Ablation of Transient Receptor Potential Vanilloid 1 Abolishes Endothelin-Induced Increases in Afferent Renal Nerve Activity
Mechanisms and Functional Significance
Chaoqin Xie, Donna H. Wang

Abstract—Endothelin 1 (ET-1) and its receptors, ETA and ETB, play important roles in regulating renal function and blood pressure, and these components are expressed in sensory nerves. Activation of transient receptor potential vanilloid (TRPV) 1 channels expressed in sensory nerves innervating the renal pelvis enhances afferent nerve activity (ARNA), diuresis, and natriuresis. We tested the hypothesis that ET-1 increases ARNA via activation of ETB, whereas ETA counterbalances ETB in wild-type (WT) but not TRPV1–null mutant mice. ET-1 alone or with BQ123, an ETA antagonist, perfused into the left renal pelvis increased ipsilateral ARNA in WT but not in TRPV1–null mutant mice, and ARNA increases were greater in the latter. [Ala1, 3,11,15]-endothelin 1, an ETB agonist, increased ARNA that was greater than that induced by ET-1 in WT mice only. [Ala1, 3,11,15]-endothelin 1–induced increases in ARNA were abolished by chelerythrine, a protein kinase C inhibitor, but not by H89, a protein kinase A inhibitor. Chelerythrine, H89, and BQ788, an ETB antagonist, did not affect ARNA triggered by capsaicin in WT mice. Substance P release from the renal pelvis was increased by [Ala1, 3,11,15]-endothelin 1 in WT mice only, and the increase was abolished by chelerythrine but not by H89. Chelerythrine, H89, and BQ788 did not affect capsaicin-induced substance P release. Our data show that ET1 increases ARNA via activation of ETB, whereas ETA counterbalances ETB in WT but not in TRPV1-null mutant mice, suggesting that TRPV1 mediates ETB-dependent increases in ARNA, diuresis, and natriuresis possibly via the protein kinase C pathway. (Hypertension. 2009;54:1298-1305.)

Key Words: TRPV1 ■ ET-1 ■ ETB receptors ■ PKC ■ afferent renal nerve activity

The transient receptor potential vanilloid type 1 (TRPV1) channel is mainly expressed in sensory nerves of unmyelinated C-fibers or thinly myelinated Aδ-fibers that innervate the cardiovascular and kidney tissues. Activation of TRPV1 causes release of a variety of sensory neuropeptides, including substance P (SP) and calcitonin gene-related peptide, which have profound effects on the modulation of cardiovascular and renal function (Figure 1).1–3 For example, the renal pelvis is densely innervated by TRPV1-positive sensory nerves.4 Agonist-induced activation of TRPV1 expressed in the unilateral renal pelvis leads to increases in ipsilateral afferent renal nerve activity (ARNA) and contralateral urinary sodium and water excretion via the renorenal reflex, which can be abolished by renal denervation (Figure 1).5,6 Hypertonic saline perfusion of the renal pelvis or increased renal pelvis pressure as a mean of mechanostimulation may activate TRPV1, leading to increased ARNA and diuresis and natriuresis, a sequence of events that depends on TRPV1-mediated SP release and subsequent SP activation of the neurokinin 1 (NK1) receptors expressed in sensory nerves.5–8 Given the important role of TRPV1 in mediating renal function, deletion of TRPV1 results in the loss of protection against renal injury.9 Indeed, ablation of TRPV1 exaggerates renal functional and tissue damage induced by deoxycorticosterone acetate-salt hypertension.9

Endothelin 1 (ET-1), a potent vasoconstrictor, is found as a neurotransmitter in primary afferent neurons and their nerve terminals.10 Immunocytochemistry results show that its receptor subtypes, endothelin A (ETA) and endothelin B (ETB) receptors, are present in medium- and large-sized cell bodies of human trigeminal ganglia.11 In rats, ET-1 perfusion into the renal pelvis increases ARNA via activation of ETB when a high-salt diet is given and decreases ARNA via activation of ETA in the face of salt deprivation.12 Colocalization of TRPV1 and ETA has been found in a subpopulation of primary sensory neurons, whereas ET-1 sensitizes capsaicin (CAP)-induced TRPV1 current in this population of neurons.13 In HEK293 cells, ET-1–induced potentiation of TRPV1 action depends on activation of ETA but not ETB via a protein kinase C (PKC)–dependent pathway.13 Moreover,
activation of TRPV1 via the ETA-PKC pathway contributes to ET-1–induced thermal hyperalgesia.\textsuperscript{14}

Despite the fact that TRPV1 and the components of the ET-1 system are coexpressed in primary afferent nerves and the fact that activation of ETB expressed in renal tubules mediates ET-1–induced diuresis and natriuresis,\textsuperscript{15–18} it is unknown whether TRPV1 plays a role in ET-1–induced changes in renal function. Understanding the interaction between TRPV1 and the ET1 system may provide insight into the mechanism underlying ET-1–mediated pathological changes in diseases and may identify downstream targets for drug development. The present study tests the following hypotheses: (1) ET-1 perfusion into the renal pelvis increases ARNA in wild-type (WT) but not TRPV1-null mutant (TRPV1\textsuperscript{−/−}) mice; (2) ET1-induced increases in ARNA are mediated by activation of ETB, whereas ETA plays a countercurrent role in WT but not in TRPV1\textsuperscript{−/−} mice; and (3) ETB-mediated increases in ARNA are via activation of the PKC but not the protein kinase A pathway in WT but not TRPV1\textsuperscript{−/−} mice (Figure 1).

**Methods**

**In Vivo Study**

All of the experimental protocols were approved by the institutional animal care and use committee of Michigan State University. Ten-week–old male TRPV1\textsuperscript{−/−} strain B6.129S4-TRPV1\textsuperscript{m12ul} and C57BL/6 mice (WT; Jackson Laboratories, Bar Harbor, ME) were used in the experiments (total 170 mice). Mice were anesthetized by IP administration of pentobarbital sodium at 50 mg/kg. A phycocyanin 10 catheter was inserted into the left carotid artery for monitoring mean arterial pressure with a Statham 231D pressure transducer coupled to a Gould 2400s recorder (Gould Instrument System). Two MD-2000 microdialysis tubes (ID 0.18/OD 0.22 mm; BASi) were bonded together and placed inside the left ureter via a midline incision. One of the tubes, of which the tip extended 1 to 2 mm into the renal pelvis compared with the other, was used for drug perfusion, whereas the other was used for urine draining. The perfusion was performed at a rate of 20 µL/min, at which time the pelvis pressure did not change, and the drugs were perfused into the renal pelvis for 3 minutes for ARNA measurement.\textsuperscript{6} Mice were given the following treatments (n=5 to 6 in each group): (1) 10\textsuperscript{−7} M or 10\textsuperscript{−7} M ET-1 (Sigma-Aldrich) perfused into the left renal pelvis of WT mice; (2) 10\textsuperscript{−7} M ET-1 with or without 5×10\textsuperscript{−6} M BQ123 (Sigma-Aldrich), a selective ETA antagonist, given into the renal pelvis in WT and TRPV1\textsuperscript{−/−} mice; (3) 10\textsuperscript{−7} M [Ala1,3,11,15]-ET-1 (4 Ala-ET-1; Sigma-Aldrich), a selective ETB agonist, given into the renal pelvis of WT and TRPV1\textsuperscript{−/−} mice; (4) 4×10\textsuperscript{−6} M BQ788 (Sigma-Aldrich), a selective ETB antagonist, with 4×10\textsuperscript{−6} M CAP administrated into the renal pelvis of WT mice; (5) 10\textsuperscript{−5} M chelerythrine (CHE; Tocris Bioscience), a PKC inhibitor, given with 10\textsuperscript{−7} M 4 Ala-ET1 or 4×10\textsuperscript{−6} M CAP into the renal pelvis of WT mice.

The renal nerves were isolated at the angle between the abdominal aorta and the renal artery via a left flank incision with the use of a stereoscopic disecting microscope. The nerves were placed on the bipolar platinum electrodes to record multifiber nerve activity. The electrode was connected to a high-impedance probe (HIP-511; Grass Instruments). The signals were amplified ×20,000, filtered with a high-frequency cutoff at 1000 Hz and a low-frequency cutoff at 100 Hz by a Grass model P511 AC amplifier, and recorded by a Gould 2400s recorder (Gould Instrument System). After the renal nerve surgery. The basal value of ARNA was recorded 10 minutes before the treatment, and the recovery value of ARNA was recorded 10 minutes after the treatment. The postmortem renal nerve activity recorded as the background of renal nerve activity was subtracted from all of the values. Average responses of ARNA were used for analysis and ARNA was expressed in the percent of its basal value.\textsuperscript{6,19}

**In Vitro Study**

**SP Release From the Renal Pelvis**

The renal pelvis wall was removed from anesthetized mice and incubated in 37°C HEPES buffer (HEPES, 25.0 mmol/L; NaCl, 135.0 mmol/L; KCl, 3.5 mmol/L; CaCl\textsubscript{2}, 2.5 mmol/L; MgCl\textsubscript{2}, 1.0 mmol/L; d-glucose, 3.3 mmol/L; and ascorbic acid, 0.1 mmol/L; pH 7.45) with 95% O\textsubscript{2}/5% CO\textsubscript{2}. The pelvis was incubated with drugs for 1 hour after it was equilibrated in the HEPES buffer for 30 minutes. The incubation solution was collected and measured by radioimmunoassay (rat radioimmunoassay kits; Peninsula Laboratories Inc), as described previously, and the SP concentration was normalized by kidney weight.\textsuperscript{10}

**Immunofluorescence Staining**

Frozen kidney sections obtained from WT and TRPV1\textsuperscript{−/−} mice were fixed with formalin for 15 minutes and washed with PBS-0.01% Tween 20 for 5 minutes. After blocking nonspecific binding sites with 5% normal donkey serum for 30 minutes, tissues were incubated with goat anti-TRPV1 (1:100; Santa Cruz), rabbit anti-ETB receptor (1:200; Santa Cruz), or rabbit anti-ETA receptor (1:200; Santa Cruz) diluted with 5% normal donkey serum at 4°C overnight, whereas negative controls were incubated with serum overnight only. The sections were rinsed with PBS-0.01% Tween 20 and incubated with donkey-antigoat fluorescein isothiocyanate–labeled IgG or donkey-antirabbit Cy3-labeled IgG for 1 hour at room temperature. The sections were washed, dehydrated with 95% and 100% ethanol, and covered with antifade mounting medium and
coverslips. In the double immunofluorescence staining study, the sections were incubated with the mixture of primary antibodies overnight at 4°C and then incubated with the mixture of secondary antibodies after rinse.

**Statistical Analysis**

All of the values were expressed as mean ± SE. The differences of ARNA among groups were analyzed using 1-way ANOVA, followed by the Tukey-Kramer multiple comparison tests. The unpaired Student t test was used to determine the difference of SP levels between groups. Differences were considered statistically significant at P < 0.05.

**Results**

There was no difference in the body weight between WT (29.4 ± 0.5 g) and TRPV1−/− (28.8 ± 0.6 g) mice in all of the groups. The mean arterial pressure between WT (95 ± 6 mm Hg) and TRPV1−/− (97 ± 4 mm Hg) mice was not statistically different, and it maintained at these levels before, during, and after the treatments.

**In Vivo Results**

To examine the role of ET-1 in the regulation of ARNA in WT and TRPV1−/− mice, ET-1 was perfused into the left renal pelvis. Ipsilateral ARNA was increased by ET-1 perfusion at the concentrations of 10−8 M (119 ± 9%; P < 0.05) or 10−7 M (136 ± 11%; P < 0.01) in WT mice (Figure 2). In contrast, ARNA was not altered (Figure 2; 99 ± 11%; P > 0.05), even when the higher dose of ET-1 (10−7 M) was perfused into the left renal pelvis in TRPV1−/− mice.

To examine the role of the ETA receptor in ET-1–induced increases in ARNA, an ETA receptor antagonist, BQ123, was perfused into the left renal pelvis with or without 10−7 M ET-1. BQ123 alone did not change ARNA in WT (Figure 3;
106±7%; P>0.05) or TRPV1−/− (98±8%; P>0.05) mice. BQ123 combined with ET-1 perfusion into the left renal pelvis increased ARNA to 166±18% in WT mice (Figure 3; P<0.01), but it had no effect on ARNA in TRPV1−/− mice (105±13%; P>0.05). Furthermore, the increase in ARNA induced by BQ123 plus 10−7 M ET-1 (Figure 3; 166±18%) was higher than that induced by 10−7 M ET-1 alone (Figure 2; 136±11%) in WT mice (P<0.05).

To examine the role of the ETB receptor in the regulation of ARNA in WT and TRPV1−/− mice, an ETB receptor agonist, 4 Ala-ET-1, was perfused into the left renal pelvis. 4 Ala-ET-1 increased ARNA in WT mice (Figure 4; 177±35%; P<0.01) but not in TRPV1−/− mice (106±18%; P>0.05). Moreover, the increase in ARNA induced by 10−7 M 4 Ala-ET-1 (Figure 4; 177±35%) was higher than that induced by 10−7 M ET-1 alone (Figure 2; 136±11%) in WT mice (P<0.05).

To determine whether the ETB receptor mediates CAP-induced increases in ARNA, an ETB receptor antagonist, BQ788, was perfused into the left renal pelvis with or without CAP. CAP increased ARNA (Figure 4; 201±21%; P<0.01) in WT mice. CAP combined with BQ788 also increased ARNA (202±23%; P<0.01) in WT mice, but the magnitude of the increases in ARNA was the same between CAP alone and CAP plus BQ788 (P>0.05).

To examine the role of PKC and PKA in ETB-induced increases in ARNA in WT mice, PKC or PKA inhibitors were perfused into the left renal pelvis with or without the ETB receptor agonist. The PKC inhibitor, CHE, perfused alone did not alter ARNA (Figure 5; 102±9%; P>0.05) in WT mice. However, CHE abolished 4 Ala-ET-1–induced increases in ARNA (Figure 5; 109±4%; P>0.05) in WT mice. In contrast, CHE had no effect on CAP-induced increases

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**Figure 4.** Ipsilateral ARNA response to 4 Ala-ET-1, an ETB receptor agonist, perfused into the left renal pelvis in WT or TRPV1−/− mice or response to CAP, a TRPV1 receptor agonist, with or without BQ788, a selective ETB receptor antagonist, perfused into the left renal pelvis in WT mice. A, ARNA at the basal, response to 4 Ala-ET-1 or CAP with or without BQ788 or recovery in WT or TRPV1−/− mice. B, Representative recording of ARNA. n=5 to 6 in each group. **P<0.01 vs basal values of each group.

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**Figure 5.** Ipsilateral ARNA response to CHE, a PKC inhibitor, with or without 4 Ala-ET-1, an ETB receptor agonist, or CAP, a TRPV1 receptor agonist, perfused into the left renal pelvis in WT mice. A, ARNA at the basal, response to CHE with or without 4 Ala-ET-1 or CAP, or recovery in WT mice. B, Representative recording of ARNA. n=5 to 6 in each group. **P<0.01 vs basal values of each group.
in ARNA (200±23%; P<0.01). The PKA inhibitor H89 perfused alone did not alter ARNA (Figure 6; 105±11%; P>0.05) in WT mice. Neither 4 Ala-ET-1- nor CAP-induced increases in ARNA were affected by H89 (Figure 6; 173±19% and 199±14%, respectively; P<0.01).

In Vitro Results

Radioimmunoassay was used to determine the level of SP released from the renal pelvis incubated in vitro (Figure 7). The SP levels were not different between WT and TRPV1−/− mice at the baseline (0.57±0.18 pg/g per minute; P>0.05), or treated with BQ123 combined with ET-1 (0.72±0.23 versus 0.67±0.11 pg/g per minute; P>0.05) but not in TRPV1−/− (0.58±0.13 versus 0.57±0.18 pg/g per minute; P>0.05) mice compared with their respective base-lines. 4 Ala-ET-1-induced SP release in WT mice was abolished by CHE (0.83±0.10 versus 0.58±0.07 pg/g per minute; P<0.05) but not by H89 (0.83±0.10 versus 0.81±0.08 pg/g per minute; P>0.05). CAP increased SP release (0.84±0.26 versus 0.57±0.15 pg/g per minute; P<0.05) compared with the baseline in WT mice, and CAP-induced increases in SP release in WT mice were not affected by BQ788 (0.85±0.16 pg/g per minute), CHE (0.92±0.11 pg/g per minute), or H89 (0.90±0.21 pg/g per minute).

Immunofluorescence staining was performed to determine the expression and coexpression of TRPV1, ETA, and ETB receptors in the renal pelvis of WT and TRPV−/− mice. TRPV1-positive nerve fibers were detected in the epithelial layer in the pelvis wall of WT but not TRPV1−/− mice (Figure 8). ETA staining was not observed in the renal pelvis wall in either WT or TRPV1−/− mice. In contrast, ETB was expressed in nerve fibers innervating the epithelial layer of the renal pelvis in both WT and TRPV−/− mice (Figure 8). Moreover, ETB colocalized with TRPV1 in the nerve fibers, innervating the pelvis wall in WT mice.

Discussion

It has been reported that urine ET-1 levels are much higher than that of plasma.23 In normal rats, the plasma ET-1 level is at 28±3 fmol/mL, whereas the ET-1 concentration in the urine is ≈4.7±0.3 pmol/24 hours and in the kidney tissue, 2.6 fmol/mg of protein.24,25 Evidence shows that little circulating ET-1 is excreted into the urine, and the most urinary ET is renal in origin.26 The preparation used in the present study, namely, renal pelvis perfusion, allows for renal afferent nerve exposure to perfused drugs, similar to urine. It has been shown that affinity of 4 Ala-ET-1 binding to ETB is 1700 times higher than that binding to ETA.27 ET-1 induces vasoconstriction with EC50 10−9 M, which is abolished by the ETA antagonist, whereas 4 Ala-ET-1 with a concentration ≤10−6 M has no effect.28 These data indicate that 4 Ala-ET-1 is unlikely to activate the ETA receptor at a concentration <10−6 M.27,28 Furthermore, ET-1 incubated with cultured

Figure 7. Levels of SP released into the incubation buffer from the isolated renal pelvis in WT or TRPV1−/− mice. n=7 to 8 in each group. *P<0.05 vs control groups. #P<0.05 vs 4 Ala-ET-1-treated group.

Figure 6. Ipsilateral ARNA response to H89, a PKA inhibitor, with or without 4 Ala-ET-1, an ETB receptor agonist, or CAP, a TRPV1 receptor agonist, perfused into the left renal pelvis in WT mice. A, ARNA at the basal value, response to H89 with or without 4 Ala-ET-1 or CAP, or recovery in WT mice. B, Representative recording of ARNA. n=5 to 6 in each group. **P<0.01 vs basal values of each group.
juxtaglomerular cells (IC$_{50}$ $3 \times 10^{-9}$ M) inhibits renin release, an effect that is mimicked by $10^{-6}$ M 4 Ala-ET-1 but not affected by BQ123.29 Taken together, these results provide rationales for the selection of doses of ET-1 and 4 Ala-ET-1 used for renal pelvis perfusion that ensures effectiveness and avoids nonspecific binding.

All of the components of the ET-1 system, including ET-1, ETA, and ETB, have been found to express in the sensory nervous system.11,30 Ablation of ET-1 leads to an elevation in the resting renal sympathetic nerve activity (RSNA), and an attenuation in hypercapnia-induced increases in RSNA,31 suggesting that endogenous ET-1 governs the basal and reflex controls of RSNA. Moreover, ET-1 injected into the hind paw of rats induces pain that is transmitted by sensory nerve fibers expressing ETA and ETB, which play distinct roles in mediating the pain pathway.32,33 The ETA-PKC pathway contributes to ET-1–induced thermal hyperalgesia.14 In contrast, the ETB-PKC pathway contributes to ET-1–mediated, mechanical-induced hypernociception.33 It has been shown that activation of ETA increases RSNA, whereas activation of ETB inhibits RSNA.34,35 In contrast, activation of ET-1 expressed in the renal pelvis suppresses ARNA in low-salt–treated rats, whereas activation of ETB enhances ARNA in high-salt–fed rats.12,36 These studies indicate that the ET system also plays a key role in the control of sensory nerve function and function of organs/tissues innervated by sensory nerves. Our data show that ET-1 perfused into the renal pelvis increases ARNA in WT but not in TRPV1$^{-/-}$ mice, indicating that ET-1–induced increases in ARNA require the presence or activation of TRPV1. In addition, activation of ETB increases ARNA, and inhibition of ETA potentiates ET-1–induced increases in ARNA in WT but not in TRPV1$^{-/-}$ mice. These results indicate that TRPV1 mediates ETB–induced increases and ETA–induced suppression of ARNA.

ET-1 may modulate renal sodium and urine excretion,17,37,38 and this effect may be mediated by interaction with renal nerves.37 An ETA and ETB antagonist, bosentan, given into the kidney causes a reduction in urine flow in both normal and hypertensive rats, whereas bosentan-mediated decreases in renal excretory function are abolished after renal denervation,35 indicating a role for ET-1 receptors expressed in renal nerves in the regulation of renal function. Furthermore, decreased expressions of ET-1, ETA, and ETB have been found in the kidney of spontaneously hypertensive rats, where downregulation of ETB may contribute to excessive sodium retention in spontaneously hypertensive rats.37 Indeed, ETB in the kidney is involved in ET-1–induced inhibitory effects on antiurea.39 The ETB agonist, sarafotoxin, given into the kidney causes enhanced diuresis in anesthetized dogs, whereas excretion of sodium and the glomerular filtration rate remain unchanged.38 Activation of ARNA also results in diuresis and natriuresis.40 It has been shown that increased renal pelvis perfusion pressure leads to activation of ipsilateral ARNA, which causes an inhibitory renal sympathetic reflex and leads to diuresis and natriuresis via suppression of contralateral renal sympathetic nerve activity.40,41 Our previous data show that activation of TRPV1 by CAP perfused into the unilateral renal pelvis leads to activation of...
Ipsilateral ARNA and bilateral diuresis and natriuresis via the renorenal reflex.5,6 Our data in the present study show that activation of ETB increases ARNA in WT but not in TRPV1−/− mice, whereas blockade of ETB has no effect on CAP-induced increases in ARNA in WT mice. Taken together, these data indicate that TRPV1 mediates ETB-dependent increases in ARNA induced by ET-1 and thereby contributes to ETB-induced increases in sodium and water excretion.

The primary sequence of TRPV1 contains many putative phosphorylation sites, and PKC- and PKA-mediated phosphorylation of TRPV1 is critical for its functions.14,42–44 PKC-mediated phosphorylation of TRPV1 has been shown to increase TRPV1-mediated effects.14,42–44 Activation of PKC potentiates or sensitizes TRPV1 responses to heat, protons, or its agonists and increases TRPV1-mediated SP and calcitonin gene-related peptide release.43,44 PKC also participates in ET-1–induced pain sensation.14,33 Previous data show that activation of ETB by ET-1 leads to hypernociception induced by mechanical stimulation via activation of the PKC pathway.33 Our data in the present study show that the PKC inhibitor but not the PKA inhibitor perfused into the renal pelvis abolishes ETB-induced increases in ARNA, whereas CAP-induced increases in ARNA are not affected by the PKC or PKA inhibitors. These data indicate that the ETB-PKC pathway mediates the ET-1 effect on activation of TRPV1 and ARNA.

SP release has been shown to be regulated by several factors,42,43 and mechanoinduced increases in ARNA and SP release are abolished when NK1 receptors45 or TRPV1 channels8 are blocked. Our previous data show that TRPV1-induced increases in ARNA and renal excretory function depend on NK1 receptor activation by SP on its release.6 Data in the present study show that SP release is elevated when ETB is activated in WT but not in TRPV1−/− mice, which is abolished by the PKC but not PKA inhibitors, whereas neither the PKC nor PKA inhibitors affect CAP-induced increases in SP release. Taken together, these data indicate that TRPV1 mediates ETB-induced increases in SP release that are PKC dependent.

TRPV1 is mainly expressed in small- and medium-sized neurons in dorsal root and trigeminal ganglia and is transported to both the central and peripheral terminals of these primary afferent neurons.1 TRPV1 has been found primarily in unmyelinated C-fibers or thinly myelinated Aδ-fibers in the periphery.1 TRPV1-containing sensory nerves heavily innervate the upper ureter, the pelvis wall presenting in between uroepithelial and smooth muscle layer, and in the tubular cells of distal tubules and collecting ducts in the cortex and medulla.46,8 The data in the present study show that TRPV1 expresses in nerve fibers innervating the epithelial layer of the renal pelvis wall in WT but not in TRPV1−/− mice. Similarly, both ETA and ETB have been found in medium- to large-sized neurons in the trigeminal ganglia.11 In the kidney, ETA that mainly locates in glomeruli and renal vasculature15,46,47 mediates the cortical and medullary vasoconstriction.15,46 ETB that is mainly distributed in the renal tubules contributes to ET-1-induced water and sodium excretion.15,46 Our immunofluorescence data show that ETB, colocalized with TRPV1, expresses in nerve fibers innervating the renal pelvis wall in both WT and TRPV1−/− mice. The reason that ETA staining was undetectable is unknown at the present time. However, it is likely that the staining method was not sensitive enough for detecting low abundance of ETA expressed in the renal pelvis wall in WT and TRPV1−/− mice.15,46,47

In conclusion, the data in the present study show that deletion of TRPV1 abolishes ET-1–induced increases in ARNA and SP release that is via activation of ETB, whereas activation of ETA plays a counterbalancing role. Moreover, ETB-induced activation of TRPV1 is mediated through a PKC but not a PKA pathway. These findings indicate for the first time that TRPV1 may govern or contribute to ETB-mediated control of water and sodium excretion in the kidney.

**Perspectives**

TRPV1-containing sensory nerves, when activated, regulate diuresis and natriuresis of the kidney.5 Neonatal degeneration of this population of sensory nerves leads to increased salt sensitivity and arterial pressure.48 Similarly, ET-1 also plays an important role in regulating renal excretory function through activation of the ETB receptors.49 Collecting duct-specific knockout of the ETB receptors causes sodium retention and hypertension.18 The data in the present study indicate that TRPV1 colocalizes with ETB in sensory nerve fibers innervating the renal pelvis, mediates ETB-induced increases in ARNA, and, therefore, may contribute to ETB-governed renal excretory function that is sensory nerve dependent. The molecular interaction of ETB and TRPV1 depends on activation of the PKC pathway. On the other hand, activation of ETA conveys an inhibitory role, which counterbalances ETB stimulatory action, in the regulation of ET-1–induced increases in ARNA. Thus, it is conceivable that, in the cases when single or multiple dysfunction(s) of components in the ETA- or ETB-PKC-TRPV1 pathway in sensory nerves innervating the kidney occur, they may lead to impaired renal sensory nerve function and disturbed sodium and water homeostasis, as depicted in Figure 1.

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**Disclosures**

None.

**References**


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