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Role of AMP-activated protein kinase α1 in angiotensin-II-induced renal Tgfß-activated kinase 1 activation

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ABSTRACT

Angiotensin-II is a key factor in renal fibrosis. Obstructive nephropathy induces an isoform shift from catalytic Ampkα2 towards Ampkα1 which contributes to signaling involved in renal tissue injury. The present study explored whether the Ampkα1 isoform contributes to the renal effects of angiotensin-II. To this end, angiotensin-II was infused by subcutaneous implantation of osmotic minipumps in gene-targeted mice lacking functional Ampkα1 (Ampkα1$^{-/-}$) and corresponding wild-type mice (Ampkα1$^{+/+}$). Western blotting and qRT-PCR were employed to determine protein abundance and mRNA levels, respectively, in renal tissue. In Ampkα1$^{+/+}$ mice, angiotensin-II increased renal Ampkα1 protein expression without significantly modifying renal Ampkα2 protein expression. The renal phosphorylated Ampkα (Thr^{172}) protein abundance was not affected by angiotensin-II in neither genotypes, but was significantly lower in Ampkα1$^{-/-}$ mice than Ampkα1$^{+/+}$ mice. Angiotensin-II increased the phosphorylation of Tak1 (Ser^{412}) in renal tissue of Ampkα1$^{+/+}$ mice, an effect virtually absent in the Ampkα1$^{-/-}$ mice. Furthermore, angiotensin-II treatment significantly increased renal protein and mRNA expression of α-smooth muscle actin (αSma) as well as Tak1-target gene expression: *Cox2*, *Il6* and *Pai1* in Ampkα1$^{+/+}$ mice, all effects significantly less pronounced in Ampkα1$^{-/-}$ mice. In conclusion, angiotensin-II upregulates the Ampkα1 isoform in renal tissue. Ampkα1 participates in renal Tak1 activation and Tak1-dependent signaling induced by angiotensin-II.

1. Introduction

The pleotropic effects of angiotensin-II include the triggering of renal fibrosis [1–3], which could ultimately progress to end-stage renal failure [4]. In renal fibrosis extracellular matrix proteins are accumulated by myofibroblasts [5], which could originate from several sources [5–7]. Myofibroblasts are identified by expression of mesenchymal proteins including α-smooth muscle actin (α-Sma) [8]. Angiotensin-II-induced renal fibrosis involves expression of diverse further genes [3] including cyclooxygenase 2 (*Cox2*) [9], interleukin 6 (*Il6*) [10] and plasminogen activator inhibitor 1 (*Pai1*) [11].

Signaling molecules modifying the proinflammatory and profibrotic effect of renal injury include AMP-activated protein kinase (Ampk) [12], a kinase composed of a catalytic α subunit and regulatory β and gamma γ subunits [13]. Two catalytic Ampkα isoforms have been identified, which differ in targets and thus effects [14,15]. The Ampkα1 isoform is ubiquitously expressed, while the Ampkα2 isoform is expressed mainly in muscular and cardiac tissue [13,16].

In renal tissue, an isoform shift from the catalytic Ampkα2 towards Ampkα1 was observed in a mouse model of obstructive nephropathy [17], which contributes to signaling involved in renal tissue injury [12]. Ampkα1 stimulates renal transforming growth factor β (Tgfß) -activated kinase 1 (Tak1) [12], a key factor in the response to renal injury [18]. Angiotensin-II increases Ampkα1 expression in cardiac tissue [19] and can stimulate Tak1 [20].

The present study therefore explored whether the catalytic Ampkα1 isoform is involved in Tak1 activation in renal tissue and in renal effects of angiotensin-II. To this end, angiotensin-II was infused in gene-targeted mice lacking functional Ampkα1 and corresponding wild-type mice for two weeks.

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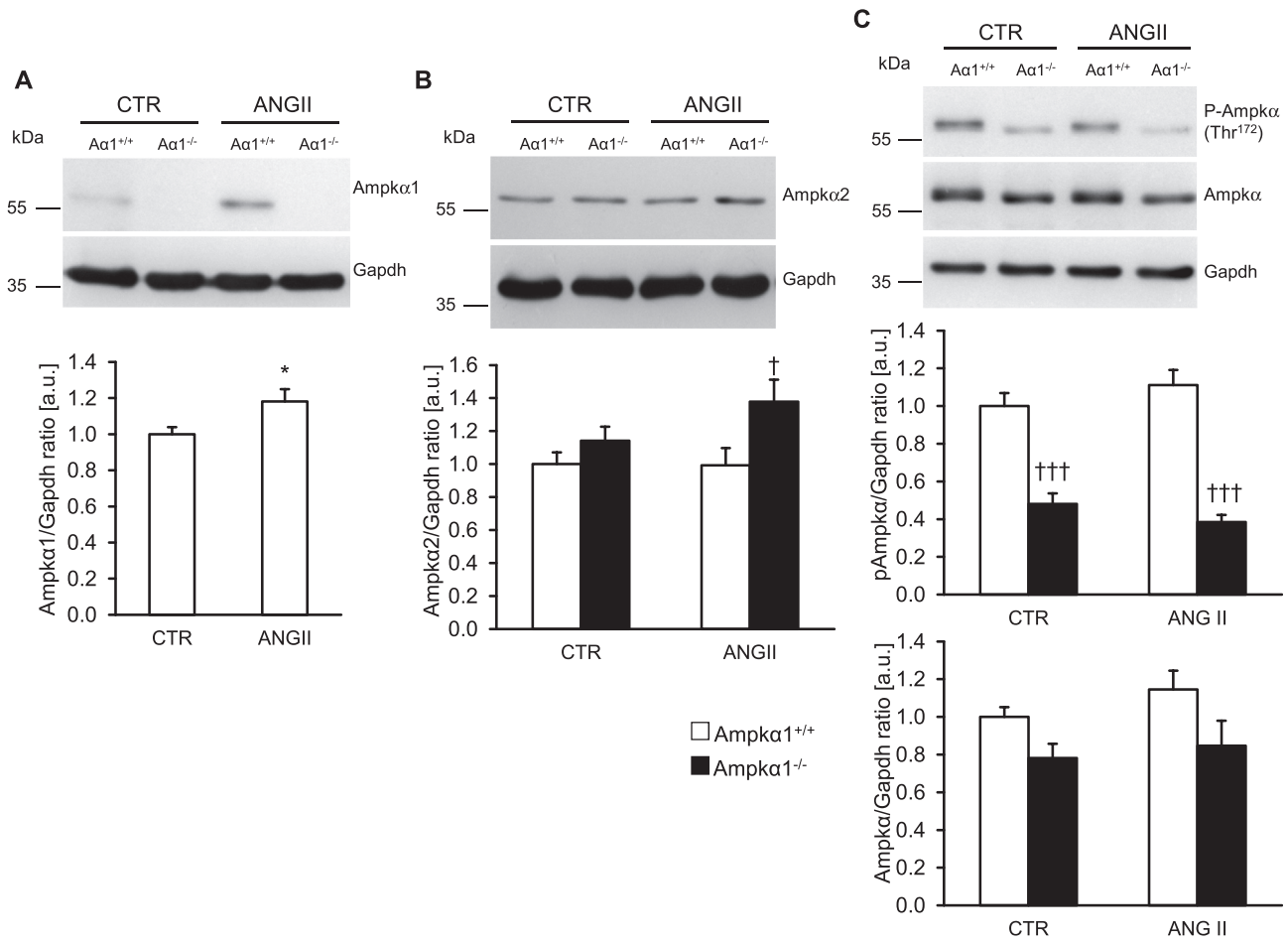

Fig. 1. Effect of angiotensin-II on renal Ampkα isoforms and phosphorylated and total Ampkα protein expression in Ampkα1 knockout mice. Representative original Western blots and arithmetic means ± SEM (arbitrary units, a.u.) of normalized Ampkα1/Gapdh (**A,** n = 12), Ampkα2/Gapdh (**B,** n = 12), phosphorylated Ampkα (Thr^{172})/Gapdh and total Ampkα/Gapdh protein ratio (**C,** n = 10) in renal tissue from Ampkα1 knockout mice (Ampkα1$^{-/-}$) and corresponding wild-type mice (Ampkα1$^{+/+}$) following control (CTR) or angiotensin-II infusion (ANGII). *($p < 0.05$) statistically significant vs. control treated wild-type mice; †($p < 0.05$), †††($p < 0.001$) statistically significant vs. respective wild-type mice.

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