

## 11 $\beta$ -Hydroxysteroid dehydrogenase in the heart of normotensive and hypertensive rats<sup>☆</sup>

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### Abstract

Corticosteroids have been shown to play a role in cardiac remodeling, with the possibility of a direct effect of overexpression of 11 $\beta$ -hydroxysteroid dehydrogenase (11HSD) isoform 2 at the level of the cardiomyocytes. The aim of this study was to examine cardiac steroid metabolism in hypertensive rats with hearts that are hypertrophied and fibrotic and have structural alterations in the coronary circulation. To assess possible alterations of cardiac steroid metabolism the expression and activity of both isoforms of 11 $\beta$ -hydroxysteroid dehydrogenase (11HSD) were studied in spontaneously hypertensive rats (SHR), their normotensive controls Wistar–Kyoto (WKY), and in Dahl salt-sensitive (DS) and salt-resistant rats (DR) kept on a low- or high-salt diet. Using real-time quantitative RT-PCR and enzyme activity assay we found strain-dependent differences in cardiac metabolism of glucocorticoids. In Dahl rats expression of 11HSD1 and 11HSD2 mRNA was lower in DS than in DR rats and was not influenced by dietary salt intake; 11HSD1 mRNA was expressed at higher level than 11HSD2 mRNA. NADP<sup>+</sup>-dependent cardiac 11HSD activity showed similar distribution as 11HSD1 mRNA—lower activity in DS than in DR rats and no effect of salt intake. In SHR and WKY strains 11HSD2 mRNA expression was significantly higher in WKY than in SHR but no differences were observed in 11HSD1 mRNA abundance and NADP<sup>+</sup>-dependent 11HSD activity. These results show that the heart is able to metabolize glucocorticoids and that this metabolism is strain-dependent but do not support the notion of association between cardiac hypertrophy and changes of 11HSD1 and 11HSD2 expression.

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### 1. Introduction

Glucocorticoid and mineralocorticoid hormones act on various tissues including the cardiovascular system. Previous studies have established that mammalian heart possesses both glucocorticoid (GR) and mineralocorticoid receptors (MR) and may not only produce endogenous corticosteroids such as aldosterone and corticosterone but also metabolize glucocorticoids via 11 $\beta$ -hydroxysteroid dehydrogenase (11HSD) [1–4]. The studies of Weber and others have shown that aldosterone has an important role in inducing cardiac hypertrophy and fibrosis [1,2,5]. Several lines of evidence suggest that this

is a direct effect via MR rather than an indirect effect due to hemodynamic changes [5–7].

The direct effect of aldosterone on MR requires the co-expression of MR and 11HSD isoform 2 (11HSD2), which prevents activation of MR by glucocorticoids. 11HSD2 is a NAD<sup>+</sup>-dependent oxidase that converts the endogenous glucocorticoids, corticosterone and cortisol, to inactive 11-oxo metabolites, 11-dehydrocorticosterone and cortisone [8]. Recent studies have reported the presence of 11HSD2 in cardiac tissue [9–11]. In addition to 11HSD2, the heart also expresses substantial levels of isoform 1 (11HSD1) that appears to act predominantly as a NADPH-dependent reductase in vivo, but as both a dehydrogenase and reductase in broken cell system [12,13], and is localized in interstitial cardiac fibroblasts [14].

Overexpression of 11HSD2 in cardiomyocytes of transgenic mice is associated with cardiac hypertrophy and fibrosis [15] so that the ability of the heart to modulate local concentrations of glucocorticoids via 11HSDs might thus play

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a role in cardiac remodeling. Although such remodeling is associated with various forms of experimental hypertension, the capacity of the heart to metabolize glucocorticoids and the relationship between experimental cardiac hypertrophy and 11HSDs have not been addressed in hypertensive rats in detail. We have, therefore, determined the expression of 11HSD1 and 11HSD2, and their activity, in spontaneously hypertensive (SHR) and Dahl salt-sensitive rats (DS), and in their normotensive counterparts Wistar–Kyoto (WKY) and Dahl salt-resistant rats (DR).

## 2. Materials and methods

### 2.1. Animals

Male SHR and WKY rats and inbred Dahl rats of both phenotypes (DS, DR) were obtained from the breeding colony of the Institute of Physiology, Czech Academy of Sciences, Prague. SHR and WKY rats were maintained on standard chow, whereas Dahl rats were fed a low-salt diet (0.2% NaCl) until the age of 6 weeks. These rats were then allocated into two groups. Two groups were fed the low-salt diet (0.2% NaCl; DS–LS, DR–LS) and two groups the high-salt diet (8% NaCl; DS–HS, DR–HS) for next 7 weeks. The animals were then killed, the hearts excised immediately, weighed and the tissue used for RNA analysis and 11HSD activity measurement. Blood pressure was measured by direct puncture of the carotid artery under light ether anaesthesia just before sacrifice. In weighing the heart the ventricles were considered as the total cardiac mass. The wet weight was normalized to the individual rat's body weight and expressed as the heart mass index (mg/g). The study was approved by the Animal Ethics Committee of the Institute of Physiology.

### 2.2. RNA isolation and analysis

Total RNA was extracted from the heart by the guanidium thiocyanate method. To remove DNA, the isolated RNA was treated with DNAase (Promega, Madison, WI) and the contamination of RNA samples by DNA was detected by PCR followed by electrophoresis on a 2% agarose gel as described previously [16].

cDNA was synthesized from about 5 µg of RNA and M-MLV Reverse Transcriptase reagents (Invitrogen GmbH, Lofer, Austria) in a total volume of 20 µl containing oligo (dT) primer, 0.5 µg; reaction buffer (1×); DTT, 0.01 M; dNTP mix, 0.5 mM; RNaseOUT (recombinant ribonuclease inhibitor), 40 units and M-MLV reverse transcriptase, 200 units. RNA was first heated with oligo (dT) to 70 °C for 10 min and quickly chilled on ice. After cooling, the remaining chemicals were added, the incubation continued 60 min at 37 °C and then was stopped by heating to 95 °C for 5 min.

Expression of three different genes was quantified with a LightCycler-FastStart DNA Master SYBR Green I kit (Roche, Mannheim, Germany), and a LightCycler instrument and primers (VBC-GENOMICS, Wien, Austria) with the following sequences (5' → 3'): for 11HSD1, sense GAGTTCA-GACCAGAAATGCTCC and antisense TGTGTGATGT-GATTGAGAATGAGC [16]; for 11HSD2 sense CCG-GTTGTGACACTGGTTTTG and antisense GGGGTATG-GCATGTCTCCTG; for β-actin sense CCGTAAAGACCTC-TATGCCA and antisense AAGAAAGGGTGTAACGCA [16]. PCR was performed in a total volume of 10 µl containing 1 µl five-fold diluted cDNA; 5 mM (β-actin), 4 mM (11HSD2), or 3 mM (11HSD1) MgCl<sub>2</sub>; 0.5 µM of each primer and PCR reaction mix (1×). The LightCycler was programmed as follows: pre-incubation and denaturation of the template cDNA for 10 min at 95 °C; followed by 45 cycles of amplification: 95 °C for 15 s, followed by 55 °C for 10 s (11HSD1, β-actin) or 60 °C for 5 s (11HSD2), and then 72 °C for 15 s (11HSD1, β-actin) or 20 s (11HSD2). The melting curve analysis was performed at 95 °C for 0 s, 65 °C for 60 s and 99 °C for 0 s. The temperature slope was always 20 °C/s, with the exception of the last step of melting analysis when it was 0.1 °C/s. For quantification, we prepared standard curves for each pair of primers from serial dilutions of kidney cDNA containing both isoforms of 11HSD. The results were calculated as relative expression of 11HSD1 mRNA and 11HSD2 mRNA to β-actin mRNA.

### 2.3. Assay of 11HSD activity

The heart was homogenized in an ice-cold buffer containing 200 mM sucrose and 10 mM Tris–HCl at pH 8.5 (1:9, w/v) on a Polytron homogenizer. The homogenate was centrifuged at 1000 × g for 10 min at 4 °C, the protein concentration determined by the Coomassie blue method, and the homogenate used immediately for measurement of enzyme activity by a radiometric conversion assay as described previously [16,17]. Briefly, to measure 11β-hydroxysteroid dehydrogenase activity, the tissue homogenate equivalent to 1 mg protein was incubated with tritiated corticosterone and NAD<sup>+</sup> or NADP<sup>+</sup>. The reaction was stopped by cooling, steroids were extracted on C<sub>18</sub> reverse-phase Sep-Pak columns (Waters, Milford MA), dried under nitrogen and stored at –20 °C. [<sup>3</sup>H]Corticosterone and [<sup>3</sup>H]11-dehydrocorticosterone were separated and quantified by HPLC.

### 2.4. Statistical analysis

All data are expressed as mean ± S.E.M. The mean values for Dahl rats were compared between strain and treatment by ANOVA with subsequent application of the Newman–Keuls multiple range test to determine significant differences between individual means. Values of SHR and WKY rats were compared by Student's *t*-test. Differences of *p* < 0.05 were considered significant.

Table 1  
Arterial blood pressure and heart weight

	SBP (mmHg)	Heart weight (mg/g BW)
SHR	149 ± 3*	3.73 ± 0.08*
WKY	131 ± 3	3.01 ± 0.08
DS-LS	141 ± 4 <sup>+</sup>	2.75 ± 0.03
DS-HS	200 ± 5 <sup>+,**</sup>	4.07 ± 0.08 <sup>+,**</sup>
DR-LS	129 ± 3	2.89 ± 0.03
DR-HS	128 ± 5	2.96 ± 0.06

Values are the mean ± S.E.M. SBP, systolic blood pressure; SHR, spontaneously hypertensive rats; WKY, Wistar–Kyoto rats; DS-LS and DR-LS, Dahl salt-sensitive and salt-resistant rats kept on a low-salt diet; DS-HS and DR-HS, Dahl salt-sensitive and salt-resistant rats kept on a high-salt diet.

\* Significantly different from WKY rats ( $p < 0.01$ ).

\*\* Significantly different from DS-LS ( $p < 0.01$ ).

<sup>+</sup> Significantly different from DR rats ( $p < 0.01$ ).

### 3. Results

#### 3.1. Hypertension and cardiac changes

Blood pressure was significantly elevated in SHR and DS rats kept on both high- and low-salt diet in comparison with WKY and DR rats (Table 1). In SHR and DS-HS rats the relative heart weight was significantly higher than in WKY and DS-LS rats. In contrast with the DS rats, an augmented salt intake did not result in any changes of cardiac index in DR rats.

#### 3.2. Expression of 11HSD1 and 11HSD2 mRNA

Investigation of cardiac mRNA demonstrated readily detectable transcripts for both isoforms of 11HSD in all heart samples, but the lower level of 11HSD2 mRNA suggests that this enzyme is present in much lesser amounts than 11HSD1. As is illustrated in Fig. 1, two-way ANOVA proved significant differences between DS and DR strain but no effect of dietary salt intake on abundance of both isoforms of 11HSD mRNA. 11HSD1 mRNA levels were significantly lower in DS than in DR rats and a similar pattern was also observed in 11HSD2 mRNA. In contrast with Dahl rats, there was no strain difference in the expression of 11HSD1 between SHR and WKY rats (Fig. 2). The 11HSD2 message was low in

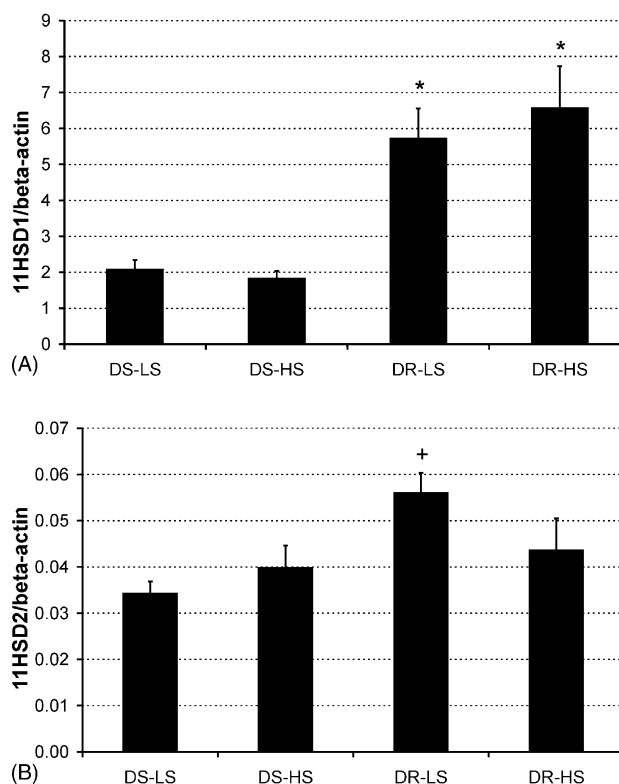


Fig. 1. Strain-specific expression of 11HSD1 mRNA (A) and 11HSD2 mRNA (B) in the heart of Dahl rats kept on a low- or high-salt diet. For further details see description of Table 1. Analysis of variance proved significant differences between strains but no effect of diet. \*  $p < 0.01$  and <sup>+</sup>  $p < 0.05$  between DS and DR rats.

both strains but the level of 11HSD2 mRNA was twice as high in WKY than in SHR.

#### 3.3. 11HSD activity

11HSD activity was assayed in heart homogenates in the presence of cofactors NAD<sup>+</sup> and NADP<sup>+</sup>. Values in the presence and in absence of NAD<sup>+</sup> were similar and much lower than in the presence of NADP<sup>+</sup>. As shown in Table 2, conversion of [<sup>3</sup>H]corticosterone to [<sup>3</sup>H]11-dehydrocorticosterone was significantly lower in DS than in DR rats. Thus, it

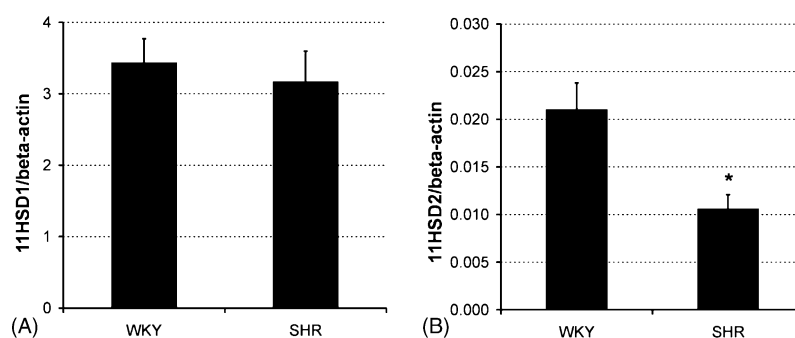


Fig. 2. Expression of 11HSD1 mRNA (A) and 11HSD2 mRNA (B) in the heart of spontaneously hypertensive rats (SHR) and their normotensive counterpart Wistar–Kyoto rats (WKY). \*  $p < 0.01$ .

Table 2

11 $\beta$ -Hydroxysteroid dehydrogenase activity in the hearts of normotensive and hypertensive rats

	NIL	NAD <sup>+</sup>	NADP <sup>+</sup>
SHR	4.2 $\pm$ 0.6	3.6 $\pm$ 0.5	16.8 $\pm$ 1.5
WKY	5.1 $\pm$ 0.6	5.9 $\pm$ 0.7	16.1 $\pm$ 1.9
DS-LS	4.8 $\pm$ 0.6	5.9 $\pm$ 0.6	18.1 $\pm$ 1.7
DS-HS	3.9 $\pm$ 0.5	4.3 $\pm$ 0.4	21.7 $\pm$ 1.1
DR-LS	5.4 $\pm$ 0.3	5.2 $\pm$ 0.4	26.7 $\pm$ 3.2*
DR-HS	4.2 $\pm$ 0.5	5.0 $\pm$ 0.6	28.9 $\pm$ 3.1*

Values are the mean  $\pm$  S.E.M. Enzyme activity was determined as described in Section 2 in the absence (NIL) and presence of 0.4 mM NAD<sup>+</sup> or NADP<sup>+</sup>. The data are expressed as percentage of conversion of corticosterone to 11-dehydrocorticosterone by heart homogenates. For further details see Table 1.

\*  $p < 0.05$  Between DS and DR rats.

appears that the NADP<sup>+</sup>-dependent enzyme is present in the rat heart and its activity correlates with the abundance of 11HSD1 mRNA. The absence of NAD<sup>+</sup>-dependent oxidation of [<sup>3</sup>H]corticosterone indicates that 11HSD2 activity is probably below the limit of detection of our assay.

#### 4. Discussion

In the present paper, we have demonstrated strain-dependent expression of 11HSD1 and 11HSD2. Our observation that the rat heart expresses 11HSD2 contrasts with earlier reports that were unable to prove the presence of cardiac 11HSD2 using RNase protection analysis or in situ hybridization [12,18] when the presence of 11HSD1 could be demonstrated at the level of mRNA, protein and enzyme activity [12–14,19]. The results of the present study are, however consistent with several studies examining 11HSD2 in the human heart [2,9,10] and with recent findings of Konishi et al. [11] in rats. The data suggest the preponderance of net 11HSD reductase activity catalyzing conversion of 11-dehydrocorticosterone to corticosterone as 11HSD1 mRNA expression and NADP<sup>+</sup>-dependent 11HSD activity are higher than 11HSD2 mRNA expression and undetectable NAD<sup>+</sup>-dependent 11HSD activity.

When considering the low expression of 11HSD2 and much higher expression of 11HSD1 in the rat heart, there are several questions concerning the physiological role of these isoforms. First, both human and animal studies have shown that corticosteroids play a pathophysiological role in the generation of cardiac hypertrophy and fibrosis [1,4,5,20]. Second, 11HSD1, an enzyme that operates in vivo as a reductase increasing the concentration of biologically active glucocorticoids, has been reported in both cardiac myocytes and fibroblasts in some studies [12], though its presence in cardiomyocytes was not found by others [14]. Third, carbenoxolone, the inhibitor of 11HSD, induces cardiac hypertrophy similar to that induced by exogenous excess of mineralocorticoids and high dietary salt intake [21]. Fourth, the transgenic mice over-

expressing 11HSD2 in cardiomyocytes spontaneously develop cardiac hypertrophy, fibrosis, and heart failure [15]. In agreement with these findings Funder [22] has proposed, on the basis of his previous studies, that glucocorticoids act in the heart as antagonists rather than agonists of MR. In agreement with this notion, overexpression of 11HSD2 in cardiomyocytes allows activation of MR by aldosterone leading to cardiac hypertrophy, fibrosis, and heart failure [15].

In an attempt to explore whether modulation of 11HSD1 and 11HSD2 are factors that play a role in experimental cardiac hypertrophy and fibrosis we have studied these enzymes in two models of hypertension, SHR and DS rats, in which the hearts are hypertrophied and remodeled [23–25]. Although there is a clear variability among the strains we found no overall relation between cardiac hypertrophy and changes in 11HSD isoforms. First, neither 11HSD1 mRNA nor 11HSD2 mRNA expression and 11HSD activities changed in DS rats on high- versus low-salt intake, whereas there was an about 50% increase in heart weight. Second, high salt intake was also without any effect on both isoforms of 11HSD in normotensive DR rats, in which heart weight did not change with high-salt treatment. Nevertheless, in the present study we have found strain differences between DS and DR rats. In considering the possibility that glucocorticoids act as antagonists rather than agonists of MR in the heart [15,22], then the availability of glucocorticoids in DS rats might be lower than in DR rats. Third, the expression of 11HSD1 mRNA in SHR is similar and 11HSD2 even lower than in their normotensive WKY counterparts, whereas heart weight is higher in SHR than in WKY rats. Our interpretation of these findings is that overexpression of 11HSD2 in cardiomyocytes is not necessary for the development of cardiac hypertrophy and fibrosis. This concept is supported by studies that showed absence of stimulatory effect of high-salt dietary intake on glucocorticoid metabolism. Although aldosterone in combination with high salt intake produces experimental hypertension, and cardiac fibrosis and hypertrophy [5], Niepel et al. [26] did not report any effect of high salt diet on renal and hepatic 11HSD activities and Pácha and Mikšík [27] and Norregaard et al. [28] found a decrease of colonic 11HSD2 in response to increased salt intake. In addition, the recent data of Melander et al. [29] indicate that the relative importance of 11HSD for salt-induced blood pressure seems to be minor.

In summary, mineralocorticoids and overexpression of cardiomyocyte 11HSD2 have been reported to provide cardiac hypertrophy, fibrosis, and heart failure and aldosterone blockers to ameliorate this effect [5,15]. Consistent with these findings it was suggested that glucocorticoids protect heart MR against activation and 11HSD2 diminishes this protection. However, our study does not support this notion. The data show that neither 11HSD2 nor 11HSD1 represent etiologic factors that might play a role in pathophysiology of experimental cardiac hypertrophy.

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