RESEARCH ARTICLE
doi: 10.2306/scienceasia1513-1874.2018.44.332

ScienceAsia 44 (2018): **332-339**

Nongenomic effect of aldosterone on angiotensin II type 1 receptor dimerization in human renal proximal tubular cells: Implications for endoplasmic reticulum stress

Kittisak Sinphitukkul[a,g], **Krissanapong Manotham**[b], **Somchai Eiam-Ong**[c], **Masaomi Nangaku**[d], **Reiko Inagi**[e], **Somchit Eiam-Ong**[f,*]

[a] Graduate Division, Faculty of Medicine, Chulalongkorn University, Bangkok 10330 Thailand
[b] Renal Unit, Department of Medicine, Lerdsin General Hospital, Bangkok 10330 Thailand
[c] Department of Medicine (Division of Nephrology), Faculty of Medicine, Chulalongkorn University, Bangkok 10330 Thailand
[d] Division of Nephrology and Endocrinology, University of Tokyo Graduate School of Medicine, Tokyo 113-8655 Japan
[e] Division of Chronic Kidney Disease Pathophysiology, University of Tokyo Graduate School of Medicine, Tokyo 113-8655 Japan
[f] Department of Physiology, Faculty of Medicine, Chulalongkorn University, Bangkok 10330 Thailand
[g] Faculty of Medicine, Siam University, Bangkok 10160 Thailand

[*]Corresponding author, e-mail: eiamong@yahoo.com

Received 6 Jan 2018
Accepted 17 Sep 2018

ABSTRACT: In vitro studies have showed that aldosterone increases oxidative stress molecules through a nongenomic effect. Oxidative stress induces angiotensin II type 1 receptor (AT_1R) dimerization and endoplasmic reticulum (ER) stress, leading to renal tubular damage. However, the nongenomic effect of aldosterone on AT_1R dimerization and ER stress in renal cells has not been determined. Here, we examined the nongenomic action of aldosterone in renal proximal tubular epithelial cells (PTECs) to better understand the underlying mechanisms. HK-2 cells, human renal PTECs, were exposed to vehicle or aldosterone for 30 min. In two additional groups, the cells were pretreated with eplerenone, a mineralocorticoid receptor (MR) blocker or apocynin, an NADPH oxidase inhibitor, for 30 min before aldosterone incubation. Protein abundances of dimeric/monomeric forms of AT_1R, p47phox (a cytosolic part of NADPH oxidase), and activating transcription factor 4 (ATF4), a transcription factor responsive to ER stress, were determined by Western blotting. Aldosterone nongenomically increased plasma membrane protein expression of AT_1R dimeric forms in a time- and dose-dependent manners. The levels of the cytosolic p47phox protein declined while the membranous protein level was enhanced following aldosterone treatment. The aldosterone induced alteration in these two proteins was abolished by pretreatment with eplerenone or apocynin. In addition, aldosterone (100 nM) induced nuclear ATF4 protein accumulation in a time-dependent fashion, which was blocked by apocynin and partially attenuated by eplerenone. Aldosterone nongenomically increased AT_1R dimerization and nuclear ATF4 protein accumulation dependent on MR and NADPH oxidase activation. Hence aldosterone could induce AT_1R dimerization and activate the endoplasmic reticulum stress response.

KEYWORDS: nongenomic action

INTRODUCTION

Aldosterone is the main mineralocorticoid hormone and the last hormone axis of the renin-angiotensin-aldosterone system. This hormone plays an important role in controlling blood pressure by influencing sodium and water homeostasis in the kidney through genomic and nongenomic mechanisms[1]. In the genomic pathway, aldosterone first binds to the mineralocorticoid receptor (MR), and then the hormone-receptor complex is transferred to the nucleus where it interacts to hormone-responsive elements in the promoter of target genes[2]. Recently, several investigators examined the nongenomic effects of aldosterone[3–5], which is a rapid onset, tak-

ing only seconds to minutes[6,7].

A previous in vitro study demonstrated aldosterone-induced dimerization of angiotensin II type 1 receptor (AT_1R) by a nongenomic effect[8]. Dimerization of AT_1R plays a significant role in various physiological conditions through G-protein-coupled receptor activation[9,10]. Furthermore, enhanced AT_1R dimerization is also involved in many pathological conditions[11,12]. Conditions of oxidative stress molecules can cause inappropriate activation through cross-linking of AT_1R monomers forming dimers in human embryonic kidney cells[13]. In porcine proximal tubular cells, aldosterone rapidly increased oxidative stress via NADPH oxidase activation[14]. This enzyme requires translocation of p47phox from the cytosol to the plasma membrane in order to bind to the membranous subunit of NADPH oxidase and is then capable of contributing to oxidative stress[14]. These studies suggest that aldosterone-stimulated NADPH oxidase activity may lead to AT_1R dimerization. In addition, aldosterone can promote endoplasmic reticulum (ER) stress in human renal proximal tubular epithelial cells (PTECs)[15]. ER stress is characterized by a lack of cellular protein homeostasis due to the accumulation of unfolded proteins in the ER[16]. It has been documented that ER stress leads to alterations in the structure and function of kidney cells, resulting in the development and progression of kidney diseases[16]. A previous study reported that oxidative stress enhances nuclear accumulation of activating transcription factor 4 (ATF4), a protein marker responsive to ER stress, in cultured mouse cerebral cortex[17]. Hence it is plausible that aldosterone-induced oxidative stress production from NADPH oxidase might rapidly stimulate ATF4 protein accumulation in the nucleus.

Both MR and AT_1R proteins are expressed in cultured human renal PTECs, HK-2 cells[18,19]. To date, in vitro data demonstrating a nongenomic effect of aldosterone on protein abundance of AT_1R dimers and nuclear ATF4 in cultured HK-2 cells is not available. The roles of MR and NADPH oxidase in regard to this nongenomic effect are still unknown. To better understand this process, the present study examined HK-2 cells following a 30-min incubation with vehicle or aldosterone. In addition, the effects of aldosterone following eplerenone (MR blocker) or apocynin (NADPH oxidase inhibitor) pretreatment, at appropriate concentrations for 30 min before aldosterone administration were monitored. Protein abundance of plasma membrane dimeric/-monomeric forms of AT_1R, p47phox, and of nuclear ATF4 protein were investigated by using immunoblot analysis.

MATERIALS AND METHODS

Reagents

Aldosterone and dimethyl sulphoxide were purchased from Sigma (St. Louis, MO, USA). Eplerenone and apocynin were obtained from Santa Cruz Biotechnology (Dallas, Texas, USA). Prestained protein molecular marker was provided from Bio-Rad Laboratories (Hercules, CA, USA). The ECL Western blotting substrate Plus was obtained from Thermo Fisher Scientific (Rockford, IL, USA).

Cell culture

Human renal PTECs (HK-2 cells, American Type Culture Collection, Manassas, VA, USA) were grown in a 1:1 mixture of Dulbecco's Modified Eagle's Medium (DMEM) and Ham's F-12 Nutrient mixture supplemented with 10% fetal bovine serum (Sigma)[20]. The cells were grown at 37 °C in a humidified 5% CO_2 incubator and subcultured at 50–80% confluence into 150 mm dishes. HK-2 cells were growth arrested for 48 h in serum-free medium without adding growth factors[21]. To prepare solutions, aldosterone was diluted in ethanol to yield the doses of 1, 10, and 100 nM[14], whereas dimethyl sulphoxide (DMSO) was used to dissolve eplerenone (10 μM) and apocynin (100 μM)[18,22]. Each solution was applied at 4–5 μl for each intervention. On the day of the experiment, the HK-2 cells were incubated in serum-free medium with ethanol or DMSO (control) or aldosterone (Aldo) for 30 min, or with pretreated of eplerenone (Ep.), an MR blocker, or apocynin (Apo.), an NADPH oxidase inhibitor, for 30 min before aldosterone administration (Ep.+Aldo and Apo.+Aldo, respectively)[14,18].

Protein extraction and immunoblotting

The HK-2 cells (5×10^6) were rinsed with phosphate-buffered saline (pH 7.4), and moved to HGNT buffer (20 mM HEPES, 150 mM NaCl, 10% glycerin, 20 mM NaH_2PO_4, 1% Triton X-100, pH 7.4), containing complete protease inhibitor cocktail (Roche Diagnostics, Mannheim, Germany), and then centrifuged at $18\,000\times g$ for 20 min at 4 °C. The supernatant was used as the whole cell protein lysate[8,23]. Subcellular fractions (cytosolic, plasma membrane, and nuclear proteins) were separated using a Qproteome Cell Compartment kit (Qiagen,

Hilden, Germany) following the manufacturer's instructions. Protein concentrations were measured with a Bio-Rad DC protein assay (Bio-Rad Laboratories). For immunoblotting analysis, protein samples (6 μg/lane for dimeric and monomeric forms of AT_1R and p47phox; 9 μg/lane for ATF4) were separated using 10% sodium dodecyl sulphate polyacrylamide gel electrophoresis, blotted on polyvinylidene difluoride m embranes, blocked for 2 h with 5% nonfat dry milk in Tris-buffered saline (TBS) (pH 7.4) with 0.1% (v/v) Tween 20 and incubated overnight at 4 °C with mouse monoclonal primary antibody to AT_1R (1E10-1A9: sc-81671; 1:500; Santa Cruz Biotechnology), rabbit polyclonal primary antibody to p47phox (H-195: sc-14015; 1:500; Santa Cruz Biotechnology); or blocked for 2 h with 3% bovine serum albumin in TBS, followed by incubation overnight at 4 °C with rabbit polyclonal primary antibody to ATF4 (C-20: sc-200; 1:200; Santa Cruz Biotechnology). Appropriate horseradish peroxidase-linked secondary antibody (Bio-Rad Laboratories) were utilized for detection using chemiluminescence (Thermo Fisher Scientific), and documented using the LAS4000 luminescence imager (Fujifilm, Tokyo). Equal loading and transfer of proteins among lanes were verified u sing l oading c ontrols a s f ollows, C D26 with rabbit polyclonal primary antibody (H-270: sc-9153; 1:500; Santa Cruz Biotechnology), a plasma membrane enzyme in proximal tubular cells (for plasma membrane protein of AT_1R) [24], or actin with rabbit polyclonal primary antibody to actin (C11 A2066; 1:2000; Sigma) (for whole cell protein lysate, cytosolic and plasma membrane proteins of p47phox) [25], or histone H1 with mouse monoclonal primary antibody to histone H1 (AE-4: sc-8030; 1:500; Santa Cruz Biotechnology) (for nuclear protein of ATF4) [26]. Band intensities were measured using densitometric analysis with NIH Image J software (version 1.49). The number of replicates for each experiment was four ($n = 4$).

Statistical analysis

Results for dimeric/monomeric forms of AT_1R, p47phox, and nuclear ATF4 protein were expressed as mean ± SD. Statistical analysis was performed by one-way ANOVA with post-hoc comparison by Tukey's test where appropriate. A p value of < 0.05 was considered statistically significant. Statistical tests utilized SPSS 22.0 (SPSS Inc., Chicago, IL, USA).

RESULTS

Effect of aldosterone on AT_1R dimerization

Fig. 1a showed Western blot gel illustrating representative bands of plasma membrane AT_1R protein in the absence or presence of 100 nM aldosterone at 5, 15, and 30 min. The intensity of AT_1R monomeric forms presented at 54 kDa were lessen by aldosterone at 5 min and no signals were detected after 15 min. Higher molecular weight dimeric forms of AT_1R (110 kDa) started to appear at 5 min, and were highest at 30 min after aldosterone treatment. The dimeric form of AT_1R protein increased to 136 ± 15% in the 5-min group ($p = 0.99$), 5888 ± 152% in the 15-min group ($p < 0.001$; compared with the control and 5-min groups), and to 8070 ± 179% in the 30-min group ($p < 0.001$; compared with all groups).

To examine whether aldosterone-induced AT_1R dimerization through a dose-dependent mechanism, several concentrations of aldosterone were applied (1, 10, or 100 nM). After 30-min incubation, significant increases of AT_1R dimeric forms were observed at doses of 10 nM from 100% in the control to 3558 ± 106%; $p < 0.001$ and of 100 nM to 8342 ± 176% ($p < 0.001$) (Fig. 1b). For the positive control, H_2O_2 treatment was used as a form of oxidative stress to induce AT_1R dimerization. Aldosterone could mimic the action of H_2O_2-induced AT_1R dimerization (Fig. 1b). Formation of AT_1R dimers following aldosterone exposure is time- and dose-dependent (Fig. 1a, 1b). Pretreatment with eplerenone or apocynin blocked the effects of aldosterone-induced AT_1R dimerization as compared with the control group (control = 100%; Aldo = 7998 ± 170%, $p < 0.001$; Ep.+Aldo = 112.5 ± 7.8%, $p = 0.25$; Apo.+Aldo = 116.7 ± 6.8%, $p = 0.12$) (Fig. 1c).

Effect of aldosterone on p47phox protein abundances

We also examined the effects of aldosterone on p47phox protein levels in whole cell lysate, cytosol, and plasma membrane fractions. As shown in Fig. 2a, aldosterone decreased the p47phox protein level in the cytosol to half of the vehicle control (control = 42.4 ± 2.9%; Aldo = 18.7 ± 0.6%, $p < 0.01$). However, the membranous protein was increased to approximately two times as compared with the control group (control = 12.1 ± 0.6%; Aldo = 29.7 ± 1.3%, $p < 0.05$). In contrast, aldosterone treatment had no effect on the p47phox protein levels in whole cell lysates

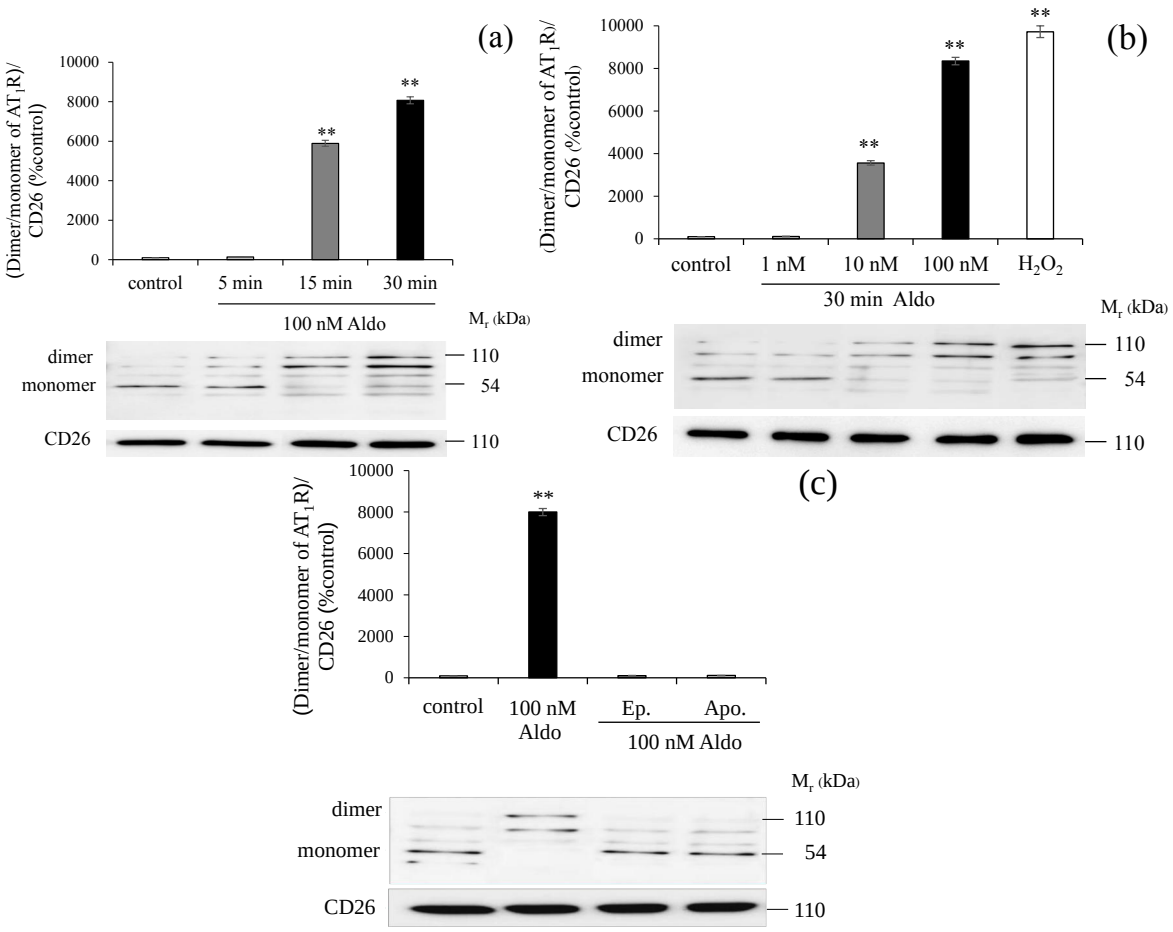

Fig. 1 Aldosterone enhanced plasma membrane protein abundance of AT_1R dimeric form in (a) time-dependent and (b) dose-dependent manners. (c) Aldosterone stimulated AT_1R dimerization via MR dependence and NADPH oxidase activation. Histogram bars show densitometric analysis ratios of the dimer/monomer forms of AT_1R to CD26 intensity. Representative immunoblot photographs are shown. Data are means ± SD of 4 independent experiments. ** $p < 0.001$ compared with the control group. H_2O_2 (200 μM) was used for a positive control.

(Fig. 2a). These results suggested that aldosterone enhances membranous translocation of p47phox proteins. In addition, aldosterone-induced membranous protein accumulation of p47phox was abolished by pretreatment with eplerenone or apocynin (control = 100%; Aldo = 243.1 ± 8.1%, $p < 0.001$; Ep.+Aldo = 110.1 ± 5.8%, $p = 0.23$; Apo.+Aldo = 106.0 ± 6.4%, $p = 0.61$) (Fig. 2b).

Effect of aldosterone on nuclear ATF4 protein abundances

Fig. 3a shows representative bands (38 kDa) of the nuclear ATF4 protein in the absence and presence of aldosterone exposure at 5, 15, and 30 min. The nuclear ATF4 protein level was highest at 30 min after aldosterone treatment as compared with the control group, from 100% to 113.1 ± 7.8% in the 5-min group ($p = 0.23$), 156.7 ± 4.7% in the 15-min group ($p < 0.01$), and 207 ± 10% in the 30-min group ($p < 0.001$). Dose-dependent responses were monitored using aldosterone treatment at 1, 10, or 100 nM. After a 30-min incubation, only a dose of 100 nM could significantly increase the nuclear ATF4 protein level to 197.1 ± 6.6% ($p < 0.001$) relative to vehicle control, (Fig. 3b). A positive control utilized tunicamycin was used as an inducer of endoplasmic reticulum stress to increase nuclear accumulation of ATF4 protein. Aldosterone could mimic the effect of tunicamycin-induced ATF4 protein accumulation in the nucleus. Accumulation of nuclear ATF4 protein-activated by aldosterone was a time-dependent manner at the dose of 100 nM

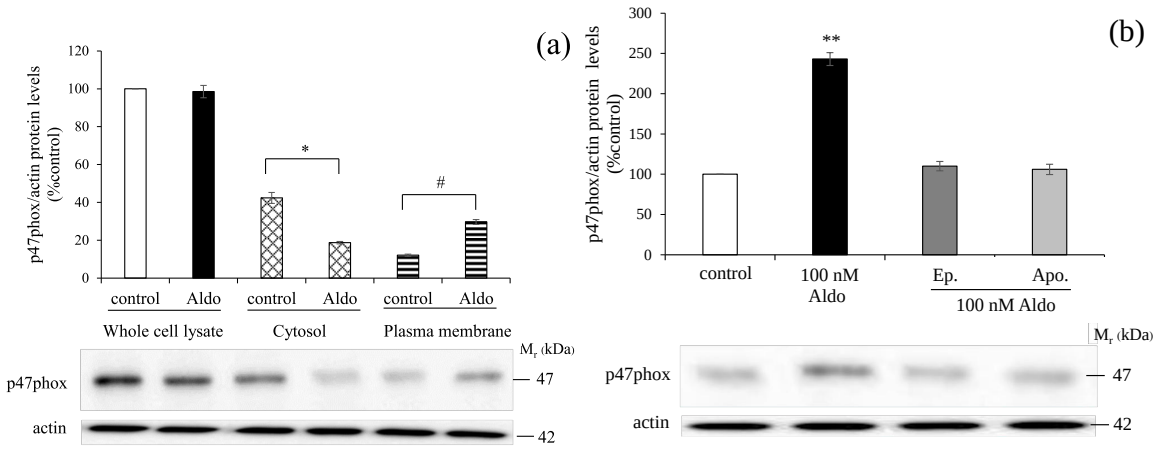

Fig. 2 (a) Aldosterone at dose of 100 nM increased plasma membrane protein level of p47phox but the cytosolic protein content was decreased. (b) Aldosterone-enhanced plasma membrane protein abundances of p47phox were mediated through MR dependence and NADPH oxidase activation. Histogram bars show densitometric analysis ratios of p47phox to actin intensity. Representative immunoblot photographs are shown. Data are means ± SD of 4 independent experiments. * $p < 0.01$ compared with the cytosolic fraction from the control group, # $p < 0.05$ compared with the plasma membrane fraction from the control group, ** $p < 0.001$ compared with the control group.

aldosterone (Fig. 3a). In addition, nuclear ATF4 protein level induced by aldosterone exposure was completely inhibited by pretreatment with apocynin, but only partially abrogated by eplerenone (control = 100%; Aldo = 202.6 ± 7.5%, $p < 0.001$; Ep.+Aldo = 155.0 ± 6.0%, $p < 0.01$; Apo.+Aldo = 101.4 ± 4.6%, $p = 0.98$) (Fig. 3c).

DISCUSSION

In this study, we show the first in vitro investigation into the mechanism of the nongenomic effects of aldosterone. Increased abundance of dimerized-AT_1R proteins from aldosterone exposure was time- and dose-dependent in cultured human renal PTECs (Fig. 1a, 1b). Essential roles of AT_1R dimerization are receptor activation, signalling, and functions through G-protein activation[9]. Several studies indicated that AT_1R dimerization plays important roles in both physiological and pathological conditions[10,12,27]. Under physiological conditions, AT_1R dimerization could stimulate Na^+-ATPase activity in microdissected rat proximal tubules, resulting in sodium reabsorption[10]. Furthermore, AT_1R dimerization also activated the Ca^{2+}-ATPase activity of the sarco(endo)plasmic reticulum in cultured porcine proximal tubular cells, leading to alterations in calcium homeostasis in renal cells[27]. Under pathological conditions, an increase in AT_1R dimerization occurred in the renal cortex in the preeclampsia rat model[12].

Our investigation has demonstrated that aldosterone increased AT_1R dimers and decreased AT_1R monomers (Fig. 1c). In contrast, a previous study using vascular mouse smooth muscle cells revealed that while AT_1R dimers were elevated after aldosterone administration, monomers were unchanged[8]. This discrepancy may be due to the difference in animal species and the cell types used. A mechanism of cellular G-protein-coupled receptor dimerization has been proposed[28]. Oxidative stress-induced dimerization of AT_1R was enhanced while monomers were decreased in human embryonic kidney cells transfected with AT_1R[13]. Hence aldosterone might increase the formation of AT_1R dimers from monomers in renal PTECs, resulting in a decrease in AT_1R monomers.

Our first observation indicated that aldosterone-induced AT_1R dimerization occurred through an MR-dependent pathway with the activation of NADPH oxidase (Fig. 1c), since NADPH oxidase is a major source of oxidative stress in the kidney, including PTECs[29,30]. Our study demonstrated that aldosterone reduced cytosolic p47phox and activated its insertion into the plasma membrane (Fig. 2a). These findings are consistent with the hypothesis that NADPH oxidase contributes to oxidative stress production after aldosterone treatment[14]. Furthermore, aldosterone-induced membranous translocation of p47phox was markedly attenuated by eplerenone (Fig. 2b). These results

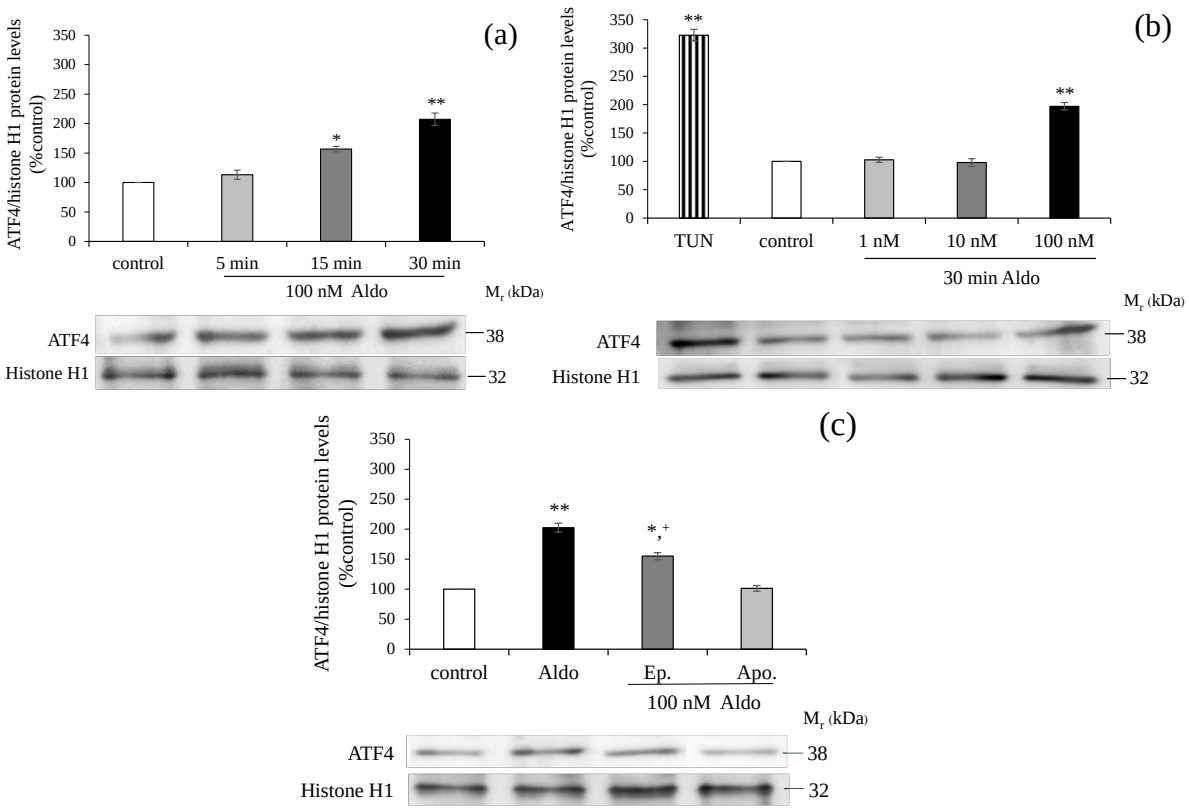

Fig. 3 (a) Aldosterone elevated nuclear protein content of ATF4 in a time-dependent manner. (b) After 30-min incubation, only the dose of 100 nM aldosterone significantly enhanced nuclear ATF4 protein level. (c) Aldosterone induced ATF4 protein accumulation through MR dependence and NADPH oxidase activation. Histogram bars show densitometric analysis ratios of ATF4 to histone H1 intensity, and the representative immunoblot photographs are presented. Data are means ± SD of 4 independent experiments. * $p < 0.01$ compared with the control group, ** $p < 0.001$ compared with the control group, + $p < 0.01$ compared with the Aldo group. Tunicamycin (TUN, 2 μg/ml) was used for a positive control.

indicate that, in the MR pathway, aldosterone has the potential stimulation on p47phox which is an adaptor protein to regulate the assembly of the NADPH oxidase complex. Regarding the mechanism of aldosterone in this milieu, aldosterone in the MR pathway exerts p47phox-induced NADPH oxidase activation and stimulates AT_1R dimerization in human renal PTEC cells.

The present study provides the first in vitro data which showed aldosterone increases nuclear ATF4 protein abundance in a time-dependent manner at the dose of 100 nM aldosterone (Fig. 3a). ATF4, a transcription factor responded to ER stress induced by unfolded protein, plays a role in the regulation of downstream genes and proteins involved in the maintenance of protein homeostasis in the ER [16, 31]. In a previous study, it has been reported that homocysteate, an oxidative stress inducer, increased nuclear ATF4 protein accumulation in cultured mouse cerebral cortex [17]. Aldosterone has also been reported to nongenomically elevate oxidative stress in porcine proximal tubular cells and in Madin Darby canine kidney cells (representing cells of distal tubule) [32]. Furthermore, aldosterone has been shown to rapidly enhance oxidative stress through NADPH oxidase [14]. These data suggested that aldosterone might contribute to the accumulation of nuclear ATF4 protein through activation of the NADPH oxidase pathway. Nevertheless, the effect of nuclear ATF4 protein accumulation by aldosterone has not been examined. In the present study, we provide evidence that aldosterone-induced nuclear ATF4 protein accumulation is mediated through NADPH oxidase activation since the accumulation was abolished by apocynin, an inhibitor of NADPH oxidase, (Fig. 3c). Collectively, aldosterone nonge-

nomically activated NADPH oxidase through an MR-dependent pathway that may induce ER stress and consequently stimulated nuclear ATF4 protein accumulation in HK-2 cells.

In conclusion, the present in vitro study in human renal PTECs demonstrates that aldosterone nongenomically increases AT_1R dimerization and nuclear ATF4 protein accumulation through MR and NADPH oxidase. Our findings suggest that, in proximal tubular cells, aldosterone induces AT_1R dimerization and activates endoplasmic reticulum stress.

Acknowledgements: This work was supported by the Royal Golden Jubilee PhD scholarship under the Grant number PHD/0104/2556 awarded to Mr Kittisak Sinphitukkul by the Thailand Research Fund. Our appreciation is expressed to the Graduate Division, Department of Physiology, Faculty of Medicine, Chulalongkorn University. The grateful acknowledgement is also expressed to the Division of Nephrology and Endocrinology and Division of Chronic Kidney Disease Pathophysiology, The University of Tokyo Graduate School of Medicine.

REFERENCES

1. Dooley R, Harvey BJ, Thomas W (2012) Non-genomic actions of aldosterone: from receptors and signals to membrane targets. *Mol Cell Endocrinol* **350**, 223–34.
2. Rozansky DJ (2006) The role of aldosterone in renal sodium transport. *Semin Nephrol* **26**, 173–81.
3. Thomas W, Harvey BJ (2011) Mechanisms underlying rapid aldosterone effects in the kidney. *Annu Rev Physiol* **73**, 335–57.
4. Winter C, Schulz N, Giebisch G, Geibel JP, Wagner CA (2004) Nongenomic stimulation of vacuolar H^+-ATPases in intercalated renal tubule cells by aldosterone. *Proc Natl Acad Sci USA* **101**, 2636–41.
5. Eiam-Ong S, Sinphitukkul K, Manotham K, Eiam-Ong S (2014) Rapid action of aldosterone on protein expressions of protein kinase C alpha and alpha1 sodium potassium adenosine triphosphatase in rat kidney. *J Steroids Horm Sci* **5**, 1–5.
6. Vinson GP, Coghlan JP (2010) Expanding view of aldosterone action, with an emphasis on rapid action. *Clin Exp Pharmacol Physiol* **37**, 410–6.
7. Grossmann C, Benesic A, Krug AW, Freudinger R, Mildenberger S, Gassner B, Gekle M (2005) Human mineralocorticoid receptor expression renders cells responsive for nongenotropic aldosterone actions. *Mol Endocrinol* **19**, 1697–710.
8. Yamada M, Kushibiki M, Osanai T, Tomita H, Okumura K (2008) Vasoconstrictor effect of aldosterone via angiotensin II type 1 (AT_1) receptor: possible role of AT_1 receptor dimerization. *Cardiovasc Res* **79**, 169–78.
9. Lyngsø C, Erikstrup N, Hansen JL (2009) Functional interactions between 7TM receptors in the renin-angiotensin system–dimerization or crosstalk? *Mol Cell Endocrinol* **302**, 203–12.
10. Dias J, Ferrão FM, Axelband F, Carmona AK, Lara LS, Vieyra A (2014) ANG-(3-4) inhibits renal Na^+-ATPase in hypertensive rats through a mechanism that involves dissociation of ANG II receptors, heterodimers, and PKA. *Am J Physiol* **306**, F855–63.
11. AbdAlla S, Lother H, Langer A, el Faramawy Y, Quitterer U (2004) Factor XIIIA transglutaminase crosslinks AT_1 receptor dimers of monocytes at the onset of atherosclerosis. *Cell* **119**, 343–54.
12. Anguiano-Robledo L, Reyes-Melchor PA, Bobadilla-Lugo RA, Pérez-Alvarez VM, López-Sánchez P (2007) Renal angiotensin-II receptors expression changes in a model of preeclampsia. *Hypertens Pregnancy* **26**, 151–61.
13. AbdAlla S, Lother H, el Massiery A, Quitterer U (2001) Increased AT_1 receptor heterodimers in preeclampsia mediate enhanced angiotensin II responsiveness. *Nat Med* **7**, 1003–9.
14. Queisser N, Schupp N, Stopper H, Schinzel R, Oteiza PI (2011) Aldosterone increases kidney tubule cell oxidants through calcium-mediated activation of NADPH oxidase and nitric oxide synthase. *Free Radic Biol Med* **51**, 1996–2006.
15. Ding W, Yang L, Zhang M, Gu Y (2012) Reactive oxygen species-mediated endoplasmic reticulum stress contributes to aldosterone-induced apoptosis in tubular epithelial cells. *Biochem Biophys Res Commun* **418**, 451–6.
16. Inagi R, Ishimoto Y, Nangaku M (2014) Proteostasis in endoplasmic reticulum–new mechanisms in kidney disease. *Nat Rev Nephrol* **10**, 369–78.
17. Lange PS, Chavez JC, Pinto JT, Coppola G, Sun CW, Townes TM, Geschwind DH, Ratan RR (2008) ATF4 is an oxidative stress-inducible, prodeath transcription factor in neurons in vitro and in vivo. *J Exp Med* **205**, 1227–42.
18. Zhang A, Jia Z, Guo X, Yang T (2007) Aldosterone induces epithelial-mesenchymal transition via ROS of mitochondrial origin. *Am J Physiol* **293**, F723–31.
19. Shalamanova L, Wilkinson MC, McArdle F, Jackson MJ, Rustom R (2010) Characterisation of the expression of the Renin-Angiotensin system in primary and immortalised human renal proximal tubular cells. *Nephron Exp Nephrol* **116**, e53–61.
20. Muratsu-Ikeda S, Nangaku M, Ikeda Y, Tanaka T, Wada T, Inagi R (2012) Downregulation of miR-205 modulates cell susceptibility to oxidative and endoplasmic reticulum stresses in renal tubular cells. *PLoS One* **7**, e41462.
21. Shalamanova L, McArdle F, Amara AB, Jackson MJ, Rustom R (2007) Albumin overload induces adaptive

responses in human proximal tubular cells through oxidative stress but not via angiotensin II type 1 receptor. *Am J Physiol* **292**, F1846–57.
22. Miyata K, Rahman M, Shokoji T, Nagai Y, Zhang GX, Sun GP, Kimura S, Yukimura T, et al (2005) Aldosterone stimulates reactive oxygen species production through activation of NADPH oxidase in rat mesangial cells. *J Am Soc Nephrol* **16**, 2906–12.
23. Kawakami T, Inagi R, Takano H, Sato S, Ingelfinger JR, Fujita T, Nangaku M (2009) Endoplasmic reticulum stress induces autophagy in renal proximal tubular cells. *Nephrol Dial Transplant* **24**, 2665–72.
24. Girardi AC, Knauf F, Demuth HU, Aronson PS (2004) Role of dipeptidyl peptidase IV in regulating activity of Na^+/H^+ exchanger isoform NHE3 in proximal tubule cells. *Am J Physiol* **287**, C1238–45.
25. Kitada M, Koya D, Sugimoto T, Isono M, Araki S, Kashiwagi A, Haneda M (2003) Translocation of glomerular p47phox and p67phox by protein kinase C-beta activation is required for oxidative stress in diabetic nephropathy. *Diabetes* **52**, 2603–14.
26. Chiang CK, Nangaku M, Tanaka T, Iwawaki T, Inagi R (2013) Endoplasmic reticulum stress signal impairs erythropoietin production: a role for ATF4. *Am J Physiol* **304**, C342–53.
27. Ferrão FM, Lara LS, Axelband F, Dias J, Carmona AK, Reis RI, Costa-Neto CM, Vieyra A, et al (2012) Exposure of luminal membranes of LLC-PK1 cells to ANG II induces dimerization of AT_1/AT_2 receptors to activate SERCA and to promote Ca^{2+} mobilization. *Am J Physiol* **302**, F875–83.
28. Devi LA (2001) Heterodimerization of G-protein-coupled receptors: pharmacology, signaling and trafficking. *Trends Pharmacol Sci* **22**, 532–7.
29. Schreck C, O'Connor PM (2011) NAD(P)H oxidase and renal epithelial ion transport. *Am J Physiol* **300**, R1023–9.
30. Gill PS, Wilcox CS (2006) NADPH oxidases in the kidney. *Antioxid Redox Signal* **8**, 1597–607.
31. Inagi R (2010) Endoplasmic reticulum stress as a progression factor for kidney injury. *Curr Opin Pharmacol* **10**, 156–65.
32. Queisser N, Oteiza PI, Stopper H, Oli RG, Schupp N (2011) Aldosterone induces oxidative stress, oxidative DNA damage and NF-kB-activation in kidney tubule cells. *Mol Carcinog* **50**, 123–35.