Hijacking epidermal growth factor receptors by angiotensin II: new possibilities for understanding and treating cardiac hypertrophy


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Abstract. Activation of the type 1 angiotensin II receptor (AT1R) is associated with the aetiology of left ventricular hypertrophy, although the exact intracellular signalling mechanism(s) remain unclear. Transactivation of the epidermal growth factor receptor (EGFR) has emerged as a central mechanism by which the G protein-coupled AT1R, which lacks intrinsic tyrosine kinase activity, can stimulate the mitogen-activated protein kinase signalling pathways thought to mediate cardiac hypertrophy. Current studies support a model whereby AT1R-dependent transactivation of EGFRs on cardiomyocytes involves stimulation of membrane-bound metalloproteases, which in turn cleave EGFR ligands such as heparin-binding EGF from a plasma membrane-associated precursor. Numerous aspects of the ‘triple membrane-passing signalling’ paradigm of AT1R-induced EGFR transactivation remain to be characterised, including the identity of the specific metalloproteases involved, the intracellular mechanism for their activation and the exact EGFR subtypes required. Here we examine how ‘hijacking’ of the EGFR might explain the ability of the AT1R to elicit the temporally and qualitatively diverse responses characteristic of the hypertrophic phenotype, and discuss the ramifications of delineating these pathways for the development of new therapeutic strategies to combat cardiac hypertrophy.

Key words. Type 1 angiotensin II receptor (AT1R); transactivation; EGFR; metalloprotease; HB-EGF; cardiac hypertrophy.

Introduction

Cardiac hypertrophy enables terminally differentiated cardiomyocytes to adapt to the increased workload initiated by a variety of stimuli, such as growth factors, cytokines, haemodynamic stress and G protein-coupled receptor (GPCR) agonists. Although initially a positive homeostatic mechanism, prolonged cardiac hypertrophy can be maladaptive and lead to a variety of cardiopathies, including left ventricular hypertrophy (LVH). LVH is a significant risk factor for cardiac morbidity and mortality [1, 2], and elucidation of the signalling pathways leading to aberrant heart growth remains a primary obstacle to rational therapeutic targets for cardiac diseases.

Angiotensin II and cardiac hypertrophy

The peptide hormone angiotensin II (AngII) and its cognate GPCR (AT1R), are well recognised for their critical role in arterial blood pressure regulation, water balance and electrolyte homeostasis. Increasingly apparent, however, is the contribution of AngII to the development and...
maintenance of LVH. In humans and animals, angiotensin-converting enzyme (ACE) inhibitors (which prevent the synthesis of AI1) and AT,R antagonists have been shown to prevent or regress the development of hypertrophy [3–6]. AT,Rs are upregulated in experimentally induced cardiac hypertrophy [7–9], and AngII has been shown to cause hypertrophy by direct action on cardiomyocytes; stimulation of isolated cardiomyocytes leads to the induction of a characteristic hypertrophic phenotype, including protein synthesis, cell growth and re-expression of a foetal gene programme in the absence of proliferation [10–12]. Transgenic animals expressing human AT,Rs exclusively on cardiomyocytes further demonstrate that upregulation of AT,Rs, in the absence of haemodynamic change, is sufficient to cause significant hypertrophy, and in at least one case, premature death due to cardiac failure [13, 14].

‘Classical’ AT,R signalling, responsible for acute AngII actions such as vasoconstriction, is thought to occur via the G protein Gq, leading to activation of phospholipase C-β and the subsequent generation of second messengers diacylglycerol and inositol trisphosphate, which in turn stimulate protein kinase C (PKC) and mobilise intracellular calcium [15]. However, the hypertrophic effect of AngII in cardiac and non-cardiac cells is delayed (requires hours to days) and appears to involve the sequential and parallel activation of a variety of protein kinases that are normally associated with signalling pathways downstream of tyrosine kinase receptors. Kinases typically implicated in mediating cardiac growth include mitogen-activated protein kinases (MAPKs), such as extracellular signal-regulated kinases 1 and 2 (ERK1/2), the phosphatidylinositol 3-kinase (PI3K)-dependent signalling kinases, Akt/PKB and the mTOR/S6 kinase axis [12, 16, 17]. Activation of some of these pathways can be readily explained by ‘classical’ GPCR signalling, for example, PKC-dependent activation of ERK1/2. In contrast, mobilisation of additional growth-dependent kinases by the AT,R, which lacks intrinsic tyrosine kinase activity, has been more difficult to elucidate. Axel Ullrich’s group provided the first evidence for a GPCR-mediated ‘transactivation’ of the epidermal growth factor receptor (EGFR), potentially explaining ‘non-classical’ GPCR signalling outcomes related to proliferation [18]. Indeed, consistent with this paradigm, it now appears that AngII also ‘hijacks’ intracellular growth machinery by usurping EGFR signalling pathways to cause cardiac and smooth muscle hypertrophy [19–22].

**GPCR transactivation paradigm**

In 1996, Daub et al. established a pathway by which a variety of GPCR agonists could cause phosphorylation and activation of the plasma membrane-bound EGFR [18]. Furthermore, using the selective EGFR inhibitor tyrphostin AG1478 and a dominant-negative EGFR, the authors demonstrated that GPCR-mediated growth and proliferation were dependent upon EGFR ‘transactivation’. Initially attributed to an intracellular signalling mechanism, the group subsequently demonstrated that EGFR transactivation also required matrix metalloprotease (MMP)-dependent extracellular cleavage of pro-heparin-binding EGF (HB-EGF), which liberated a soluble HB-EGF that could activate the EGFR (fig. 1) [23]. Accordingly, this ‘triple membrane-passing signalling’ (TMPS) paradigm has been verified for a variety of GPCRs in different cellular backgrounds (for reviews see [22, 24–28]). In particular, TMPS is pertinent to AngII-mediated cell proliferation and growth, including cardiac hypertrophy (table 1), although the exact mechanisms involved in MMP mobilisation and HB-EGF liberation are far from established. Given the critical function of the EGFR and its subtypes, matrix metalloproteases and HB-EGF in the developing and mature heart, it is reasonable to expect a predominant role of TMPS in AngII-mediated cardiac hypertrophy.

**EGFR transactivation in the heart**

An important clue to the physiological function of the EGFR and its family members (HER2, HER3, HER4) in the myocardium came inadvertently from the anti-HER2 breast cancer drug, Herceptin, which caused dilated cardiomyopathy in a subset of patients [29]. Consistent with this, transgenic mice lacking HB-EGF [30], HER2 [31] or EGFR [32] in the heart developed a similar phenotype of cardiomyopathy, cardiac hypertrophy and premature death. One of the first direct demonstrations of AngII-mediated TMPS in the heart was provided by Thomas et al. (2002), who showed that AngII administration caused hypertrophy in primary cultures of neonatal cardiomyocytes in an MMP/EGFR-dependent manner [12]. Asakura et al. (2002) provided evidence for a functional role in vivo for the TMPS by demonstrating that ADAM12, a disintegrin MMP, was linked to the release of HB-EGF and subsequent EGFR transactivation in the intact heart following AT,R and other GPCR stimulation [20]. The requirement for Gq in GPCR-mediated transactivation and the nature of the signalling molecules that link the receptor to EGFR transactivation are controversial [26, 27, 33, 34]. In the studies of Thomas et al. (2002), a Gq inhibitory peptide prevented the induction of the hypertrophic marker, atrial natriuretic peptide, in accordance with the established hypertrophic effect of cardiospecific overexpression of constitutively active Gq [35–38]. In contrast, recent studies using G protein-uncoupled AT,Rs indicate that EGFR transactivation may occur independent of Gq, although it would appear that