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E Prostanoid-1 receptor regulates renal medullary αENaC in rats infused with angiotensin II

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ABSTRACT

E Prostanoid (EP) receptors play an important role in urinary Na^+ excretion. In the kidney, the epithelial sodium channel (ENaC) is the rate-limiting-step for Na^+ reabsorption. We hypothesized that activation of EP1/EP3 regulates the expression of ENaC in the face of renin–angiotensin–aldosterone-system (RAAS) activation. In primary cultures of inner medullary collecting duct (IMCD) cells, sulprostone (EP1 > EP3 agonist, 1 μM) and 17 Phenyl trinor (17 Pt, EP1 agonist, 10 μM) prevented the up-regulation of αENaC mRNA induced by aldosterone (10 nM). In Sprague–Dawley rats infused with angiotensin II (0.4 μg/kg/min), αENaC expression was up-regulated in renal cortex and medulla coincidently with high plasma aldosterone levels. Sulprostone and/or 17 Pt prevented this effect in renal medulla but not in cortex. Immunocytochemistry demonstrated that IMCD cells express EP1. Our results suggest that specific activation of EP1 receptor during RAAS activation antagonizes the action of aldosterone on αENaC expression in the renal medulla.

Introduction

Renal prostaglandins (PGs) participate in the maintenance of renal blood flow and the regulation of urinary salt excretion [1]. Prostaglandin E_2 (PGE_2) is the major PG produced by cyclooxygenase metabolism in the nephron and is particularly abundant in the rat kidney medulla [2,3]. PGE_2 elicits its biological effects through interactions with the E-prostanoid family of G protein-coupled cell surface receptors (EP1, EP2, EP3 and EP4). EP2 and EP4 receptors are mainly expressed in *vasa recta* and afferent arteriole [4]. EP1 is detected in the medullary collecting ducts (CD), whereas EP3 is predominantly in thick ascending limb and cortical CD [4,5].

Activation of the renin–angiotensin–aldosterone-system (RAAS) has been suggested to cause induction of PGE_2 synthesis in the renal medulla [6,7]. On the other hand, experimental and clinical evidence have shown that inhibition of PGE_2 synthesis cause salt retention in humans [7–10]. Therefore, in the kidney, the action of the RAAS could be modulated by PGE_2 acting as a buffer against the RAAS antinatriuretic effects [11].

Since Na^+ reabsorption in CD is mainly modulated by aldosterone, the activation of EP1 and/or EP3 could modify Na^+ transport proteins [12]. The rate-limiting-step for Na^+ reabsorption in the CD is the activity of the apical epithelial sodium channel (ENaC). ENaC is composed by α, β and γ subunits and among the three subunits; the production of the α subunit is the rate-limiting for assembly of the mature ENaC complexes [13]. Aldosterone is the main regulator of ENaC, increasing αENaC gene expression and the abundance of the functional channel present in plasma membrane of principal CD cells [14–16].

Although EP1 and EP3 are suggested to be expressed in CD, the mechanism through which PGE_2 inhibits Na^+ transport in the distal nephron is not well understood. Experimental data obtained in isolated rabbit cortical CD demonstrated that PGE_2 inhibits Na^+ transport. Studies with specific EP agonists suggested that the EP1 receptor activation mediates the inhibition of Na^+ transport through a Ca^{2+}-coupled mechanism [17,18,12].

We hypothesized that activation of EP1 and/or EP3 can down-regulate the expression of ENaC in the renal medulla in the context of RAAS activation. To test this hypothesis we evaluated changes of ENaC expression and plasma membrane protein abundance in primary cultures of inner medullary collecting duct (IMCD) cells incubated with RAAS effectors in the presence or absence of EP agonists; sulprostone an EP1 > EP3 agonist or 17 Phenyl trinor (17 Pt), an EP1 specific agonist. To evaluate the effect of EP1 and/or EP3 activation in the context of RAAS activation *in vivo*, male Sprague–Dawley rats were infused with angiotensin II (Ang II), Ang II + sulprostone, Ang II + 17 Pt or Ang II + SC19220 (EP1 antagonist) for 5 days. We studied the expression of ENaC subunits in the renal cortex and medulla and the effect on Na^+ excretion.

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Materials and methods

Primary cultures of inner medullary collecting duct (IMCD) cells. Primary cultures were prepared as described previously [19]. Aldosterone (Sigma, St. Louis, MO) was used at 10 nM [20], Ang II at 0.1 nM [21], sulprostone at 1 μM [18], 17 Pt and SC19220 at 10 μM [18,22]. Ethanol was used as vehicle. Immunofluorescence studies for aquaporin-2 (AQP-2) detection were performed as described previously [19]. For immunocytochemical studies, cells were fixed by immersion in Bouin's solution for 24 h followed by the same procedure described below.

RNA isolation and reverse transcription-polymerase chain reaction (RT-PCR). Total RNA was extracted from 35 mm dishes of primary cultured cells, according to the Chomczynski and Sacchi method [23] Single-stranded cDNA was synthesized from 0.3 μg of total RNA by RT-PCR using the ImProm-II™ Reverse Transcription System (Promega, Madison, WI). The same procedure was performed for kidney cortex and renal medulla (15 mg).

Quantitative real-time RT-PCR. After RT-PCR, 3 μL of RT-PCR product was heated to 95 °C for 5 min and then subjected to quantitative PCR analysis in the Mx3000P® real-time PCR system (Stratagene, La Jolla, CA) with Brilliant SYBR green and 10 mM of the appropriate primers. Primers used for RT-PCR were; αENaC: 5′-TCCTGCTTCCAGG AGAACAT-3′ and 3′-GAGCTTTGCAACTCCGTTTC-5′; βENaC: 5′-CTA CACCTACAAGGAGCTGCTAGT-3′ and 3′-CACAGCACTGTACTTGTAA GGGTTGATA-5′; γENaC: 5′-CCTCTGCTGTGGATCGCGTTCAC-3′, and 3′-CACAGCACTGTACTTGTAAGGGTTGATA-5′; EP1: 5′-CGGACATGAG GGTTGAGATT-3′ and 3′-CCCTTTCAGATCCCACTTCA-5′; EP2: 5′-AC CTTATTCGCATGCAGCTT-3′ and 3′-TTTCCTTTCGGGAAGAGGTT-5′; EP3: 5′-TGTCTAGGCTTGCTGGCTCT-3′ and 3′-TGCGTCTTGCATTGCT CTAC-5′; EP4: 5′-ATGAGCATTGAGCGCTACCT-3′ and 3′- ATGTAAGA GAAGGCGGCGTA-5′; 18S: 5′-CGACGACCCATTCGAACGTCT-3′ and 3′-GCTATTGGAGCATGGAATTACCG-5′.

IMCD cells immunoblots. After 16 h treatment, cells were lysed directly in Sample buffer (100 mM Tris–HCl, pH 6.8, 200 mM dithiothreitol, 4% SDS, 0.2% bromophenol blue, 20% glycerol). Preliminary gels were run for the entire set of a given experiment on 10% polyacrylamide/SDS gels, and stained with Coomassie blue dye to assess equality of loading. Blots were scanned, and densitometric analysis was performed using NIH Image v1.61 (US NIH, http://rsb.info.nih.gov/nih-image).

Biotinylation of membrane proteins. Biotinylation was performed as described previously [24]. After biotinylation, total protein concentration was determined and 300 μg was combined with streptavidin beads (Pierce, Rockford, IL) and incubated overnight at 4 °C. Samples were collected and subjected to Western blot analysis.

Animals. Adult male Sprague–Dawley rats (180–200 g) were housed in individual metabolic cages 12:12-h light–dark cycle with 3 days of habituation before treatment. All rats were maintained at the University Animal Care Facilities in accordance with institutional and international standards for the human care and use of laboratory animals (Animal Welfare Assurance Publication A5427-01, Office for Protection from Research Risks, Division of Animal Welfare, National Institutes of Health). Food and water were supplied at equal quantities.

Drugs and treatment. Ang II was used at 0.4 μg/kg/min (Sigma, St. Louis, MO), sulprostone at 20 μg/kg/d [25], 17 Pt at 30 μg/kg/d [26] and SC19220 at 60 μg/kg/d. EP agonists and antagonists were obtained from Cayman Chemical Co., Ann Arbor, MI, all administered by constant infusion using osmotic mini-pumps (Alzet, Cupertino, CA) implanted subcutaneously. Control rats were sham-operated. Losartan (AT1 receptor blocker) was used at 40 mg/kg/d [27] and spironolactone at 400 mg/kg/d [13]; both were administered by gavage. Systolic blood pressure was measured daily by tail-cuff method. Animals were sacrificed on day 5 of treatment and urine and blood collected.

Biochemical parameters. Serum aldosterone determination were performed by commercially available radioimmunoassay kit (Euro/DPC Ltd, Caernarfon, UK), serum and urine electrolytes were assayed using an ion selective electrolyte analyzer 9180 (Roche Diagnostic, Mannheim, Germany). Serum and urinary creatinine was measured using Creatinine Analyzer 2 (Beckman Coulter, Inc., Fullerton, CA). PGE_2 was measure using a commercial kit (Cayman Chemical Co., Ann Arbor, MI).

Renal tissue preparation and immunoblotting. Extracts from renal medulla and cortex (30 mg) were homogenized in phosphate buffer saline solution containing 250 mM sucrose, 10 mM triethanolamine, adjusted to pH 7.6. Protein concentration was determined by Bradford method (Bio-Rad, Hercules, CA). Western blotting was performed as described previously [28]. Western blots were run with the total samples in each time-period ($n = 4–8$ for each group). Selected blots are representative of each group.

Immunohistochemistry and double immunolabeling in the same tissue sections. This technique was performed as previously described [29]. After immunohistochemistry procedure, samplers were dehydrated and cleared with xylene and then coverslipped without hematoxylin counterstaining (except Fig. 4A, C and E).

Antibodies. EP1 (catalog 101740) and EP3 (catalog 101760) polyclonal antibodies were obtained from Cayman (Ann Arbor, MI). Antibodies against ENaC subunits were obtained from Chemicon International, Inc. (Temecula, CA; according catalog number; α:AB3530P, β:AB3532P and γ:AB3534P). Antibody against rabbit polyclonal anti-aquaporin-2 (AQP-2) was obtained from Chemicon International, Inc. (Temecula, CA; according catalog number AB3066).

Statistical analysis. ANOVA was used to detect differences between three or more groups. Mann–Whitney rank-sum tests were used when variances were significantly different between groups. P-value <0.05 was considered significant. Data are presented as mean ± SE. For quantitative RT-PCR and protein abundances values are normalized by the mean of the control group, which was defined as 100%.

Results

Expression of EP receptors in IMCD cells

Primary cultures of rat IMCD cells expressed aquaporin-2 (AQP-2), a principal cell specific protein (Fig. 1A). The presence of EP receptors was first evaluated by RT-PCR. We found EP1 and low levels of EP3 transcripts in IMCD cells; EP2 and EP4 mRNAs were not detected (Fig. 1B). As expected, immunoblots from total protein obtained from IMCD primary cultures confirmed the expression of EP1 and low levels of EP3 (Fig. 1C). Immunocytochemistry in IMCD cells detected the presence of EP1 but not EP3 (Fig. 1D).

EP1 receptor activation prevents the aldosterone-induced αENaC up-regulation in IMCDs cells

We evaluated the effect of aldosterone (10 nM) on the abundance of αENaC mRNA. After 16 h, aldosterone caused the up-regulation of αENaC mRNA as compared to control cells (197 ± 38% *vs.* 100 ± 15% $P < 0.05$). Spironolactone, a mineralocorticoid receptor antagonist (1 μM) blunted the up-regulation of αENaC (112 ± 36%, NS). Since reports have suggested that the activation of AT1 receptor in CD could directly up-regulate αENaC expression [13], we evaluated the effect of Ang II (0.1 nM) on αENaC mRNA abundance. As showed in Fig. 2A we observed that Ang II did not affect the αENaC mRNA abundance as compared to control (121 ± 20%, P = NS). As depicted

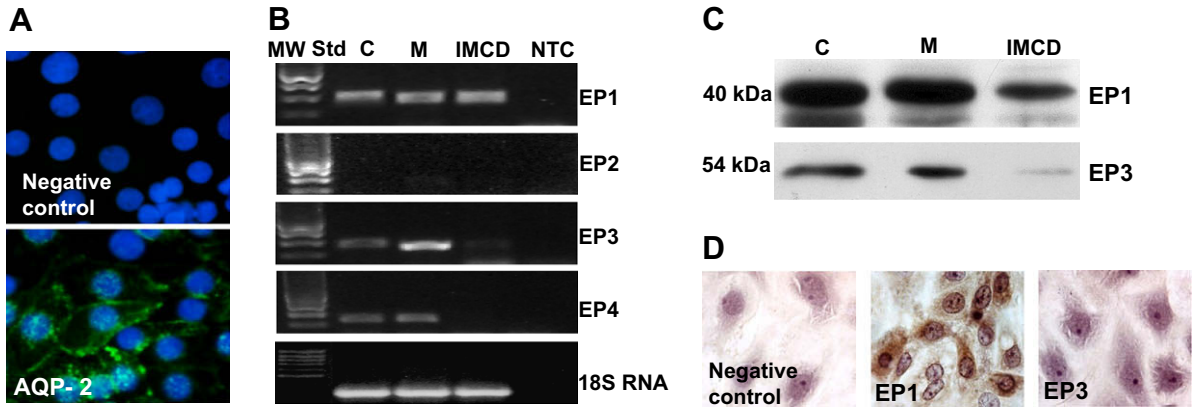

Fig. 1. (A) Immunofluorescence of primary cultures demonstrated the expression of AQP-2 a principal cell specific protein (green: AQP-2; blue: nucleus staining performed with 4′,6-diamidino-2-phenylindole, dihydrochloride (DAPI). (B) Detection of EP1, EP2, EP3 and EP4 transcripts by RT-PCR in renal cortex (C), renal medulla (M) and IMCD cells. (C) Immunoblots of EP1 and EP3 in kidney and IMCD cells. (D) Detection of EP1 and EP3 through immunocytochemistry. Negative controls were performed by omission of the primary antibody. NTC, non template control; MW Std, molecular weight standard.

in Fig. 2B, sulprostone alone did not modify αENaC mRNA abundance (122 ± 16%, P = NS). Interestingly, sulprostone and 17 Pt impaired the aldosterone-dependent up-regulation of αENaC mRNA (127 ± 27% and 125 ± 36%, respectively, P = NS). The EP1 antagonist SC19220 (10 μM) blocked this effect (175 ± 23% and 163 ± 13%, respectively, $P < 0.05$) (Fig. 2B). Immunoblotting of total protein homogenates from IMCD cells demonstrated that aldosterone significantly increased αENaC protein abundance (169 ± 7% *vs.* 100 ± 4%, $P < 0.05$). As hypothesized, 17 Pt prevented the aldosterone-induced up-regulation of αENaC protein (120 ± 10%, P = NS). Neither aldosterone nor 17 Pt affected the expression of βENaC or γENaC (Fig. 2C).

EP1 activation prevents the membrane destination of ENaC subunits

We evaluated if EP1 activation would blunt the effect of aldosterone on ENaC expression in plasma membrane. Biotinylation experiments demonstrated that aldosterone caused a significant increase in the abundance of all subunits in plasma membrane of IMCD cells, as compared with control (αENaC = 164 ± 9%, βENaC = 140 ± 8% and γENaC = 148 ± 16%, $P < 0.05$). Interestingly, 17 Pt impaired the increase of ENaC subunits in plasma membrane induced by aldosterone (αENaC = 104 ± 8%, βENaC = 114 ± 10% and γENaC = 115 ± 10%, P = NS) (Fig. 2D).

Effect of RAAS activation on ENaC subunit abundance in vivo

To activate the RAAS *in vivo* we infused Sprague–Dawley rats with Ang II. After 5 days of Ang II infusion we evaluated changes in ENaC subunit abundance in kidney cortex and medulla. Ang II caused a significant increase in the abundance of αENaC protein in the cortex compared to control animals (186 ± 17% *vs.* 100 ± 9%, $P < 0.05$). In the renal medulla, we observed a similar effect (167 ± 12% *vs.* 100 ± 13%, $P < 0.05$). No changes were seen

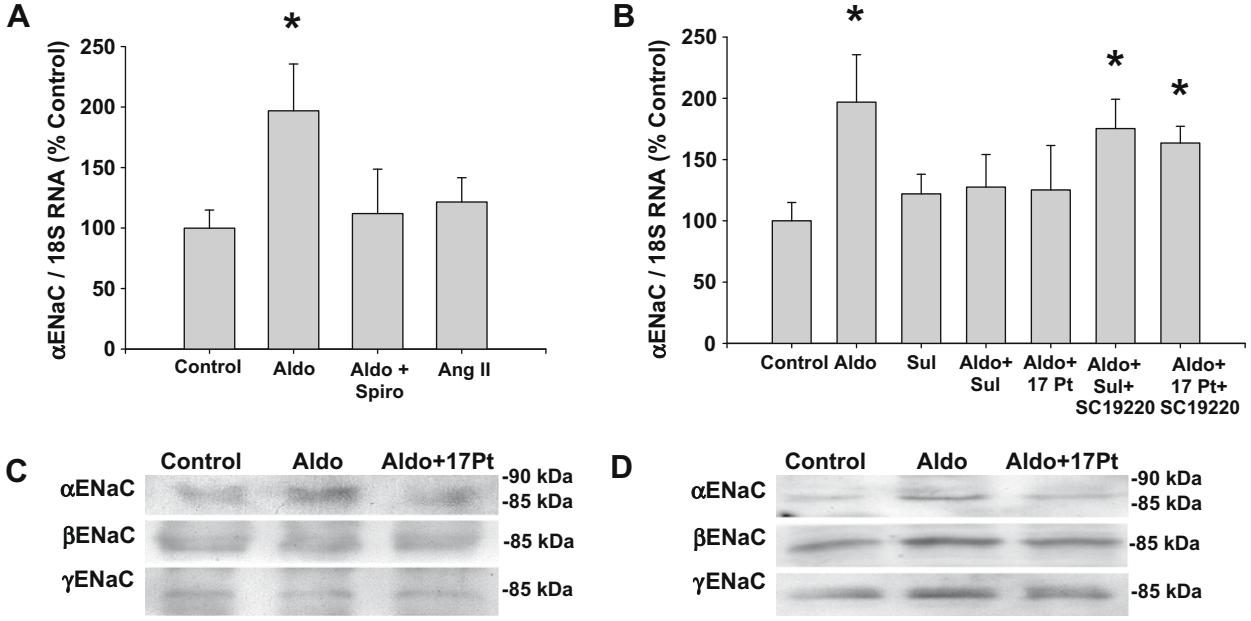

Fig. 2. Effect of EP1/EP3 activation in aldosterone stimulated IMCD cells on αENaC mRNA expression. (A) Aldosterone (Aldo, 10 nM) up-regulates the αENaC mRNA levels, this effect was blunted by spironolactone (Spiro). Ang II, (0.1 nM) did not show a significant effect. (B) Sulprostone (Sul, 1 μM) and 17 Phenyl trinor (17 Pt, 10 μM) were able to prevent the aldosterone-induced αENaC up-regulation. (C) Representative immunoblot of IMCD showing that the aldosterone-induced αENaC up-regulation was blunted by 17 Pt. (D) Biotinylation of three ENaC subunits demonstrated that 17 Pt prevented the increase of the abundance of all three subunits mediated by aldosterone (n = 8 for immunoblots and n = 5 for biotinylation experiments).

in βENaC or γENaC in cortex and medulla. The increase in αENaC protein abundance caused by Ang II infusion was prevented by losartan (cortex: 95 ± 8%; medulla: 98 ± 5%, *P* = NS) and spironolactone treatment (cortex: 108 ± 5%; medulla: 95 ± 7%, *P* = NS).

Sulprostone prevented the up-regulation of αENaC caused by Ang II in the renal medulla but not in cortex

In the kidney cortex, sulprostone infusion did not affect the up-regulation of αENaC protein levels in response to Ang II (Fig. 3A). By contrast, in the renal medulla, sulprostone was able to prevent the up-regulation of αENaC protein levels (119 ± 9%, *P* = NS) (Fig. 3B). Consistent with these results, sulprostone ameliorated the up-regulation of αENaC mRNA induced by Ang II in the renal medulla (control: 100 ± 28%, Ang II: 302 ± 54%, *P* < 0.05 *vs.* control, Ang II + sulprostone: 140 ± 30%, *P* = NS *vs.* control). No changes were seen in βENaC or γENaC mRNAs. Previous experiments demonstrated that sulprostone alone did not modify α, β or γENaC protein levels in the renal cortex and medulla (data not shown).

Expression of EP1 and EP3 in the rat kidney

Since we observed differential effects of sulprostone in kidney cortex and renal medulla, we evaluated the distribution of EP1 and EP3 receptor in renal cortex and medulla by immunohistochemistry. Kidney sections showed specific labeling for EP1 receptor in afferent arterioles (Fig. 4A and B, upper panels) and EP3 restricted to cortical CD (Fig. 4C and D, upper panels). By using AQP-2 as a specific marker for CD, we observed that EP1 was present mainly in medullary CD with a basolateral distribution (Fig. 4A and B, lower panels). We did not observe co-localization of EP3 and AQP-2 in the medulla (Fig. 4C and D, lower panels).

EP1-specific activation prevents the αENaC up-regulation caused by Ang II infusion

In a new set of experiments we evaluated the effect of EP1 specific agonists/antagonists on cortical and medullary ENaC expression in a context of activated RAAS. 17 Pt did not affect the up-regulation of αENaC abundance induced by Ang II in renal cortex as compared to control rats (212 ± 40% *vs.* 100 ± 15%, *P* < 0.05) (Fig. 3C). However, αENaC protein abundance in renal medulla of rats infused with Ang II plus 17 Pt was not changed (126 ± 16% *vs.* 100 ± 10%, *P* = NS) (Fig. 3D). 17 Pt caused similar effects on medullary αENaC mRNA abundance as compared to control (144 ± 69 *vs.* 100 ± 28%, *P* = NS). Interestingly, the infusion of the EP1 antagonist (SC19220) in Ang II infused rats did not modify the up-regulation of medullary αENaC protein and mRNA. No changes were seen in βENaC or γENaC mRNAs and protein levels.

The EP1 activation causes natriuresis in rats infused with the Ang II

To evaluate the effect of EP1 and EP1/EP3 activation on sodium balance at day 5 of treatment, four male Sprague–Dawley rats were infused either with Ang II (0.4 μg/kg/min), Ang II + sulprostone, Ang II + 17 Pt or Ang II + SC19220. Table 1 summarizes relevant physiological parameters by treatment at day 5. Ang II infusion causes an increase in systolic blood pressure. Arterial pressure was not modified by co-infusion of sulprostone, 17 Pt or SC19220. Chronic infusion of Ang II + sulprostone or Ang II + 17 Pt caused an increase in Na^+ excretion, as compared to control rats (Table 1), this effect was observed at day 5 of treatment.

Discussion

The main finding of the present study is that the activation of EP1 receptor in cultured IMCD cells and in the renal medulla *in vivo* prevented the aldosterone-induced up-regulation of αENaC. We showed that aldosterone up-regulates both, αENaC mRNA and protein and increases the abundance of all three ENaC subunits in the plasma membrane. The EP1 activation in IMCD cells prevented the up-regulation of αENaC mRNA and blunted the increase of all three ENaC subunits present at the plasma membrane of IMCD cells caused by aldosterone, suggesting reduced assembly of the mature ENaC complexes. The aldosterone-induced up-regulation of αENaC mRNA was also prevented by sulprostone (EP1 > EP3 agonist), however, this effect can be blunted *in vitro* by SC19220 (specific EP1 antagonist), confirming an EP1 receptor-dependent action. In addition to observing that the EP1 activation in IMCD cells directly prevents the aldosterone-dependent ENaC up-regulation, we found that sulprostone did not alter ENaC expression in the absence of aldosterone.

Based in our *in vitro* results, we tested the hypothesis that EP1 activation would modulate the expression of ENaC under RAAS activation. As expected, Ang II infusion caused an increase in plasma aldosterone (≈10 nmol/L) and induced a significant

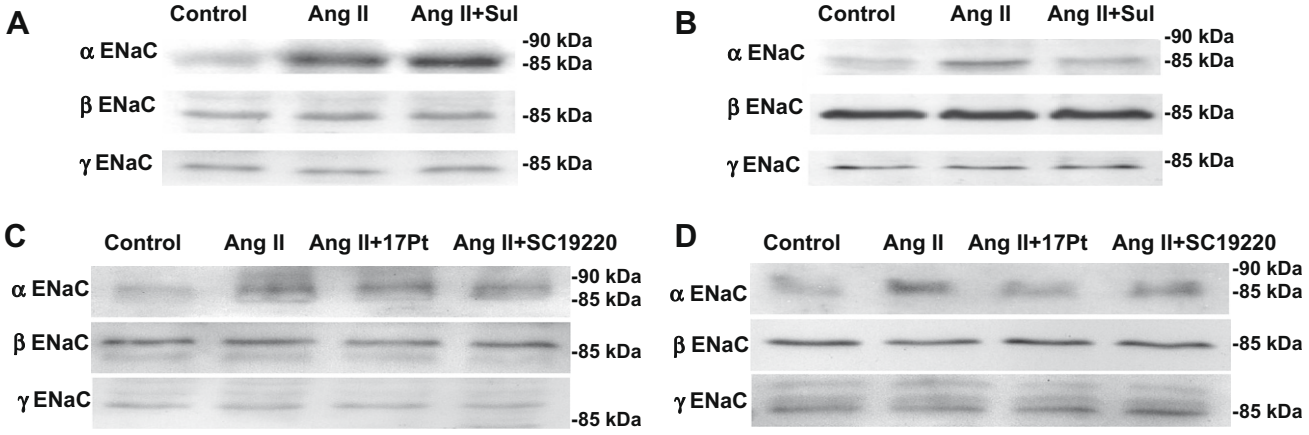

Fig. 3. Effect of angiotensin II (Ang II) infusion and EP1/EP3 activation on ENaC subunit protein abundances. Immunoblots from renal homogenates showed that Ang II infusion (*n* = 8) causes the up-regulation of αENaC in renal cortex (A) and medulla (B) without changes in β and γ-ENaC. Rats infused with Ang II + sulprostone (Sul, *n* = 6) showed an increase in αENaC protein abundance in cortex (A); however, in renal medulla αENaC abundance was not altered (B). Rats infused with Ang II + 17 Phenyl trinor (17 Pt, *n* = 5) showed a similar pattern of sulprostone in cortex (C) and medulla (D). SC19220 an EP1 antagonist did not alter αENaC up-regulation caused by Ang II infusion. See Results section for details.

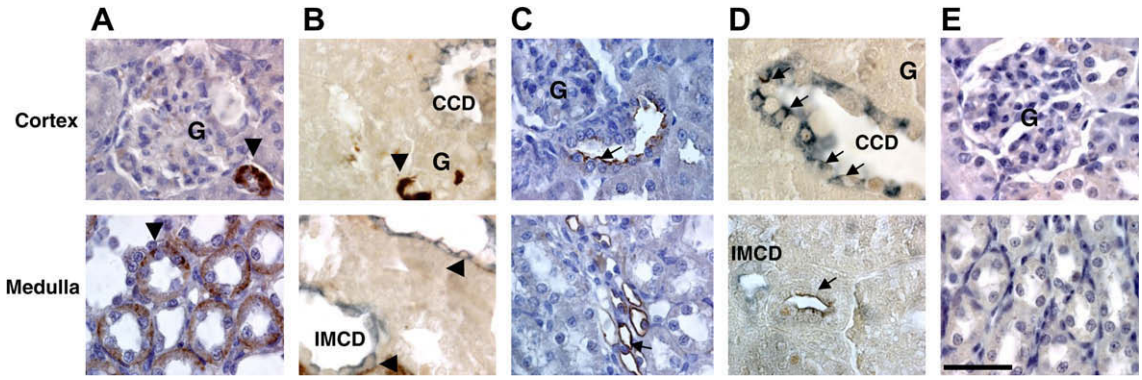

Fig. 4. (A) EP1 immunolabeling in normal kidneys (brown), (B) double immunolabeling for EP1 (brown reaction and arrowhead) with AQP-2 (collecting duct principal cell, blue reaction) showing EP1 expression in medullary collecting ducts, and afferent arteriole in cortex. (C) EP3 immunolabeling (brown). (D) Double immunolabeling for EP3 (brown reaction and arrow) with AQP-2 (blue reaction) demonstrates co-localization of EP3 and AQP-2 in cortical collecting ducts but not medullary collecting duct. (E) Negative control performed by omission of the primary antibody. G, glomeruli; CCD, cortical collecting duct; IMCD, inner medullary collecting duct. Scale bar = 50 μm.

Table 1
Physiological parameters at day 5 of treatment.

	Control	Ang II	Ang II + Sul	Ang II + 17 Pt	Ang II + SC19220
n	4	4	4	4	4
Systolic blood pressure (mmHg)	127 ± 2	190 ± 7*	185 ± 8*	186 ± 5*	188 ± 4*
Creatinine clearance (mL/min/100 g)	0.86 ± 0.09	0.80 ± 0.11	0.73 ± 0.12	0.71 ± 0.20	0.86 ± 0.22
Serum concentrations:					
Aldosterone (nmol/L)	0.6 ± 0.1	10.1 ± 2.1*	7.2 ± 2.0*	8.1 ± 2.1*	6.1 ± 1.8*
Na^+ (mEq/L)	139 ± 1	137 ± 1	137 ± 1	138 ± 1	136 ± 2
K^+ (mEq/L)	3.2 ± 0.1	2.9 ± 0.2	3.1 ± 0.1	3.2 ± 0.2	2.8 ± 0.2
Urinary excretion:					
Na^+ (μEq 24 h/g)	2.7 ± 0.2	2.9 ± 0.4	4.2 ± 0.5*	3.9 ± 0.4*	2.8 ± 0.6
K^+ (μEq 24 h/g)	7.5 ± 0.4	7.5 ± 0.4	7.1 ± 0.5	7.3 ± 0.6	7.7 ± 0.7

* $P < 0.05$ *vs.* control group.

increase in αENaC mRNA and protein in kidney cortex and medulla. The *in vivo* effect of Ang II infusion on αENaC expression was blocked by co-administration of spironolactone, indicating an MR-dependent effect.

Interestingly, we observed that sulprostone was able to prevent the up-regulation of αENaC induced by aldosterone in the medulla but not in cortex. Previous studies have demonstrated EP1 mRNA expression in medullary CD, whereas EP3 mRNA is predominantly expressed in the thick ascending limb and cortical and outer medullary CD [4,5,11]. Our immunolabeling studies, using EP1/EP3 antibodies combined with AQP-2 antibody as a principal CD cell marker demonstrated the expression of EP1 in medullary, but not in cortical CD (Fig. 4B and D). Thus, we conclude that *in vivo* chronic activation of the EP1 present in medullary CD prevents the aldosterone-induced up-regulation of αENaC. These results indicate that activation of EP1 receptor under high plasma levels of aldosterone or Ang II could activate cellular pathways antagonizing αENaC up-regulation in the renal medulla.

Several studies have suggested natriuretic effect of PGE_2 in cortical CD [9–12]. We observed that both, sulprostone and 17 Pt increased urinary Na^+ excretion at day 5 of treatment in rats infused with Ang II, coincidently with highest arterial pressure values. We did not observe the effect on arterial pressure at the early phase of the treatment. Also, we found in previous experiments that sulprostone infusion in normotensive rats did not cause changes in medullary αENaC protein levels and Na^+ excretion (data not shown); supporting the hypothesis that EP1 activation would be able to modulate ENaC expression when the RAAS is active.

Our results provide new evidence that contributes to a better understanding of the role of EP1 and EP3 receptors in the regulation of Na^+ handling and ENaC expression in the setting of RAAS activation. *In vitro* and *in vivo* studies strongly suggest that the activation of EP1 can modify the aldosterone-mediated up-regulation of αENaC in the renal medulla.

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