Novel Receptor Binding Protein ATRAP in the Regulation of Renal Sodium Reabsorption and Blood Pressure Response

Salt and Health

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Abstract

Pathological activation of renal angiotensin II (Ang II) type 1 receptor (AT1R) signaling stimulates renal tubular sodium transporters including epithelial sodium channel to increase renal sodium reabsorption. In the course of an investigational search for a means to functionally and selectively modulate AT1R signaling for that purpose, a molecule directly interacting with the carboxyl-terminal cytoplasmic domain of AT1R was identified by employing yeast two-hybrid screening of a mouse kidney cDNA library and named AT1R-associated protein (ATRAP). We showed that ATRAP promotes constitutive AT1R internalization so as to inhibit pathological AT1R activation in response to certain stimuli. In the kidney, ATRAP is abundantly distributed in epithelial cells along the renal tubules. The results employing genetic engineered mice with modified ATRAP expression showed that ATRAP plays a key role in the regulation of renal sodium handling and the modulation of blood pressure in response to pathological stimuli such as chronic Ang II infusion, dietary high salt loading and 5/6 nephrectomy, and suggest ATRAP to be a target of interest.

Introduction

It is certain that physiological activity of the renin-angiotensin system is necessary for the organ development, such as the kidney, and the maintenance of cardiovascular and renal homeostasis[1]. However, chronically exacerbated activation of the renin-angiotensin system exerts detrimental effects to provoke hypertension and cardiovascular and renal disease [1]. Most

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of biological actions of the renin-angiotensin system are mediated mainly by binding of the active peptide Ang II to AT1R or by physical stretch of AT1R. Accumulating scientific evidence *in vitro* and *in vivo* has shown that changes in AT1R expression and/or AT1R signaling have physiological and pathophysiological relevance [1]. With respect to the mechanisms involved in these changes, the AT1R expression is reportedly regulated by gene transcription [2], positively via various factors, including cytokines, growth factors, and reactive oxygen species, but negatively via nitric oxide-mediated S-nitrosylation of NF-□B through activation of purinergic $P2Y_2$ receptor [3]. In addition, heterodimerization of AT1R and other G protein-coupled receptors (GPCRs) confers activation of atypical signaling [4], and phosphoryaltion/dephosphorylation of AT1R as well as conformational changes such as by post-translational modification, affect its downstream signaling pathways as post-transcriptional control [5, 6].

The various GPCRs certainly play an important role in the regulation of many biological functions in physiology and pathophysiology. The GPCRs interact with different classes of intracellular proteins, including heterotrimeric G proteins, kinases, and arrestins [7, 8]. Recently, GPCRs are shown to support a diversity of pharmacological profiles, a concept broadly referred to as functional selectivity [9]. Thus, on-going research to develop new drugs targeting GPCRs is no longer limited to seeking agonists or antagonists to stimulate or block downstream signaling pathways of a particular receptor and includes investigation of biased ligands [10]. Several novel molecules, which are capable of directly binding to AT1R and possibly modulating receptor-mediated functions, are recently identified by employing molecular cloning strategy [11-13]. Presently precise functional role in physiology and putative pathophysiological role in disease states of these AT1R-interacting molecules are targets of considerable research interest [2, 14-16]. In this review we focus on functional characterization of the AT1R-associated protein ATRAP in the renal sodium handling and blood pressure (BP) regulation in pathophysiology, by employing genetic engineered mice with modified ATRAP expression.

Analysis of functional role of ATRAP in renal sodium handling and BP regulation employing ATRAP knockout mice

Renal tubules play critical roles in the regulation of electrolyte homeostasis and hence in the maintenance of circulatory system. Accumulated evidence also indicates that the renin–

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angiotensin system has a pivotal role in the modulation of renal tubular function [17]. Among tissue forms of the renin-angiotensin system, activation of AT1R signaling in the renal tubules has a key role in altered renal sodium handling that occurs in hypertension. The exaggerated activation of renal tubule AT1R signaling is suggested to provoke defective renal sodium handling, with a consequent dysregulation of body fluid volume which, in turn, leads to development of hypertension. Since ATRAP is widely and abundantly distributed along the renal tubules in normal kidney [18, 19], it is highly probable that ATRAP in the renal tubules exerts functionally modulating effects on the function of renal tubular AT1R *in vivo*. With regard to the role of ATRAP in salt-mediated BP regulation, sustained recovery of repressed renal ATRAP expression contributed to the long-term therapeutic effects of prepubertal transient treatment with an AT1R blocker in dietary high salt loading–mediated hypertension in Dahl Iwai salt-sensitive rats, a representative animal model of human salt-sensitive forms of hypertension [20].

Although ATRAP is abundantly and broadly expressed in the renal epithelial cells from proximal to distal tubules, the accumulated experimental results indicate that ATRAP does not affect physiological renal sodium handling and BP regulation under normal condition [16]. Sodium reabsorption through renal proximal and distal tubules is reportedly regulated by the activity of the intrarenal Ang II–AT1R axis [17], and chronic Ang II infusion to control wild-type (WT) C57BL6 mice provokes significant reduction in renal ATRAP expression concomitant with the increase in renal sodium reabsorption and the development of hypertension [21]. Thus, a targeted gene disruption strategy was employed to produce systemic ATRAP-knockout (ATRAP-KO) mice on a genetic background of C57BL/6, and to examine the effects of total deletion of ATRAP at baseline on renal sodium handling and BP regulation under normal condition and in response to pathological stimuli such as chronic Ang II infusion and 5/6 nephrectomy [22, 23]. With respect to renal sodium handling and BP regulation under normal condition in ATRAP-KO mice, although previous genetic inactivation of the renin-angiotensin system components, such as angiotensinogen (AGT), renin, and AT1R, has been reported to result in renal morphological alteration and pathological BP changes even under normal conditions, ATRAP-KO mice did not exhibit any renal morphological alteration and changes in renal sodium handling and baseline BP [22].

In contrast to the lack of any evident change in baseline BP in ATRAP-KO mice under normal condition, profiles of renal sodium reabsorption and BP regulation were changed by

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chronic Ang II stimulation and 5/6 nephrectomy. Metabolic cage analysis showed that day-by-day sodium balance and the extent of cumulative sodium balance were significantly increased in ATRAP-KO mice compared with control WT mice by chronic Ang II infusion [22]. In addition, ATRAP-KO mice exhibited the exacerbation of Ang II-mediated hypertension as revealed by direct BP measurement performed by radiotelemetric method, concomitant with an increased sodium balance compared with WT mice [22]. In addition, while the BP response and renal expression of the epithelial sodium channel (ENaC), a major sodium transporter in the distal tubules, were not affected by chronic aldosterone infusion, stimulation of the renal expression and activity of ENaC was significantly enhanced by chronic Ang II infusion. These results indicate that ATRAP deficiency exacerbated Ang II-mediated hypertension via a pathological activation of renal tubular AT1R by Ang II, which directly stimulates ENaC in the distal tubules and enhances sodium retention in an aldosterone-independent manner [22].

Renal ATRAP also plays a critical role in suppressing hypertension in a mouse remnant kidney chronic kidney disease (CKD) model (5/6 nephrectomy) [23]. The effects of 5/6 nephrectomy on endogenous ATRAP expression were analyzed in the kidney of C57BL/6 and 129/Sv mice. While 129/Sv mice that underwent 5/6 nephrectomy showed decreased renal ATRAP expression and developed hypertension, C57BL/6 mice exhibited increased renal ATRAP expression and resistance to progressive hypertension[23]. Consequently, it was hypothesized that down-regulation of renal ATRAP expression is involved in pathogenesis of remnant kidney CKD model-related hypertension. To investigate this, 5/6 nephrectomy was performed in ATRAP-KO mice on the hypertension-resistant C57BL/6 background [23]. ATRAP-KO mice that underwent 5/6 nephrectomy showed hypertension with increased plasma volume [23]. Moreover, in ATRAP-KO mice compared with wild-type C57BL/6 mice after 5/6 nephrectomy, renal ENaC expression was significantly enhanced, concomitant with increased plasma membrane AT1R in the kidneys. These results indicate that renal ATRAP down-regulation is involved in onset and progression of BP elevation caused by renal mass reduction, and implicates ATRAP as a therapeutic target for hypertension in CKD [23].

Analysis of functional role of ATRAP in renal sodium handling and BP regulation employing ATRAP transgenic mice

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The results using systemic ATRAP-KO mice suggest that the AT1R-ENaC axis in the renal tubules play an important role in the Ang II-mediated hypertension. Thus, in order to examine the functional role of renal tubule ATRAP in pathological conditions such as chronic Ang II infusion and high salt loading, we generated transgenic mice dominantly expressing ATRAP in the renal tubules, including distal tubules[24]. The renal ATRAP transgenic mice (renal ATRAP-TG mice) exhibited no significant change in physiological renal sodium handling and baseline BP under normal condition. However, in the renal ATRAP-TG mice compared with wild-type mice, the following took place: (1) suppression of the Ang II-mediated hypertension, (2) reduction in the extent of daily positive sodium balance during chronic Ang II infusion, and (3) inhibition of the Ang II-induced activation of ENaC [24, 25].

In addition, in renal ATRAP-TG mice, the dietary high salt loading-mediated BP elevation was suppressed compared to wild-type mice, in spite of a similar baseline BP. Urinary sodium excretion in response to dietary high salt loading was significantly enhanced in the renal ATRAP-TG mice[26]. Furthermore, functional transport activity of ENaC was significantly decreased under saline volume-expanded conditions in renal ATRAP-TG mice compared to wild-type mice, without any evident change in ENaC protein expression[26]. Plasma membrane AT1R expression in the kidney of renal ATRAP-TG mice was also decreased compared with wild-type mice[26]. These results indicate that renal tubule–dominant ATRAP activation provokes no evident effects on BP under normal condition, but exerts an inhibitory effect on the pathological increase in renal sodium reabsorption and the exacerbated BP elevation in response to chronic Ang II stimulation and dietary high salt loading[27].

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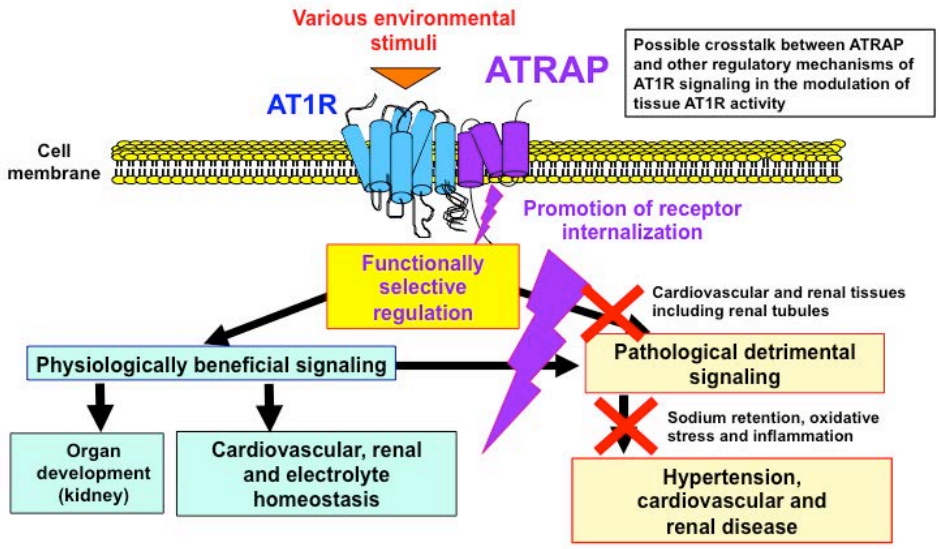
Conclusions

Cardiovascular and renal diseases are the most common chronic disease entity worldwide. Hypertension plays an important role as an essential trigger of cardiovascular and renal disease



and it is a multifactorial disease in which genetic and environmental factors are intricately intertwined. Understanding the mechanism underlying hypertension is thus extremely complex, and caution should be used in interpreting the findings of the studies using in genetic engineered mice in terms of the pathophysiology of human hypertension. Nevertheless, the findings of these studies do provide a useful basis for the further investigation of the *in vivo* functional roles of renal tubule ATRAP in BP regulation in response to pathological stimuli and also suggest the potential benefit of an activation strategy of renal tubule ATRAP, by such as transcriptional activation of the ATRAP gene[28, 29]. Furthermore, possible crosstalk between ATRAP and other regulatory mechanisms of AT1R expression and signaling by gene transcription and post-transcriptional control in the modulation of tissue AT1R activity in pathophysiology should be investigated (**Figure 1**). **Figure 1: ATRAP may exert a functionally selective inhibition on pathological detrimental AT1R signaling in tissues**

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