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Endocrinology 2009 150:4270-4277 originally published online May 21, 2009; , doi: 10.1210/en.2008-1493

Chronic Intermittent Hypoxia Induces 11β-Hydroxysteroid Dehydrogenase in Rat Heart

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Corticosteroids are known to not only regulate electrolyte homeostasis but also play a role in the cardiovascular system, including myocardial remodeling. Because transgenic mice that overexpress 11β-hydroxysteroid dehydrogenase (11HSD) type 2 in cardiomyocytes have been shown to spontaneously develop cardiac hypertrophy and fibrosis, we investigated whether changes in the cardiac metabolism of glucocorticoids accompany remodeling of the heart under physiological conditions. In the present study, glucocorticoid metabolism and 11HSD2 were explored in the hearts of rats exposed to chronic intermittent hypobaric hypoxia (CIH), which induces hypertrophy and fibrosis of the right and less of the left ventricle. We first demonstrated that adaptation to CIH led to a significant increase in 11HSD2 transcript levels and activity in the myocardium. In contrast, neither 11HSD1 activity and mRNA level nor the abundance of mineralocorticoid and glucocorticoid receptor mRNA were up-regulated. The adaptation to CIH also led to an increase of 11HSD2 mRNA in isolated cardiomyocytes, whereas 11HSD1, glucocorticoid receptor, and mineralocorticoid receptor mRNA levels were not changed in comparison with the cardiomyocytes of control normoxic rats. The changes in cardiac metabolism of glucocorticoids were accompanied by inflammatory responses. The expression levels of the proinflammatory markers cyclooxygenase-2 and osteopontin were significantly increased in both the myocardium and the cardiomyocytes isolated from rats exposed to CIH. These findings suggest that myocardial remodeling induced by CIH is associated with the up-regulation of cardiac 11HSD2. Consequently, local metabolism of glucocorticoids could indeed play a role in cardiac hypertrophy and fibrosis. **(*Endocrinology* 150: 4270–4277, 2009)**

The administration of exogenous mineralocorticoids together with increased dietary sodium intake promotes cardiac hypertrophy, fibrosis, and inflammation in experimental animal models (1–3). The effect of mineralocorticoid/salt excess appears to be direct and humoral rather than being an indirect process that is dependent on hemodynamics. It has been found that mineralocorticoid antagonists are able to block cardiac pathophysiological changes at doses that do not decrease blood pressure (4–6). Cardiomyocytes, fibroblasts, vascular smooth muscle, and endothelial cells all express mineralocorticoid receptor (MR) (7–9), even though not all studies confirm the expression of MR in cardiac fibroblasts (7).

It is well known that MR shows high affinity for not only aldosterone but also the glucocorticoids corticosterone and cortisol (10, 11). In epithelial cells, coexpression of MR and 11β-hydroxysteroid dehydrogenase type 2 (11HSD2) is essential in conferring aldosterone selectivity on the MR. 11HSD2 is an enzyme that converts corticosterone and cortisol to their biologically inactive 11-oxo derivatives that have low affinity for MR (11). In contrast with the epithelia, 11HSD2 mRNA and activity are very

ISSN Print 0013-7227 ISSN Online 1945-7170
Printed in U.S.A.

doi: 10.1210/en.2008-1493 Received October 22, 2008. Accepted May 11, 2009.
First Published Online May 21, 2009
* P.K. and L.R. contributed equally to the study.

Abbreviations: CIH, Chronic intermittent hypoxia; COX, cyclooxygenase; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; GR, glucocorticoid receptor; 11HSD1, 11β-hydroxysteroid dehydrogenase type 1; 11HSD2, 11β-hydroxysteroid dehydrogenase type 2; LV, left ventricle; MR, mineralocorticoid receptor; NADPH, nicotinamide adenine dinucleotide phosphate, reduced form; OPN, osteopontin; P_B, barometric pressure; RV, right ventricle; SHR, spontaneously hypertensive rats.

low in the heart, whereas the enzyme 11β-hydroxysteroid dehydrogenase type 1 (11HSD1) that converts 11-oxo derivatives back to active glucocorticoids is expressed at much higher levels (7, 12–14). This means that cardiac MR are primarily occupied by glucocorticoids and that the steroids bound to cardiac MR probably operate in tonic inhibitory mode (15). Because 11HSD1 and 11HSD2 are the major determinants of the intracellular concentration of glucocorticoids, the activities of these two enzymes control the access of glucocorticoids to MR and thus are able to affect aldosterone binding to MR and its activation. In this context, changes in 11HSD2 expression may contribute to the mineralocorticoid-induced myocardial remodeling and pathogenesis of heart failure. Interestingly, transgenic mice that overexpress 11HSD2 in cardiomyocytes develop cardiac hypertrophy and fibrosis that can be partially antagonized by the aldosterone antagonist eplerenone (16). Similar pathological changes can be observed in the hearts of transgenic mice that overexpress MR (17).

These findings raise the question of whether cardiac fibrosis and hypertrophy are associated with the up-regulation of 11HSD2 under physiological and/or pathophysiological conditions. A previous study of low-aldosterone hypertensive rats demonstrated the beneficial effects of the MR antagonist eplerenone on the development of cardiac hypertrophy and failure but did not examine whether changes of 11HSD2 are involved (18). To address this question, we used a rat model of chronic intermittent hypoxia (CIH) using a hypobaric chamber; adaptation to this environment leads to pulmonary hypertension and subsequently to right ventricular (RV) hypertrophy. This response is usually lacking or less expressed in the left ventricle (LV), depending on the severity of hypoxia. However, increased accumulation of fibrillar collagen occurs in both ventricles (19, 20). To examine the relationship between cardiac glucocorticoid metabolism and myocardial remodeling induced by CIH, we analyzed the cardiac expression of 11HSD1, 11HSD2, MR, and glucocorticoid receptor (GR), and 11β-oxidase and 11-reductase activity in ventricular myocardium and isolated cardiomyocytes. We found a significant increase in 11HSD2 expression and activity associated with hypertrophy and inflammation.

Materials and Methods

Animal model

Adult male Wistar rats (230–260 g body weight) were exposed to chronic intermittent hypobaric hypoxia (CIH) corresponding to the altitude of 7000 m for 8 h/d, 5 d/wk. Barometric pressure (P_B) was lowered stepwise so that the level equivalent to the altitude of 7000 m (P_B = 41 kPa; PO_2 = 8.6 kPa) was reached after 13 exposures. The total number of exposures was 24 (21). The control group of animals was kept for the same period of time at P_B and PO_2 equivalent to an altitude of 200 m (P_B = 99 kPa; PO_2 = 20.7 kPa). All rats had free access to water and a standard laboratory diet. The study was conducted in accordance with the Guide for the Care and Use of Laboratory Animals published by the National Institutes of Health (Publication No. 85-23, revised 1996). Animals were studied on the day after the last hypoxic exposure. Rats were decapitated and their hearts rapidly excised, washed in ice-cold saline, and dissected into RV and LV free ventricular walls and septum. All tissues were weighed and ventricles were frozen in liquid nitrogen and stored at −80 C until use.

Isolation of ventricular cardiomyocytes

Hearts from separate groups of heparinized normoxic and chronically hypoxic rats were perfused through the aorta, for 3–5 min with Ca^{2+}-supplemented Tyrode solution (millimoles per liter): 140 NaCl, 5.4 KCl, 10 HEPES, 1 $MgCl_2$, 1 $CaCl_2$, and 10 glucose (pH 7.4) at 37 C (22) and then with Ca^{2+}-free Tyrode solution for 5 min before digestion with the same solution containing collagenase (20 mg, 700 U/mg; Yakult, Tokyo, Japan) and protease (type XIV, 0.2 mg/ml) for 9–11 min. Collagenase was subsequently removed by perfusion with Ca^{2+}-free tyrode solution (pH 7.4) for 5 min. All solutions were gassed with 100% O_2 for 5 min before use. Atria and blood vessels were removed and the free RV and LV walls dissected. Cells were dissociated by gentle mechanical shaking in 3 ml of tyrode solution and filtered through a nylon mesh to remove nondissociated tissue. Filtered cells were kept at room temperature for 8 min to obtain a purer fraction of myocytes at the bottom. The upper part of the preparation, containing mostly smaller cell types and dead myocytes, was removed. Myocytes were resuspended in 1.5 ml of Tyrode solution, rapidly frozen in liquid nitrogen and stored at −80 C until use. The purity of isolated cardiomyocytes was evaluated by several methods and was not significantly different between normoxic and hypoxic group (for details, see the supplemental data, published as supplemental data on The Endocrine Society's Journals Online web site at http://endo.endojournals.org).

Plasma analysis

Blood samples were collected between 1000 and 1200 h from the carotid artery of rats anesthetized with 2% isoflurane and used for isolation of cardiomyocytes. Blood was centrifuged, plasma samples stored at −20 C until measurement, and the concentrations of corticosterone and aldosterone were measured with RIA kits (MP Biomedicals, Irvine, CA; Beckman Coulter, Immunotech, Prague, Czech Republic).

Reverse transcription and quantitative real-time PCR

Total RNA from the ventricular samples was extracted by the guanidinium thiocyanate method and isolated RNA treated with deoxyribonuclease (Sigma, St. Louis, MO) to remove potential contamination by genomic DNA (23). First-strand cDNA was synthesized from 5 μg of RNA with Moloney murine leukemia virus reverse transcriptase reagents (Invitrogen GmbH, Lofer, Austria) and priming with oligo(dT) (Roche, Mannheim, Germany). Total RNA from the isolated cardiomyocytes was obtained with a GeneElute mammalian total RNA miniprep kit (Sigma). First-strand cDNA was synthesized as above.

The genes analyzed in this study were 11HSD1, 11HSD2, MR, GR, cyclooxygenase (COX)-2, and osteopontin (OPN).

Levels of 11HSD2, MR, GR, COX-2, and OPN mRNA were measured with the ABI PRISM 7000 sequence detection system (Applied Biosystems, Foster City, CA). The probes and primers used for these experiments were developed as TaqMan gene expression assays by Applied Biosystems, specifically: 11HSD2 (Rn00492539-m1), MR (NR3C2) (Rn00565562-m1), GR (NR3C1) (Rn00561369-m1), COX-2 (Ptgs2) (Rn00568225-m1), and OPN (Spp1) (Rn01449972-m1). The reaction was carried out in a final volume of 20 μl using TaqMan universal PCR master mix with AmpEraseUNG (Applied Biosystems) and expression assay. For 11HSD1, amplification of the target cDNA was performed in the LightCycler (Roche) with LightCycler Fast Start DNA Master SYBR green I, as previously reported (14). For a panel of candidate reference genes, amplification of the target cDNA was performed in the ABI PRISM 7000 sequence detection system (Applied Biosystems) using DyNAmo HS SYBR green quantitative PCR kit (Finnzymes, Espoo, Finland). The primers for glyceraldehyde-3-phosphate dehydrogenase (GAPDH), ubiquitin C, cytochrome c-1, ATP synthase β-polypeptide, β-actin, and malate dehydrogenase 1 were delivered by PrimerDesign, London, UK (normalizing gene detection kit).

Gene-specific calibration curves were generated from serial dilutions of standard cDNA. For a stability comparison of candidate reference genes throughout the adaptation to hypoxia, we used the NormFinder software that calculates the stability values of individual genes (24). mRNA levels of all the genes of interest were normalized to the most stable reference gene.

Measurement of 11HSD activity

The cardiac tissue was homogenized in an ice-cold buffer containing 200 mM sucrose and 10 mM Tris/HCl (pH 8.5) (1:9 wt/vol) in a Polytron homogenizer (Kinematica AG, Littau, Switzerland). Homogenates were centrifuged at 400 × *g* for 10 min at 4 C to remove cell debris. Supernatants were then centrifuged at 800 × *g* for 10 min and again at 9000 × *g* for 10 min to remove the nuclear and mitochondrial fractions. Finally, the resultant supernatant was centrifuged at 100,000 × *g* for 60 min to obtain the microsomal fraction, and the pellet was resuspended and sonicated. Protein concentrations were determined by the Bradford method and the homogenates were used immediately for the measurement of 11HSD activity.

11HSD1 and -2 activities were measured by radiometric conversion assays as reported previously (25). Briefly, the conversion of [^{3}H]corticosterone (final concentration 14.5 nmol/liter) to [^{3}H]11-dehydrocorticosterone was measured in incubation buffer [100 nmol/liter KCl, 50 nmol/liter Tris/HCl (pH 8.5)] containing 400 μmol/liter of nicotinamide adenine dinucleotide (NAD^+) and 600 μg membrane protein. In some experiments the incubation buffer also contained Thiram (20 μmol/liter), a specific inhibitor of 11HSD2 (26). 11HSD1 was measured as the conversion of [^{3}H]11-dehydrocorticosterone to [^{3}H]corticosterone in the incubation buffer [100 mmol/liter KCl, 50 mmol/liter Tris/HCl (pH 7.5)] containing reduced nicotinamide adenine dinucleotide phosphate (NADPH)-regenerating system (1.3 mmol/liter glucose-6-phosphate, 2 U glucose-6-phosphate dehydrogenase), NADPH (400 μmol/liter), and 600 μg membrane protein. The reaction was stopped by cooling, the samples extracted with a Strata-X C_{18} cartridge, and the steroids quantified by HPLC.

Statistical analysis

Results are presented as means ± SEM and analyzed by an unpaired Student's *t* test. In all cases, a probability level of $P < 0.05$ was considered significant.

Results

In line with our previous studies, adaptation of rats to CIH resulted in hypertrophy accompanied by a significant retardation of body growth compared with age-matched normoxic animals. The RV weight normalized to body weight increased to 180% and that of the LV and septum to 136 and 127% of the respective normoxic values. Plasma corticosterone levels were significantly increased in all hypoxic rats, whereas plasma aldosterone levels were not changed (Table 1). To determine whether the heart adaptive changes are associated with changes in glucocorticoid metabolism and signaling, we measured the expression of 11HSD1, 11HSD2, GR, and MR mRNA, and 11HSD1 and 11HSD2 enzyme activities, in the myocardium.

The prerequisite for normalization and comparative quantification of the amount of RNA is the choice of an appropriate internal standard gene or housekeeping gene. Consequently, we focused on the expression of six candidate genes during CIH. The NormFinder program calculated the stability value in the ranking order for GAPDH (stability value 0.114), ubiquitin C (0.144), malate dehydrogenase (0.144), cytochrome c-1 (0.187), ATP synthase β-polypeptide (0.199), and β-actin (0.516). Because high expression stability is indicated by a low stability value (24), GAPDH was used for normalization. Compared with control normoxic rats, the hypertrophied RV of hypoxic rats increased 11HSD2 mRNA 8-fold, whereas expression of 11HSD1 did not change significantly (Fig. 1).

TABLE 1. Body weight, heart weight parameters, and plasma corticosterone and aldosterone levels

Parameter	Normoxia	Hypoxia
n	8	7
BW (g)	384 ± 6	306 ± 8[a]
HW (mg)	819 ± 18	940 ± 47
LVW (mg)	452 ± 16	490 ± 28
RVW (mg)	184 ± 5	264 ± 14[a]
SW (mg)	183 ± 5	185 ± 12
LVW/BW (mg/g)	1.18 ± 0.04	1.60 ± 0.08[a]
RVW/BW (mg/g)	0.48 ± 0.02	0.86 ± 0,04[a]
SW/BW	0.48 ± 0.02	0.61 ± 0.03[a]
Cort. (ng/ml)	239 ± 17	358 ± 33[a]
Aldo. (pg/ml)	85 ± 12	75 ± 8

Values are means ± SEM. BW, Body weight; HW, heart weight; LVW, LV weight; RVW, RV weight; SW, septum weight; LVW/BW, relative LV weight; RVW/BW, relative RV weight; SW/BW, relative septum weight; Cort., plasma level of corticosterone; Aldo., plasma level of aldosterone.

[a] $P < 0.05$ *vs.* normoxic group.

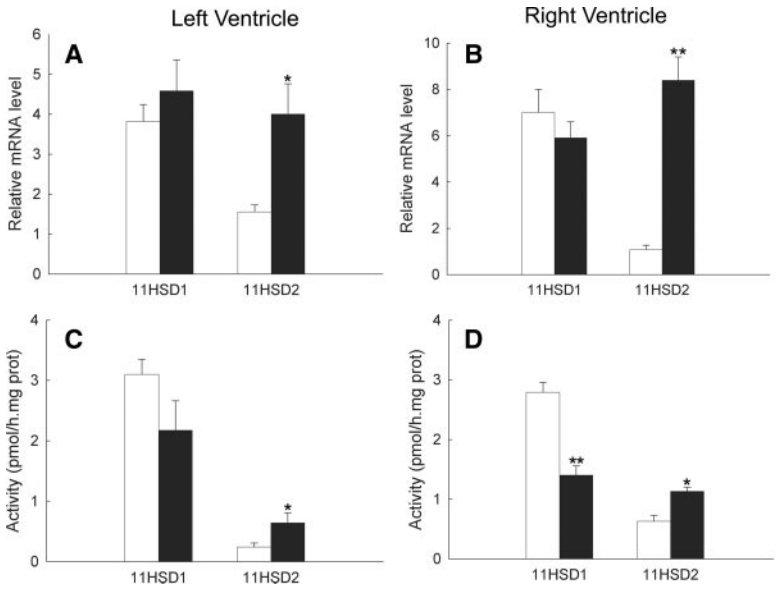

FIG. 1. Effect of chronic hypoxia on 11HSD1 and 11HSD2 in the LV and RV myocardium. A and B, Expression of 11HSD1 and 11HSD2 mRNA in LV and RV of normoxic (*open bars*) and chronically hypoxic rats (*filled bars*) normalized to GAPDH (six to eight animals in each group). C and D, 11HSD1 and 11HSD2 activities in LV and RV of normoxic (*open bars*) and chronically hypoxic rats (*filled bars*). The enzyme activities were expressed as the amount of the metabolite, 11-dehydrocorticosterone, or corticosterone, respectively, per hour and milligram of protein (prot; six to seven animals in each group). Data are expressed as mean ± SEM. *, $P < 0.05$; **, $P < 0.01$.

In LV, changes in 11HSD2 were much less pronounced. 11HSD1 mRNA expression relative to total RNA in RV and LV of normoxic rats was 130- and 180-fold lower than the expression in liver. In comparison with kidney, 11HSD2 mRNA levels were 1000-fold lower in LV and 310-fold lower in RV than in rat kidney. However, during exposure to CIH, the heart 11HSD2 mRNA level relative to kidney was decreased in LV from 1000- to 620-fold and in RV from 310- to 90-fold, respectively.

To determine whether remodeling of the heart also results in a shift of local glucocorticoid metabolism, we assessed the 11β-oxidase activity of 11HSD2 and the 11-reductase activity of 11HSD1. As illustrated in Fig. 1, 11HSD2 activity increased in both ventricles, whereas 11HSD1 activity was without any significant changes in LV and decreased in RV. The activity of 11HSD2 was completely abolished by Thiram, whereas 11HSD1 activity was not affected. At present we do not have any explanation for the discrepancy between 11HSD1 mRNA and 11HSD1 activity in RV of normoxic and hypoxic rats. Notwithstanding this discrepancy, it appears that myocardial remodeling is associated with decreased tissue availability of corticosterone.

In contrast with 11HSD1 and -2, corticosteroid receptor density was not altered in myocardium of hypoxic rats. Both GR and MR were expressed in the myocardium of normoxic and chronically hypoxic rats, but expression was not changed during hypoxia-induced heart remodeling (Fig. 2). Because cardiac hypertrophy and fibrosis induced in uninephrectomized rats by mineralocorticoid/salt excess is preceded by inflammation (3, 27, 28), we also examined the expression of proinflammatory molecules COX-2 and OPN in normoxic and hypoxic rats. Figure 2 shows that the expression of both OPN and COX-2 was significantly increased above the very low levels observed in the normoxic group.

Previous studies have suggested that 11HSDs are not expressed homogenously in heart cells such as cardiomyocytes, fibroblasts, and endothelial cells. To eliminate the possibility that the observed effects might be restricted to fibroblast and/or coronary vessels, we used isolated cardiomyocytes. Figure 3 shows that cardiomyocytes of control normoxic rats express 11HSD1 mRNA, but 11HSD2 mRNA is nearly absent. Compared with these animals, the cardiomyocytes of rats adapted to CIH have markedly increased 11HSD2 expression with no significant changes in 11HSD1 expression and this 11HSD2 up-regulation in RV was double that in LV. Although 11HSD2 expression was increased, no significant changes in expression of GR and MR were observed in isolated cardiomyocytes (Fig. 2). Consistent with the myocardium, OPN and COX-2 expression was also increased in the cardiomyocytes of rats exposed to CIH (Fig. 2). Taken together, our data indicate that remodeling of the heart induced by CIH is associated with inflammation and modulation of glucocorticoid metabolism without changes in corticosteroid receptor density.

Discussion

In this study we investigated cardiac metabolism of glucocorticoids during adaptation to CIH that results in RV hypertrophy associated with abnormal accumulation of interstitial collagen, in particular collagen type I, as shown previously. Moreover, in this model fibrosis is also present in the LV myocardium of chronically hypoxic rats (19, 20).

The study yielded three major findings. The first is that hypertrophy and fibrosis induced by CIH is associated with up-regulation of 11HSD2 in the heart. Although cardiac hypertrophy due to chronic hypoxia is an adaptive response to increased pressure load, hypertension is not always associated with an up-regulation of 11HSD2. We and others have shown recently that cardiac hypertrophy in spontaneously hypertensive rats (SHR), uremic rats, and low-aldosterone hypertensive Dahl-salt sensitive rats

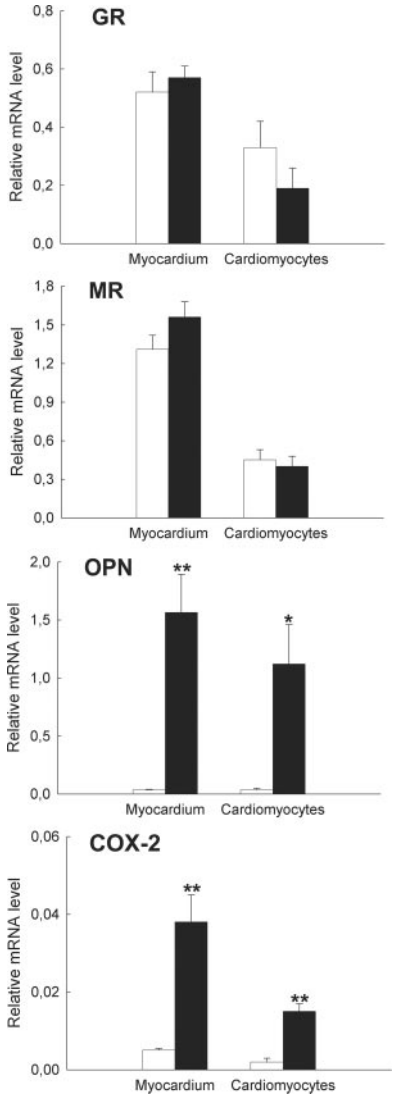

FIG. 2. Expression of GR and MR and proinflammatory molecules OPN and COX-2 in the RV myocardium and RV cardiomyocytes of normoxic (*open bars*) and chronically hypoxic rats (*filled bars*). Results were normalized to GAPDH and are expressed as mean ± SEM (seven or eight animals in each group). *, $P < 0.05$; **, $P < 0.01$.

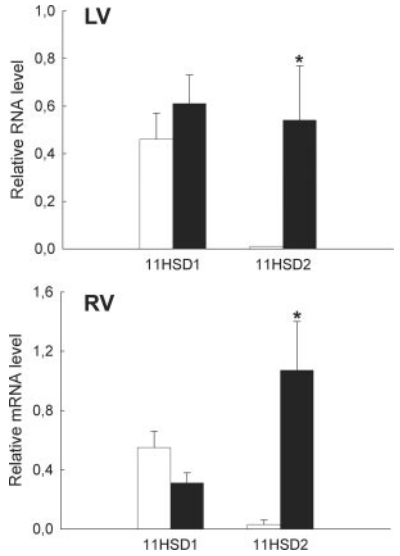

FIG. 3. Expression of 11HSD1 and 11HSD2 mRNA in the LV and RV cardiomyocytes of normoxic (*open bars*) and chronically hypoxic rats (*filled bars*). Results were normalized to GAPDH and are expressed as mean ± SEM (eight animals in each group). *, $P < 0.05$.

fed a high-salt diet is not accompanied with changes of 11HSD2 expression (14, 18, 29, 30). Up-regulation of 11HSD2 mRNA together with collagen type I mRNA was observed only in malignant stroke-prone SHR (29). The mechanism responsible for the induction of 11HSD2 in CIH that is absent in many animal models of heart hypertrophy and fibrosis remains unknown. Given that plasma corticosterone level is increased in CIH, it is possible that 11HSD2 is stimulated by this hormone. However, no effect of glucocorticoid hormones was evidenced on rat 11HSD2 (31, 32). Taken together, the data argue for physiological differences in the adaptation changes leading to heart hypertrophy and fibrosis in various animal models or for methodological differences in the experiments. 11HSD2 was studied in whole heart or LV that is less sensitive to stimulation of 11HSD2 than RV (Fig. 1) and different techniques were used for quantification of 11HSD2 mRNA.

The second major finding is the demonstration that up-regulation of 11HSD2 in cardiomyocytes is not limited to the artificial transgenic model (16) but is also active during physiological adaptation-heart remodeling induced by chronic intermittent hypoxia. However, we must acknowledge one limitation of this study. Our investigation was primarily designed to examine metabolism of glucocorticoids in the hypertrophied and fibrotic heart rather than expression of 11HSD2 in cardiomyocytes. It is therefore beyond the scope of the current study to determine whether the observed up-regulation of 11HSD2 is associated exclusively with cardiomyocytes. Nevertheless, the presence of 11HSD2 mRNA in the cardiomyocytes of hypoxic rats, and its absence in the suspension of cardiomyocytes isolated from normoxic hearts, suggests that cardiomyocytes may contribute to the up-regulation of cardiac 11HSD2 during adaptation to hypoxia. This up-regulation seems to be limited to the heart and other non-mineralocorticoid target tissues. This is because chronic hypoxia down-regulates 11HSD2 in mineralocorticoid target tissues, in which 11HSD2 protects MR against activation by glucocorticoids (33).

Comparison of 11HSD2 mRNA level in heart ventricles and isolated cardiomyocytes of hypoxic rats shows that 11HSD2 mRNA level relative to GAPDH mRNA is 7- and 8-fold lower in cardiomyocytes than in LV and RV myocardium, respectively. This discrepancy seems to reflect the problem of normalization across different tissues and cell types. A 15-fold difference in GAPDH mRNA copy numbers was observed between the highest and low-

est expressing tissue types in humans (34), and a similar difference was observed also in rat tissues (35). These variations in expression of GAPDH reflect the role of GAPDH, a key enzyme of glycolysis; in the cells, the highest GAPDH mRNA expression, protein, and activity was found in tissues with high energy demands such as muscle cells (35). The recent demonstration that cellular makeup of the rat heart contains about 70% noncardiomyocyte cell population and only 30% cardiomyocytes (36) argues for the lower expression level of 11HSD2 mRNA in cardiomyocytes than myocardium containing the majority of cells with lower energy demands. A second possible source of disparity between 11HSD2 mRNA expression in ventricle and cardiomyocytes might lie in cardiac vessels that express both types of 11HSD and in which inflammation might modulate glucocorticoid metabolism (37).

Studies of transgenic mice with cardiomyocyte overexpression of 11HSD2 suggest that the induction of 11β-oxidase activity in cardiomyocytes is one mechanism by which aldosterone and/or the changes in the ratio of nicotinamide adenine dinucleotide (NAD^+)/NADPH could exert their pathophysiological effects (16). A similar explanation may be valid in our model, but it cannot distinguish whether corticosterone or aldosterone contributes to myocardial remodeling. The simple effect of increased levels of corticosterone activating MR does not seem to be important. There is increasing evidence that normal levels of endogenous glucocorticoids are able to activate MR due to tissue injury/altered intracellular redox state in some rat models of cardiac hypertrophy and fibrosis (38). Overexpression of 11HSD2 reduces not only the local concentration of corticosterone but also occupancy of GR (11). Thus, the decreased corticosterone occupancy of GR, rather than aldosterone occupancy of MR, may play a role. However, evidence suggests that cardiac hypertrophy and fibrosis are caused by MR but not GR action and that the severity of these changes can be reduced by MR blockade (1, 2, 39, 40). On the other hand, activation of either MR or GR in cardiomyocytes *in vitro* had only a small effect on cell hypertrophy, even when MR and GR expression was increased (41). Because the cellular response to corticosteroids depends on the number of receptors, we tested whether CIH modulates their expression. Our results from both the myocardium and isolated cardiomyocyte experiments endorse the idea that changes in corticosteroid receptors are not involved in this model of cardiac hypertrophy and remodeling.

Our third major finding showed that adaptation to CIH is accompanied by not only hypertrophic/fibrotic (19, 20) but also a proinflammatory phenotype. This response is reminiscent of that seen in mineralocorticoid/salt-treated rats. There the vascular inflammatory effects associated with cardiac hypertrophy could be mimicked by the administration of carbenoxolone, an 11HSD inhibitor, and reversed with eplerenone, indicating that the endogenous glucocorticoid effects are mediated through MR activation (29, 30). Normally cardiomyocytes do not express 11HSD2 but only 11HSD1, which increases the local concentration of corticosterone. This hormone has higher affinity for MR than GR (11), and thus, MR must be occupied but not activated by corticosterone (15). However, the expression of 11HSD2 during adaptation to CIH decreases the local concentration of corticosterone and the tonic inhibition of MR operating in nonmineralocorticoid target tissues that express MR (15, 39). We cannot determine from this study whether the activation of MR with aldosterone or the changes in the redox potential due to tissue damage are responsible for the observed up-regulation of OPN and COX-2. There are several *in vitro* studies that suggest connection between activation of MR and expression of COX-2 and OPN. In rat cardiomyocytes, aldosterone is able to induce COX-2 expression, and this effect can be inhibited by actinomycin D or spironolactone (42). Similarly, the transcriptional regulation of OPN expression by aldosterone and its inhibition by spironolactone was found in renal fibroblasts and mesangial cells (43, 44). In addition, intermittent hypoxic stress not only results in cardiac hypertrophy and interstitial fibrosis but also stimulates the myocardial superoxide production via NADPH oxidase (45), an enzyme that is activated by aldosterone in certain cells (46).

In summary, we found that adaptation to CIH leading to myocardial hypertrophy and remodeling is associated with an up-regulation of 11HSD2 expression and is accompanied with inflammation. It is speculated that changes in glucocorticoid metabolism in heart may contribute to adaptive myocardial remodeling.

Acknowledgments

We thank Dr. J. Bryndová for her help in pilot experiments with cardiomyocytes and I. Muricová for technical assistance.

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This work was supported by Grant AVOZ 50110509 from the Academy of Sciences and Czech Science Foundation Grants 305/07/0328 (to J.P.) and 305/07/0875 (to F.K.).

Disclosure Summary: P.K., L.Ř., G.B., K.V., J.N., P.E., I.M., F.K. and J.P. have nothing to declare.

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