Commentary

Soluble (pro)renin receptor: a novel ligand for angiotensin II type 1 receptor?

Keiichi Torimoto and Satoru Eguchi
Cardiovascular Research Center, Lewis Katz School of Medicine at Temple University, Philadelphia, PA, U.S.A.
Correspondence: Satoru Eguchi (seguchi@temple.edu)

This commentary highlights the study entitled ‘Soluble (pro)renin receptor induces endothelial dysfunction and hypertension in mice with diet-induced obesity via activation of angiotensin II type 1 receptor’ presented by Fu et al. published in Clinical Science (Clin Sci (Lond) (2021) 135(6), https://doi.org/10.1042/CS20201047). The authors evaluated the role of the soluble (pro)renin receptor (sPRR), a cleavage product of the prorenin receptor (PRR) by the site 1 protease, as a ligand for angiotensin II type 1 receptor (AT1R). They presented for the first time that sPRR directly interacts with AT1R, causing nuclear factor-κB activation, inflammation, apoptosis, and endothelial dysfunction in primary human umbilical vein endothelial cells (HUVECs). Furthermore, the interaction between sPRR and AT1R was responsible for endothelial dysfunction and hypertension in diet-induced obesity mice. These results provide a potential mechanism for obesity-induced endothelial dysfunction and hypertension. Thus, the sPRR/AT1R complex may be a novel therapeutic target for cardiovascular diseases associated with endothelial dysfunction.

The renin–angiotensin–aldosterone system (RAAS) is involved in regulating many physiological functions, including blood pressure and fluid volume. In addition, angiotensin II (Ang II), a component of the RAAS, and its signaling pathways have been studied for many important pathophysiological mechanisms such as hypertension [1]. The Ang II type 1 receptor (AT1R) is found in various tissues including vascular smooth muscle, endothelium, heart, brain, kidney, adrenal gland, and adipose tissue [2]. When Ang II binds to AT1R, AT1R interacts with heterotrimeric G proteins and produces second messenger signals leading to the activation of downstream effectors such as phospholipases C. In addition, AT1R activates a variety of intracellular protein kinases, including receptor and non-receptor tyrosine kinases, mitogen-activated protein kinase (MAPK) family kinases, and serine/threonine kinases such as Akt and protein kinase C [3].

The prorenin receptor (PRR) is a multifunctional protein working as a receptor for renin and prorenin, triggering intracellular signaling such as MAPK activation, acting as a vacuolar proton ATPase, and constructing Wnt signaling receptor complex [4]. Structurally, it is a single transmembrane protein with a protease cleavage site in the N-terminal domain near the transmembrane domain [5]. When PRR is cleaved by furin [5] or ADAM19 [6], the N-terminal fragment dissociates as a soluble PRR (sPRR). Interestingly, plasma sPRR levels were reported to be elevated in patients with atherosclerosis [7], heart failure [8], and hypertension [9]. Although plasma sPRR levels are clinically important, the study by Fu et al. [10] appears to be a stepping-stone to understanding the molecular mechanism by which sPRR may contribute to CVD.

Up to now, the studies on RAAS signaling have mainly focused on the stimulation of AT1R by Ang II. This is because the activation of AT1R has been thought to be mostly dependent on Ang II, the major physiological ligand [3]. However, Fu et al. [10] challenged this conventional view by evaluating the role of sPRR as a ligand for the AT1R. When sPRR was administered to human umbilical vein endothelial cells (HUVECs), the production of Nox4-derived H₂O₂ was increased, leading to inflammation, apoptosis, and decreased nitric oxide production (Figure 1A). These sPRR-dependent responses were alleviated by...
Figure 1. The main findings by Fu et al. demonstrating direct AT1R activation by sPRR

(A) A new role of sPRR for renin–angiotensin system. Fu et al. [10], demonstrated a novel important role of sPRR, a product of site-1 protease-mediated cleavage of PRR, as a direct ligand for the AT1R. Treatment of primary HUVECs with sPRR increased the production of Nox4-derived H2O2 and activity of NFκB. Nox4-derived H2O2 production resulted in inflammation, apoptosis and inhibition of NO production. These sPRR-evoked responses were attenuated by AT1R antagonist but not ACE inhibitor. Immuno-precipitation and radioactive ligand competitive assays further demonstrated that sPRR directly interacts with AT1R via Lys199 and Asp295. (B) The interaction between sPRR and AT1R contributes to endothelial dysfunction and hypertension in obese mice. The authors showed that the endothelial dysfunction caused by sPRR was protected by an AT1R antagonist in obese C57/BL6 mice. Furthermore, sPRR increased blood pressure, which was attenuated by AT1R antagonist but not by ACE inhibitor.

inhibition of AT1R with AT1R antagonist, but not by angiotensin-converting enzyme (ACE) inhibitor. Moreover, the present study is the first report demonstrating the direct interaction between sPRR and AT1R.

AT1R is a seven transmembrane protein belonging to the G protein-coupled receptor superfamily. AT1R has several contact sites for Ang II binding, where Lys199 is one of the important amino acid residues. AT1R also has a sodium-binding pocket where Asn111 and Asn295, present in transmembrane domains III and VII, respectively, promote receptor activation [11]. In addition, Ser109 and Asn295 are important for the binding of AT1R to an AT1R
antagonist, losartan. By immunoprecipitation and radioligand competitive binding, Fu et al. [10] showed that sPRR interacts directly with AT1R via Lys\textsuperscript{199} and Asn\textsuperscript{295}. Therefore, it is likely that sPRR shares binding sites of AT1R with Ang II and losartan. In addition to Lys\textsuperscript{199}, there are several other residues in the AT1R that interact with Ang II, which were not examined in the present study.

Ang II has been clinically implicated in the development and progression of atherosclerosis by causing endothelial dysfunction, inflammation, oxidative stress, thrombosis, and plaque destabilization [3]. Fu et al. [10] showed that AT1R inhibitor is protective against endothelial dysfunction in obese C57/BL6 mice. Furthermore, sPRR increased blood pressure in obese C57/BL6 mice, and this effect was reversed by treatment with an AT1R antagonist but not with an ACE inhibitor. These findings suggest that AT1R mediates sPRR function in obesity-associated hypertension (Figure 1B). However, there was no significant difference in endothelial function between DIO mice and DIO plus sPRR-infused mice. It remains unclear whether the AT1R inhibitor inhibited DIO itself or the sPRR effect, which deserves further investigation.

There are several interesting points which deserve further discussion. Why blood pressure in DIO mice was not higher than in lean mice despite the increased plasma sPRR levels and why AT1R-mediated ‘Ang II-like’ effect produced hypertension only in DIO mice are two important questions. One potential explanation is that the endothelial co-receptor may be expressed or enhanced in DIO but not control mice. In addition, whether the findings seen in the animal model of obesity are applicable to human remains obscure. As far as we are aware, there has been no report that ARBs are more efficient than ACE inhibitors in lowering blood pressure in obese hypertensive patients [12]. Although the authors used losartan as an ARB to inhibit the sPRR effects, it is known that losartan has several AT1R-independent effects [13], which may have influenced the results of the study.

Several key questions remain to be answered. The authors discussed that the mechanism seems to be specific for endothelial cells as sPRR did not activate AT1R in vascular smooth muscle cells. How about in other cell types or organs such as brain and kidney in which Ang II/AT1R functions are critical? Both Ang II-dependent and Ang II-independent effects of PRR have been reported [14]. It was reported that sPRR did not show diurnal or postural variability, and was not associated with renin, prorenin, or aldosterone concentrations [15]. Recently, it was also reported that renin and aldosterone dynamics were invariant in sPRR knockout mice [16]. These findings suggest that the effects of sPRR on kidney and adrenal gland are AT1R-independent. Thus, the question arises in kidney and adrenal gland whether Ang II/AT1R interaction in the endothelium is negligible. However, to better understand the exact relations between the RAAS and sPRR, it is necessary to evaluate the relationship not only in the circulations but also in organs and cells (such as in kidney and adrenal gland). While the authors suggested that sPRR might define target cells by binding to co-receptors expressed specifically on endothelial cells, whether the AT1R forms sPRR receptors in select vascular beds require further investigation.

The author stated that PRR is abundantly and specifically expressed in VSMCs but not in endothelial cells, and VSMC-derived sPRR may act in a paracrine fashion to induce endothelial dysfunction. On the other hand, ACE mainly converts Ang I into Ang II on the endothelial surface. Therefore, it is likely that Ang II and sPRR compete for the endothelial AT1R binding, which may shift Ang II actions to non-endothelial AT1R such as those in VSMCs. This possibility should be further tested in both physiological (endogenous Ang II) and pathophysiological (such as Ang II infusion) settings.

While commonly studied AT1R signaling pathways were confirmed with sPRR stimulation, it is likely that the modes of AT1R utilization may be distinct from those used by Ang II. The possibilities may include distinct regulations in internalization, durations in the signaling pathways and additional signaling and functions. Further research on this interesting topic in novel RAAS signaling is highly desired. In particular, the authors’ data suggest that sPRR activates Nox4 via Gq/PKC signaling, but it did not activate Nox2. However, it has been reported that Ang II mainly activates Nox2 and Nox4 subunits in endothelial cells, and that Ang II-induced endothelial dysfunction can be inhibited in Nox2 knockout mice [17]. The activation of Nox2 by Ang II requires Rac-1 [3], which may be different from the sPRR-mediated AT1 receptor signaling.

Finally, the author stated that sPRR may be a novel therapeutic target for the prevention of atherosclerosis. However, global knockout of the PRR is lethal in mice [18]. In humans, mutations in the PRR gene develop epilepsy with mental retardation [19]. Since similar risks are possible when targeting sPRR in humans, we need to carefully consider the safety and efficacy of sPRR-targeted drugs and compare them with existing drugs targeting the classical RAAS pathway.

### Competing Interests
The authors declare that there are no competing interests associated with the manuscript.
Abbreviations
AT1R, Ang II type 1 receptor; CVD, cardiovascular disease; DIO, diet-induced obesity; MAPK, mitogen-activated protein kinase; NOX, NADH/NADPH oxidase; PRR, prorenin receptor; RAAS, renin–angiotensin–aldosterone system; sPRR, soluble (pro)renin receptor; VSMC, vascular smooth muscle cell.

References