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Sodium chloride regulation of the α epithelial amiloride-sensitive sodium channel (αENaC) gene requires syntheses of new protein(s)

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Abstract

The epithelial amiloride-sensitive sodium channel (ENaC) plays a central role in sodium homeostasis and blood pressure control. The molecular effect of high sodium intake on the ENaC gene is not well known. This study examined the effects of high salt (HS) intake on αENaC gene transcription in rat kidney. Rats were injected intraperitoneally with hypertonic (1.5 M NaCl) or normal saline solution (three rats per group). The serum sodium concentration of rats injected with hypertonic saline increased significantly 30 min after injection (158 ± 2 mM versus 140 ± 1 mM for normal saline injected rats and 139 ± 1 mM for uninjected rats). At 3 h after injection, serum sodium decreased (144 ± 1 mM) but remained above the control values (139 ± 1 mM for normal saline injected rats, 139 ± 1 mM for uninjected rats). The serum aldosterone decreased 1.5 and 3 h after the hypertonic saline injection (217 ± 10 and 139 ± 23 pg/ml for hypertonic saline injected rats, 358 ± 2 pg/ml for uninjected rats). The kidney cortex was dissected macroscopically and total RNA was isolated at 1.5 and 3 h after treatment. Semi-quantitative RT-PCR studies revealed that following hypertonic saline treatment, αENaC mRNA levels were dramatically downregulated, compared with controls, as early as 1.5 h. Western blot analysis showed similar patterns of protein downregulation. Inhibition of protein synthesis by cycloheximide (CHX) blocked the sodium chloride-induced αENaC mRNA downregulation, 3 h after treatment. This indicates that synthesis of new, uncharacterized protein(s) is required for sodium chloride-mediated inhibition of αENaC gene transcription.

Keywords: Sodium chloride; αENaC

1. Introduction

The role of dietary sodium as a risk factor in the development of hypertension has been extensively reported in both animals and humans [1]. One essential kidney function is to maintain salt homeostasis in the body; the kidney increases reabsorption of salt filtered in the glomerulus in salt deficiency states and decreases reabsorption during salt overload [2]. The rate limiting step in sodium reabsorption occurs via the epithelial amiloride-sensitive sodium channel (ENaC) in the cortical and medullary collecting ducts of the mammalian kidney [3]. ENaC, a heterotetrameric channel complex composed of at least three homologous but distinct α, β, and γ subunits [4], plays an important role in sodium homeostasis and blood pressure regulation [5].

The physiological regulation of sodium transport by cells of the colon and renal collecting duct is also influenced by circulating aldosterone levels in response to dietary sodium intake [6–8]. A few reports have demonstrated a direct correlation between ENaC activity and aldosterone levels. Aldosterone binds to the mineralocorticoid receptor, which then translocates to the nucleus where the aldosterone-occupied receptor modulates transcription of target genes [9,10]. A recent study elucidated the aldosterone-induced αENaC transcription mechanism. Elevated circulating aldosterone levels increase the abundance of αENaC mRNA in the connecting tubule and principal cells of the cortical collecting duct via mineralocorticoid receptor binding sites located at the 5′ flanking region of αENaC [5,11,12]. Transcriptional upregulation of αENaC by glucocorticoids, which does not require protein synthesis, has also been shown in human and rat epithelia [13–15].

Recent study reported physiologic regulation of αENaC by chronic dietary sodium intake. Low sodium

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administration for 3 weeks increased the levels of aldosterone and the α subunit, and induced a shift of the β and γ subunits from the cytoplasm to the apical plasma membrane [3]. However, no previous study has shown the short-term effects of sodium on αENaC in vivo. Long-term in vivo studies involve various metabolic pathways, which complicate interpretation of the mechanism of action. The purpose of this study was to examine the short-term effects of high salt (HS) administration on αENaC transcription in kidney cortex. Our data show that in the rat kidney cortex, high salt administration dramatically downregulates αENaC at mRNA levels as early as 1.5 h, the earliest time point examined. It is possible that this downregulation is an indirect effect caused by the decreased serum aldosterone induced by increased salt intake. However, the salt-induced αENaC downregulation was blocked by the protein synthesis inhibitor cycloheximide (CHX), indicating that newly synthesized protein(s) other than mineralocorticoid receptor are required in the regulation.

2. Materials and methods

2.1. Animals

All animal procedures were conducted according to recommendations of the Animal Care Committee of the Samsung Biomedical Research Institute and the Use Committee. Female Sprague–Dawley rats (7 weeks old), weighing 175–185 g, were purchased from Daehan Laboratory Animal Research Center. They received standard rat chow (Cheil Jedang) with free access to tap water. Rats were injected (10 ml/kg i.p.) with normal saline (0.15 M) or hypertonic (1.5 M) saline solution (three rats per group) as was previously reported at the same time [16]. After intraperitoneal injection, animals were killed by cervical dislocation at different times. Left kidneys were quickly removed, cut into longitudinal halves, and macroscopically subdivided into cortex and medulla. Those were immediately frozen on a bed of powdered dry ice and stored at –80 °C until extraction of total RNA and protein.

2.2. Determination of serum sodium and aldosterone concentration

Rats were anesthetized with ether and blood was obtained from the abdominal aorta. Serum sodium concentrations were determined by a commercially available ion-selective electrodes method at Seoul Clinical Laboratories and serum aldosterone concentrations were determined using a radioimmunoassay kit (Diagnostic Products).

2.3. Semi-quantitative RT-PCR

Total RNA was extracted using Trizol Reagent (GIBCO-BRL) according to the manufacturer's instruction. RNA pellets were dissolved in diethylpyrocarbonate-treated water. The yield of RNA was quantified by spectroscopy at 260 nm. Samples were aliquoted and stored at −80 °C until further processing. To synthesize first strand cDNA, 5 μg total RNA was incubated at 70 °C for 5 min with 0.5 μg of random hexamer and deionized water (up to 11 μl). The reverse transcription reaction was performed using 40 units of M-MuLV reverse transcriptase (Promega) in 5× reaction buffer (250 mmol/l Tris–HCl; pH 8.3, 375 mM KCl, 15 mM $MgCl_2$, 50 mM DTT), RNase inhibitor at 1 unit/μl, and 1 mM dNTP mixtures at 37 °C for 60 min. The reaction was terminated by heating at 70 °C for 10 min, followed by cooling at 4 °C. The resulting cDNA was added to the PCR reaction mixture containing 10× PCR buffer (100 mM Tris–HCl; pH 8.3, 500 mM KCl, 15 mM $MgCl_2$), 25 units of rTaq polymerase (TaKaRa), 4 μl of 2.5 mM dNTP mixtures, and 100 pM mole of primers each. The final volume was 50 μl. Samples were amplified at 94 °C for 5 min, 30 cycles of 94 °C for 45 s, 55 °C for 45 s, and 72 °C for 45 s using Mastercycler gradient (eppendorf). Ribosomal protein L19 (RPL19) was amplified for 20 cycles, followed by 72 °C for 10 min. The sequences of the αENaC primers were αENaCa (5′-TACGCGACAACAATCCCCAAGT-3′) and αENaCb (5′-ATGGAGGACATCCAGAGATTGGAG-3′). Preproendothelin (ET)-1 primers were preproET-1a (5′-CTAGGTCTAAGCGATCCTTG-3′) and preproET-1b (5′-TTCTGGTCTCTGTAGAGTTC-3′). RPL19 primers were RPL19a (5′-CTGAAGGTCAAAGGGAATGTG-3′) and RPL19b (5′-GGACAGAGTCTTGATGATCTC-3′). COX-2 primers were COX-2a (5′-ACACTCTATCACTGGCATCC-3′) and COX-2b (5′-GAAGGGACACCCTTTCACAT-3′). The expected size of amplicons for αENaC, preproET-1, COX-2, and RPL19 are 300, 300, 500, and 194 bp, respectively. The PCR products were visualized and quantified using a bio-imaging analyzer (Bio-Rad), and band density was normalized to the intensity of band of RPL19 mRNA.

2.4. Western blotting

Protein extracted from rat kidney was isolated by serial passage through 18- and 22-gauge needles in radioimmune precipitation buffer (150 mM NaCl, 50 mM Tris–HCl, 5 mM EDTA, 1% Nonidet P-40, 0.5% deoxycholate, 1% SDS) with protease inhibitor cocktail (Sigma) on ice for 1 h and then centrifuged for 20 min at 13,000 × *g*. Supernatant was collected and protein concentrations were measured using the Bradford method (Bio-Rad). Fifty micrograms of kidney protein was dissolved in sample buffer and boiled for 5 min prior to loading onto a 8% acrylamide gel. After SDS–PAGE, proteins were transferred to a polyvinylidene difluoride membrane, blocked with 5% nonfat dry milk in Tris-buffered saline containing 0.1% Tween-20 (TBST), and incubated with rabit anti-polyclonal antibody to αENaC (affinity bioreagents) overnight at 1:500. After washing with TBST, blots were incubated with goat anti-rabbit horseradish peroxidase-conjugated secondary antibody

and visualized with enhanced chemiluminescence kits (Amersham).

2.5. Statistical analysis

Results of density measurements of PCR products and Western blots are presented as means ± S.E.M. Significance of differences between means was evaluated using Student's *t*-test.

3. Results

3.1. Serum sodium and aldosterone concentration

Rats were injected with 1.5 M saline solution and sacrificed 0.5, 1.5, and 3 h later. At the time of death, serum was separated from blood cells and frozen at −20 °C until assayed. At 30 min after injection with hypertonic saline, serum sodium levels were 158 ± 2 mM compared with 140 ± 1 mM in rats injected with normal saline and 139 ± 1 mM in uninjected rats. At 1.5 and 3 h after injection, serum sodium decreased but remained above the control values (Table 1). The serum aldosterone concentration was highly significantly decreased at 1.5 and 3 h after hypertonic saline injection, but did not decrease at 0.5 h after hypertonic saline injection (Table 1).

3.2. Effects of acute high salt on αENaC expression in the kidney cortex

We analyzed the changes induced by high salt on αENaC gene expression in the left kidney cortex at the mRNA and protein levels. We used left kidney for all experiments performed in this study to control for differences in expression between left and right kidneys [17]. We initially measured

Table 1
Serum sodium and aldosterone concentrations

	Uninjected ($n = 3$)	NS ($n = 3$)	HS ($n = 3$)
Serum sodium (mM, 30 min)	139 ± 1	140 ± 1	158 ± 2*
Serum sodium (mM, 1.5 h)	139 ± 1	140 ± 1	149 ± 1**
Serum sodium (mM, 3 h)	139 ± 1	139 ± 1	144 ± 1**
Serum aldosterone (pg/ml, 30 min)	358 ± 2	317 ± 64	323 ± 44
Serum aldosterone (pg/ml, 1.5 h)	358 ± 2	161 ± 70**	217 ± 10**
Serum aldosterone (pg/ml, 3 h)	358 ± 2	173 ± 35**	139 ± 23*

* $P < 0.001$ vs. uninjected.
** $P < 0.05$ vs. uninjected.

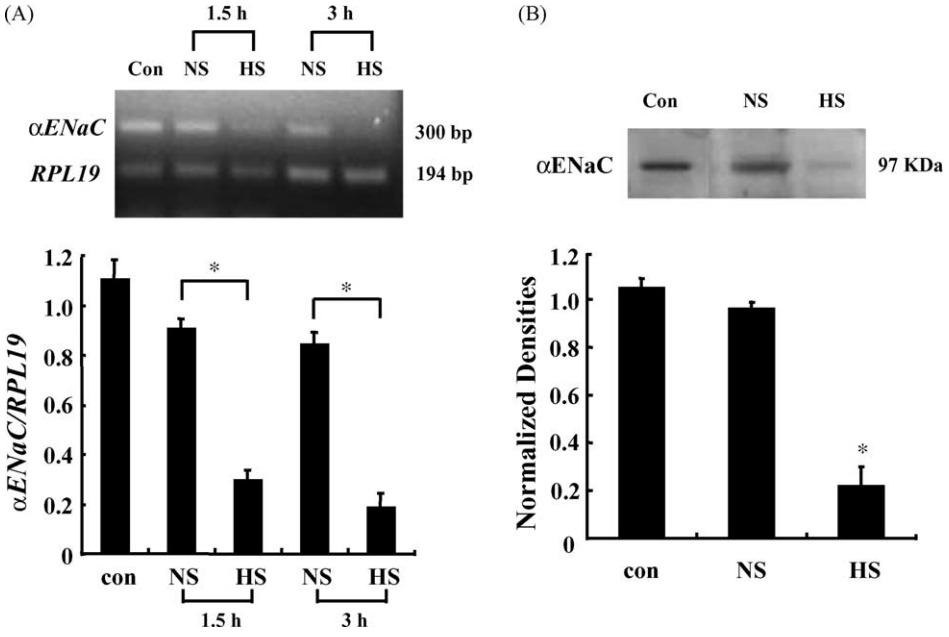

Fig. 1. (A) RT-PCR analysis of the effect of normal salt (NS) or high salt (HS) on left kidney cortex αENaC mRNA at 0 (con), 1.5, and 3 h after injection. The αENaC mRNA band density was normalized to the RPL19 mRNA band. Values are means ± S.E.M. of four experiments with triplicate samples. *$P < 0.005$ vs. NS Student's *t*-test. (B) Western blot analysis of the rat kidney cortex αENaC expression after salt treatment. Fifty micrograms of rat kidney cortex isolated after 3 h of salt injections ($n = 3$) were immunoblotted with αENaC antibody. Values are means ± S.E.M of four separate experiments. *$P < 0.005$ vs. con or NS, Student's *t*-test.

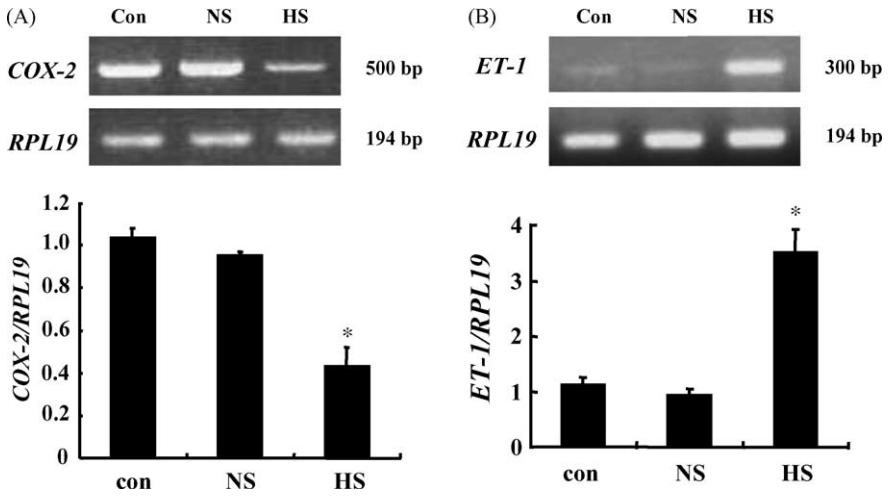

Fig. 2. RT-PCR analysis of the effect of high salt on the left kidney cortex cyclooxygenase-2 mRNA (A) and medulla ET-1 (B) 3 h after injection. The density of the COX-2 and ET-1 mRNA bands were normalized to the RPL19 mRNA band. Values are means ± S.E.M. of four experiments with triplicate samples. $*P < 0.005$ vs. control (con) or normal salt (NS), Student's *t*-test.

αENaC mRNA levels in the kidney cortex after hypertonic saline injection by semi-quantitative RT-PCR. Constitutively expressed rat RPL19 was used as an internal control [18,19]. Rats were injected with normal saline or hypertonic saline and sacrificed after 1.5 or 3 h. At 3 h after hypertonic saline injection αENaC mRNA levels decreased (7.8-fold) in kidney cortex compared with rats injected with normal saline. This downregulation was also observed 1.5 h after treatment, the earliest time examined (3.7-fold decrease) (Fig. 1 A). αENaC protein levels were also significantly decreased in the kidney cortex of hypertonic saline-injected rats compared with control rats (Fig. 1 B). To confirm that intraperitoneal injection of hypertonic saline is comparable to dietary salt intake (except for the duration), we examined ET-1 and COX-2 gene expression in our model system. We found that salt injection increased ET-1 mRNA expression in the kidney medulla and decreased COX-2 mRNA expression in the kidney cortex, consistent with previous findings [20–22] (Fig. 2). This result shows that the salt-induced gene identified by long-term dietary study is under similar regulation despite different administration routes. These data eliminate a possibility that αENaC downregulation by high salt is due to injection of 1.7–2 ml of saline solution into the intraperitoneal space.

3.3. Effects of cycloheximide on salt-mediated αENaC regulation

To examine whether salt directly regulates αENaC mRNA, we determined whether regulation occurred regardless of protein synthesis. The protein synthesis inhibitor cycloheximide was co-injected with salt into rats at a concentration of 1.5 mg/kg body weight [23]. While cycloheximide alone had no effect on αENaC mRNA levels, it completely abolished the regulatory effect of salt on αENaC mRNA levels (Fig. 3). These results suggest that de novo protein synthesis is required for salt-mediated regulation of αENaC gene transcription; αENaC regulation occurs through newly synthesized regulatory proteins triggered by high salt.

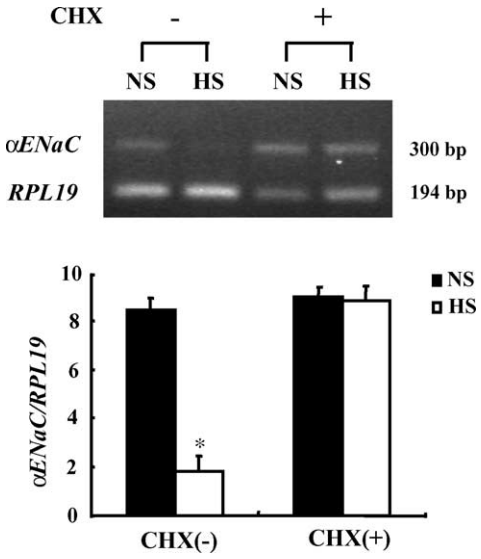

Fig. 3. αENaC mRNA levels in response to high salt injection in the presence of the protein synthesis inhibitor cycloheximide (CHX) 3 h after injections. Values represent the mean ± S.E.M. of four separate experiments. $*P < 0.005$ vs. NS, Student's *t*-test.

4. Discussion

Sodium homeostasis is important for the maintenance of extracellular volume and blood pressure, and ENaC regulation is critical for homeostasis [24]. Despite progress in understanding signaling pathways and transcriptional

mechanisms governing αENaC regulation at the molecular level, it is still unclear how salt regulates αENaC at the transcriptional level.

Sodium intake, aldosterone levels, and the number of functional sodium channels in the apical plasma membrane are interrelated [3]. Aldosterone is the main hormone controlling ENaC activity at the cell surface [25] and several groups reported ENaC regulation by aldosterone. Aldosterone increased mRNA levels of the β and γ subunits, but little of α subunit [26]. A controversial result was reported in an increase only in the α subunit mRNA [27]. Aldosterone has also been shown by different groups to regulate ENaC at both translational and posttranslational levels [28–30]. A recent study reported that aldosterone stimulation of αENaC expression occurs via mineralocorticoid receptor-responsive elements in the 5′-flanking region of the αENaC gene [12]. As stated, plasma aldosterone levels are usually inversely correlated with dietary sodium intake and a high sodium diet also regulates ENaC activity. A few studies have shown the effects of long-term dietary salt on ENaC in vivo, but it is not clear whether the results were due to changes in aldosterone levels or to high salt intake alone. Under our experimental conditions, serum aldosterone was decreased 3 h after the hypertonic saline injection. One possible explanation for this observasion is that αENaC is down regulated through an indirect mechanism by reduced levels of aldosterone-occupied mineralocorticoid receptors. However, it should be pointed out that even with normal saline injection, we observed decreases in aldosterone without changes in αENaC mRNA expression. We observed αENaC downregulation only with hypertonic saline treatment. At 30 min after initiation of hypertonic saline injection, serum sodium was significantly raised without a substantial change in circulating aldosterone (Table 1). These results point to the possibility that a major portion of the initial decrease in αENaC regulation by salt may be due to Na^+ itself rather than aldosterone. A recent study reported that extracellular Na^+ itself can reduce ENaC activity [31]. It has been suggested that short-term regulation mechanisms for sodium transport may be directly related to changes in sodium concentration [32,33]. In addition, if the downregulation is due to aldosterone, a protein synthesis inhibitor should not influence salt-induced αENaC downregulation.

In conclusion, we found that αENaC is downregulated by salt at the mRNA level by newly synthesized protein(s). Further study is needed to identify the upstream protein(s) involved in salt-mediated transcriptional regulation and increase our understanding of salt-triggered cellular signaling pathways.

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References

[1] A. Pomeranz, T. Dolfin, Z. Korets, A. Eliakim, B. Wolach, Increased sodium concentrations in drinking water increase blood pressure in neonates, J. Hypertens. 20 (2002) 203–207.
[2] K. Wolf, H. Castrop, G.A. Riegger, A. Kurtz, B.K. Kramer, Differential gene regulation of renal salt entry pathways by salt load in the distal nephron of the rat, Pflugers Arch. 442 (2001) 498–504.
[3] J. Loffing, L. Pietri, F. Aregger, M. Bloch-Faure, U. Ziegler, P. Meneton, B.C. Rossier, B. Kaissling, Differential subcellular localization of ENaC subunits in mouse kidney in response to high- and low-Na diets, Am. J. Physiol. Renal. Physiol. 279 (2000) F252–F258.
[4] C.M. Canessa, L. Schild, G. Buell, B. Thorens, I. Gautschi, J.D. Horisberger, B.C. Rossier, Amiloride-sensitive epithelial Na^+ channel is made of three homologous subunits, Nature 367 (1994) 463–467.
[5] Y.R. Su, A.G. Menon, Epithelial sodium channels and hypertension, Drug Metab. Dispos. 29 (2001) 553–556.
[6] C.P. Bastl, J.P. Hayslett, The celluar action of aldosterone in target epithelia, Kidney Int. 42 (1992) 250–264.
[7] H. Garty, Molecular properties of epithelial, amiloride-blockable Na^+ channels, FASEB J. 8 (1994) 522–528.
[8] F. Verrey, Transcriptional control of sodium transport in tight epithelia by adrenal steroids, J. Mebmr. Biol. 144 (1995) 93–110.
[9] M.K. Agarwal, Perspectives in receptor-mediated mineralocorticoid hormone action, Pharmacol. Rev. 46 (1994) 67–87.
[10] J.W. Funder, Glucocorticoid and mineralocorticoid receptors: biology and clinical relevance, Annu. Rev. Med. 48 (1997) 231–240.
[11] S. Masilamani, G.H. Kim, C. Mitchell, J.B. Wade, M.A. Knepper, Aldosterone-mediated regulation of ENaC α, β, and γ subunit proteins in rat kidney, J. Clin. Invest. 104 (1999) R19–R23.
[12] E.M. Verity, A.I. Omar, W.L. Randy, F.H. Russell, J.S. Thomas, P.T. Christie, The α-subunit of the epithelial sodium channel is an aldosterone-induced transcript in mammalian collecting ducts, and this transcriptional response is mediated via distinct cis-elements in the 5′-flanking region of the gene, Mol. Endo. 15 (2001) 575–588.
[13] R. Sayegh, S.D. Auerbach, X. Li, R. Loftus, R. Husted, J.B. Stokes, C.P. Thomas, Glucocorticoid induction of epithelial sodium channel expression in lung and renal epithelia occurs via trans-activation of a hormone response element in the 5′-flanking region of the human epithelial sodium channel alpha subunit gene, J. Biol. Chem. 274 (1999) 12431–12437.
[14] H.H. Lin, M.D. Zentner, H.L. Ho, K.J. Kim, D.K. Ann, The gene expression of the amiloride-sensitive epithelial sodium channel α-subunit is regulated by antagonistic effects between glucocorticoid hormone and ras pathways in salivary epithelial cells, J. Biol. Chem. 274 (1999) 21544–21554.
[15] G. Otulakowski, B. Rafii, H.R. Bremner, H. O'Brodovich, Structure and hormone responsiveness of the gene encoding the (subunit of the rat amiloride-sensitive epithelial sodium channel, Am. J. Respir. Cell. Mol. Biol. 20 (1999) 1028–1040.
[16] P.J. Shiromani, M. Magner, S. Winston, M.E. Charness, Time course of phosphorylated CREB and Fos-like immunoreactivity in the hypothalamic supraoptic nucleus after salt loading, Brain Res. Mol. Brain Res. 29 (1995) 163–171.
[17] J.L. Wang, H.F. Cheng, R.C. Harris, Cyclooxygenase-2 inhibition decreases renin content and lowers blood pressure in a model of renovascular hypertension, Hypertension 34 (1999) 96–101.
[18] D.A. Schreihofer, M.H. Stoler, M.A. Shupnik, Differential expression and regulation of estrogen receptors (ERs) in rat pituitary and cell lines: estrogen decreases ERalpha protein and estrogen responsiveness, Endocrinology 141 (2000) 2174–2184.
[19] A. Mukherjee, J. Urban, P. Sassone-Corsi, K.E. Mayo, Gonadotropins regulate inducible cyclic adenosine 3′,5′-monophosphate early repressor in the rat ovary: implications for inhibin alpha subunit gene expression, Mol. Endocrinol. 12 (1998) 785–800.
[20] T. Ikeda, H. Ohta, M. Okada, N. Kawai, R. Nakao, P.K. Siegl, T. Kobayashi, S. Maeda, T. Miyauchi, M. Nishikibe, Pathophysiolo-

gical roles of endothelin-1 in Dahl salt-sensitive hypertension, Hypertension 34 (1999) 514–519.
[21] T.J. Hsieh, S.R. Lin, Y.J. Lee, S.J. Shin, Y.H. Lai, C.H. Hsu, J.H. Tsai, Increased renal medullary endothelin-1 synthesis in prehypertensive DOCA- and salt-treated rats, Am. J. Physiol. Renal Physiol. 279 (2000) F112–F121.
[22] T. Yang, I. Singh, H. Pham, D. Sun, A. Smart, J.B. Schnermann, J.P. Briggs, Regulation of cyclooxygenase expression in the kidney by dietary salt intake, Am. J. Physiol. 274 (1998) F481–F489.
[23] H.I. Osipova-Goldberg, V.A. Rogozkin, B.I. Feldkoren, Properties of free and occupied androgen receptor in rat skeletal muscle cytosol: effect of testosterone, J. Steroid. Biochem. Mol. Biol. 78 (2001) 481–492.
[24] P.M. Snyder, D.R. Olson, B.C. Thomas, Serum and glucocorticoid-regulated kinase modulates Nedd4-2-mediated inhibition of the epithelial Na^+ channel, J. Biol. Chem. 277 (2001) 5–8.
[25] S. Renard, N. Voilley, F. Bassilana, M. Lazdunski, P. Barbry, Localization and regulation by steroids of the alpha, beta and gamma subunits of the amiloride-sensitive Na^+ channel in colon, lung and kidney, Pfluegers Arch. 430 (1995) 299–307.
[26] C. Asher, H. Wald, B.C. Rossier, H. Garty, Aldosterone-induced increase in the abundance of Na^+ channel subunits, Am. J. Physiol. 271 (1996) C605–C611.
[27] J.B. Stokes, R.D. Sigmand, Regulation of rENaC mRNA by dietary NaCl and steroids: organ, tissue, and steroid heterogeneity, Am. J. Physiol. 274 (1998) C1699–C1707.
[28] D. Alvarez de la Rosa, H. Li, C.M. Canessa, G.K. Fyfe, P. Zhang, Structure and regulation of amiloride-sensitive sodium channels, Annu. Rev. Physiol. 62 (2000) 573–594.
[29] H. Garty, L.G. Palmer, Epithelial sodium channels: function, structure, and regulation, Physiol Rev. 77 (1997) 359–396.
[30] F. Verrey, Early aldosterone action: toward filling the gap between transcription and transport, Am. J. Physiol. Renal. Physiol. 277 (1999) F319–F327.
[31] E. Babini, H.S. Geisler, M. Siba, S. Grunder, A new subunit of the epithelial Na^+ channel identifies regions involved in Na^+ self-inhibition, J. Biol. Chem. 278 (2003) 28418–28426.
[32] S.K. Woo, S.D. Lee, K.Y. Na, W.K. Park, H.M. Kwon, TonEBP/NFAT5 stimulates transcription of HSP70 in response to hypertonicity, Mol Cell Biol. 22 (2002) 5753–5760.
[33] J.D. Ferraris, C.K. Williams, P. Persaud, Z. Zhang, Y. Chen, M.B. Burg, Activity of the TonEBP/OREBP transactivation domain varies directly with extracellular NaCl concentration, Proc. Natl. Acad. Sci. U.S.A. 99 (2002) 739–744.