Renal Tubule Angiotensin II Type 1 Receptor–Associated Protein Promotes Natriuresis and Inhibits Salt-Sensitive Blood Pressure Elevation

（腎尿細管におけるアンジオテンシン II1 型受容体直接結合因子 ATRAP は
ナトリウム利尿を促進し，食塩感受性の血圧上昇を抑制する）

Kazushi Uneda
畝田 一司

Departments of Medical Science and Cardiorenal Medicine
Yokohama City University Graduate School of Medicine
横浜市立大学 大学院医学研究科 医科学専攻 病態制御内科学

（Doctoral Supervisor: Satoshi Umemura, Professor）

（指導教員: 梅村 敏 教授）
Renal Tubule Angiotensin II Type 1 Receptor–Associated Protein Promotes Natriuresis and Inhibits Salt-Sensitive Blood Pressure Elevation

http://jaha.ahajournals.org/content/4/3/e001594.long

Introduction
The interaction between genetic and environmental factors, especially the excessive intake of dietary salt typical in many diets and inappropriate sodium retention by the kidney, plays a critical role in pathologically increased BP (O'Shaughnessy KM and Karet FE, 2004). Activation of the RAS promotes sodium reabsorption in the distal tubules through angiotensin II (Ang II) type 1 receptor (AT1R)–mediated stimulation of the thiazide-sensitive Na⁺Cl⁻ cotransporter (NCC) and amiloridesensitive epithelial Na⁺ channel (ENaC) (Nguyen MT et al., 2013; van der Lubbe N et al., 2013). The AT1R-associated protein (ATRAP; Agtrap gene) was identified as a molecule that directly binds to the carboxyl-terminal domain of AT1R and selectively suppresses Ang II–mediated pathological activation of AT1R signaling (Daviet L et al., 1999; Tamura K et al., 2013). We previously showed that sustained recovery of repressed renal ATRAP expression contributed to the longterm therapeutic effects of prepubertal transient treatment with an AT1R blocker in dietary high salt (HS) loading–mediated hypertension in Dahl Iwai salt-sensitive rats (Dejima T et al., 2011). Little is known, however, about the functionally causal role of ATRAP in HS-mediated BP regulation. We investigated the effects of dietary HS loading on renal sodium handling and BP regulation in the context of renal distal tubule–dominant enhancement of ATRAP, using transgenic mice on a C57BL/6J background known as a salt-sensitive animal model (Lantelme P et al., 2002).

Methods
Renal ATRAP transgenic (rATRAP-Tg) mice dominantly expressing hemagglutinin-tagged ATRAP in the renal distal tubules were generated on a C57BL/6J (Wt) background, as described previously (Wakui et al., 2013). The rATRAP-Tg mice and Wt mice were subjected to dietary HS loading (4% NaCl) for 7 days. During the loading period, blood pressure was measured by the radiotelemetric method and metabolic cage analysis was performed. In
addition, we compared renal angiotensin II levels, functional activities and expressions of the major renal sodium transporters and renal plasma membranous AT1R expressions between rATRAP-Tg and Wt mice.

Results
At baseline, there were no significant differences in body weight, heart rate, urine volume, creatinine clearance, and plasma physiological parameters, including serum osmolality and aldosterone concentration between the rATRAP-Tg and Wt mice on the normal salt (NS) diet. However, the dietary HS loading–mediated blood pressure elevation in the dark period was suppressed in rATRAP-Tg mice compared with Wt mice (HS vs NS; rATRAP-Tg, 129.8 ± 3.1 vs 127.7 ± 2.9 mmHg, not significant; Wt, 135.8 ± 3.7 vs 126.3 ± 2.4 mmHg, \( P < 0.05 \)). Although renal Ang II levels were comparable in rATRAP-Tg and Wt mice with and without HS loading, urinary sodium excretion in response to HS loading was significantly enhanced in the rATRAP-Tg mice. Moreover, under saline volume-expanded conditions, functional transport activity of ENaC was significantly decreased in rATRAP-Tg mice compared with Wt mice without any significant difference in protein expression. In addition, plasma membrane AT1R expression in the kidney of rATRAP-Tg mice was decreased compared with Wt mice.

Discussion
In contrast to the lack of any evident change in baseline BP in the rATRAP-Tg mice, dietary HS loading–mediated BP elevation was significantly suppressed in the rATRAP-Tg mice, concomitant with increased cumulative sodium excretion. With respect to the mechanisms involved in the suppression of salt-sensitive BP elevation, stimulated sodium reabsorption in the distal tubules plays a critical role in salt-sensitive hypertension (Reilly RF and Ellison DH, 2000; Ronzaud C et al., 2013). Regarding the distal tubules in renal sodium handling, activation of NCC and ENaC in the distal nephron segments reportedly plays a crucial role in the stimulation of sodium reabsorption by the Ang II–AT1R axis (Zhao D et al., 2009; van der Lubbe N et al., 2011; Castaneda-Bueno M et al., 2012; Mamenko M et al., 2013; Zaika O et al., 2013; Moes AD et al., 2014). Although these rATRAP-Tg mice did not exhibit any evident change in renal tissue Ang II level under dietary HS loading, dietary HS loading reportedly provokes salt-induced BP elevation concomitant with activation of the intrarenal renin–
angiotensin system in several models of salt-sensitive hypertension (Lantelme P et al., 2002). The rATRAP-Tg mice exhibited a predominantly high expression pattern of the hemagglutinin-tagged ATRAP transgene in the distal tubules from the distal convoluted tubule to the connecting tubule, in which both NCC and ENaC are abundant. The results of the diuretic tests using specific inhibitors of the respective sodium transporters showed that the functional transport activity of ENaC was significantly decreased in rATRAP-Tg mice compared with Wt mice. These results indicate that inhibition of the functional activity of ENaC is critically involved in suppression of HS-induced BP elevation in rATRAP-Tg mice. Considering that protein expression of the ENaC subunits was not altered in the rATRAP-Tg mouse kidney, posttranslational modifications that alter trafficking or function of the channel, rather than modulation of ENaC expression, may contribute to reduced ENaC function. Further studies are needed to investigate these and other possible mechanisms.

It was reportedly shown that the phenotype of salt sensitivity was not altered by renal proximal tubule–specific deletion of AT1R, regardless of the decrease in baseline BP (Gurley SB et al., 2011). Furthermore, the rATRAP-Tg mice exhibited significant amelioration of Ang II–induced hypertension, despite no evident change in baseline BP, via suppression of upregulation of renal ENaC expression by Ang II stimulation in the absence of any dietary HS loading (Wakui et al., 2013). Collectively, these results indicate that renal distal tubule–dominant ATRAP enhancement exerts an inhibitory effect on dietary HS loading–mediated BP elevation via suppression of the distal tubule AT1R–sodium transporter axis. Indeed, the plasma membrane AT1R level was significantly decreased in the kidney of rATRAP-Tg mice compared with Wt mice, suggesting that enhancement of renal tubule ATRAP expression beyond baseline promotes AT1R internalization to reduce distal tubule AT1R activity.

In conclusion, the present study shows that renal tubule–dominant enhancement of ATRAP suppressed the functional activity of ENaC in the distal tubules, promoting sodium excretion and contributing to inhibition of dietary salt-sensitive BP elevation.


Reilly RF, Ellison DH. Mammalian distal tubule: physiology, pathophysiology, and molecular anatomy. (2000). *Physiol Rev.* 80, 277–313.


論文目録

I 主論文
Renal Tubule Angiotensin II Type 1 Receptor–Associated Protein Promotes Natriuresis and Inhibits Salt-Sensitive Blood Pressure Elevation.

(Hiromichi Wakui and Kazushi Uneda contributed equally to this work.)

II 副論文
1. Comparison of Direct Renin inhibitor and Angiotensin II Receptor Blocker on Clinic and Ambulatory Blood Pressure Profiles in Hypertension with Chronic Kidney Disease.


2. Effects of single pill-based combination therapy of amlodipine and atorvastatin on within-visit blood pressure variability and parameters of renal and vascular function in hypertensive patients with chronic kidney disease.

(Kengo Azushima and Kazushi Uneda contributed equally to this work.)

III 参考論文
1. Effects of aliskiren-based therapy on ambulatory blood pressure profile, central hemodynamics, and arterial stiffness in nondiabetic mild to moderate hypertensive patients.


2. Aliskiren induced remarkable hypertriglyceridemia.


3. L/N-type calcium channel blocker cilnidipine added to renin-angiotensin inhibition improves ambulatory blood pressure profile and suppresses cardiac hypertrophy in hypertension with chronic kidney disease.


5. Upstream stimulatory factors 1 and 2 mediate the transcription of angiotensin II binding and inhibitory protein.

6. Addition of aliskiren to Angiotensin receptor blocker improves ambulatory blood pressure profile and cardiorenal function better than addition of benazepril in chronic kidney disease.


7. Angiotensin Receptor-Binding Protein ATRAP/Agtrap Inhibits Metabolic Dysfunction With Visceral Obesity.


8. Activation of angiotensin II type 1 receptor-associated protein exerts an inhibitory effect on vascular hypertrophy and oxidative stress in angiotensin II-mediated hypertension.


9. Bofu-tsu-shosan, an oriental herbal medicine, exerts a combinatorial favorable metabolic modulation including antihypertensive effect on a mouse model of human metabolic disorders with visceral obesity.


10. Effects of Ang II Receptor Blocker Irbesartan on Adipose Tissue Function in Mice with Metabolic Disorders.


11. Effects of the Angiotensin receptor blocker olmesartan on adipocyte hypertrophy and function in mice with metabolic disorders.


12. Deletion of the angiotensin II type 1 receptor-associated protein enhances renal sodium reabsorption and exacerbates angiotensin II-mediated hypertension.

