is not prevented by spironolactone, indicating that it does not involve the classical mineralocorticoid receptor. Much recent attention has been focused on the possibility that steroid hormones may act through genomic and nongenomic mechanisms simultaneously, whereby rapid activation of nongenomic signals by a steroid may, in turn, modulate its genomic response (8–10, 12). Our results broaden this concept by demonstrating that the activation of nongenomic pathways by one steroid can modify the genomic response to another, dissimilar steroid. These findings identify novel mechanisms through which aldosterone may influence electrolyte transport in the kidney and through which tissue-specific regulation by aldosterone could be achieved. In addition, they present new therapeutic options whereby the biological response to a particular steroid such as 1,25(OH)\(_{2}\)D\(_{3}\) could be modified through pharmacological manipulation of nongenomic mechanisms activated by a different steroid hormone in the same cell.

The mechanism(s) by which aldosterone potentiates regulation by 1,25(OH)\(_{2}\)D\(_{3}\) remain to be defined, but some possibilities can be considered on the basis of findings in other systems. The interaction could involve cross talk between the aldosterone-induced ERK pathway and PKC, which appears to be an essential component of the 1,25(OH)\(_{2}\)D\(_{3}\) regulatory pathway. Alternatively, the ERK-dependent pathway activated by aldosterone could alter the transcriptional activity of the VDR through receptor phosphorylation or by alteration of the activity of coactivator proteins (28). One such coactivator may be the retinoid X receptor, which heterodimerizes with the VDR and is essential for its DNA-binding and transcriptional activity (7, 28). Recent work suggests that the retinoid X receptor can be specifically phosphorylated and regulated through the Ras-MEK-ERK cascade (11, 27). These and other possible mechanisms need to be tested experimentally in the future. Our results suggest, however, that ERK is an important mediator of cross talk between nongenomic and genomic steroid regulatory pathways.

**Physiological Significance and Clinical Implications**

The direct action of 1,25(OH)\(_{2}\)D\(_{3}\) to inhibit HCO\(_{3}\)\(^{-}\) absorption in the MTAL and the ability of aldosterone to enhance 1,25(OH)\(_{2}\)D\(_{3}\)-induced regulation have important implications on several levels. 1) As discussed above, increased MTAL HCO\(_{3}\)\(^{-}\) absorption in response to decreased 1,25(OH)\(_{2}\)D\(_{3}\) levels may facilitate acid excretion in metabolic acidosis. 2) Infusion of HCO\(_{3}\)\(^{-}\) and induction of bicarbonaturia in acidic animals decreased renal Ca\(^{2+}\) excretion and enhanced Ca\(^{2+}\) absorption by the distal convoluted tubule independent of PTH, consistent with an effect of luminal HCO\(_{3}\)\(^{-}\) to directly enhance Ca\(^{2+}\) absorption (42). It is therefore possible that the primary inhibition of HCO\(_{3}\)\(^{-}\) absorption by 1,25(OH)\(_{2}\)D\(_{3}\) in the MTAL could secondarily enhance Ca\(^{2+}\) absorption in the distal convoluted tubule and other distal nephron segments by increasing luminal HCO\(_{3}\)\(^{-}\) delivery to those downstream segments. 3) Extracellular fluid volume has an important influence on renal Ca\(^{2+}\) handling, with volume depletion decreasing and volume expansion increasing Ca\(^{2+}\) excretion (42). Our findings suggest the novel possibility that direct aldosterone-1,25(OH)\(_{2}\)D\(_{3}\) interactions could contribute to this relation. For example, 1,25(OH)\(_{2}\)D\(_{3}\)-dependent stimulation of Ca\(^{2+}\) absorption in distal nephron segments could be potentiated by increased aldosterone levels during volume depletion. Conceivably that interaction could promote hypercalcemia that may contribute to vasoconstriction in volume-contracted states. Direct tests of these hypotheses will require future studies of the interacting effects of aldosterone and 1,25(OH)\(_{2}\)D\(_{3}\) on Ca\(^{2+}\) absorption in multiple nephron segments and direct analysis of the role of luminal HCO\(_{3}\) as a determinant of distal nephron Ca\(^{2+}\) absorption.

The discovery of a functional interaction between 1,25(OH)\(_{2}\)D\(_{3}\) and aldosterone has additional, broader implications. In addition to its role in Ca\(^{2+}\) and phosphate homeostasis, 1,25(OH)\(_{2}\)D\(_{3}\) influences a wide variety of other biological processes, including the immune response, insulin secretion, cardiovascular function, and blood pressure (7, 24, 28, 40, 47). Several 1,25(OH)\(_{2}\)D\(_{3}\) target tissues, including intestinal epithelial cells and the cardiovascular system, also have been identified as sites of nongenomic aldosterone regulation (12, 22, 33). Our results raise the possibility
that aldosterone could influence a broad range of physiological and pathophysiological processes, such as intestinal Ca\textsuperscript{2+} absorption, vascular inflammation and calcification, and blood pressure, by modifying the response of target tissues to 1,25(OH)\textsubscript{2}D\textsubscript{3} stimulation. This possibility is supported by recent work indicating that aldosterone may influence blood pressure by acting through nongenomic mechanisms to modulate vascular responses to adrenergic stimulation (33, 38). Finally, 1,25(OH)\textsubscript{2}D\textsubscript{3} has been identified recently as a regulator of the renin-angiotensin-aldosterone system (32). In normal mice, inhibition of 1,25(OH)\textsubscript{2}D\textsubscript{3} synthesis increased, whereas 1,25(OH)\textsubscript{2}D\textsubscript{3} administration decreased, renin production. Furthermore, in VDR-null mice, renin production was elevated, leading to increased angiotensin II levels and hypertension (32). Our results suggest that increased aldosterone levels induced by 1,25(OH)\textsubscript{2}D\textsubscript{3} deficiency could enhance 1,25(OH)\textsubscript{2}D\textsubscript{3} actions in the kidney and other target tissues, thereby maintaining 1,25(OH)\textsubscript{2}D\textsubscript{3}-dependent regulation despite reduced circulating hormone levels. In this way, aldosterone could serve as the effector in a feedback system whereby the renin-angiotensin-aldosterone system helps mitigate against the highly detrimental systemic consequences of 1,25(OH)\textsubscript{2}D\textsubscript{3} deficiency.

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DISCLOSURES
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