Cardiac modulations of ANG II receptor expression in rats with hypoxic pulmonary hypertension

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Adamy, Christophe, Patricia Oliviero, Saadia Eddahibi, Lydie Rappaport, Jane-Lise Samuel, Emmanuel Teiger, and Catherine Chassagne. Cardiac modulations of ANG II receptor expression in rats with hypoxic pulmonary hypertension. Am J Physiol Heart Circ Physiol 283: H733–H740, 2002. First published April 18, 2002; 10.1152/ajpheart.01088.2001.—Right ventricular myocardial hypertrophy during hypoxic pulmonary hypertension is associated with local renin-angiotensin system activation. The expression of angiotensin II type 1 (AT1) and type 2 (AT2) receptors in this setting has never been investigated. We have therefore examined the chronic hypoxia pattern of AT1 and AT2 expression in the right and left cardiac ventricles, using in situ binding and RT-PCR assays. Hypoxia produced right, but not left, ventricular hypertrophy after 7, 14, and 21 days, respectively. Hypoxia for 2 days was associated in each ventricle with a simultaneous and transient increase (P < 0.05) in AT1 binding and AT1 mRNA levels in the absence of any significant change in AT2 expression level. Only after 14 days of hypoxia, AT2 binding increased (P < 0.05) in the two ventricles, concomitantly with a right ventricular decrease (P < 0.05) in AT2 mRNA. Along these data, AT1 and AT2 binding remained unchanged in both the left and hypertrophied right ventricles from rats treated with monocrotaline for 30 days. These results indicate that chronic hypoxia induces modulations of AT1 and AT2 receptors in both cardiac ventricles probably through direct and indirect mechanisms, respectively, which modulations may participate in myogenic (at the level of smooth or striated myocytes) rather than in the growth response of the heart to hypoxia.

chronic hypoxia; heart ventricles; angiotensin II; AT 1 and AT 2 receptor subtypes

CHRONIC HYPOXIA-INDUCED pulmonary hypertension is characterized by an increased pulmonary vascular resistance due to pulmonary artery thickening that impedes ejection of blood by the cardiac right ventricle (RV) and leads to RV hypertrophy (1, 20, 21, 33). Evidence that chronic hypoxia increases the plasma levels of renin (9), angiotensin-converting enzyme (ACE) (24), and angiotensin II (ANG II) (42) has stimulated interest in the contribution of the renin-angio-

tensin system to the hypoxia-induced cardiopulmonary changes. This hypothesis is supported by data from chronically hypoxic rats showing that ACE inhibitor treatment reduces pulmonary arterial pressure (5, 14, 26, 27, 34, 41), pulmonary artery thickening (5, 26, 27, 41), and RV hypertrophy (14, 26, 32, 41).

Two pharmacologically distinct subtypes of receptors, designated as AT1 and AT2, participate in regulating ANG II activity. Both have been described based on their affinity for selective receptor antagonists and their sensitivity to reducing agents (6). Whereas AT1 mediates the vasoconstrictor and growth-promoting effects of ANG II, AT2 has been suggested to mediate vasodilator effects and to exert antiproliferation action within many cell types, including vascular smooth muscle cells, endothelial cells, cardiac fibroblasts, and myocytes (6, 12, 22, 23). We have shown in the rat hypoxic lung that AT1 and AT2 receptor expression is upregulated concomitantly with the muscularization of peripheral small pulmonary arteries (3), suggesting a participation of these receptors in the remodeling process. Whether exposure to chronic hypoxia also induces cardiac changes in ANG II receptor expression concomitantly with the development of RV hypertrophy has never been investigated.

The purpose of this study was to directly analyze the time courses of ANG II receptor expression in the chronically hypoxic rat RV and left ventricle (LV) and to compare them with the enlargement capacity of the RV and LV, respectively. Moreover, to ensure the specificity of hypoxia exposure in regulating ANG II receptor expression, we also examined the levels of AT1 and AT2 receptors in the ventricles of rats with monocrotaline-induced pulmonary hypertension.

The levels of AT1 and AT2 receptors were quantitated in the RV and LV of rats subjected to chronic normobaric hypoxia for 1–21 days or treatment with monocrotaline (MCT) for 30 days, by in situ autoradiographic binding assays. The levels of AT1 and AT2 transcripts were quantitated in the ventricles of rats subjected to hypoxia by RT-PCR assays. Assessment of

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ANG II RECEPTOR AND mRNA EXPRESSION IN HYPOXIC HEARTS

hypoxia- and MCT-induced RV hypertrophy was provided by measurements of the Fulton index at 7, 14, and 21 days and at 30 days, respectively.

MATERIALS AND METHODS

Experimental models. All animals were studied in accordance with procedures established by the Animal Care and Use Committee of our institution. Male Wistar rats were continuously exposed to hypoxia for 1, 2, 3, 7, 14, and 21 days and studied within 1 h of removal from the hypoxic chamber (hypoxic groups) or to room air for 1 to 21 days (normoxic groups). Hypoxia (10% O₂) was obtained in a ventilated chamber (500 liters; Flufrance; Cachan, France) as previously described (2) and monitored using an oxygen analyzer (model OA150, Servomex; Crowborough, UK). The chamber was opened once a day for 1 h to clean the cages and replenish food and water stores. Other rats were treated with either MCT or saline and were maintained to room air for 30 days. MCT (Sigma Chemical) was dissolved in phosphate-buffered saline (PBS), and the pH was adjusted to 7.4 with 0.5 N HCl. The MCT solution was given as a single subcutaneous injection (60 mg/kg). MCT control rats were injected with the same volume of saline. All groups were kept in the same room and subjected to the same light-dark cycle. Rat Chow and tap water were provided ad libitum.

Assessment of RV hypertrophy. The rats were anesthetized by intraperitoneal injection of pentobarbital sodium (20 mg/kg). The thorax was opened, the heart was quickly removed, and the ventricles were dissected free of atrial tissue and large blood vessels. In some experiments, the ventricles en bloc were blotted dry and immediately frozen at −80°C for receptor binding assays. In other experiments, the RV was carefully separated from the LV and septum (LV+S). The fresh ventricular tissues were immediately blotted dry and weighed separately to determine the degree of RV hypertrophy based on two parameters, i.e., the RV free wall weight-to-body weight ratio (RV/BW) and RV weight-to-LV+S weight ratio (RV/LV+S). The LV+S weight-to-body weight ratio (LV+S/BW) allows excluding specific changes in the growth capacity of the LV. Because neither RV/BW nor RV/LV+S differed among normoxic groups within the time of experiments, only one normoxic group was considered.

In situ autoradiographic quantitative receptor binding assay. Transverse heart cryosections (10 μm) were used for ANG II binding studies, which were performed as previously described (28), with (3-[125]I)iodotyrosyl-Sar-Ile8-ANG II (specific activity, 2,000 Ci/mmol; Flufrance; Cachan, France) and the AT1- and AT2-selective antagonists losartan (Merck Sharp & Dohme-Chibret; Paris, France) and PD-123319 (0.5 μM) or unlabeled ANG II (0.5 μM, Sigma). Finally, the sections were incubated for 90 min in the presence of 32 pM (3-[125]I)iodotyrosyl-Sar-Ile8-ANG II with or without losartan, PD-123319, or unlabeled ANG II (in the same concentrations as above) to identify AT2, AT1, and ANG II specific total binding sites, respectively. After being washed and dried, the sections were exposed to imaging plates with a range of known amounts of (3-[125]I)iodotyrosyl-Sar-Ile8-ANG II from the same medium as that used for cardiac section incubation and were finally analyzed and quantified using the Bio-Imaging Analysis system (Fuji Mac BAS 1000, Fuji Medical Systems, Ray-test; Courbevoie, France) as previously described (28). Pixels accumulated within the entire RV or LV section were normalized for surface area and converted to femtomoles per squared millimeter by direct density comparison with the 3-[125]Iiodotyrosyl-Sar-Ile8-ANG II calibration curve. Thus the data are means of receptor binding capacity per unit of surface (expressed in fmol/mm²). (3-[125]I)iodotyrosyl-Sar-Ile8-ANG II specific total binding corresponds to the total (AT) minus the nonspecific (NS) labeling revealed with unlabeled ANG II. AT1-selective binding was revealed in the presence of PD-123319, and AT2-specific binding was revealed in the presence of losartan.

RNA preparation and quantitative RT-PCR assay. RNA was extracted according to the procedure described by Chomczynsky and Sacchi (4) from the RV or LV. RNA concentration was determined using standard spectrophotometric techniques and RNA quality was assessed by visual inspection of ethidium bromide-stained denaturing agarose gels.

The RT-PCR assay allowing evaluation of AT1 and AT2 mRNA levels was similar to that previously described (28) with a few modifications. For each ventricle sample, 7 μg of total RNA previously denatured by a 2-min exposure to 95°C were reverse transcribed for 30 min at 42°C using 800 units of Moloney murine leukemia virus reverse transcriptase (Life Technologies) in 80 μl H₂O/diethylylpyrocarbonate containing 4 μg of the oligo(dT) primers (Boehringer), 80 units of RNase inhibitor (Promega), 5 mM deoxynucleotide triphosphate (dNTP)-Na salts (Boehringer), 20 mM MgCl₂ and 8 μl of PCR buffer (×10) (RT solution). Heating the mixture for 5 min at 99°C stopped the RT reaction. Total pool (80 μl) of reverse-transcribed products (complementary DNAs [cDNAs]) was divided into aliquots corresponding to increasing concentrations of total RNA (100, 200, 300, 400, and 500 ng), with each aliquot being adjusted to 20 μl volume with RT solution. All aliquots were then amplified using the AT1 or AT2 sense and antisense primers (Oligo Express; Paris, France), whose DNA sequences are given by Chassagne et al. (3). The amplification reaction was performed with 5 units of Taq DNA polymerase (Life Technologies) in 100 μl of an aqueous mixture containing 300 ng of each of AT1 or AT2 sense and antisense primers, 0.2 nM dNTP-Na salts (Boehringer), 20 mM MgCl₂ and 10 of PCR buffer (×10). Thirty-one amplification cycles were performed in a Perkin-Elmer apparatus, and signals of PCR products were analyzed on ethidium bromide-stained gels using densitometry with a computer-based imaging system (Gel Doc 1000, Bio-Rad), as previously described (28).

Statistical analysis. Results for the various groups of rats are expressed as means ± SE, and differences across groups were evaluated using one-way analysis of variance (ANOVA), and group-to-group comparison were done using Scheffe’s test or t-test. For whole results, the accepted level of significance was P < 0.05.

RESULTS

Assessment of hypoxia- and MCT-induced RV hypertrophy. Final body weight did not vary in hypoxic rats within the 21 days of experiments (Table 1), whereas it was significantly (P < 0.05) lower in MCT-treated rats than in saline controls 30 days after the injection. Compared with rats exposed to room air or treated with saline, rats exposed to hypoxia or given MCT showed significant (P < 0.05 to P < 0.001) increases in
RV weight-to-body weight ratio in the absence of any change in LV weight-to-body weight ratio (Table 1). In hypoxic rats, the degree of RV hypertrophy was significantly increased by 1.4-fold \((P < 0.05)\), 1.7-fold \((P < 0.001)\), and 2-fold \((P < 0.001)\) after 7, 14, and 21 days, respectively, compared with normoxic values, and in MCT-treated rats the degree of RV hypertrophy was increased by 1.7-fold \((P < 0.05)\) compared with saline.

**Ligand binding studies.** Figure 1 shows representative pictures of \((3-^{125}I\text{iodotyrosylSar}^1\text{Ile}^8)\text{ANG II}\) binding in heart sections from rats exposed to room air or to hypoxia for 2 and 14 days, (Fig. 1A), or from rats treated with saline or MCT for 30 days (Fig. 1B). As can be seen in Fig. 1A, total \(^{125}\text{I}\)-labeled ANG II binding (column AT) was present throughout the two ventricles under normoxic conditions (row N), increased uniformly after 2 days of hypoxia (row H-2d), and decreased after 14 days (row H-14d) versus 2 days of hypoxia. Cotreatment of heart sections with \(^{3}\text{H}\)ANP reduced radioligand binding, revealing a threefold decrease in binding level during the first fortnight of hypoxia that reached significant values \((P < 0.05)\) beyond 14 days of hypoxia (Fig. 3B). Compared with controls, AT\(_2\) receptor density after 14 days and at 21 days was higher of about 2.9-fold in the LV \((4.3 \pm 0.5\text{ fmol/mm}^2)\) and 1.4-fold in the RV \((0.291 \pm 0.06\text{ fmol/mm}^2)\) at 14 days; 4.0 \(\pm 0.4\text{ vs. } 3.0 \pm 0.7\text{ fmol/mm}^2\) at 21 days) and of about 6.2-fold in the LV \((4.8 \pm 0.4\text{ vs. } 0.7 \pm 0.4 \times 10^{-3}\text{ fmol/mm}^2)\) at 14 days; 4.0 \(\pm 0.6\text{ vs. } 0.7 \pm 0.4 \times 10^{-3}\text{ fmol/mm}^2)\) at 21 days).

**ANG II receptor subtype mRNA accumulation in hypoxic hearts.** Figure 4A shows representative gels obtained from the analysis by the AT\(_1\)-PCR and AT\(_2\)-PCR assays of various amounts of RNA extracted from either RV or LV of normoxic or 2- or 14-day hypoxic hearts. As can be seen, whatever the tissues, i.e., RV or LV, and the amount of RNA (100, 200, 300, 400, or 500 ng), the intensity of the AT\(_1\)-related signals increased after 2 days but no more after 14 days of hypoxia. The intensity of AT\(_1\)-related signals did not vary in LV RNA after 2 and 14 days of hypoxia but was decreased in RV RNA beyond 14 days of hypoxia. To obtain normalized results, we considered the slope of the linear part of the AT\(_1\) or AT\(_2\) amplification curve, which linear part systematically fitted with initial RNA concentrations from 100 to 500 ng (Fig. 4B). For each independent RNA sample, the relative concentration of AT\(_1\) or AT\(_2\) mRNA was the mean of slopes from a duplicate determination and is expressed as arbitrary units (AU).

Complete analysis of ATR mRNA concentrations confirmed that in normoxic hearts the levels of AT\(_1\) and AT\(_2\) mRNA were nearly similar in each ventricle (see in Fig. 5, A and B), but in the RV their levels was twofold more abundant than in the LV, according to previous findings (10, 28). A transient increase \((P < 0.05)\) in AT\(_1\) mRNA level was detected in either ventricle at hypoxia 2 days (Fig. 5A). The increase was 1.4-fold in the RV \((0.291 \pm 0.022\text{ vs. } 0.201 \pm 0.010\text{ AU})\) in each ventricle transiently increased \((P < 0.05)\) within the first 3 days of hypoxia, reaching a maximum at day 2 compared with rats exposed to room air (Fig. 3A). The increases were 1.8-fold in the RV \((13.8 \pm 0.4\text{ vs. } 7.6 \pm 1.2 \times 10^{-3}\text{ fmol/mm}^2)\) and 1.6-fold in the LV \((10.5 \pm 1.6\text{ vs. } 6.6 \pm 0.9 \times 10^{-3}\text{ fmol/mm}^2)\). Afterward, AT\(_1\) binding decreased in both ventricles, tending toward values below baseline until hypoxia 7 days. Regarding AT\(_2\), each ventricle exhibited a progressive increase in binding level during the first fortnight of hypoxia that reached significant values \((P < 0.05)\) beyond 14 days of hypoxia (Fig. 3B).

**Table 1. Body weight and heart weight in rats used for experiments**

<table>
<thead>
<tr>
<th>Time (Days)</th>
<th>Body Weight, g</th>
<th>RV/BW, mg/g</th>
<th>(LV + S)/BW, mg/g</th>
<th>RV/(LV + S)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normoxia</td>
<td>395 ± 8</td>
<td>0.44 ± 0.05</td>
<td>1.7 ± 0.2</td>
<td>0.26 ± 0.01</td>
</tr>
<tr>
<td>Hypoxia</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>7 days</td>
<td>392 ± 9</td>
<td>0.63 ± 0.04</td>
<td>1.7 ± 0.2</td>
<td>0.37 ± 0.01</td>
</tr>
<tr>
<td>14 days</td>
<td>392 ± 11</td>
<td>0.69 ± 0.03</td>
<td>1.6 ± 0.3</td>
<td>0.43 ± 0.03</td>
</tr>
<tr>
<td>21 days</td>
<td>391 ± 12</td>
<td>0.85 ± 0.04</td>
<td>1.6 ± 0.2</td>
<td>0.50 ± 0.02</td>
</tr>
<tr>
<td>Saline (30)</td>
<td>390 ± 10</td>
<td>0.54 ± 0.06</td>
<td>2.0 ± 0.3</td>
<td>0.27 ± 0.02</td>
</tr>
<tr>
<td>MCT (30)</td>
<td>345 ± 12</td>
<td>0.85 ± 0.34</td>
<td>1.8 ± 0.4</td>
<td>0.47 ± 0.05</td>
</tr>
</tbody>
</table>

Values are means ± SE; \(n = 5–6\) rats per group. Rats exposed to hypoxia for variable periods of time (7, 14, or 21 days) and rats treated with monocrotaline (MCT) for 30 days had a significant increase in the right ventricular (RV)-to-body weight (BW) ratio. LV + S, left ventricle plus septum. *\(P < 0.05\); †\(P < 0.01\); ‡\(P < 0.001\) compared with normoxic or saline rat groups.
in controls) and 2.6-fold in the LV (0.219 ± 0.002 vs. 0.084 ± 0.005 AU in controls) and returned to baseline at 7 days. Whereas AT1 mRNA levels remained unchanged in the RV until the end of hypoxic period, they tend to increase progressively in the LV, reaching more doubling (2.6-fold) values at 21 days of hypoxia. AT2 mRNA level did not vary in the LV within the 21 days of hypoxia but decreased significantly (P < 0.05) in the RV after 7 to 21 days compared with controls (Fig. 5B). The decrease was of about 40% at 7 days (0.131 ± 0.005 vs. 0.210 ± 0.024 AU), at 14 days (0.119 ± 0.007 vs. 0.210 ± 0.024 AU), and at 21 days (0.145 ± 0.007 vs. 0.210 ± 0.024 AU).

**DISCUSSION**

The present results show that exposure to chronic hypoxia and treatment with MCT, both providing RV hypertrophy in rats, are associated with different patterns of expression of cardiac AT1 and AT2 receptors.
Whereas MCT-treated rats showed no change in RV and LV levels of AT₁ and AT₂ receptors, rats exposed to hypoxia showed an increase in AT₁ and AT₂ receptor levels in both ventricles, with this increase being early and transient for AT₁ but delayed and sustained for AT₂. Moreover, in chronically hypoxic ventricles, AT₁ receptors accumulated simultaneously with AT₁ mRNA, whereas AT₂ receptor accumulation is associated with a decrease in AT₂ mRNA in the right ventricle. This study is, to our knowledge, the first to report a differential regulation of AT₁ and AT₂ receptor gene expression in heart ventricles during chronic hypoxia.

Previous studies have examined renin-angiotensin system involvement in RV hypertrophy during development of hypoxia-induced pulmonary hypertension. Increased ACE activity and expression in the RV have been reported during chronic hypoxia (25). In addition, the benefit of ACE inhibitors and AT₁ receptor antagonists against the development of RV hypertrophy (13, 14, 26, 32, 41) point for an important role of ANG II in the process. However, whether RV hypertrophy development depends on local action of ANG II is not known, and the expression pattern of AT₁ and AT₂ receptors and of corresponding transcripts has never been investigated in hypoxic hearts.

We found that the density of AT₁ receptors was seven to eight higher than of those of AT₂ receptors in normoxic ventricles, whereas the levels of their corresponding transcript were very closed. AT₁ receptor binding capacity increased transiently and simultaneously with AT₁ mRNA within the first 2 days of hypoxia. On the other hand, AT₂ receptor density increased lately (after day 14) together with a simultaneous decrease in RV AT₂ mRNA and stable level in the LV. A meaningful observation from our in situ binding studies was a relatively uniform increase in AT₁ and AT₂ radioactive signal throughout the entire RV and LV sections at 2 and 14 days of hypoxia, respectively, suggesting a homogeneous accumulation of both receptor subtypes throughout the hypoxic ventricles. Our finding of an increased AT₁ receptor expression during the early stage (first 2 days) of hypoxia argues for a direct regulatory role of hypoxia. Accounting that, the simultaneous accumulation of AT₁ mRNA and AT₁ receptors indicates that hypoxia-induced transcriptional mechanisms may activate the AT₁ gene via a hypoxia-response DNA element. This hypothesis is supported by previous reports of an activation of the AT₁ gene in the rat aorta under hypoxic condition (16) and the presence in the AT₁ gene promotor of an AP1 response element (12) that would respond to hypoxia (40). Regarding AT₂, our finding of ventricular changes during the late stage of hypoxia, but not in the MCT-treated rat, suggests that AT₂ expression regulation might be associated with hypoxia- rather than overload-induced responses. The increase in AT₂ receptors in both ventricles together with no change (LV) or a decrease (RV) in AT₂ mRNA level suggests that increased binding capacity is mainly due to posttranscriptional mechanisms. Several lines of evidence support this hypothesis. First, chronic hypoxia has been reported to increase the ANG II circulating levels (42). Second, ANG II has been reported in vitro to increase the levels of AT₂ receptors through translational and not through transcriptional mechanisms (17). At least it is noteworthy that chronic hypoxia increases the RV but not LV content, and activity of protein kinase C (PKC) (35) whose activation decreased AT₂ receptor mRNA stability and AT₂ receptor gene transcription as well in vitro (15). Thus it is conceivable that increased levels of circulating ANG II might participate in accu-

Fig. 2. AT₁ and AT₂ binding levels in the right (RV; A) and left ventricle (LV; B) from rats studied 30 days after saline (S) or MCT injection. Values are means ± SE of n = 4 animals.

Fig. 3. Time course of AT₁ receptor (A) and AT₂ receptor binding (B) in the RV (●) and LV (▲) from rats exposed to hypoxia. Values are means ± SE of n = 4–6 animals. *P < 0.05 vs. normoxic group.
mulating AT$_2$ receptors in both ventricles, meanwhile PKC activation in RV causes a decrease in AT$_2$ receptor mRNA levels.

The presence and increase of AT$_1$ and AT$_2$ receptors in hypoxic hearts raise questions regarding the role of both subtypes. There is ample evidence now that AT$_1$ mediates the vasoconstrictor and growth-promoting effects of ANG II, whereas AT$_2$ was suggested to participate in vasodilator effects and to exert antigrowth action in many cardiovascular cell types (6). Although the cellular localization of AT$_1$ and AT$_2$ receptors was not examined, our finding of their homogeneous distribution throughout the two hypoxic ventricles, but unchanged expression in the hypertrophied RV from MCT-treated rats, suggests that AT$_1$ and AT$_2$ do not mainly contribute to the growth-adaptive response of the RV to hypoxia. This concept is in apparent discrepancy with the demonstration of other groups that overexpression of AT$_1$ receptor in cardiomyocytes promotes LV hypertrophy in basal condition (29) as well in the face of pressure and/or volume overload (11). Interestingly, cardiomyocyte hypertrophy was recently shown to be proportional to AT$_1$ receptor density (38). Our finding of RV enlargement in the absence of sustained expression of AT$_1$ receptor indicates that RV hypertrophy may be a consequence of pressure overload-associated mechanical factors rather than an ANG II local action. Thus the accumulation of AT$_1$ receptors within the first 2 days may counteract the acute vasodilatation of the heart in the face of decreased P$_O2$ levels in arterial blood at this early stage of hypoxia. Conversely, those of AT$_2$ receptors may rather coincide with completion of slow processes ensuring durable changes in heart function. Chronic hypoxia induces inducible nitric oxide synthase expression and activity in the cardiomyocytes of both ventricles (35), as well as a nitric oxide (NO)-dependent coronary vasodilatation (39). Because the vasodilator effect of AT$_2$ receptor has been reported to be mediated mainly through NO production in vivo (18, 37), the increase in AT$_2$ receptor density during the late stage of hypoxia may contribute to NO activity pathways in the heart, leading to both deleterious (such as contractile dysfunction of cardiomyocytes) and beneficial (such cardiac vasodilatation) effects in the heart.

Fig. 4. A: typical pictures of ethidium bromide-stained gels obtained from the analysis by the AT$_1$- and AT$_2$-PCR assays of various amounts of RNA extracted from the either RV or LV of rats exposed to room air (N) and to hypoxia for 2 days (H-2d) or 14 days (H-14d). B: representative graphs showing the AT$_1$ and AT$_2$ amplification curves obtained from the analysis of normoxic and 2- or 14-day hypoxic RV RNA samples. AU, arbitrary units.

Fig. 5. Time course expression of AT$_1$ mRNA (A) and AT$_2$ mRNA (B) in the RV (●) and LV (▲) from rats exposed to hypoxia. Values are means ± SE of n = 4–6 animals. *P < 0.05 vs. normoxic group.
In conclusion, this is, to our knowledge, the first study designed to investigate the cardiac expression of ANG II receptor subtypes during normobaric chronic hypoxia. Our finding of a differential increase in AT₁ and AT₂ receptors in both ventricles may provide a new basis for understanding the mechanisms by which ANG II plays a role in processes ensuring adaptation of heart to hypoxia.

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