Pressure-overload hypertrophy is unabated in mice devoid of AT$_{1A}$ receptors

Masayoshi Hamawaki, Thomas M. Coffman, Andrew Lashus, Masaaki Koide, Michael R. Zile, Michael I. Oliverio, Gilberto DeFreyte, George Cooper IV, and Blase A. Carabello. Pressure-overload hypertrophy is unabated in mice devoid of AT$_{1A}$ receptors. Am. J. Physiol. 274 (Heart Circ. Physiol. 43): H868–H873, 1998.—Mechanisms controlling cardiac growth are under intense investigation. Among these, the renin-angiotensin system has received great interest. In the current study, we tested the hypothesis that the renin-angiotensin system was not an obligate factor in cardiac hypertrophy. We examined the left ventricular hypertrophic response to a pressure overload in mice devoid of the AT$_{1A}$ receptor, the putative major effector of the growth response of the renin-angiotensin system. Aortic banding produced similar transband gradients in wild-type and AT$_{1A}$ knockout mice. The left ventricular mass-to-body weight ratio increased from 3.44 ± 0.08 to 5.62 ± 0.25 in wild-type ascending aortic-banded mice. The response in the knockout mice was not different (from 2.97 ± 0.13 to 5.24 ± 0.37). We conclude that the magnitude of cardiac hypertrophy is not affected by the absence of the AT$_{1A}$ receptor and its signaling pathway and that this component of the renin-angiotensin system is not necessary in cardiac hypertrophy.

HYPERTROPHY IS one of the fundamental mechanisms by which the myocardium responds to a hemodynamic overload. Initially, hypertrophy is viewed as a compensatory mechanism because it facilitates ejection performance of the overloaded ventricle. In pressure overload, facilitation occurs because concentric hypertrophy normalizes wall stress (4). Although initially hypertrophy is compensatory, if the overload is severe and prolonged, muscle dysfunction develops. Accordingly, there has been great interest in the mechanisms and systems controlling cardiac growth in response to a hemodynamic overload and in those mechanisms responsible for the transition from compensatory to pathological hypertrophy. Among various neurohumoral systems, the renin-angiotensin system (RAS) has received intense study of its role in controlling hemodynamically initiated cardiac growth. It is clear that angiotensin can stimulate neonatal and adult cardiocytes to grow (10, 15, 16, 20). On the other hand, in vivo infusions of angiotensin and in vivo blockade of the RAS either at the converting enzyme level or at the AT$_{1}$-receptor level have yielded widely disparate results regarding the effects of the RAS on cardiac hypertrophy. In one study, angiotensin infusion caused cardiac growth in the absence of a well-defined increase in load, suggesting a primary role for the RAS in regulating myocardial growth (2). This role was reinforced by other studies (3, 7, 14), which found that RAS blockade blocked cardiac growth or permitted hypertrophy regression even when a hemodynamic stimulus was present. However, in other studies (19, 21, 24, 25), RAS blockade failed to block myocardial or cardiocyte growth in the presence of a hemodynamic stimulus. In still other studies (8, 9), the effect of angiotensin-converting enzyme (ACE) inhibition derived from increased bradykinin rather than interference with the RAS. The conflicting outcomes of these studies might stem from transient unrecognized increases in load, because load needs to be only briefly increased to cause hypertrophy (12), or from a lack of certainty regarding the extent of RAS activation and degree of blockade. Thus, although it is clear that the RAS can stimulate cardiocyte and presumably myocardial growth, it is unclear whether this system has an obligate role in cardiac growth or is merely one of many systems that can modulate this response.

Whereas several receptors for angiotensin II have been identified, it is the AT$_{1}$ receptor that is responsible for growth regulation. In the current study, we tested the hypothesis that the RAS working through the AT$_{1}$ receptor was not obligate in the hypertrophic response of the heart to a hemodynamic load by examining cardiac hypertrophy in response to a pressure overload in mice devoid of AT$_{1A}$ receptors, the putative initiators of RAS-induced cardiac hypertrophy.

METHODS

Three pairs of mouse groups were studied: wild-type control and aortic-banded wild-type mice, heterozygous controls and heterozygous banded mice, and homozygous AT$_{1A}$ knockout mice and AT$_{1A}$ knockout banded mice. The left ventricles were weighed at death and indexed to body weight in controls and in banded mice 3 wk after banding.

Preparation of AT$_{1}$ knockout mice. The AT$_{1}$ knockout mice were prepared by homologous recombination in stem cells as described previously (6). The genotype of each mouse was established by Southern blotting using previously developed diagnostic probes (Fig. 1). The effect of AT$_{1A}$ knockout was confirmed by 10.220.33.1 on June 28, 2017 http://ajpheart.physiology.org/ Downloaded from
Two different banding techniques were employed. In one group the band was placed around the ascending aorta. However, because this technique required left ventricular puncture for measurement of proximal pressure (no catheter could be passed across the tiny residual orifice), a second group of animals was banded at the aortic arch between the carotid arteries. By catheterizing the upstream and downstream carotid arteries, upstream and downstream pressure and the gradient between them could be obtained.

Mice were anesthetized with a combination of ketamine (50 mg/kg) and xylazine (2.5 mg/kg) given intraperitoneally. The skin was cleaned with iodine solution, and the operation was performed under sterile conditions. The animals were placed supine, and a midline cervical incision was made to expose the trachea for direct intubation with a 22-gauge plastic catheter. The catheter was connected to a volume-cycled ventilator supplying supplemental oxygen with a tidal volume of 2.5 ml and a respiratory rate of 120 beats/min. A right thoracotomy was performed. For ascending aorta banding, a 6-0 nylon suture was placed around the proximal aorta over a 26-gauge needle, causing complete occlusion of the aorta. The needle was then removed, restoring a lumen with a severely stenotic aortic orifice. Transverse arch banding was performed similarly with a 27-gauge needle, causing a more severe but more distal stenosis. After banding, the thoracotomy and trachea were repaired and air was evacuated from the chest.

Echocardiography. Echocardiography was performed with the use of a 9.0-MHz transducer at baseline and 3 wk after banding. Echocardiography was used to determine left ventricular performance and to measure left ventricular wall thickness, ventricular dimension, and mass.

Hemodynamics. At the final study after 3 wk of banding in the aortic arch-banded groups, 24-gauge plastic catheters were placed in both carotid arteries and the pressures were obtained. In the ascending aortic-banded model, pressures were obtained via direct left ventricular puncture and a carotid artery (Fig. 3). After hemodynamic and echocardiographic measurements were made, the heart was removed while the animal was under deep anesthesia. The atria and right ventricle were dissected away, and the left ventricle was weighed.

Left ventricular weight. Left ventricular wet weights were obtained directly after the left ventricle was dissected free of the atria and right ventricle. Left ventricular dry weight was obtained by desiccation in an oven at 93°C for 48 h.

Statistics. Three pairs of animals were compared, with six groups in total. Therefore, analysis of variance was used to detect whether differences among the six groups were present. If a difference was found, a Newman-Keuls test was used to determine where the differences were located. Dispersion from the mean is noted as standard error (SE).

RESULTS

Animal weights for controls and banded mice are shown for the transverse aortic-banded model (Fig. 4A) and for the ascending aortic-banded model (Fig. 4B).
Wild-type (+/+ ) mice were slightly but significantly heavier than the homozygous knockout (−/−) mice. Left ventricular mass is shown for the transverse aortic-banded model (Fig. 5A) and for the ascending aortic-banded model (Fig. 5B). At baseline, left ventricular mass was less than that in the wild-type mice, presumably because resting left ventricular pressure was lower (6). After mice were banded, mass was also lower in the transverse arch-banded −/− mice (but not in the ascending aortic-banded −/− mice), also possibly because left ventricular pressure was lower in −/− mice (see Table 1). However, the percent increase in left ventricular mass from baseline actually tended to be higher in the −/− mice. Thus an increase in mass of 87% occurred in ascending aortic-banded −/− mice compared with that in −/− controls, despite lower left ventricular pressure. Figure 6 demonstrates the left ventricular mass-to-body weight ratio for the three groups of banded mice and their respective nonbanded controls for the ascending aortic-banded (Fig. 6A) and the aortic arch-banded models (Fig. 6B). Although the homozygous knockout mice had lower left ventricular-to-body weight ratios at baseline, no differences existed after banding and the percent increase in relative mass was similar among all groups. The left ventricular dry weight-to-body weight ratio for the two banding positions is shown in Fig. 7. These confirmatory results indicate that left ventricular mass increases were not due to edema. Cross sections of left ventricles from controls and banded mice are shown in Fig. 8.

The left ventricular pressures, aortic pressures, and transband pressure gradients for the transverse arch-banded and ascending aortic-banded groups are shown in Table 1. Although there were no differences in...
gradient among the groups, pressures were higher in the +/+ mice. The ventricular geometry data obtained echocardiographically are given in Table 2 for +/− mice. Although dimension did not change, both wall thickness and relative wall thickness increased after banding.

DISCUSSION

The role of the RAS in regulating cardiac growth in response to a hemodynamic overload is controversial. It is clear that in vitro stimulation of cardiocytes by angiotensin II increases protein synthesis. Several laboratories, including our own, have made this observation (10, 15, 16, 20, 23), although in our laboratory the growth response was less than the response that occurred due to contraction alone. Less clear is the role of this system in vivo. Assessment of the in vivo response is made harder by the difficulty in divorcing a direct effect of RAS stimulation or blockade from the hemodynamic effects of these maneuvers. A variety of in vivo and whole ventricle in vitro models have been used in attempts to define this role. The experimental strategies include 1) angiotensin II infusion compared with angiotensin II infusion plus blockade of AT1 receptors or use of an antihypertensive agent to abolish the hypertensive effect of angiotensin II and 2) pressure overload compared with pressure overload plus RAS blockade at either the ACE or AT1 levels. In some of these studies low-dose blockade was used in an attempt to block the RAS without altering the hemodynamic load.

In two studies employing angiotensin infusion, either protein synthesis increased (18) or left ventricular hypertrophy occurred (2) in the absence of an obvious pressure overload. AT1-receptor blockade blocked the increase in protein synthesis (18). These studies are congruent with the in vitro cardiocyte data (10, 15, 16, 20, 23), indicating a tropic effect of angiotensin II.

More disparate are the studies of pressure overload during RAS blockade. On one hand, ACE inhibition (24) or AT1 blockade (5) had no effect on the development of pressure-overload right ventricular hypertrophy. Also, in some studies of left ventricular hypertrophy, ACE inhibition failed to block hypertrophy (11, 25). On the other hand, in other studies ACE inhibition either blocked hypertrophy (7) or its transition to heart failure (22). In still other studies ACE inhibition seemed more dependent on increased bradykinin than RAS blockade in blunting the hypertrophic response (8, 9). In studies of left ventricular pressure overload using AT1 blockade, AT1 blockade reduced hypertrophy in some studies (3, 14, 23) but failed to do so in others (19, 21).

The current study helps to clarify these conflicting results inasmuch as the AT1A receptor, the major receptor involved in hypertrophy, was absent. Our data show conclusively that this receptor is not necessary for the full hypertrophic response to be elicited. Although the AT1B receptor was not ablated in the current studies, this receptor has been demonstrated to be present in only tiny levels in mammalian hearts (17). Furthermore, as we have shown, the overall physiological
importance of this receptor appears quite small (13). Despite the fact that the RAS is highly activated in \( 2/2 \) mice, left ventricular mass was still less than that of wild-type controls at baseline. Thus extensive stimulation of AT1B receptors by angiotensin II levels up to five times normal (1) appears incapable of normalizing left ventricular mass in the face of the reduced systemic blood pressure (6) in controls. It is possible that AT1B-mediated responses contributed to the hypertrophy found in our study. However, the fact that the complete absence of AT1A receptors had no effect on the amount of hypertrophy that developed suggests that the hypertrophy in this study developed at least in part through a non-RAS mechanism. The conclusion is further supported by studies showing that losartan, which blocks both AT1A and AT1B receptors, had no effect on the hypertrophy that developed (19, 21). If our study is taken in context with the others noted above, it is likely that there are multiple systems involved in the hypertrophic process, one of which is the RAS. Other mechanisms of transduction of mechanical load into the signal for hypertrophy include activation through ion channels and through activation at focal adhesion complexes. The effect of blocking the RAS may be determined by how much it was initially activated and to what extent other mechanisms activate hypertrophic growth. However, blockade of the AT1A receptor of this

Table 2. Left ventricular geometry

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<thead>
<tr>
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<th>Baseline</th>
<th>Final</th>
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<tr>
<td>R, mm</td>
<td>1.92 ± 0.04</td>
<td>1.89 ± 0.06</td>
</tr>
<tr>
<td>Th, mm</td>
<td>0.71 ± 0.02</td>
<td>0.95 ± 0.02*</td>
</tr>
<tr>
<td>Th/R</td>
<td>0.36 ± 0.01</td>
<td>0.51 ± 0.02*</td>
</tr>
<tr>
<td>FS</td>
<td>0.41 ± 0.01</td>
<td>0.41 ± 0.01</td>
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Values are means ± SE. R, left ventricular axis dimension; Th, posterior wall thickness; Th/R, relative wall thickness; FS, fractal shortening. *P < 0.1 vs. baseline.
system is not adequate to prevent or even reduce the hypertrophy response due to pressure overload.

Limitations. Our observations were made in only one species and at only one point in time. It is conceivable that differences in hypertrophy might be present in other species or in mice more chronically observed. Furthermore, we do not have data regarding AT1A-receptor density. It is possible that the absence of AT1A upregulated AT1B-receptor density in the heart [it does not do so in the kidney (6)], allowing AT1B receptors to act permissively on cardiac growth regulation. If so, this only occurs during pressure overload because left ventricular mass in the absence of pressure overload is subnormal.

In conclusion, pressure-overload hypertrophy occurs normally in mice devoid of the AT1A receptor. Although our data do not rule out a role for the RAS in mediating hypertrophy in this model, they do suggest that other systems are active in producing the hypertrophy that we found.

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