Cardiac angiotensin II: does it have a function?

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ANGIOTENSIN (ANG) II, in addition to its vasoconstrictor effects, is believed to be a direct mediator of left ventricular hypertrophy and cardiac fibrosis, thus explaining the beneficial cardiac effects of renin-angiotensin system (RAS) blockers in humans. Yet, simultaneously, it has been argued that these effects can simply be attributed to the blood pressure-lowering capacity of RAS blockers. A critical review recently concluded that the bulk of evidence, obtained in genetically modified experimental animals [e.g., overexpressing angiotensinogen, angiotensin-converting enzyme (ACE), or ANG II type 1 (AT1) receptors in the heart], does not support a direct role of the RAS in left ventricular hypertrophy (18). In fact, even transgenic mice expressing an engineered fusion protein designed to directly release ANG II in the heart (using the α-myosin heavy chain promoter), despite having 20- to 50-fold elevated cardiac ANG II levels, showed no evidence of cardiac hypertrophy (27). At most, a slight increase in interstitial fibrosis occurred. One possibility is that the deleterious effects of ANG II become apparent only in combination with another stimulus (e.g., a rise in blood pressure). In this issue of the journal, Xu et al. (31) have evaluated this possibility, using an elegant approach. The above transgenic mice were treated with deoxycorticosterone acetate (DOCA)-salt to simultaneously induce a blood pressure rise and to suppress their endogenous RAS. Under those conditions, aggravated cardiac remodeling did occur. But is it truly due to heart-derived ANG II?

Cardiac ANG II: its origin. Despite early studies supporting the concept that the heart synthesizes renin, thus allowing angiotensin production at cardiac tissue sites, the current view is that cardiac renin originates in the kidney, even under pathological conditions. Cultured cardiac myocytes and fibroblasts do not release renin or its inactive precursor, prorenin, into the medium (11, 29). Renin activity cannot be demonstrated in cardiac tissue following a bilateral nephrectomy (5, 12), nor does the isolated perfused rat Langendorff heart release or contain renin (8). Moreover, the cardiac tissue levels of renin closely correlate with the plasma levels of renin, both under normal and pathological conditions (5, 10, 13). Yet, cardiac renin levels (expressed per g wet wt) are too high to be explained based on the amount of (renin- and prorenin-containing) blood plasma (~5%) in cardiac tissue. A unifying concept is that the heart actively sequesters circulating, kidney-derived renin and/or prorenin (5, 6, 12, 17). In other words, circulating renin and prorenin not only diffuse into the cardiac interstitial space (22) but also bind to cardiac (pro)renin receptors (1, 19). Such uptake allows ample angiotensin production in the heart. Indeed, kinetic studies with 125I-labeled angiotensins have revealed that most, if not all, cardiac ANG II is derived from local synthesis (24, 26).

The site of such synthesis has been hotly debated, and both intracellular (14) and extracellular/cell surface ANG II production (3, 20) have been proposed. A recent study, making use of mice deficient for one, two, or all three angiotensin receptors (AT1A, AT1B, and AT2), has provided the definite answer (23). Tissue ANG II levels in the absence of AT receptors are close to zero and are, at most, as high as expected on the basis of the angiotensin levels in the extracellular fluid [interstitial fluid (ISF) and blood] that is present in tissue. With AT receptors, tissue ANG II levels are much higher. Given the prolonged half-life of ANG II when bound to AT receptors (25, 28), it can therefore be concluded that tissue ANG II predominantly represents ANG II bound to cell surface AT receptors and/or ANG II that has been internalized following AT1 receptor binding (AT2 receptors do not internalize). Without this mechanism, tissue angiotensins are rapidly degraded by angiotensinases (21) and remain low. The same situation can be reached during (near-complete) AT1 receptor blockade (15). Thus ANG II occurs intracellularly only when AT1 receptors are functionally active, and the most likely site(s) of angiotensin generation are the ISF and/or the cell surface. The latter may involve the recently discovered (pro)renin receptor (2).

The model. In this issue of the American Journal of Physiology-Heart and Circulatory Physiology, the model used by Xu et al. (31) depends on the release of ANG II via the secretory pathway from cardiomyocytes after cleavage from its fusion protein by furin. This ANG II release occurs in a RAS-independent manner, i.e., it does not require renin, angiotensinogen, or ACE. A critical issue when using this approach is of course to what degree it truly allows ANG II release in the cardiac interstitial space to act on AT1 receptors. The coronary perfusate of hearts expressing ANG II, when mounted in the Langendorff setup for 25 min (at a constant flow of 2 ml/min), contained 30–200 pg ANG II. This represents <2% of the total amount of ANG II detected in such hearts (12.4 ± 1.6 ng/g). Although this is in agreement with the observation that the plasma ANG II levels in transgenic mice (290 ± 97 pg/ml) are not different from those in control mice (126 ± 25 pg/ml), it also implies that either the cardiac ISF ANG II levels of these hearts are several orders of magnitude above the plasma levels without apparent ANG II release in plasma and/or that a substantial percentage of this elevated cardiac ANG II is still located at an intracellular location. Given the rapid exchange of angiotensins between blood plasma and cardiac ISF, normally allowing their levels to be of comparable magnitude (7, 9), the virtual lack of ANG II release from the isolated perfused heart supports the latter possibility. Thus the actual levels to which the cardiac AT1 receptors are exposed in the ANG II transgenic mice are unlikely to be 20- to 50-fold higher than normal. Studies allowing the collection of cardiac ISF are required to solve this issue (7). In agreement...
with a more modest rise in the cardiac ISF ANG II levels, the cardiac AT1 receptor density and affinity were unaltered in this model (27). Such modest rises are more in the range of rises that will occur under pathophysiological conditions (13, 26).

The DOCA-salt treatment applied by Xu et al. (31) greatly suppressed the endogenous RAS, as evidenced by a substantial decrease in plasma renin concentration. Unfortunately, no data on cardiac ANG II under these conditions have been provided, although it seems reasonable to assume that these levels were still high.

The findings. Under basal conditions, in full agreement with previous observations (27), elevated cardiac ANG II, except for some modest fibrotic effects, had no detectable consequences. Yet, following DOCA-salt treatment, the transgenic mice displayed enhanced cardiac remodeling, as evidenced by a greater ventricular hypertrophy, cardiac fibrosis, inflammation, oxidative stress, and apoptosis compared with nontransgenic, DOCA-salt-treated mice. AT1 receptor blockade with valsartan partially prevented these phenomena, without affecting blood pressure. Unexpectedly, cardiac AT1 receptor expression was upregulated following DOCA-salt exposure and was not affected by valsartan. The authors speculate that this relates to the enhanced mechanical stretch in the DOCA-salt model. An alternative explanation is of course that it is the consequence of the reduced endogenous RAS activity, again raising the question whether the cardiac ANG II expression truly results in high ISF ANG II levels.

DOCA is a mineralocorticoid receptor (MR) agonist, and its effects will thus primarily be due to MR activation. This explains why valsartan only partially blocked the effects in the transgenic mice, and was without effect in the wild-type mice exposed to DOCA-salt. Importantly, synergistic effects of aldosterone and ANG II are known to occur (4, 16), and thus the enhanced cardiac effects in the transgenic mice may well be the consequence of such potentiation. Moreover, aldosterone has been reported to upregulate AT1 receptors and/or to induce the formation of AT1 receptor dimers (32), thereby causing AT1 receptor-dependent effects that do not necessarily involve ANG II. Thus, the upregulated AT1 receptor expression may also be a direct effect of DOCA itself, and valsartan will obviously only block effects that depend on such AT1 receptor upregulation. DOCA-salt did not (significantly) upregulate cardiac AT1 receptor expression in wild-type mice. Because stretch activates AT1 receptors even without the involvement of ANG II (33), it might be argued that the DOCA-salt-induced, AT1 receptor-mediated effects in the ANG II transgenic mice do not rely on ANG II. Valsartan is capable of upregulating cardiac AT1 receptor expression even in the absence of ANG II (33, 34), which might be explained by the greater ventricular hypertrophy in normotensive mice. Because stretch activates AT1 receptors even without the involvement of ANG II (33), it might be argued that the DOCA-salt-induced, AT1 receptor-mediated effects in the ANG II transgenic mice do not rely on ANG II. Valsartan is capable of upregulating cardiac AT1 receptor expression even in the absence of ANG II (33, 34). This leaves the question why DOCA upregulates AT1 receptors only in the transgenic mice.

Conclusion and perspective. The current study is suggestive for cardiac effects of locally generated ANG II, either in conjunction with MR activation or on top of elevated blood pressure, but not alone. Nevertheless, to obtain definite proof for this conclusion, it is crucial to demonstrate the location of the elevated cardiac ANG II levels and to exclude the possibility that the DOCA-salt effects are merely the consequence of AT1 receptor upregulation, per se, not requiring ANG II.

DISCLOSURES
No conflicts of interest are declared by the author.

REFERENCES

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