Regulation of the epithelial Na\(^{+}\) channel by endothelin-1 in rat collecting duct

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Submitted 19 May 2008; accepted in final form 24 July 2008

Bugaj V, Pochynyuk O, Mironova E, Vandewalle A, Medina JL, Stockand JD. Regulation of the epithelial Na\(^{+}\) channel by endothelin-1 in rat collecting duct. Am J Physiol Renal Physiol 295: F1063–F1070, 2008. First published July 30, 2008; doi:10.1152/ajprenal.90321.2008.—We used patch-clamp electrophysiology to investigate regulation of the epithelial Na\(^{+}\) channel (ENaC) by endothelin-1 (ET-1) in isolated, split-open rat collecting ducts. ET-1 significantly decreases ENaC open probability by about threefold within 5 min. ET-1 decreases ENaC activity through basolateral membrane ET\(_{B}\) receptors but not ET\(_{A}\) receptors. In rat collecting duct, we find no role for phospholipase C or protein kinase C in the rapid response of ENaC to ET-1. ET-1, although, does activate src family tyrosine kinases and their downstream MAPK1/2 effector cascade in renal principal cells. Both src kinases and MAPK1/2 signaling are necessary for ET-1-dependent decreases in ENaC open probability in the split-open collecting duct. We conclude that ET-1 in a physiologically relevant manner rapidly suppresses ENaC activity in native, mammalian principal cells. These findings may provide a potential mechanism for the natriuresis observed in vivo in response to ET-1, as well as a potential cause for the salt-sensitive hypertension found in animals with impaired endothelin signaling.

salt-sensitive hypertension; systemic blood pressure

ENDOTHELIN-1 (ET-1) is a powerful vasoconstricting peptide hormone that is an important regulator of systemic blood pressure (53). Independent of its vascular effects, ET-1 also affects renal Na\(^{+}\) and water handling favoring natriuresis and diuresis. While circulating ET-1 arises from endothelial cells, local ET-1 systems also exist. For instance in the kidney, the collecting duct produces significant amounts of ET-1 (8, 25, 38, 51). ET-1 targets cells through two distinct receptor subtypes, ET\(_{A}\) and ET\(_{B}\) (32, 41). Renal collecting duct cells have both types of receptors and are able to bind ET-1 (26, 49, 50). Thus, collecting duct-derived ET-1, acting in a paracrine/autocrine manner, is an important regulator of renal Na\(^{+}\) handling (2, 20, 26, 42).

Regulated Na\(^{+}\) reabsorption in the renal collecting duct, in part, controls blood pressure. Here, activity of the aldosterone-sensitive epithelial Na\(^{+}\) channel (ENaC) is limiting for Na\(^{+}\) transport (reviewed in Refs. 19, 30, 31). Dysfunction and inappropriate regulation of ENaC consequently result in improper renal Na\(^{+}\) handling and thus, blood pressure disorders. For instance, gain of ENaC function in rodents and humans is causative for hypertension associated with the hallmarks of low plasma renin activity and aldosterone levels (1, 22, 23, 45, 46). Amiloride, an ENaC blocker, ameliorates this hypertension.

Spotted lethal (sl) rats have a naturally occurring null mutation of ET\(_{B}\) (17). These rats, when rescued from lethal intestinal aganglionosis by directed ET\(_{B}\) transgene expression in the enteric nervous system, are particularly sensitive to DOCA and salt-induced hypertension (18, 33, 34). Similarly, mice with collecting duct-specific knockout of the ET\(_{B}\) receptor have elevated blood pressure that further increases with high salt feeding (20). Collecting duct-specific ET-1 knockout, moreover, leads to hypertension exacerbated by high salt (2, 42). Plasma renin activity and aldosterone levels are low in these models of hypertension. Moreover, amiloride reduces blood pressure in collecting duct-specific ET-1 knockout mice and rescued sl/sl rats (2, 18, 42). Together, these findings provide compelling evidence that in the collecting duct, ET-1 regulation of ENaC plays a physiological role in control of renal Na\(^{+}\) reabsorption and blood pressure with dysfunction of this regulation resulting in hyperactive ENaC leading to inappropriate salt retention and hypertension. This is supported by findings from the immortalized amphibian kidney A6 cell line where ET-1 rapidly decreased ENaC open probability through basolateral ET\(_{B}\) receptors; and by findings from an overexpression system where ET-1 via ET\(_{B}\) rapidly decreased the open probability of recombinant ENaC through a transduction cascade involving src family kinases (16, 21). The evidence that ET-1 modulates ENaC in mammalian collecting duct, although, is only circumstantial for regulation of this channel by ET-1 in the mammalian collecting has not yet been tested directly. In addition, the specific cellular and molecular mechanisms possibly coupling endothelin receptors to ENaC in the mammalian kidney remain obscure. Here, we test the hypothesis that in mammalian collecting duct principal cells, ET-1 via ET\(_{B}\) receptors decreases ENaC activity. We find that ET-1 through basolateral ET\(_{B}\) receptors rapidly decreases ENaC open probability through a signaling cascade involving activation of src family tyrosine kinases and stimulation of its downstream MAPK1/2 effector cascade. This is the first report, which we are aware of, directly showing ET-1 regulation of ENaC in the mammalian collecting duct. This investigation also places MAPK1/2 downstream of src kinases in the ET-1 to ENaC signaling cascade and confirms dynamic decreases in ENaC open probability in response to stimulation of basolateral ET\(_{B}\) receptors.

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EXPERIMENTAL DESIGN AND METHODS

Materials. All chemicals and materials were purchased from Calbiochem (San Diego, CA), BioMol (Plymouth Meeting, PA), Bio-Rad (Hercules, CA), or Sigma (St. Louis, MO) unless noted otherwise and were of reagent grade. Rabbit polyclonal anti-MAPK1/2 and mouse monoclonal anti-phospho-MAPK1/2 antibodies were from Upstate Biotechnology. Anti-mouse and anti-rabbit horseradish peroxidase-conjugated 2° antibodies were from Kirkegaard-Perry Laboratories (Gaithersburg, MD). ECL reagents were from PerkinElmer Life Sciences (Boston, MA).

Tubule isolation and cell culture. Whole cell lysates from cultured principal cells were used for Western blot experiments. The immortalized mouse cortical collecting duct principal cells (mpkCCD-14) used for these experiments were grown in defined medium on permeable supports (Costar Transwells, 0.4-µm pore, 24-mm diameter) as described previously (3, 35, 47). Cells were maintained with FBS and corticosteroids until they polarized and formed monolayers with high resistances and avid, amiloride-sensitive Na+ transport.

Patch-clamp electrophysiology was used to assess ENaC activity in isolated, split-open rat cortical collecting ducts. This preparation has been described (29, 47, 48). In brief, pathogen-free Sprague-Dawley rats of either sex (4–6 wk) were purchased from Charles River Laboratories. Rats were allowed to settle upon arrival for up to a week and then were maintained on a nominally Na+-free diet (<0.01 [Na+]; TD.90228) for 5–7 days to increase the surface expression and activity of ENaC. Rats were killed by cervical dislocation and the kidneys were immediately removed. Kidneys were cut into thin slices (<1 mm) with slices placed into ice-cold physiologic saline solution (pH 7.4). Collecting ducts were mechanically isolated from these slices by microdissection using watchmaker forceps under a stereomicroscope. Isolated cortical collecting ducts were allowed to settle onto 5 × 5-mm coverglass coated with poly-L-lysine. Coverglass containing collecting ducts were placed within a perfusion chamber mounted on an inverted Nikon TE2000 microscope and superfused with a physiologic saline solution buffered with HEPES (pH 7.4). Collecting ducts were split-open with a sharpened micropipette controlled with a micromanipulator to gain access to the apical membrane. Collecting ducts were used within 1–2 h of isolation. Animal use and welfare adhered to the National Institutes of Health Guide for the Care and Use of Laboratory Animals following a protocol reviewed and approved by the Institutional Laboratory Animal Care and Use Committee at the University of Texas Health Science Center at San Antonio.

Electrophysiology. Cell-attached patches were made under voltage-clamp conditions (−Vp = −60 mV) on the apical plasma membranes of principal cells in isolated, split-open rat collecting ducts using standard procedures (35, 37, 47). Recording pipettes had resistances of 10–15 MΩ. Typical bath and pipette solutions were (in mM) 155 NaCl, 1 CaCl2, 2 MgCl2, 5 glucose, and 10 HEPES (pH 7.4); and 140 LiCl, 2 MgCl2, and 10 HEPES (pH 7.4), respectively. In some instances, 5 mM NaCl in the bath solution was replaced with 5 mM KCl. This had no effect on the observations made in the current study. Gap-free single-channel current data from gigahm seals were acquired (and subsequently analyzed) with an Axopatch 200B (Axon Instruments) or EPC-9 (HEKA Instruments) patch-clamp amplifier interfaced via a Digidata 1322A (Axon Instruments) to a PC running the pClamp 9.2 suite of software (Axon Instruments). Currents were low-pass filtered at 100 Hz with an eight-pole Bessel filter (Warner Instruments). Unitary current (i) and the number of ENaC in a patch, N, were determined from all-point amplitude histograms. Channel activity defined as NPo was calculated using the following equation: \( NPo = \sum (t1 + t2 + \cdots + n0) \), where \( t0 \) is the fractional open time spent at each of the observed current levels. Po was calculated by normalizing NPo for the number of channels observed within a patch under control conditions. For paired experiments (as are all the experiments in this study), control conditions were before addition of ET-1 or other agents. Only patches containing five channels or fewer were used to estimate Po.

Western blot analysis. Western blot analysis was performed using standard procedures described previously (4, 36). In brief, polarized mpkCCD-14 cells were lysed in gentle lysis buffer [76 mM NaCl, 50 mM Tris-HCl (pH 7.4), 2 mM EGTA, 10% glycerol, and 1.0% NP-40] in the presence of standard protease (1 mM phenylmethylsulfonyl fluoride) and phosphatase inhibitors (0.1 mM Na3P04, 0.5 mM NaF, 0.1 mM Na2MoO4, 0.1 mM ZnCl2, and 0.04 mM Na3VO4). Whole cell lysates were cleared and normalized for total protein concentration. Normalized lysates were suspended in Lamellia sample buffer (0.005% bromphenol blue, 10% glycerol, 3% SDS, 1 mM EDTA, 77 mM Tris-HCl) and 20 mM DTT and heated at 85°C for 10 min. Lysate protein was separated by size on 10% polyacrylamide gels (100 µg total protein/well) with SDS-electrophoresis. Proteins were subsequently transferred to nitrocelluloose and then probed with antibody in tris-buffed saline supplemented with 5% dried milk (Nestlé; Solon, OH) and 0.1% Tween 20. Statistics and data treatment. All summarized data were reported as means ± SE. Summarized data were compared with the Student’s (2-tailed) t-test. P ≤ 0.05 was considered significant. For presentation, current data from some cell-attached patches were subsequently software filtered at 50 Hz and slow baseline drifts were corrected. Western blots were quantified with densitometry using SigmaGel (Jandel Sci.). The flood method with the highest practical threshold was used for densitometry.

RESULTS

ET-1 acutely decreases ENaC open probability in isolated, split-open rat collecting ducts. The goals of this study were to test for physiological regulation of ENaC by ET-1 in the mammalian collecting duct and to define the signaling pathway coupling this hormone to the channel. Figure 1 documents the acute actions of 20 nM ET-1 on ENaC activity in native rat collecting duct principal cells. As is clear in the representative current traces of ENaC that are shown in Fig. 1A, ET-1 rapidly decreases the activity of ENaC within the apical plasma membrane of principal cells. As summarized in Fig. 1B, ET-1 significantly decreased ENaC open probability within 5 min from 0.56 ± 0.04 to 0.18 ± 0.05 (n = 12; N = 8 rats). ET-1 actions were reversible with activity partially recovering from 0.13 ± 0.05 to 0.33 ± 0.08 (n = 3; N = 3 rats) after 3 min of washout [see supplemental Fig. S1A (the online version of this article contains supplemental data)]. Moreover, acute regulation of ENaC by ET-1 in rat collecting duct principal cells was not affected by the presence or absence of KCl in the bath solution with Po being 0.63 ± 0.04 and 0.12 ± 0.03 (n = 4; N = 3 rats) before and after addition of ET-1 with 5 mM KCl substituted with KCl (see supplemental Fig. S1B).

ETα receptors couple ET-1 to ENaC. We next tested which endothelin receptors were involved in transducing the actions of ET-1 to ENaC in collecting duct. Representative gap-free current traces of ENaC before and after inhibiting ETα receptors with 1 µM BQ-123 and following subsequent ET-1 treatment are shown in Fig. 2A. Inhibiting ETα receptors alone had an insignificant effect modestly decreasing ENaC open probability from 0.64 ± 0.05 to 0.55 ± 0.05. In the presence of inhibited ETα, although, ET-1 retained its ability to markedly and rapidly decrease ENaC activity. As reported in the summary graph in Fig. 2B, ENaC open probability significantly decreased from 0.55 ± 0.05 to 0.26 ± 0.06 following ET-1 treatment in the continued presence of BQ-123 (n = 7; N = 6 rats). These results suggest that ETα receptors do not play a
significant role in acute regulation of ENaC by nanomolar concentrations of ET-1 in the freshly isolated rat collecting duct. To define possible involvement of ETB receptors, we employed a similar strategy. Representative current traces of ENaC before and after application of 20 nM ET-1 to the bath solution. This patch was formed on the apical membrane of a principal cell within a freshly isolated, split-open rat collecting duct. The seal was voltage clamped to $-V_h = -60$ mV. At this holding potential and with our recording solutions, inward Li$^+$ current is downward. Areas under the gray bars over the continuous trace (top) are shown below at an expanded time scale. Dashed lines indicate the respective current levels shown to the right. B: summary graph of ENaC open probability changes in response to ET-1 from paired patch-clamp experiments performed on isolated, split-open rat collecting duct. Circles represent data from individual experiments with means shown as bars. *Significant decrease compared with before addition of ET-1.

ET-1 decreases ENaC open probability via src family kinases. Endothelin receptors, including ETB, are coupled to G proteins and belong to the GPCR superfamily (11, 24, 39). ETB, through its cognate G protein alpha subunits ($G_\alpha$ and $G_\beta$), activates a number of signaling cascades, including those mediated by PLC/PKC and src family tyrosine kinases (11, 24, 28, 39, 40, 43, 52, 54). Thus, we next explored a role for PLC and PKC in negative regulation of ENaC by ET-1. These paired experiments were similar to those in Fig. 2, where the effects of ET-1 on ENaC were quantified in cell-attached patches formed on the apical plasma membrane of principal cells in freshly isolated, split-open rat collecting ducts. As summarized in Fig. 3A, inhibiting PLC with 10 nM U73122 increased ENaC open probability from 0.57 ± 0.09 to 0.76 ± 0.06. This increase in activity was expected for as we demonstrated previously, inhibiting PLC results in increased channel activity due to the loss of tonic downregulation of ENaC activity by purinergic signaling promoting PIP$_2$ metabolism via G protein-coupled metabotropic P2Y receptors (35). Nevertheless, subsequent treatment with ET-1 decreased ENaC open probability in the continued presence of PLC inhibition. Open probability significantly decreased from 0.76 ± 0.06 to 0.33 ± 0.05 following ET-1 application in the presence of U73122 ($n = 7$; $N = 5$ rats). Similarly, ET-1 decreased ENaC open probability in the presence of inhibited PKC. As summarized in Fig. 3B (see also supplemental Fig. 1S), ET-1 rapidly decreased ENaC $P_o$ from 0.69 ± 0.06 to 0.19 ± 0.05 in collecting ducts pretreated for 1–2 h with 200 nM PKC inhibitor Ro 31–8220 ($n = 6$; $N = 6$ rats). These results demonstrate that ET-1 is capable of decreasing ENaC activity in the absence of PLC and PKC signaling excluding this phospholipase and kinase from the ETB to ENaC transduction cascade in the rat collecting duct.

To test possible involvement of src tyrosine kinases, we used the broadspectrum src family tyrosine kinase inhibitor PP2. As summarized in Fig. 3C, treatment with 1 μM PP2 had a modest stimulatory effect on ENaC increasing open probability from 0.49 ± 0.05 to 0.61 ± 0.04. This small increase in activity was rapid and may reflect loss of tonic downregulation of ENaC by endogenous src signaling as reported previously for recombinant ENaC expressed in NIH 3T3 fibroblast cells (21). Importantly, subsequent application of ET-1 in the continued presence of PP2 failed to decrease ENaC activity. ENaC open probability was 0.61 ± 0.04 before and 0.57 ± 0.05 after ET-1 application in the presence of inhibited src tyrosine kinases ($n = 7$; $N = 5$ rats). These results are consistent with src signaling playing a dominant role in ET-1 regulation of ENaC activity in this ex vivo preparation.

Activation of MAPK1/2 signaling is necessary for regulation of ENaC by ET-1. Activation of c-src by ET-1 via ETB is known to stimulate MAPK1/2 signaling in epithelial cells (28, 39). Thus, we next tested a role for MAPK1/2 signaling in regulation of ENaC by ET-1 in collecting duct principal cells. Figure 4A summarizes the effect of ET-1 on ENaC activity when MEK1/2 is inhibited with 10 μM PD98059. As apparent from the summary graph, inhibiting MAPK1/2 signaling completely abolishes ET-1 actions on ENaC. ENaC open probability was 0.51 ± 0.06 before and 0.53 ± 0.09 after inhibiting MEK1/2 with PD98059, and 0.49 ± 0.08 following subsequent ET-1 treatment in the continued presence of PD98059 ($n = 8$; 10 rats).
N = 4 rats). These results define a critical role for MAPK1/2 signaling in ET-1-mediated regulation of ENaC. To further strengthen this conclusion, for this is the first time, we are aware of, MAPK1/2 signaling has been implicated in ET-1 regulation of ENaC, we used a structurally distinct inhibitor of MEK1/2, U0126. As shown in the summary graph of Fig. 4B, similarly to PD98059, inhibiting MAPK1/2 signaling with 5 μM U0126 completely abolished ET-1 actions on ENaC. ENaC open probability was 0.62 ± 0.08 before and 0.54 ± 0.04 after inhibiting MAPK1/2 signaling with U0126, and 0.55 ± 0.06 following ET-1 treatment in the continued presence of U0126 (n = 10; N = 4 rats). These results support the conclusion that MAPK1/2 is central to negative regulation of ENaC by ET-1 in the distal nephron.

ET-1 through basolateral ETB receptors activates MAPK1/2 signaling in a src-dependent manner. While ET-1 has been reported to activate src and MAPK1/2 signaling in several cell types, we are unaware of any study specifically demonstrating...
this to be the case in collecting duct principal cells. Thus, we asked whether ET-1 activates MAPK1/2 signaling in a src-dependent manner in principal cells. Due to the limited amount of tissue available from isolated collecting ducts, we performed these experiments in the well-characterized mouse mpkCCDc14 principal cell line. These cells, which contain the mineralocorticoid receptor and ENaC, form polarized monolayers having robust aldosterone- and amiloride-sensitive Na\(^+\)/H\(^+\) reabsorption mediated by ENaC \(3, 35\). To confirm that the response of ENaC to ET-1 in mpkCCDc14 cells is similar to that in isolated, split-open rat collecting ducts, we performed patch-clamp experiments. Figure 5A shows typical current traces of ENaC in cell-attached patches on the apical membrane of mpkCCDc14 cells before and after application of 20 nM ET-1. As is clear in this representative experiment and the summary graph of like paired experiments shown in Fig. 5B, ET-1, similar to its effects on native rat collecting duct cells, rapidly decreases channel open probability. ENaC open probability was 0.28 ± 0.05 before and 0.04 ± 0.01 after addition of ET-1 \((n = 7)\).

Figure 5C contains composite Western blots from two representative experiments \((n = 4)\) used to test ET-1 activation of MAPK1/2 signaling in mpkCCDc14 cells. These blots were
probed with anti-phospho-MAPK1/2 (top) and anti-MAPK1/2 (bottom) antibodies and contain lysate harvested from cells treated with vehicle (control) and 20 nM ET-1 applied to either the apical or basolateral membrane (for 30 min) in the absence and presence of the src kinase inhibitor PP2. PMA was used as a positive control. As summarized in SD, ET-1 significantly increased MAPK1/2 phosphorylation (activation) via basolateral endothelin receptors in a src kinase-dependent manner. These results are consistent with the electrophysiology results from isolated, split-open rat collecting ducts.

DISCUSSION

The current results confirm what has been suspected, but never definitively shown, for ET-1 regulation of ENaC in the mammalian distal nephron, as well as, provide novel information extending understanding of the cellular mechanisms underpinning this regulation. Importantly, these are the first studies directly demonstrating that indeed ET-1 negatively regulates ENaC in the mammalian collecting duct. We demonstrate that ET-1 dynamically decreases ENaC open probability via activating basolateral ETB receptors and subsequently stimulating src tyrosine kinases. This confirms for the mammalian kidney what had been demonstrated previously for ET-1 regulation of ENaC in cultured, amphibian kidney cells and for regulation of recombinant ENaC overexpressed in nonpolarized cells (16, 21). We extend understanding of this regulation by demonstrating that PLC and PKC do not play a role in the acute effects of ET-1 on ENaC mediated by the ETB receptor but that MAPK1/2 signaling downstream of src kinases is necessary for regulation.

The finding that ET-1 rapidly decreases ENaC open probability in principal cells of the mammalian collecting duct is significant. This finding supports a potential causative role for decreases in ENaC activity driving natriuresis in response to ET-1. This is consistent with findings in the isolated perfused rat kidney and microperforated rabbit CCT, respectively, where nanomolar concentrations of ET-1 increase renal Na+ excretion and hyperpolarize the luminal plasma membrane in an amiloride-sensitive manner while increasing the resistance of this membrane (15, 27). In agreement are also studies utilizing in vivo administration of endothelin receptor-specific agonists and antagonists. For instance, ET-1 administered in the presence of specific ETA but not ETB receptor inhibition uncovers a natriuretic effect and specific ETB receptor agonists increase urinary Na+ excretion (6, 7, 10, 55).

The current findings are also consistent with a pathogenic role for the loss of normal ENaC regulation by ET-1 in the elevated blood pressure associated with collecting duct-specific knockout of ET-1 and ETB receptors in mice and the spontaneous null mutation of ETB in rescued sl/sl rats (2, 18, 20, 33, 34, 42). These models of hypertension are associated with salt sensitivity and an impaired ability to excrete a Na+ load. Moreover, when tested, amiloride ameliorates elevations in a similar conclusion was reached in a prior study of ET-1 and ETB receptors support this idea (2, 20, 42). Our results while being consistent with such tone are not definitive in this regard. With src inhibition, we see a slight but marked increase in ENaC activity. In contrast, inhibition of ETB receptors in the absence of other stimulus fails to affect basal ENaC activity. We must be careful to recognize that in our preparation, tubules were isolated from rats fed a low-Na+ diet, which initially sets ENaC to a high activity, and the amount of collecting duct tissue available for endogenous ET-1 production is limited compared with the volume of the bath solution. Therefore, endogenous ET-1 actions via ETB may have been, partially, washed out or suppressed by feeding. Clearly, more investigation is required to definitively demonstrate direct control of ENaC in the mammalian collecting duct by endogenously derived ET-1.

The current findings have greater impact when considering cellular signaling cascades possibly coupling ET-1 to ENaC. We find that src kinases and MAPK1/2 signaling are necessary for ET-1 to negatively regulate ENaC via basolateral ETB receptors. In contrast, PLC and PKC do not appear to play a particular role in acute regulation of ENaC by ET-1. From this, we can propose that, at least one, mechanism of regulation likely involves basolateral ETB coupling to ENaC possibly via Gi. Our rationale is that ETB receptors are G protein-coupled receptors capable of interacting with both Gq and Gi. ET-1 signaling through either of these G proteins can ultimately activate src tyrosine kinases in many different types of cells, including epithelial cells (11, 24, 39). However, ET-1 activation of PLC and PKC is critical to stimulating src kinases in response to Gi but not Gq signaling. We expect that additional investigation will confirm this. Similar to stimulating src kinases, ENaC signaling is also recognized to activate MAPK1/2 signaling in many types of cells, including epithelial cells. This ET-1-dependent stimulation of MAPK1/2 is src kinase dependent (11, 24, 28, 39, 40, 52). The current results place MAPK1/2 signaling between src kinases and ENaC during ET-1 regulation.

MAPK1/2 signaling ultimately through actions on the nucleus modulates gene expression. Such nuclear actions, although, are unlikely to be involved in regulation of ENaC by ET-1 as observed here. This is so, because ET-1 decreases ENaC open probability rapidly over a time course of a few minutes. This seemingly prohibits changes in gene expression playing a major role leaving ET-1 via MAPK1/2 signaling most likely modulating ENaC via a posttranslation mechanism. A similar conclusion was reached in a prior study of ET-1 regulation of ENaC by src kinases in an expression system (21). Our discovery that MAPK1/2 is positioned between src kinases and ENaC in the ET-1 transduction cascade and the finding that MAPK1/2 can directly and dynamically phosphor-
ylate ENaC to influence channel activity (9, 44) allow us to refine this conclusion. MAPK1/2 signaling decreases ENaC N and P0 (4, 9, 14, 44). A reasonable conclusion now is that ET-1 signaling through first src kinase and then MAPK1/2 modifies either ENaC or a protein closely associated with ENaC to rapidly change channel activity.

As a concluding remark, we note that the current results provide compelling support for ET-1 dynamically regulating ENaC open probability in native mammalian collecting duct principal cells through a signaling pathway including src kinases and MAPK1/2. Other signaling pathways have also been implicated in ET-1-dependent regulation of distal nephron Na+ transport likely mediated by ENaC. For instance, Schneider and colleagues (42) provide strong evidence that nitric oxide signaling plays a role in ET-1 regulation of distal nephron Na+ handling. Similarly, modification of medullary blood flow and increased production of locally derived signaling factors, including P450 metabolites, in response to ET-1 have been implicated in ET-1-dependent regulation of collecting duct Na+ transport (5, 12, 13). The relative physiological importance and the relationship between these possible mechanisms of ET-1 control of ENaC remain unclear. The current results, although, provide convincing evidence that ET-1 can have significant effects on collecting duct Na+ transport in the mammalian kidney by directly affecting ENaC activity via principal cell ETB receptors and src kinase-MAPK1/2 signaling.

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