RESEARCH HIGHLIGHT

New mechanism leading to alleviation of salt-sensitive hypertension by a powerful angiotensin receptor blocker, azilsartan

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Hypertension is one of the most life-threatening health problems in the modern world. Particularly, salt-sensitive hypertension is often associated with cardiovascular disease and defects in the circadian rhythm of the blood pressure. To date, the effects of angiotensin receptor blocker (ARB) against salt sensitivity and the blood pressure's circadian rhythm have been obscure. A strong ARB, azilsartan, was previously reported to improve the circadian rhythm of blood pressure in hypertensive patients. In a recently published study, we investigated the mechanism by which azilsartan brought about this reaction. We speculated that azilsartan modulated sodium transporters located in the renal tubules because the circadian rhythm of blood pressure is linked to salt handling in the kidney. We discovered that one sodium transporter, NHE3 protein, in the proximal tubules was greatly attenuated in the kidneys of 5/6 nephrectomized mice that had been treated with azilsartan, although the expression of other sodium transporter proteins remained unchanged. The genetic expression of NHE3, however, was not changed by azilsartan. In a subsequent in vitro study using OKP cells, we found that NHE3 protein reduction was induced by enhanced protein degradation by proteasomes, not lysosomes, leading to enhanced sodium excretion. It is suggested that diminished salt sensitivity in the 5/6 nephrectomized mice treated with azilsartan was due to a change in sodium handling induced by the reduction of NHE3 protein in the proximal tubules. These mechanisms underlying the decreased salt sensitivity by azilsartan treatment may lead to totally new drug discoveries.

Keywords: angiotensin receptor blocker; salt-sensitive hypertension; circadian rhythm of blood pressure; sodium transporter; NHE3; proteasome

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Salt-sensitive hypertension and circadian rhythm defect in the blood pressure

Hypertension is one of the most important risks for the development of cardiovascular and kidney diseases. About 25% of the adult populations in westernized countries are affected by hypertension [1]. Sodium ingestion has been demonstrated to be closely related to its development [2]. Sodium sensitivity in normotensive patients is defined as a decrease in the mean arterial pressure of at least 3 mmHg following reduced salt intake [3]. Salt-sensitive hypertension patients have a greater tendency to experience cardiovascular and renal events than non-salt-sensitive patients [4]. Disturbed circadian rhythm of the blood pressure is reported to be an independent predictor of cardiovascular events [5-10] and to be highly associated with salt sensitivity [11, 12]. Patients whose blood pressure does not decrease during the night (non-dipper patients) are often found to be salt sensitive.

Causes of salt sensitivity

There are mainly two causes of salt sensitivity related to the kidney: reduced glomerular sodium filtration and increased tubular reabsorption of sodium. In the former case, salt sensitivity derives from impaired sodium excretion due to reduced nephron mass in individuals with chronic kidney disease [13] or those with naturally fewer nephrons (African Americans [14] or patients born with low birth weight [15]). In the latter, salt sensitivity is implicated in increased renin-angiotensin-aldosterone activity [16], hyperinsulinemia [17], insulin resistance [18], and sympathetic hyperactivity [19]. From a molecular viewpoint, sodium channels or sodium transporters are shown to be related to salt sensitivity. To date, four sodium transporters have been identified in nephron segments: NHE3 in the proximal tubules, Na^+-K^+-Cl^- cotransporter-2 (NKCC2) in the thick ascending loop of Henle, Na^+-Cl^- cotransporter (NCC) in the ductal convoluted tubules, and Enacs in the collecting ducts. It has been reported that NKCC2 causes salt sensitivity in the Milan hypertensive strain of rats [20]. The development of salt-sensitive hypertension in C57B6 mice and Dahl rats was associated with α_2-adrenergic receptor, WNK lysine-deficient protein kinase-4, and NCC [19]. The major sodium transporter in the proximal tubules, NHE3, has been only modestly evaluated as a regulator of salt sensitivity, although transgenic mice overexpressing NHE3 in all body parts were reported to have salt-sensitive hypertension [21].

Renin-angiotensin system blockers and salt sensitivity

Renin-angiotensin system (RAS) blockers -angiotensin-converting enzyme inhibitor and angiotensin receptor blocker (ARB)-remain the main choices for hypertension therapy, particularly to prevent organ damage induced by hypertension [22, 23]. RAS blockers, however, have been considered unsuitable for treating salt-sensitive hypertension because salt loading in hypertensive patients erases the antihypertensive effects of RAS blockers [24-26], suggesting that RAS blockers may even enhance salt sensitivity [27, 28]. Although negative effects of RAS blockers on salt sensitivity have been reported, the new strong ARB, azilsartan, seems to react differently to salt-sensitive hypertension. Recent studies in hypertensive patients demonstrated that azilsartan treatment brought about a more significant and persistent reduction in blood pressure for 24 hours than other ARBs. It also changed the non-dipper pattern of blood pressure (nocturnal hypertension) into a dipper pattern more effectively than candesartan. These findings suggested that azilsartan could improve the circadian rhythm of blood pressure [29–31]. Such interesting observations prompted us to elucidate the mechanisms by which azilsartan was able to improve the circadian rhythm of blood pressure.

Unique properties of the strong ARB, azilsartan

Azilsartan has high selectivity for AT1R, with ≥10,000-fold affinity for AT1R compared with for AT2R. It was also shown that azilsartan could function as an inverse agonist [32]. Azilsartan has a unique side chain, 5-oxo-1,2,4-oxadiazole, in place of the tetrazole ring in candesartan, which accounts for its strong antagonistic activity and higher affinity for AT1R [33, 34].

New ARB, azilsartan, effect on salt sensitivity in 5/6 nephrectomized mice with high-salt diet

We selected 5/6 nephrectomized mice with a high-salt diet (8% NaCl) as a salt-sensitive hypertension model. Azilsartan treatment lowered blood pressure and diminished salt sensitivity in the 5/6 nephrectomized mice more effectively than candesartan, which was confirmed on pressure-natriuresis curves. The detailed examination of sodium transporters in the remnant kidney tissues revealed that the quantity of the main sodium transporter in proximal tubules, NHE3 protein, was decreased in azilsartan-treated murine kidneys, whereas other sodium transporters located in the other nephron segments were unchanged. NHE3 mRNA expression, however, was not changed by azilsartan. Hence, we speculated that this reduction of NHE3 by azilsartan caused the change in sodium handling in nephrons, leading to the alleviation of salt sensitivity [35].

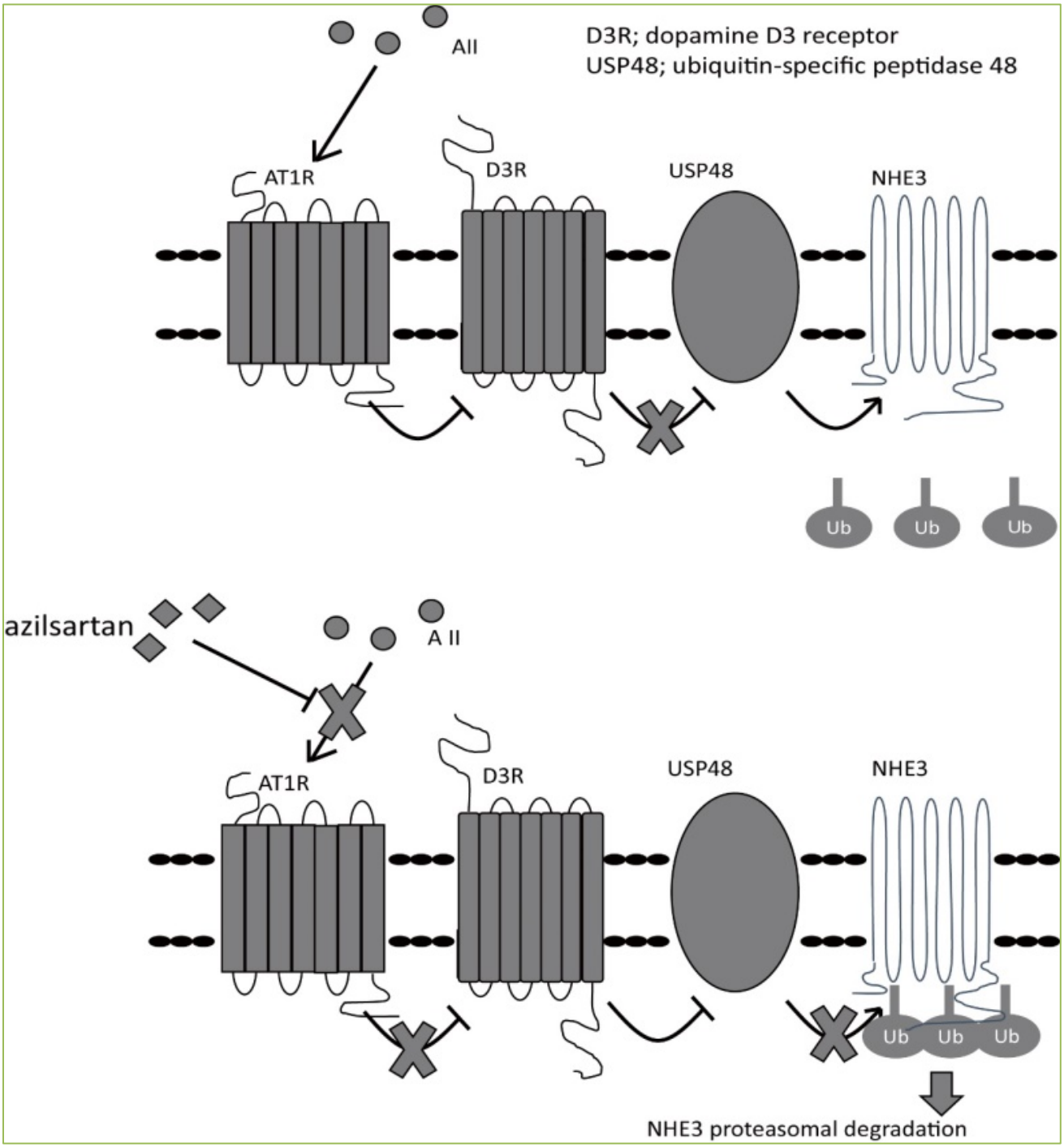

Figure 1. Proposed mechanism of NHE3 protein degradation through AT1R blockade. NHE3 ubiquitination is in equilibrium with the NHE3 de-ubiquitination by USP48 in the basal state, which is inhibited by the D3 receptor. The D3 receptor is also antagonized by angiotensin II-stimulated AT1R (the upper figure). When azilsartan blocks the AT1R signal, the D3 receptor goes toward inhibition of USP48 expression. NHE3 de-ubiquitination by USP48 is inhibited, so NHE3 subjects to proteasomal degradation (the lower figure).

Factors affecting NHE3 protein quantity

NHE3 protein reduction due to factors other than mRNA reduction has been reported in several cases. Chronic administration of dopamine in mice decreased NHE3 protein caused by degradation via a ubiquitin–proteasomal pathway [36]. Acute kidney ischemia–reperfusion injury induces NHE3 protein and transcript reduction, which is also mediated by degradation via a ubiquitin–proteasomal pathway. The key factors mediating these phenomena could be proteins or

proteolipid complexes [37]. Our in vitro study confirmed that azilsartan treatment with angiotensin II induced NHE3 degradation via a ubiquitin–proteasomal pathway as well [35]. These data are consistent with those of a previous report on experiments using proximal tubule-specific AT1 receptor (AT1R) knockout mice [38].

Proposed mechanism of NHE3 protein degradation through AT1R blockade

In a previous study, AT1R formed a protein complex with dopamine D3 receptor [39]. Recently, it was reported that ubiquitin-specific peptidase (USP) 48 was implicated in NHE3 degradation via dopamine. The dopamine-stimulated dopamine D3 receptor inhibits USP48 function, which leads to the promotion of NHE3 degradation via a ubiquitin–proteasomal pathway [40]. Thus, we hypothesized that NHE3 ubiquitination is in equilibrium with the NHE3 de-ubiquitination by USP48 in the basal state, which is inhibited by the D3 receptor. The D3 receptor is also antagonized by angiotensin II-stimulated AT1R. When azilsartan blocks the AT1R signal, the D3 receptor starts the course toward inhibition of USP48 expression. NHE3 de-ubiquitination by USP48 is blocked, so NHE3 proceeds to proteasomal degradation (Figure 1). Thus, NHE3 function decreases, which results in increased sodium excretion and alleviated salt sensitivity. In the future, it may be possible for the new drug for salt-sensitive hypertension to be developed using these mechanisms as targets.

Conflicting interests

The authors have declared that no conflict of interests exist.

Author contributions

S.T., Y.I. and H.R. conceived the paper. J.Y.K wrote the paper. M.H., S.Y. and N.I. edited the paper.

Abbreviations

ARB: angiotensin receptor blocker; AT1R: angiotensin type 1 receptor; AT2R: angiotensin type 2 receptor; NCC: Na^+-Cl^- cotransporter; NHE3: Na^+/H^+ exchanger; NKCC2: Na^+-K^+-Cl^- cotransporter-2; OKP: opossum kidney, clone P; RAS: Renin-angiotensin system; USP: ubiquitin-specific peptidase; WNK: with no lysine.

References

1. Lewington S, Clarke R, Qizilbash N, Peto R, Collins R. Age-specific relevance of usual blood pressure to vascular mortality: a meta-analysis of individual data for one million adults in 61 prospective studies. Lancet 2002; 360:1903-1913.
2. Yu HC, Burrell LM, Black MJ, Wu LL, Dilley RJ, Cooper ME, *et al*. Salt induces myocardial and renal fibrosis in normotensive and hypertensive rats. Circulation 1998; 98:2621-2628.
3. Weinberger MH. Salt sensitivity of blood pressure in humans. Hypertension 1996; 27:481-490.
4. Morimoto A, Uzu T, Fujii T, Nishimura M, Kuroda S, Nakamura S, *et al*. Sodium sensitivity and cardiovascular events in patients with essential hypertension. Lancet 1997; 350:1734-7.
5. Dolan E, Stanton A, Thijs L, Hinedi K, Atkins N, McClory S, *et al*. Superiority of ambulatory over clinic blood pressure measurement in predicting mortality: the Dublin outcome study. Hypertension 2005; 46:156-161.
6. Kario K. Morning surge in blood pressure and cardiovascular risk: evidence and perspectives. Hypertension 2010; 56:765-73.
7. Ohkubo T, Hozawa A, Yamaguchi J, Kikuya M, Ohmori K, Michimata M, *et al*. Prognostic significance of the nocturnal decline in blood pressure in individuals with and without high 24-h blood pressure: the Ohasama study. J Hypertens 2002; 20:2183-2189.
8. Sega R, Facchetti R, Bombelli M, Cesana G, Corrao G, Grassi G, *et al*. Prognostic value of ambulatory and home blood pressures compared with office blood pressure in the general population: follow-up results from the Pressioni Arteriose Monitorate e Loro Associazioni (PAMELA) study. Circulation 2005; 111:1777-1783.
9. Verdecchia P, Porcellati C, Schillaci G, Borgioni C, Ciucci A, Battistelli M, *et al*. Ambulatory blood pressure. An independent predictor of prognosis in essential hypertension. Hypertension 1994; 24:793-801.
10. Verdecchia P, Angeli F, Mazzotta G, Garofoli M, Ramundo E, Gentile G, *et al*. Day-night dip and early-morning surge in blood pressure in hypertension: prognostic implications. Hypertension 2012; 60:34-42.
11. Uzu T, Kazembe FS, Ishikawa K, Nakamura S, Inenaga T, Kimura G. High sodium sensitivity implicates nocturnal hypertension in essential hypertension. Hypertension 1996; 28:139-142.
12. Uzu T, Ishikawa K, Fujii T, Nakamura S, Inenaga T, Kimura G. Sodium restriction shifts circadian rhythm of blood pressure from nondipper to dipper in essential hypertension. Circulation 1997; 96:1859-1862.
13. Johnson RJ, Rodriguez-Iturbe B, Nakagawa T, Kang DH, Feig DI, Herrera-Acosta J. Subtle renal injury is likely a common mechanism for salt-sensitive essential hypertension. Hypertension 2005; 45:326-330.
14. Gretler DD, Fumo MT, Nelson KS, Murphy MB. Ethnic differences in circadian hemodynamic profile. Am J Hypertens 1994; 7:7-14.
15. Barker DJ. The fetal origins of adult hypertension. J Hypertens 1992; 10:S39-44.
16. Shibata S, Mu S, Kawarazaki H, Muraoka K, Ishizawa K, Yoshida S, *et al*. Rac1 GTPase in rodent kidneys is essential for salt-sensitive hypertension via a mineralocorticoid receptor-dependent pathway. J Clin Invest 2011; 121:3233-3243.
17. Rocchini AP, Key J, Bondie D, Chico R, Moorehead C, Katch V, *et al*. The effect of weight loss on the sensitivity of blood pressure to sodium in obese adolescents. New Engl J M 1989;

321:580-585.

18. Zheng Y, Yamada H, Sakamoto K, Horita S, Kunimi M, Endo Y, *et al*. Roles of insulin receptor substrates in insulin-induced stimulation of renal proximal bicarbonate absorption. JASN 2005; 16:2288-2295.
19. Mu S, Shimosawa T, Ogura S, Wang H, Uetake Y, Kawakami-Mori F, *et al*. Epigenetic modulation of the renal beta-adrenergic-WNK4 pathway in salt-sensitive hypertension. Nat Med 2011; 17:573-80.
20. Carmosino M, Rizzo F, Ferrari P, Torielli L, Ferrandi M, Bianchi G, *et al*. NKCC2 is activated in Milan hypertensive rats contributing to the maintenance of salt-sensitive hypertension. Pflug Arch 2011; 462:281-291.
21. Kuro-o M, Hanaoka K, Hiroi Y, Noguchi T, Fujimori Y, Takewaki S, *et al*. Salt-sensitive hypertension in transgenic mice overexpressing Na(+)-proton exchanger. Circ Res 1995; 76:148-153.
22. Morimoto S, Yano Y, Maki K, Sawada K. Renal and vascular protective effects of telmisartan in patients with essential hypertension. Hypertens Res 2006; 29:567-572.
23. Dahlof B, Devereux RB, Kjeldsen SE, Julius S, Beevers G, de Faire U, *et al*. Cardiovascular morbidity and mortality in the Losartan Intervention For Endpoint reduction in hypertension study (LIFE): a randomised trial against atenolol. Lancet 2002; 359:995-1003.
24. Herlitz H, Dahlof B, Jonsson O, Friberg P. Relationship between salt and blood pressure in hypertensive patients on chronic ACE-inhibition. Blood Press 1998; 7:47-52.
25. Fang Z, Sripairojthikoon W, Calhoun DA, Zhu S, Berecek KH, Wyss JM. Interaction between lifetime captopril treatment and NaCI-sensitive hypertension in spontaneously hypertensive rats and Wistar-Kyoto rats. J Hypertens 1999; 17:983-991.
26. Xu L, Brooks VL. Sodium intake, angiotensin II receptor blockade, and baroreflex function in conscious rats. Hypertension 1997; 29:450-457.
27. Endo S, Mori T, Yoneki Y, Nakamichi T, Hosoya T, Ogawa S, *et al*. Blockade of angiotensin II type-1 receptor increases salt sensitivity in Sprague-Dawley rats. Hypertens Res 2009; 32:513-519.
28. Kimura G, Deguchi F, Kojima S, Ashida T, Yoshimi H, Abe H, *et al*. Antihypertensive drugs and sodium restriction. Analysis of their interaction based on pressure-natriuresis relationship. Am J Hypertens 1988; 1:372-379.
29. Takagi H, Mizuno Y, Niwa M, Goto SN, Umemoto T. A meta-analysis of randomized controlled trials of azilsartan therapy for blood pressure reduction. Hypertens Res 2014; 37:432-437.
30. Rakugi H, Enya K, Sugiura K, Ikeda Y. Comparison of the efficacy and safety of azilsartan with that of candesartan cilexetil in Japanese patients with grade I-II essential hypertension: a randomized, double-blind clinical study. Hypertens Res 2012; 35:552-558.
31. Rakugi H, Kario K, Enya K, Igeta M, Ikeda Y. Effect of azilsartan versus candesartan on nocturnal blood pressure variation in Japanese patients with essential hypertension. Blood Press 2013; 22 Suppl 1:22-28.
32. Kurtz TW, Kajiya T. Differential pharmacology and benefit/risk of azilsartan compared to other sartans. Vasc Health Risk Manag 2012; 8:133-143.
33. Ojima M, Igata H, Tanaka M, Sakamoto H, Kuroita T, Kohara Y, *et al*. In vitro antagonistic properties of a new angiotensin type 1 receptor blocker, azilsartan, in receptor binding and function studies. J Pharmacol Exp Ther 2011; 336:801-808.
34. Miura S, Okabe A, Matsuo Y, Karnik SS, Saku K. Unique binding behavior of the recently approved angiotensin II receptor blocker azilsartan compared with that of candesartan. Hypertens Res 2013; 36:134-139.
35. Hatanaka M, Kaimori JY, Yamamoto S, Matsui I, Hamano T, Takabatake Y, *et al*. Azilsartan Improves Salt Sensitivity by Modulating the Proximal Tubular Na+-H+ Exchanger-3 in Mice. PLos One 2016; 11:e0147786.
36. Hu MC, Di Sole F, Zhang J, McLeroy P, Moe OW. Chronic regulation of the renal Na(+)/H(+) exchanger NHE3 by dopamine: translational and posttranslational mechanisms. Am J Physiol Renal Physiol 2013; 304:F1169-1180.
37. Di Sole F, Hu MC, Zhang J, Babich V, Bobulescu IA, Shi M, *et al*. The reduction of Na/H exchanger-3 protein and transcript expression in acute ischemia-reperfusion injury is mediated by extractable tissue factor(s). Kidney Int 2011; 80:822-8231.
38. Gurley SB, Riquier-Brison AD, Schnermann J, Sparks MA, Allen AM, Haase VH, *et al*. AT1A angiotensin receptors in the renal proximal tubule regulate blood pressure. Cell Metab 2011; 13:469-475.
39. Zeng C, Asico LD, Wang X, Hopfer U, Eisner GM, Felder RA, *et al*. Angiotensin II regulation of AT1 and D3 dopamine receptors in renal proximal tubule cells of SHR. Hypertension 2003; 41:724-729.
40. Armando I, Villar VA, Jones JE, Lee H, Wang X, Asico LD, *et al*. Dopamine D3 receptor inhibits the ubiquitin-specific peptidase 48 to promote NHE3 degradation. FASEB J 2014; 28:1422-1434.