Mineralocorticoid-receptor signalling in vascular smooth muscle

Jing Wu¹ and Friedrich C. Luft¹,²

¹Division of Clinical Pharmacology, Department of Pharmacology, Vanderbilt University School of Medicine, Nashville, TN, USA and ²Experimental and Clinical Research Centre, a cooperation between the Max-Delbrück Center and the Charite Medical Faculty, Berlin, Germany

Correspondence and offprint requests to: Friedrich C. Luft, ECRC, Lindenbergerweg 80, 13125 Berlin, Germany; E-mail: luft@charite.de

Keywords: aldosterone, cre recombinase, hypertension, LoxP, mineralocorticoid receptor

ABSTRACT

Human data suggest that eplerenone and spironolactone lowers blood pressure by mechanisms independent of the distal tubule. Now, a novel inducible conditional knockout model of the mineralocorticoid receptor (MR) in vascular smooth muscle cells (VSMCs) brings out understanding further.

The mineralocorticoid receptor (MR) belongs to the nuclear receptor subfamily 3, group C, member 2, (NR3C2) and is a protein that in humans is encoded by the NR3C2 gene on chromosome 4q31.1-31.2. The MR ligand is aldosterone, although the MR also has great affinity for cortisol. The MR is ‘protected’ from glucocorticoids by co-localization of an enzyme, corticosteroid 11-beta-dehydrogenase iso-enzyme 2 (11ß-HSD2), which converts cortisol to cortisone that does not bind to the MR. The MR also responds to some progestins. Spironolactone and eplerenone are approved MR antagonists. Binding of the MR to its ligand aldosterone results in its translocation to the cell nucleus, homodimerization and binding to hormone-response elements present in the promoter of certain genes.

The MR is expressed in many tissues, such as kidney, colon, heart, central nervous system (hippocampus), brown adipose tissue and sweat glands. In epithelial tissues, its activation leads to the expression of proteins regulating ionic and water transport. These proteins include the epithelial sodium channel, Na⁺/K⁺ pump, serum and glucocorticoid-regulated kinase-1, resulting in sodium reabsorption and as a consequence an increase in the extracellular volume, increase in blood pressure and excretion of potassium [1]. Ronzaud et al. used a Cre-loxP system to eliminate the MR from renal principal cells [2]. The mice developed normally and exhibited unaltered renal sodium excretion, but showed strongly elevated aldosterone levels. When subjected to a low-sodium diet, the mice exhibited increased renal sodium and water excretion, resulting in a continuous loss of body weight.

Nevertheless, the clinical effects of MR blockade extend beyond the kidney. Levy et al. reported that eplerenone lowered blood pressure via mechanisms other than those involving epithelial electrolyte and fluid transport [3]. Their interpretation seems counterintuitive; however, a recent study of the MR deleted in vascular smooth muscle cells (VSMCs) may provide a mechanistic explanation for their conclusions. McCurley et al. used an inducible Cre-loxP system targeting Nr3c2 solely in smooth muscle. When tamoxifen was given, the Cre⁺ mice eliminated the MR from their VSMCs. MR mRNA was reduced by 95% using this technology, while AT1 or AT2 receptor expression was not affected.

Adult (3- to 4-month-old) Cre⁻ and Cre⁺ mice had no difference in blood pressure; however, as the mice aged a further 10 months, Cre⁻ mice had higher blood pressure compared with Cre⁺ mice. The authors next tested mesenteric vascular resistance vessels of the mice to agonists. They found that the responses to phenylephrine were not different. However, Cre⁺ vessels responded less strongly to KCl, or a thromboxane receptor agonist. Furthermore, the vessel preparations of Cre⁺ mice dilated more strongly in response to acetylcholine than those of Cre⁻ mice, although the response to sodium nitroprusside was not different. The authors concluded that the VSMC MR has a direct role in mediating VSMC contraction and inhibits dilatation in mesenteric...
resistance vessels. The investigators next showed that renal function, responses to low- and high-salt diet and the maintenance of electrolyte regulation were normal in their Cre+ and Cre− mice. Thus, VSMC MR deletion did not affect the MR functions in the kidney. The authors also showed that the MR deletion in VSMCs did not affect the vascular structure or the mechanical distensibility of resistance vessels in their mice. Nevertheless, the MR deletion did reduce the spontaneous myogenic tone in resistance vessels by ∼40%. The authors concluded that the MR contributes to the intrinsic vascular tone, independent of structural vascular alterations.

McCurley et al. next moved to electrophysiology. They performed patch-clamp experiments and found no change in calcium-activated potassium (BKCa) channel function. However, when they examined the L-type calcium channel (Cav1.2), they observed an 80% reduction in the expression of this channel in VSMCs from Cre+ mice. A calcium channel opener showed a strongly reduced effect in VSMCs from Cre+ mice, compared with Cre− mice. Thus, the MR appeared to downregulate L-type channel expression, but did not affect potassium channel expression.

The authors then returned to blood pressure. They infused angiotensin (Ang II) into both strains of mice. The response in Cre− mice was robust, while the Cre+ mice appeared to be ‘protected’ from Ang II. The differences between Cre+ and Cre− mice were even more pronounced in aged mice. When dihydro-ethidium staining was done, Cre+ vessels showed less staining indicating less reactive oxygen species production. Thus, the VSMC MR contributes to Ang II-induced pressor response and vascular oxidative stress. The hypothesis that the VSMC MR directly regulates the vascular tone and blood pressure, independent of any renal tubular effects, appears to be substantiated. Indeed, Wang et al. have shown that Ang II upregulates Cav1.2 in cultured blood vessels via endothelial hydrogen peroxide production [4]. Presumably, some of this effect is related to the actions of the MR in VSMCs. We have attempted to summarize these complex findings (Figure 1).

Nonetheless, there are some questions open. Phenylephrine responses were normal as shown by McCurley et al. [5]. Although phenylephrine, a selective α1 agonist, angiotensin II and thromboxane all bind to G protein-coupled receptors that utilize Gq to elicit a robust calcium signal. Somehow the phenylephrine responses observed by the authors had to be independent of the L-type calcium channel Cav1.2. Another anomaly is the resistance vessel responses to acetylcholine. The vessels of Cre+ and Cre− mice responded similarly to sodium nitroprusside but differently to acetylcholine. The acetylcholine response involves endothelium, not VSMCs. The MR was presumably intact in endothelium. Why would the response to acetylcholine be different? Also unanswered is how the MR deficiency in VSMCs results in downregulation of Cav1.2. Gene expression studies should be done in Cre+ and Cre− VSMCs to elucidate this mechanism. Finally, do we combat the MR action in VSMCs by administering a calcium channel blocker? At least they do not cause hyperkalaemia.

**CONFLICT OF INTEREST STATEMENT**

None declared.
Clinical and histological predictors of long-term kidney graft survival

Pierre Galichon1,2,3, Yi-Chun Xu-Dubois3,4, Serge Finianos1, Alexandre Hertig1,2,3, and Eric Rondeau1,2,3,*

Correspondence and offprint requests to: Eric Rondeau; E-mail: eric.rondeau@tnn.aphp.fr

ABSTRACT

Renal transplantation is the best option for patients with end-stage renal disease (ESRD), but its half-life is limited to a decade. Clinical and histological markers measurable within the first year of transplantation can be used to predict its outcome. These markers are important for selecting kidneys for transplantation, for identifying the main causes of late allograft loss, for therapeutic decisions and as surrogate markers in therapeutic trials. ‘Basal state’ markers, such as age, glomerular filtration rate and fibrotic lesions, are highly predictive of allograft loss, showing that early and stable pathological mechanisms contribute considerably to this loss. On the other hand, some more dynamic predictors such as treatment, recurrence of the initial disease, inflammation and epithelial phenotypic changes offer clinicians and researchers opportunities to influence the fate of allografts.

INTRODUCTION

Renal transplantation is the best treatment for advanced chronic renal failure. It offers a better quality of life than haemodialysis, and significantly improves patient survival. It also dramatically reduces the long-term cost of medical care for these patients. However, prolonged immunosuppression is associated with several side effects, which may alter both graft and patient survival. Overall, in patients who are suitable for transplantation, renal transplantation offers a better benefit–risk ratio than dialysis. Various clinical and histological predictors of long-term kidney graft survival have been identified, and in some cases, they can be used to intervene in order to prolong the survival of the graft or of the patient. In other cases, they are simply markers that can be used in therapeutic trials as early surrogate markers for long-term efficacy.