Nuclear Hormone Receptors as Regulators of the Renin-Angiotensin-Aldosterone System

Irma Kuipers, Pim van der Harst, Gerjan Navis, Linda van Genne, Fulvio Morello, Wiek H. van Gilst, Dirk J. van Veldhuisen, Rudolf A. de Boer

The renin-angiotensin-aldosterone system (RAAS) has been identified as the main system involved in blood pressure regulation, renal hemodynamics, and sodium-volume homeostasis. Furthermore, the RAAS directly affects vascular and cardiac remodeling through proliferative and inflammatory signaling. Pharmacological targeting of the RAAS is a consolidated and evidence-based approach in the treatment of various aspects of cardiovascular disease. An exploding number of recent studies have provided novel insights into nuclear receptor biology in relation to cardiovascular (patho)physiology. In particular, members of the nuclear hormone receptor (NHR) superfamily have been identified as key molecules in various relevant cellular processes. As such, NHRs have been proposed as amenable targets for therapy, and their role in cardiovascular disease is currently explored.

This review focuses on the potential effects of NHRs on the RAAS, summarizing the extensive body of evidence from experimental, animal, and clinical studies, suggesting that NHRs and the RAAS are closely intertwined. We discuss how these findings might translate into the clinical setting and discuss the (older) trials that evaluated NHR agonists in humans. We postulate that therapies targeting NHRs will be useful in the treatment of various aspects of cardiovascular disease. An overwhelming amount of evidence exists for the role of NHRs in cholesterol, lipid, fatty acids, and cholesterol derivatives. An overwhelming amount of evidence exists for the role of NHR in cholesterol, lipid, and glucose metabolism. Several NHRs are provisionally indicated as “orphan” receptors, because their ligands are as yet unknown. After activation by their ligands, NHRs bind to DNA-responsive elements located within target gene promoters or have cross-talk with other signaling pathways. Effects are often modified by nuclear coregulators (coactivators and corepressors).

However, if and how different NHRs may affect the RAAS remain largely unknown. Angiotensin II (Ang II) is the main effector peptide of the RAAS. Other end products are generated as well, such as angiotensin IV, angiotensin (1-7), and others (detailed in Figure S1). In this review, we focus mainly on the effects of NHRs on renin, angiotensinogen, and angiotensin receptors.

NHRs and Renin

Renin is (almost) exclusively secreted from specialized juxtaglomerular (JG) cells, located in the afferent arterioles of the kidney. Renin transcription is tightly regulated. It has become clear that several NHRs regulate renin transcription through interaction with specific responsive elements in the renin promoter (Figure 1). Through specific responsive elements, NHRs can act as either positive or negative regulators of renin transcription (Table).

Liver X Receptors

Liver X receptor (LXR-α) and the highly homologous LXR-β are important modulators of lipid and glucose metabolism, inflammation, and innate immunity. LXR-α is expressed predominantly in liver, gut, heart, kidney, and adrenals, whereas LXR-β is ubiquitously expressed. Tamura et al provided evidence that LXRs play an important role in renin regulation. Previously, they described a specific responsive element in the mouse renin promoter, called cAMP-negative response element (CNRE; Figure 1). LXR-α was identified as a CNRE-binding protein that regulates renin mRNA expression. This finding was confirmed recently using a mouse in vivo model. Both LXR-α and LXR-β were shown to be regulators of renin transcription. Renal expression of LXR-α was confined to JG cells (Figure 2). Interestingly, LXR-α and LXR-β markedly differ in their control of renin...
transcription, because the latter constitutively upregulates renin mRNA expression, whereas LXR-α increases renin mRNA only in a cAMP-dependent manner. Importantly, high-renin status models were associated with prominent LXR-H9251 binding activity to the CNRE. Experiments in LXR-deficient mice confirmed that LXR-H9251 is necessary for the cAMP-dependent response of JG cells, whereas LXR-H9252 confers a basal constitutive effect on renin transcription and seems not strictly required for the functional responses of the JG apparatus.

LXR agonists are currently explored as a novel therapy aimed at enhancing reverse cholesterol transport, thereby inhibiting the progression of atherosclerosis. To our knowledge, no LXR-specific interventions have been put to test in humans yet. The observation that LXRs function as renin regulators may warrant the development of selective modulators of LXR function that circumvent renin activation while retaining the advantageous effects on lipid metabolism.

Vitamin D Receptors
Li et al7 reported direct evidence that the vitamin D receptor (VDR) acts as a negative regulatory factor of renin transcription. VDR-transfected As4.1 cells treated with calcitriol exhibited an almost complete abolishment of renin expression. Treatment of wild-type mice with vitamin D also suppressed renin expression, whereas in VDR-deficient mice, renin expression was elevated several-fold. So, vitamin D yields a direct negative regulatory effect on renin expression through a VDR-mediated mechanism. These findings are in agreement with the well-known inverse correlation between plasma vitamin D and blood pressure related to decreased plasma renin activity in humans.5

Thyroid Hormone Receptors
Thyroid hormone receptor (TR) expression is ubiquitous, including in the kidney. Ichihara et al9 showed that, in cultured rat JG cells, treatment with thyroid hormones induces renin transcription/secretion in a dose-dependent fashion. The same group described 3 candidate thyroid hormone response elements (Figure 1) located in the human renin gene and showed that, in CaLu-6 cells, thyroid hormones stimulate the transcription of the human renin gene.10 In vivo, plasma renin activity and renal renin expression are significantly reduced in hypothyroid rats and elevated in hyperthyroid rats.11 Hyperthyroidism-induced cardiac hypertrophy is accompanied by activation of the cardiac RAAS with a signif-

Table. Summary of NHRs Acting as Regulators of Renin Transcription

<table>
<thead>
<tr>
<th>NHR</th>
<th>Activator Tested</th>
<th>Positive/Negative Regulator</th>
<th>Heterodimer/Monomer</th>
<th>Binding Sequence</th>
<th>References</th>
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<tr>
<td>LXR-α</td>
<td>cAMP</td>
<td>Positive</td>
<td>Monomer</td>
<td>CNRE</td>
<td>5, 6</td>
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<td>6</td>
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<td>TR</td>
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<td>Monomer</td>
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<td>VDR</td>
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<td>Unknown</td>
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<td>Unknown</td>
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<td>RXR-α/RAR-α</td>
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<td>(Hetero/homo dimer)</td>
<td>DR2/DR3/DR4/DR5</td>
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<td>Unknown</td>
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<tr>
<td>MR</td>
<td>Aldosterone</td>
<td>Positive</td>
<td>Both</td>
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</table>

THRE indicates thyroid hormone response element; TZD, thiazolidinedione; RXR, retinoid X receptor; RAR, retinoic acid receptor; RARE, rapid acquisition with relaxation enhancement; MR, mineralcorticoid receptor.
Peroxisome Proliferator-Activated Receptors

Pharmacological targeting of peroxisome proliferator-activated receptors (PPARs; fibrates and thiazolidinediones) has been tested in the treatment of dyslipidemia, diabetes, and established cardiovascular diseases, such as heart failure and myocardial infarction. The 2 isoforms of PPAR are discussed in detail below.

Several animal studies have suggested a role for PPAR-α in the control of renin expression. PPAR-α-deficient mice put on a high-fat diet are protected from the development of hypertension. Mice deficient for both the low-density lipoprotein receptor and PPAR-α have been used to elucidate the role of PPAR-α in the development of glucocorticoid-related insulin resistance. In the absence of PPAR-α, mice were normotensive and euglycemic, despite dexamethasone treatment. When hepatic PPAR-α expression was restored by gene transfer, it not only reinduced hyperglycemia, but also increased plasma renin activity, blood pressure, sympathetic nervous activity, and renal sodium retention. Similar results were obtained in PPAR-α-deficient Tsukuba hypertensive mice. Tsukuba hypertensive mice carry the human renin and angiotensinogen genes and serve as a model of Ang II-mediated hypertension. PPAR-α deficiency in Tsukuba hypertensive mice decreases plasma renin concentration by ~50% and prevents hypertension and myocardial hypertrophy. These observations are supported by observations made in spontaneously hypertensive rats and healthy, human volunteers. The exact role of PPAR-α in the regulation of blood pressure remains unclear, because several other studies conducted in various rat models of hypertension have provided inconsistent results. It is currently not known whether renin activation associated with PPAR-α activation is a direct transcriptional effect of PPAR-α or if it should be regarded as a secondary phenomenon, attributable to concomitant blood pressure reduction or other ancillary effects of PPAR agonists (eg, antiinflammatory and antiproliferative effects on the vasculature or increased sympathetic activity).

Activation of PPAR-γ signaling has been consistently shown to lower blood pressure in various animal models and in human studies, at least in part through direct vascular effects. However, controversy remains concerning the mechanism of how PPAR-γ influences blood pressure regulation. In an in vitro study using Calu-6 cells, it was shown that activation of PPAR-γ, both by endogenous and pharmacological agonists, causes a significant increase in renin transcription. In a small clinical study, however, the PPAR-γ agonist pioglitazone reduced plasma renin levels in humans. Overall, there is scarce evidence suggesting that PPAR-γ is a candidate regulator of JG cell function; further studies should specifically address this issue.

Steroid Hormone Receptors

So far, the specific role of the estrogen receptors and progesterone receptors in renin expression has not been addressed directly. Renin levels fluctuate with the menstrual cycle, suggesting that estrogen and progesterone are regulators of renin transcription. In cultured human chorion cells, progesterone, estradiol, testosterone, and aldosterone all affect renin expression levels. Estradiol and progesterone seem to synergistically increase renin expression, because the combined treatment with both completely restores renin expression in ovariectomized rats. Prorenin and renin are released from placental cytotrophoblastic tissue. This release occurs in parallel with other placental hormones but does not appear to be regulated by steroid receptors, human chorionic gonadotrophin, intracellular calcium, or cAMP. Therefore, (pro)renin release from placenta may in fact be regulated in a different fashion from renal renin release, eg, by local factors, such as estrogen and progesterone.

Renin Receptor

In 2002, a human renin/prorenin receptor was cloned, which specifically binds renin and prorenin. Binding of renin to its receptor increases the catalytic activity of renin, and binding of prorenin renders prorenin enzymatically active, comparable to renin. Although studies revealing transcription mechanisms of the renin receptor are currently underway, thus far no “classical” NHRs have been identified as transcription factors for the renin/prorenin receptor.

NHRs and Angiotensinogen

Presently, only TRs, PPARs, and estrogen receptors have been implicated in angiotensinogen transcriptional activation and will be discussed below.

Thyroid Hormone Receptors

The thyroid hormone is a positive regulator of angiotensinogen. Hyperthyroidism, induced by treatment with thyroid hormone (T3), causes an increase of plasma angiotensinogen by 85% in rats. In parallel, hypothyroidism results in a 71% decrease of plasma angiotensinogen levels. Similar results were found in vitro. T3 treatment causes upregulation of angiotensinogen transcription in the human hepatic cell line.
HepG2. We speculate that the well-described effects of thyroid hormones on blood pressure can partially be explained through regulation of angiotensinogen expression and consequent activation of the RAAS.

**Peroxisome Proliferator-Activated Receptors**

PPAR-α acts as a positive regulator of angiotensinogen transcription. In contrast to HepG2 cells, HeLa cells treated with the PPAR-α agonist bezafibrate displayed activation of the angiotensinogen promoter.33 This difference between HepG2 and HeLa cells might be explained by the presence of hepatocyte nuclear factor-4 in HepG2. Hepatocyte nuclear factor-4 binds to the same responsive region of the angiotensinogen promoter as PPAR-α. Hepatocyte nuclear factor-4 is not present in the cervical cell line HeLa, and cotransfection of hepatocyte nuclear factor-4 expression in HeLa cells attenuated the activation of the angiotensinogen promoter by bezafibrate.

A dominant-negative form of the PPAR-γ gene was cloned to create a transgenic mouse strain.34 The used mutation was equivalent to the P467L mutation in humans causing severe insulin resistance and hypertension. The mouse strain did not develop high blood pressure nor increased kidney renin mRNA content. However, upregulations of angiotensinogen and the angiotensin type 1 receptor (AT1R) were reported. Further research in these mice revealed an increased expression of angiotensinogen in their subcutaneous adipose tissue, suggesting a role for PPAR-γ in blood pressure regulation, via the RAAS, by modulating angiotensinogen expression in fatty tissue.

**Steroid Hormone Receptors**

Early studies demonstrated that estrogens and glucocorticoids can regulate hepatic angiotensinogen production.35 Estrogen treatment results in a prompt and significant upregulation of the angiotensinogen mRNA content. However, upregulations of angiotensinogen and the angiotensin type 1 receptor (AT1R) were reported. Further research in these mice revealed an increased expression of angiotensinogen in their subcutaneous adipose tissue, suggesting a role for PPAR-γ in blood pressure regulation, via the RAAS, by modulating angiotensinogen expression in fatty tissue.

**NHRs and Angiotensin Receptors**

The effects of Ang II are mainly mediated via 2 plasma membrane receptors, AT1R and angiotensin type 2 receptor (AT2R). Most effects of Ang II are mediated by the AT1R, including an increase of arterial blood pressure, aldosterone secretion, and adverse cardiac and renal remodeling. Rodents express 2 subtypes of the AT1R (A and B), whereas humans express only 1 subtype. AT2R is expressed at high levels in fetal tissues and decreases rapidly after birth. Although the exact function of AT2Rs is less well understood than that of the AT1R, it seems that AT2Rs antagonize AT1R-mediated effects. We discuss the influence of PPARs and steroid hormone receptors on AT1Rs and AT2Rs below.

**Peroxisome Proliferator-Activated Receptors**

The role of PPARs in AT1R expression is well established. It has been convincingly shown that the expression of AT1Rs is decreased by PPAR-γ agonists in rat vascular smooth muscle cells,38 leading to functional inhibition of Ang II-induced cell proliferation via AT1Rs. Functionally, Diep et al39 showed that treatment with the PPAR-γ activators rosiglitazone and pioglitazone attenuated the detrimental effects of Ang II infusion in rats. Development of hypertension, small resistance artery remodeling, endothelial dysfunction, proinflammatory mediators, and upregulation of AT1Rs, all of which are increased by Ang II infusion, were blunted in blood vessels of rats treated with rosiglitazone or pioglitazone. Diep et al,39 furthermore, showed that PPAR-γ ligands are capable of increasing AT2R expression on a protein level. These findings are consistent with the significant increase in myocardial AT2R mRNA found in rosiglitazone-fed rats subjected to an ischemia-reperfusion model.40 Whereas in this model AT1R mRNA expression was reduced after rosiglitazone treatment, AT2R mRNA expression was increased by >100-fold.

Cross-talk between angiotensin receptors and PPAR-γ is, thus, relevant. This is further underscored by the observation that some angiotensin receptor blockers, such as telmisartan and irbesartan are in fact partial PPAR-γ agonists.41 The influence of PPAR-α agonist docosahexaenoic acid (DHA) has also been investigated in Ang II-infused rats.42 Like activation of PPAR-γ, activation of PPAR-α also attenuated the damaging effects of Ang II infusion in rats, resulting in reduction of Ang II-induced oxidative stress, expression of inflammatory mediators, blood pressure, endothelial dysfunction, and remodeling of small resistance arteries. The potential clinical implications of the interaction between PPARs and the AT1R is discussed in detail below.

**Steroid Hormone Receptors**

Sex hormones, especially estrogens, modulate the expression of angiotensin receptors. Estrogen attenuates AT1R mRNA, but increases AT2R gene expression in rat cardiac fibroblasts.43 In line with this, increased AT1R expression was found in estrogen treated ovariectomized rats and cell cultures of rat smooth muscle cells.44 Later, this group showed that progesterone addition to smooth muscle cell cultures upregulated AT1R expression.45 It may be speculated that the contrary effects of female sex hormones on angiotensin receptor expression could be involved in the changes in arterial pressure during the menstrual cycle and perhaps also postmenopausal.

**Potential Clinical Implications**

The discussed data suggest that modulation of NHR activity comodulates RAAS activity. Large-scale clinical studies with NHR agonists have been conducted and/or are underway, with potential clues on the role of NHRs in cardiovascular disease. Specific agonists of PPAR-α, PPAR-γ, VDR, and steroid hormone receptors have been put to trial, whereas other agonists are yet to enter the clinical arena. Unfortunately, most clinical trials did not include end-points directly linked to the RAAS, such as plasma renin activity or angiotensin-
converting enzyme activity. We will discuss the limited data available.

**Vitamin D Receptors**

Vitamin D acts as a negative regulator of renin and might consequently reduce cardiovascular morbidity and mortality. Currently, no large-scale mortality trials have tested the efficacy of vitamin D in reducing events in patients with established cardiovascular disease. Some clues are available from trials in patients with end-stage renal disease (ESRD), when vitamin D is primarily prescribed to treat secondary hyperparathyroidism.

In a small study, analyzing 242 chronic hemodialysis patients, patients treated with alfacalcidol (a vitamin D analogue) had a significantly lower risk of cardiovascular mortality than patients with no vitamin D treatment.\(^4\) In fact, several observational studies indicate that there may be a protective effect of vitamin D treatment against cardiovascular disease. This claim is hitherto restricted to hemodialysis patients with ESRD whose main cause of death is cardiovascular. We postulate that in patients with low serum vitamin D levels, and who are at risk for cardiovascular disease, the use of oral vitamin D substitutes may reduce the risk for future cardiovascular events.

**Peroxisome Proliferator-Activated Receptors**

PPAR-\(\alpha\) agonists have been primarily tested in patients with hypertriglyceridemia and end-points in such studies typically were coronary events and death. The clinical use of fibrates preceded the identification of PPAR-\(\alpha\) as a nuclear receptor, harboring potential ancillary effects beyond lipid lowering. As such, clinical trials with PPAR-\(\alpha\) agonists could be revisited for clues regarding the impact of presumable PPAR-\(\alpha\) activation on RAAS activity. There are observations that support the notion that other effects than lipid-lowering effect alone play an important role. For instance, in the Helsinki study, a paradoxical increase in noncoronary death was observed.\(^4\) However, to our knowledge, in large scale trials with PPAR-\(\alpha\) agonist, such as the Helsinki Heart Study, the Veteran’s Administration-HDL Intervention Trial (VA-HIT),\(^4\) and the FIELD,\(^4\) no analyses were conducted with regard to RAAS activity.

PPAR-\(\gamma\) agonists (thiazolidinediones) exert beneficial effects on the vasculature, including decreased carotid artery intima-medial thickness,\(^5\) improved endothelial function, and decreased inflammation.\(^5\) Because the RAAS is a dominant player in vascular remodeling, we speculate on some effects to be conferred via the RAAS. In previous sections, we discussed experimental data that show the interaction between PPAR-\(\gamma\) and the RAAS. This interaction is predominantly at the level of the AT1R. Some angiotensin receptor blockers are partial PPAR-\(\gamma\) agonists, whereas treatment with glitazones can block Ang II-mediated effects.\(^5\) Some metabolic effects of angiotensin-converting enzyme inhibitors and angiotensin receptor blockers, including a reduction of new-onset diabetes, as seen in the Heart Outcomes Prevention Evaluation\(^5\) and the Losartan Intervention for Endpoint Reduction in Hypertension,\(^5\) are suggestive for the existence of PPAR-\(\gamma\)-like effects of RAAS inhibition.

Several large-scale randomized, controlled trials with PPAR-\(\gamma\) agonists have been completed recently. The Prospective Pioglitazone Clinical Trial in Macrovascular Events\(^5\) showed rather favorable outcomes, such as a decrease in recurrent myocardial infarction and a decrease in recurrent stroke. The Diabetes Reduction Approaches With Ramipril and Rosiglitazone Medications Study established a decrease in progression to diabetes in prediabetic patients (with impaired glucose tolerance and/or impaired fasting glucose) in rosiglitazone-treated patients.\(^6\) Unfortunately, none of these trials reported any parameters of RAAS activity.

These outcomes may suggest that PPAR-\(\gamma\) agonists decrease cardiovascualr events over time; however, this remains speculative. Recently, PPAR-\(\gamma\) agonists have come under fire because of claims that they may increase rather than decrease the risk of myocardial infarction.\(^7\) Although the limitations of this meta-analysis have been pointed out by its authors and by others,\(^8\) controversy remains concerning the safety of thiazolidinedione treatment.

From the trials with PPAR agonists, it becomes clear that the theoretical working profile is not paralleled with a (solely) beneficial clinical efficacy. Likely, the effects that PPARs exert are more global that we currently understand: the beneficial effects may not outweigh adverse effects. We suggest putting combinatorial therapies with RAAS-inhibitors and PPAR-\(\gamma\) agonists to test in future studies, because from the theory this might be more efficacious than either therapy alone.

**Perspectives**

NHRs have emerged as a class of receptors with a wide array of physiological effects pertaining to fatty acid, lipid, and carbohydrate metabolism but also to other pathways, such as inflammation, cellular proliferation, and vascular remodeling. These processes are pivotal in cardiovascular disease, and pharmacological targeting of NHRs might provide a novel and promising treatment approach. Some agonists of NHRs have entered the clinical arena, showing a promising efficacy and safety profile. Because the RAAS is a main player in cardiovascular homeostasis, any effects of NHRs on RAAS activity may likely affect its working and safety profile.

In this review, we discussed the levels of interaction between some NHRs and the RAAS. Experimental studies have partially elucidated the molecular mechanisms of the regulation of the RAAS by NHRs. It is speculated that, by exploring the clinical value of NHRs, the effect on RAAS activation may have important repercussions for their efficacy and safety. We, therefore, recommend that future clinical studies include analyses of RAAS activation.

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**Disclosures**

None.
References


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In the *Hypertension* article by Kuipers et al (Kuipers I, van der Harst P, Navis G, van Genne L, Morello F, van Gilst WH, van Veldhuisen DJ, de Boer RA. Nuclear hormone receptors as regulators of the renin-angiotensin-aldosterone system. *Hypertension*. 2008;51:1442–1448) there is an incorrect statement in the upper right paragraph on page 1444. The statement “In a small clinical study, however, the PPAR-γ agonist pioglitazone reduced plasma renin levels in humans.” should read “In a small clinical study, the PPAR-γ agonist pioglitazone increased plasma renin levels in humans.”

The authors regret the error.
NUCLEAR HORMONE RECEPTORS AS REGULATORS OF THE RENIN-ANGIOTENSIN-
ALDOSTERONE SYSTEM

Irma Kuipers\textsuperscript{a} MSc; Pim van der Harst\textsuperscript{a} MD; Gerjan Navis\textsuperscript{b} MD; Linda van Genne\textsuperscript{a} BSc; Fulvio Morello\textsuperscript{c} MD; Wiek H. van Gilst\textsuperscript{a} PhD; Dirk J. van Veldhuisen\textsuperscript{a} MD; Rudolf A. de Boer\textsuperscript{a} MD

From the Departments of Cardiology\textsuperscript{a} and Nephrology\textsuperscript{b} from the University Medical Center Groningen, University of Groningen, PO Box 30.001, 9700 RB, Groningen, The Netherlands and from the IVth Division of Internal Medicine\textsuperscript{c}, San Vito Hospital, Strada San Vito 34, 10134 Torino, Italy.

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Please address correspondence to:
Rudolf A. de Boer, MD

Department of Cardiology, University Medical Center Groningen, University of Groningen, PO Box 30.001, 9700 RB, Groningen, The Netherlands.

Phone: +31503612355 / Fax: +31503614391 / E-mail address: r.a.de.boer@thorax.umcg.nl
Table S1: Subfamilies of mammalian nuclear receptors (adapted and modified from\textsuperscript{2}).

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<thead>
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<th>Class</th>
<th>Receptor</th>
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<td>α, β</td>
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The renin-angiotensin-aldosterone system (RAAS)

Figure S1: Schematic overview of the RAAS. Renin is the primum movens of the RAAS and cleaves angiotensinogen into angiotensin I. Subsequently, angiotensin I is converted into angiotensin II by angiotensin-converting enzyme (ACE). Angiotensin II mediates its effects via the angiotensin type 1 receptor (AT1R) and type 2 receptor (AT2R).