The Continuing Saga of the AT2 Receptor
A Case of the Good, the Bad, and the Innocuous

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Left ventricular hypertrophy (LVH) associated with hypertension puts patients at heightened risk for further cardiovascular complications. There has been lot of interest in the effects of angiotensin II on cardiac tissue because of clinical1,2 and experimental3 evidence of LVH reversal and reduction of cardiovascular risk by inhibition of the renin-angiotensin system. Central to this question is whether angiotensin II has direct effects on the heart independent of, or in addition to, its effects on blood pressure because this might significantly modify treatment targets in certain high-risk patients. As a result, investigators in the “heart-as-a-direct-target” camp have focused a lot of energy on characterizing the cellular responses that angiotensin II triggers in the heart.

In the late 1980s, it became evident that there were 2 types of high-affinity receptors for angiotensin II that differed in pharmacological properties and tissue distribution. The angiotensin type (AT1) receptor, which mediates most of the classic cardiovascular actions of angiotensin II, was found to be a 7-transmembrane G-protein–coupled receptor. A host of signaling pathways that lead to calcium mobilization, tyrosine kinase activation, and free radical generation have been described for this receptor since its discovery. Much more controversial is the other angiotensin II type II receptor (AT2). Molecular cloning of the AT2 receptor revealed that it was only loosely related (34% identity) to the AT1 receptor. Although it is believed by many that the physiological role of the AT2 receptor is to antagonize the effects of AT1, the intracellular signaling cascade and the resulting function of the AT2 receptor in the heart have been much more difficult to pin down experimentally and have been the focus of numerous commentaries and reviews in the recent scientific literature.4–8

In the current issue of Hypertension, d’Amore, Black, and Thomas fire the latest salvo in the debate by suggesting that the AT2 receptor plays a nefarious role in cardiac hypertrophy.9 The authors of the study use recombinant adenoviruses expressing the rat AT1 and AT2 receptors to infect cardiomyocytes cultured from neonatal rat hearts. By varying the ratio of the 2 viruses, they are able to carefully control the levels of the 2 receptors and set out to study their combined effects on hypertrophy of the cells in response to angiotensin II. The results showed that the AT1 receptor behaved as had been reported previously by numerous groups; it triggered hypertrophic growth of the cardiomyocytes. Surprisingly, rather than antagonizing AT1 signaling, the AT2 receptor complemented it. In addition, whereas the AT1 receptor required angiotensin II to trigger the hypertrophic response, the AT2 receptor was constitutively active and unresponsive to classic AT2 antagonists. The results unequivocally demonstrate fundamental mechanical differences in the cell biology of 2 receptors, and the authors conclude that the AT2 receptor could play a deleterious role in cardiac pathologies. If these results can be translated to the adult heart, the implications are profound: the AT2 receptor might induce LVH in a manner that would make it unresponsive to pharmacological intervention. However, some aspects of the biology of cardiac angiotensin receptors should be considered before extending these conclusions to the clinic.

In rats, AT2 is the predominant (>95%) angiotensin II receptor in the developing fetus, where it is expressed at high levels in undifferentiated mesenchyme. However, this situation changes dramatically after birth, when AT2 expression nearly disappears while AT1 expression remains similar to levels seen in the fetus. The same is true in the heart, where the relative levels of AT2 receptor drop ≈50% in cardiomyocytes and 90% in cardiac fibroblasts, resulting in roughly equal representation of AT1 and AT2 in the adult rat, human, and bovine hearts.7 Nevertheless, it is important to stress that the heart expresses extremely low levels of angiotensin II receptors compared with other tissues, both prenatally and postnataally. This fact is reinforced by the results of d’Amore et al, who found that uninfected cardiomyocytes expressed low to undetectable levels of angiotensin receptor and were unresponsive to exogenous angiotensin II.

In spite of the low AT1 and AT2 receptor content in the heart, others have shown that angiotensin II stimulates increased hypertrophy (as measured by an increase in protein/DNA ratios) in isolated neonatal rat cardiac myocytes, and this action is blocked by specific AT1 receptor antagonists. Angiotensin II effects on cardiomyocyte hypertrophy are enhanced by AT2 antagonists in tissue culture.10,11 These studies clearly demonstrate that the AT2 antagonists function in the setting of normal levels of AT2 receptor expression. However, if there is any consistency in the field, this is where it ends: inactivation or overexpression of the AT2 receptor either in cell culture or in transgenic mice has given every result imaginable from no effect to inhibition or enhancement of AT1 responses (see the introduction from the article by d’Amore et al for references). Commentators of these studies...
have struggled to explain them by citing strain differences in the animals used, differences in the relative ratios of the AT1 and AT2 receptors, and the effect of receptor “context” on response.4,5 However, none of these variables satisfactorily explain the results obtained by d’Amore et al. The strain of rats they use for their cell preparation (Sprague-Dawley) is the same as that used previously to show that AT2 antagonists modulate endogenous AT1 receptor signaling.10 Neither is the ratio of AT1 to AT2 receptor likely to explain their results because the authors tested a range of different AT1/AT2 ratios with identical results (their Figure 5).

Could the level of expression of the receptors play a role in explaining the results obtained by d’Amore et al? These investigators do in fact achieve very high receptor levels with viral infection (200 to 600 fmol/mg tissue). Because they do not detect angiotensin II receptor on the uninfected cells (normally ∼50 fmol/mg protein for each of the receptors), it is impossible to tell how much of an increase over normal this represents, although it is safe to assume this is a “shopping” increase. Could these high levels of expression affect the biology of the receptors? The converse would, in fact, be surprising. The angiotensin receptors (like all peptide hormone and growth factor receptors) signal through their interaction with binding partners including G-proteins,7 a variety of other transmembrane receptors12–15 and possibly other accessory proteins16,17 that may be present in limiting quantities. In the context of gross overexpression, one can imagine of number of consequences: siphoning up interacting proteins thereby preventing their interaction with other biologically important partners, forcing promiscuous interactions with signaling partners of normally insignificant affinity, and overwhelming downstream signaling cascades. What is perhaps most surprising is that the AT1 receptor behaves as expected at this level of expression (their Figure 3). AT2 may simply have a lower threshold for bizarre behavior because its expression is normally virtually eliminated at birth. Others have in fact shown that the AT2 receptor can homodimerize when overexpressed in cultured cells and signal in a ligand-independent fashion.18

Could such a “regulatory escape” have a physiological role? This might be possible during fetal development when AT2 expression is very high compared with the adult animal. AT2 expression also increases in cardiomyocytes after myocardial infarction19 and in response to stretch20 and in cardiac fibroblasts from animals with congestive heart failure.21 Although the increases in angiotensin receptor expression levels in these conditions are only 2- to 3-fold and nowhere near the levels achieved in the study by d’Amore, single-cell polymerase chain reaction has revealed that only a small percentage of cardiomyocytes express the AT2 receptor, making it possible that measuring AT2 changes in whole heart could underestimate changes in individual cells. Whether or not this type of regulation turns out to be clinically important for the heart remains to be proven, but one thing is certain with the AT2 receptor: it does not take well to being manipulated.

References
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