Original Article

Sexual Dimorphism of 11β-Hydroxysteroid Dehydrogenase in Hypertensive and Normotensive Rats

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To evaluate the role of sexually dimorphic tissue expression of 11β-oxidase activity of 11β-hydroxysteroid dehydrogenase (11βHSD) in gender-associated blood pressure differences, we have studied female and male hypertensive rats of two different strains and their normotensive controls: spontaneously hypertensive rats (SHR), Wistar-Kyoto rats (WKY) and Dahl salt-sensitive (SS/Jr) and salt-resistant rats (SR/Jr). In hypertensive SHR and SS/Jr, but not in normotensive strains WKY and SR/Jr, blood pressure reached a higher level in males than in females. The activity of 11βHSD was higher in the renal cortex, medulla, colon and aorta of males than of females in all investigated strains with the exception of aortic 11βHSD in SHR and WKY rats, both of which had very low 11β-oxidase activity. In contrast to gender-dependent differences, strain differences of 11βHSD were observed in a limited number of tissues only. Renal medullary 11βHSD showed significantly lower activity in WKY than in SHR, whereas no difference was observed in the renal cortex. Similarly, colonic 11βHSD activity was lower in WKY than in SHR. In Dahl rats the strain differences were observed in aortic 11βHSD that had higher activity in SR/Jr than in SS/Jr rats; no difference was observed in the kidney or colon. These data demonstrate the following. 1) Sexual dimorphism of 11βHSD activity exists in the kidney, colon, and aorta. 2) The sexual dimorphism of 11βHSD does not play a role in gender-associated differences in blood pressure. 3) The reduced 11βHSD activity in the aorta of hypertensive SS/Jr compared to SR/Jr rats suggests that this enzyme might play a role in the pathogenesis of salt-sensitive hypertension in Dahl rats. (*Hypertens Res* 2003; 26: 333−338)

***Key Words*: 11β-hydroxysteroid dehydrogenase, Dahl rats, spontaneously hypertensive rats**

Introduction

A number of epidemiological and clinical studies have confirmed that male subjects have a greater predisposition toward hypertension than premenopausal women of the same age. Such gender-associated differences in blood pressure regulation have also been documented in various hypertensive rat models, including spontaneously hypertensive and Dahl salt-sensitive rats, the males of which have higher blood pressure than age-matched females (*1*−*4*). Although the mechanisms involved in these gender-related differences have not been completely elucidated, androgens (*1*, *2*, *4*) and sex differences in the endothelial functions have been shown to promote hypertension (*5*, *6*). In addition, female rats have been shown to excrete Na^+ load more effectively than males (*2*).

As abnormalities in glucocorticoid production or metabolism have been implicated in various forms of hypertension (*7*, *8*), and sexual dimorphism of glucocorticoid metabolism

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has been described in various organs (*9*–*11*), these phenomena might also be related to gender differences in blood pressure. Even if the exact mechanisms mediating corticosteroid-induced hypertension are still not fully understood, the enzyme 11β-hydroxysteroid dehydrogenase isoform 2 (11βHSD2) seems to play an important role in this process. 11βHSD2 catalyzes the conversion of biologically active glucocorticoids cortisol and corticosterone to their 11-oxo derivatives cortisone or 11-dehydrocorticosterone, respectively (*12*). Since local glucocorticoids within the vascular wall potentiate the vasoconstrictive action of a number of pressor substances, the local metabolism of glucocorticoids mediated by 11βHSD2 seems to be involved in regulation of vascular tone (*13*). Similarly, the impairment of the renal 11βHSD2 activity seems to be involved in the elevation of blood pressure due to enhanced renal Na^+ retention (*14*, *15*).

Much as in human hypertension (*8*), abnormalities in corticosteroid production and metabolism have been found in rat models of hypertension (*16*–*19*), and changes in dietary NaCl intake have been shown to modulate 11βHSD2 (*20*–*22*). However, these studies did not analyze the contribution of gender status to peripheral metabolism of glucocorticoids. To examine the possible role of 11βHSD2 in the sexually dimorphic pattern of hypertension development, we measured the conversion of corticosterone in the vascular tissue and kidneys of spontaneously hypertensive and Dahl salt-sensitive rats and their respective normotensive controls. For purposes of comparison, 11βHSD activity was also studied in the colon, an intestinal segment in which Na^+ transport properties were similar to those of the renal collecting duct.

Methods

Animals

Male and female adult spontaneously hypertensive rats (SHR), normotensive Wistar-Kyoto rats (WKY) and Dahl salt-sensitive and salt-resistant rats (SS/Jr, SR/Jr) were obtained from the breeding colonies of the Institute of Physiology (Czech Academy of Sciences, Prague, Czech Republic). SHR and WKY were maintained on a standard chow, whereas Dahl rats (10 weeks old) were fed a low salt diet (0.2% NaCl) before being placed on a high salt diet (8% NaCl) for 6 weeks before the sacrifice. Blood pressure was measured by a direct puncture of the carotid artery under light ether anaesthesia. The experiments were approved by the Animal Care and Use Committee of the Institute of Physiology.

11βHSD Activity

The rats (age: 90–115 days) were killed by cervical dislocation, and the kidney, aorta, and colon were removed and dissected from the surrounding tissue on ice. The tissues were homogenized in a Polytron homogenizer in 9 volumes of ice-cold buffer (200 mmol/l sucrose, 10 mmol/l Tris/HCl, pH 8.5) and centrifuged at 1,000$\times g$ for 10 min. The supernatant was assayed for protein concentration using the Coomassie Blue method and 11β-oxidase activity of 11βHSD was measured by radiometric assay. *In vitro*, 11βHSD1 is a $NADP^+$(H) or NAD^+(H)-dependent 11β-oxidoreductase that has K_M in micromolar range, whereas 11βHSD2 is exclusively a NAD^+-dependent 11β-dehydrogenase that has K_M in nanomolar range (*12*). On the basis of these studies, an assay with 20 nmol/l corticosterone and NAD^+ was designed to estimate 11βHSD2 activity. The assay was done as previously described (*23*). Briefly, the dehydrogenase activity was determined by measuring the conversion of 20 nmol/l [^{3}H]-corticosterone to [^{3}H]11-dehydrocorticosterone in a buffer containing 100 mmol/l KCl, 50 mmol/l Tris/HCl and 0.4 mmol/l NAD^+ (pH 8.5). Tissue homogenates (in mg/ml: kidney, 0.125; colon, 1.0; aorta, 0.5) were incubated at 37°C for 10 (kidney), 30 (colon), or 150 min (aorta), respectively, and the reaction was terminated by cooling. The amounts of protein and the incubation times were determined in preliminary experiments to establish the optimal conditions for each tissue, in order to work in the linear portion of the enzyme reaction. The samples were centrifuged for 15 min (3,000$\times g$) and the steroids were extracted on Sep-Pak C_{18}-cartridges (Waters, Milford, USA), dried under nitrogen and stored at −20°C. Blank incubations without tissue were carried out in each experiment to determine nonspecific conversion of corticosterone and the experimental data were adjusted accordingly.

In separate experiments 11β-oxidase bioactivity of 11βHSD was measured in aseptic prepared fresh fragments of intact renal, colonic, and vascular tissues (*24*, *25*). Tissue slices of the renal cortex and medulla (100 mg), colon (250 mg), and aorta (~500 mg) were incubated in sealed vessels containing oxygenated incubation solution at 37°C for 20 (kidney), 90 (colon), or 240 min (aorta). The incubation solution contained (in mmol/l): NaCl, 119.0; $CaCl_2$, 1.2; $MgCl_2$, 1.2; $NaHCO_3$, 21.0; K_2HPO_4, 2.4; KH_2PO_4, 0.6; glucose, 10.0; glutamine, 2.5; β-hydroxybutyrate, 0.5, mannitol, 10.0; and 1.45 μmol/l corticosterone. At the end of incubation, an internal standard of deoxycorticosterone (1.45 μmol/l) was added, and the solution was chilled and extracted as described above.

Corticosterone and 11-dehydorcorticosterone were estimated by high-performance liquid chromatography as described previously (25).

Statistical Analysis

Results are given as the mean±SEM for the indicated number of rats. Statistical analysis of the data was performed by two-way analysis of variance (strain *vs*. gender) with subsequent application of Newman multiple range test to determine significant differences among individual means. Values of $p<0.05$ were considered to indicate statistical significance.

Table 1. Numbers of Animals and Mean Arterial Blood Pressure in Hypertensive and Normotensive Rats

	Number of animals		Blood pressure (mmHg)	
	Male	Female	Male	Female
SHR	14	12	$194 \pm 10^{\dagger}$	$157 \pm 6^{\dagger,*}$
WKY	14	13	130 ± 4	125 ± 3
SS/Jr	16	12	$186 \pm 7^{\dagger}$	$160 \pm 8^{\dagger,**}$
SR/Jr	12	12	123 ± 3	126 ± 4

Values are expressed as the means±SEM. SHR, spontaneously hypertensive rats; WKY, Wistar-Kyoto rats; SS/Jr, Dahl salt-sensitive rats; SR/Jr, Dahl salt-resistant rats. $^{\dagger}p<0.01$ as compared with the corresponding normotensive counterpart; $^{*}p<0.01$ or $^{**}p<0.05$ compared with males of the same strain.

Table 2. Effect of Strain and Gender on 11βHSD Activity in the Renal Cortex and Medulla of Hypertensive and Normotensive Rats

	SHR	WKY	SS/Jr	SR/Jr
Renal cortex				
Male	967 ± 74	766 ± 70	842 ± 51	742 ± 84
Female	$370 \pm 42^{*}$	$334 \pm 44^{*}$	$486 \pm 59^{*}$	$461 \pm 59^{*}$
Renal medulla				
Male	861 ± 58	$615 \pm 53^{\dagger}$	983 ± 80	809 ± 128
Female	$189 \pm 18^{*}$	$119 \pm 16^{*,\dagger}$	$387 \pm 83^{*}$	$256 \pm 34^{*}$

Values are the means±SEM; data are given in pmol of 11-dehydrocorticosterone per mg of protein per h. In the cortex, analysis of variance demonstrated significant changes between genders but no differences among strains. In the medulla, significant changes were observed between genders and strains. * Significantly different values between genders of the same strain ($p<0.01$). † Significantly different values between SHR and WKY ($p<0.05$). For further details see Table 1.

Results

Blood pressure was significantly higher in SHR than in their normotensive controls (WKY), and blood pressure in males was higher than that in females (Table 1). Similarly, a high-salt diet given for 6 weeks increased blood pressure in SS/Jr but not in SR/Jr rats, and this increase was higher in males than in females.

11βHSD activities were measured in the aorta, colon, and renal cortex and medulla of both males and females. As shown in Tables 2–4, the gender significantly influenced 11βHSD activity in all investigated tissues. In contrast, strain differences were observed in all tissues with the exception of the renal cortex. However, the strain differences reflected predominantly changes between the biochemical phenotype of SS/Jr and SR/Jr rats on one side and SHR and WKY rats on the opposite side.

Table 3. Effect of Strain and Gender on 11βHSD Activity in the Colon of Hypertensive and Normotensive Rats

	SHR	WKY	SS/Jr	SR/Jr
Male	31.9 ± 2.9	$9.3 \pm 0.8^{\dagger}$	120.6 ± 18.8	102.4 ± 15.7
Female	$11.5 \pm 0.9^{*}$	$6.7 \pm 0.7^{\dagger}$	$11.3 \pm 1.7^{**}$	$8.2 \pm 1.0^{**}$

Values are the means±SEM; data are given in pmol of 11-dehydrocorticosterone per mg of protein per h. Analysis of variance demonstrated significant changes between genders and strains. $^{*}p<0.05$ and $^{**}p<0.01$ between males and females of the same strain; $^{\dagger}p<0.05$ between SHR and WKY. For further details see Table 1.

Table 4. Effect of Strain and Gender on 11βHSD Activity in Aorta of Hypertensive and Normotensive Rats

	SHR	WKY	SS/Jr	SR/Jr
Male	6.4 ± 1.4	4.6 ± 0.7	22.9 ± 2.8	$30.8 \pm 4.3^{\dagger}$
Female	2.3 ± 0.3	2.2 ± 0.2	$2.4 \pm 0.7^{*}$	$9.1 \pm 0.4^{*,\dagger}$

Values are the means±SEM; data are given in pmol of 11-dehydrocorticosterone per mg of protein per h. Analysis of variance demonstrated significant changes between genders and strains. $^{*}p<0.01$ between males and females of the same strain; $^{\dagger}p<0.05$ between SS/Jr and SR/Jr. For further details see Table 1.

11β-Oxidase activity is not distributed homogeneously in the kidney (*12*), and therefore, 11βHSD was studied separately in the renal cortex and medulla. As shown in Table 2, the activity of renal 11βHSD was 2–5 times higher in males than in females and this gender difference was more obvious in the renal medulla than in the renal cortex. Medullary 11βHSD activity was greater in SHR than in WKY rats, but no strain differences were observed between SS/Jr and SR/Jr rats.

Table 3 shows the effect of gender and strain on colonic 11βHSD activity. The differences between males and females were observed in all strains with the exception of WKY rats, in which low colonic activity of 11βHSD was similar in males and females. The strain difference in 11βHSD activity was especially prominent when males of SHR and WKY rats were compared, the activity being more than threefold higher in SHR. The level of colonic 11βHSD activity in male Dahl rats was significantly higher than that in SHR or WKY rats ($p<0.01$).

11βHSD activity in the aorta of Dahl rats was not only gender- but also strain-dependent (Table 4). A low level of 11βHSD activity was detected in females of all investigated strains and in the aorta of SHR and WKY males. Interestingly, the aorta of male Dahl rats was found to possess a relatively high level of 11βHSD. Nevertheless, the highest enzyme activity in SR/Jr rats corresponded to only 2–4% of renal and 20–40% of colonic activity.

Using tissue fragments, experiments were performed to

Table 5. 11βHSD Bioactivity in Tissue Fragments of the Rat Renal Cortex, Renal Medulla, Colon and Aorta

	SHR	WKY	SS/Jr	SR/Jr
Renal cortex				
Male	996±105	1,008±132	1,160±138	1,240±129
Female	585±63*	610±75**	545±82**	600±90**
Renal medulla				
Male	945±79	720±63†	1,120±163	1,080±121
Female	380±30**	289±33**	652±72*	602±75**
Colon				
Male	95±13	56±8†	195±26	144±19
Female	39±5**	45±7	58±6**	49±6**
Aorta				
Male	4.3±0.8	5.2±0.6	7.1±0.7	11.8±1.5†
Female	2.9±0.4	3.5±0.5	3.2±0.4**	7.5±1.0**,††

Values are the means±SEM; data are given in pmol of 11-dehydrocorticosterone per mg of dry weight per h. *$p<0.05$ and **$p<0.01$ between genders of the same strain; †$p<0.05$ and ††$p<0.01$ between the hypertensive strain and corresponding normotensive counterpart. For further details see Table 1.

identify 11β-oxidase bioactivity of 11βHSD in intact tissue and to quantify its level (Table 5). In all investigated strains 11βHSD was significantly higher in males than in females. Strain differences were detected in colonic and renal medullary 11βHSD between SHR and WKY rats and in aortic 11βHSD between the two strains of Dahl rats. These strain differences were more obvious in males than in females. In agreement with aortic homogenates (Table 4) the intact aorta of SS/Jr rats possessed less 11β-oxidase bioactivity than in SR/Jr rats.

Discussion

It is well known that there are gender-associated differences in hypertension development (*1–4*) and that abnormalities of glucocorticoid production and metabolism have been implicated in some forms of hypertension (*7*, *8*). Several lines of evidence suggest that conversion of glucocorticoids by 11βHSDs might play an important role in the development of hypertension. First, administration of 11βHSD inhibitors to WKY rats not only inhibits 11βHSD activity but also induces hypertension (*26*). Second, a deficiency of renal 11βHSD2 causes the syndrome of apparent mineralocorticoid excess that is associated with hypertension of the salt-sensitive type (*12*). Third, inhibition of 11βHSD2 activity increases vascular tone due to stimulation of angiotensin II binding, impairment of NO formation and activation of the vascular endothelin system (*13*, *26*). Thus it seems possible that the decrease in 11βHSD2 might be associated with hypertension in experimental animals such as SHR and SS/Jr rats. In addition, sex-related differences in 11βHSD1 have been described in several organs (*9*, *10*). Therefore questions arise as to whether 1) 11βHSD2 expression and enzyme activity may depend on gender and 2) there may be a relationship between 11βHSD2 activity and the gender-associated differences in the development of hypertension.

Our study confirms that both normal and hypertensive rats show marked sexual dimorphism of 11βHSD and that this activity reflects the type 2 isoform. This contention is supported by the presence of NAD^+-dependent 11βHSD activity at a low nanomolar concentration of corticosterone and by the existence of 11β-oxidase bioactivity in intact tissue. The isoform 11βHSD1 exists as a bidirectional enzyme in tissue homogenates and microsomal fractions, but it is considered to operate only as 11-reductase in intact tissue; in contrast, 11βHSD2 always exists as 11β-oxidase (*12*). Our results are in agreement with the recent study of Condon *et al*. (*11*), who demonstrated the sexual dimorphism of mouse renal and colonic 11βHSD2 at the level of enzyme activity and mRNA expression. Although our study did not address the mechanism of sexual dimorphism, we cannot rule out the possibility that sex steroids and growth hormone may play a role (*9*, *10*). It is well known that females of hypertensive rat strains excrete more urine and sodium in association with lower plasma aldosterone (*2*, *27*), but our data exclude the possibility that the increased Na^+ reabsorption is associated with sexual dimorphism of renal glucocorticoid metabolism. 11βHSD2 prevents the binding of glucocorticoids to mineralocorticoid receptors, and thus the lower activity of 11βHSD2 could result in stimulation of mineralocorticoid receptors by corticosterone followed by an increase of Na^+ reabsorption in renal tubules. However, this concept would be compatible with the lower corticosterone metabolizing capacity in males and higher in females.

Our finding that the strain differences of renal 11βHSD do not correlate with hypertension, although changes in Na^+ retention exist between hypertensive and normotensive strains (*28*, *29*), suggests that renal 11βHSD2 does not play a role in the strain differences of blood pressure. With the exception of the difference in 11βHSD2 in the renal medulla between SHR and WKY rats, we have not observed any significant difference between 11βHSD activity in the kidney. Although a clear difference of medullary 11βHSD was found between SHR and WKY, the degradation of corticosterone was enhanced in SHR. A similar biochemical phenotype pattern of 11βHSD was also found in the colon, the Na^+ transport properties and corticosteroid specificity of which resemble those of the renal collecting ducts. Therefore, we can conclude that the differences in Na^+ retention in hypertensive and normotensive rats may be related to inherent differences between genders rather than to altered 11βHSD2 activity. In addition, the increased Na^+ sensitivity and higher blood pressure in SS/Jr appear not to correlate with 11βHSD activity (Table 2) or 11βHSD2 mRNA expression (*30*), although at least one other study has reached the opposite conclusion (*17*).

11βHSD activity in the aorta was also gender-dependent, being significantly higher in male than in female Dahl rats, and was significantly lower in SS/Jr than in SR/Jr rats. Decreased 11βHSD activity and 11βHSD2 mRNA expression were also found in mesenteric arteries of Dahl-Iwai salt-sensitive rats (*16*), and this enzyme has been considered to play a physiological role in the elevation of blood pressure (*13*, *31*). The mechanism of interaction between 11βHSD2 and vascular tone has not been fully explained, but several possibilities can be considered. First, the cyclooxygenase-derived constricting factors released from the endothelium, such as prostaglandins, are potent inhibitors of 11βHSD2 (*32*). Second, the inhibition of 11βHSD *in vivo* decreases endothelial nitric oxide synthase (NOS) and stimulates aortic endothelin expression (*26*), which is known to play an important role not only in the regulation of vascular tone but also in vascular remodeling (*33*). Defects in nitric oxide (NO) production and regulation of NOS associated with increased salt intake have been described in Dahl salt-sensitive rats (*34*, *35*) and thus the changes in NO production might influence endothelial 11βHSD2 activity. The effect of NO on 11βHSD2 was observed in human trophoblasts (*36*), but no data are available in endothelial or vascular smooth muscle cells, even though both cell types have been shown to possess 11βHSD1 and 11βHSD2 (*37*, *38*).

In summary, we have obtained clear evidence that 11βHSD2 activity is higher in males than in females. Since 11βHSD2 acts as a dehydrogenase, the conversion of corticosterone to 11-dehydrocorticosterone is enhanced in males and thus it is unlikely that 11βHSD2 is directly involved in gender-associated differences of hypertension development. Nevertheless, the finding of decreased corticosterone inactivation in the aorta of male and female hypertensive Dahl salt-sensitive rats indicates that impaired 11β-dehydrogenation might potentiate corticosterone activity in the regulation of vascular tone.

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References

1. Ganten U, Schröder G, Witt M, Zimmermann F, Ganten D, Stock G: Sexual dimorphism of blood pressure in spontaneously hypertensive rats: effects of anti-androgen treatment. *J Hypertens* 1989; **7**: 721−726.
2. McIntyre M, Hamilton CA, Rees DD, Reid JL, Dominiczak AF: Sex differences in the abundance of endothelial nitric oxide in a model of genetic hypertension. *Hypertension* 1997; **30**: 1517−1524.
3. Kähönen M, Tolvanen J-P, Sallinen K, Wu X, Pörsti I: Influence of gender on control of arterial tone in experimental hypertension. *Am J Physiol* 1998; **275**: H15−H22.
4. Reckelhoff JF, Zhang H, Granger JP: Testosterone exacerbates hypertension and reduces pressure-natriuresis in male spontaneously hypertensive rats. *Hypertension* 1998; **31**: 435−439.
5. Crofton JT, Ota M, Share L: Role of vasopressin, the renin-angiotensin system and sex in Dahl salt-sensitive hypertension. *J Hypertens* 1993; **11**: 1031−1038.
6. Hinojosa-Laborde C, Lange DL, Haywood JR: Role of female sex hormones in the development and reversal of Dahl hypertension. *Hypertension* 2000; **35**: 484−489.
7. Seckl JR, Brown RW: 11-Beta-hydroxysteroid dehydrogenase: on several roads to hypertension. *J Hypertens* 1994; **12**: 105−112.
8. Ferrari P, Lovati E, Frey FJ: The role of the 11β-hydroxysteroid dehydrogenase type 2 in human hypertension. *J Hypertens* 2000: **18**: 241−248.
9. Low SC, Chapman KE, Edwards CRW, Wells T, Robinson ICAF, Seckl JR: Sexual dimorphism of hepatic 11β-hydroxysteroid dehydrogenase in the rat: the role of growth hormone patterns. *J Endocrinol* 1994; **143**: 541−548.
10. Albiston AL, Smith RE, Krozowski ZS: Sex- and tissue-specific regulation of 11β-hydroxysteroid dehydrogenase mRNA. *Mol Cell Endocrinol* 1995; **199**: 183−188.
11. Condon J, Ricketts ML, Whorwood CB, Stewart PM: Ontogeny and sexual dimorphic expression of mouse type 2 11β-hydroxysteroid dehydrogenase. *Mol Cell Endocrinol* 1997; **127**: 121−128.
12. Stewart PM, Krozowski ZS: 11β-Hydroxysteroid dehydrogenase. *Vitam Horm* 1999; **57**: 249−324.
13. Hatakeyama H, Inaba S, Miyamori I: 11β-Hydroxysteroid dehydrogenase in cultured human vascular cells: possible role in the development of hypertension. *Hypertension* 1999; **33**: 1179−1184.
14. Lovati E, Ferrari P, Dick B, *et al*: Molecular basis of human salt sensitivity: the role of 11β-hydroxysteroid dehydrogenase type 2. *J Clin Endocrinol Metab* 1999; **84**: 3745−3749.
15. Agarwal AK, Giacchetti G, Lavery G, *et al*: CA-repeat polymorphism in intron 1 of HSD11B2: effects on gene expression and salt sensitivity. *Hypertension* 2000; **36**: 187−194.
16. Takeda Y, Miyamori I, Yoneda T, Iki K, Hatakeyama H, Takeda R: Gene expression of 11β-hydroxysteroid dehydrogenase in the mesenteric arteries of genetically hypertensive rats. *Hypertension* 1994; **23**: 577−580.
17. Takeda Y, Inaba S, Furukawa K, Miyamori I: Renal 11β-hydroxysteroid dehydrogenase in genetically salt-sensitive hypertensive rats. *Hypertension* 1998; **32**: 1077−1082.
18. Franco-Saenz R, Tokita Y, Latif S, Morris DJ: 11β-Hydroxysteroid dehydrogenase in the Dahl rat. *Am J Hypertens* 1997; **10**: 1004−1009.
19. Lloyd-MacGilp SA, Nelson SM, Florin M, *et al*: 11β-Hydroxysteroid dehydrogenase and corticosteroid action in Lyon hypertensive rats. *Hypertension* 1999; **34**: 1123−1128.
20. Pácha J, Mikšík I: 11β-Hydroxysteroid dehydrogenase in developing rat intestine. *J Endocrinol* 1996; **148**: 561−566.
21. Brem AS, Bina RB, King T, Chobanian MC, Morris DJ: Influence of dietary sodium on the renal isoforms of 11β-hydroxysteroid dehydrogenase. *Proc Soc Exp Biol Med* 1997; **214**: 340−345.
22. McKinnell J, Roscoe D, Holmes MC, Lloyd-MacGilp SA,

Kenyon CJ: Regulation of 11β-hydroxysteroid dehydrogenase enzymes by dietary sodium in the rat. *Endocr Res* 2000; **26**: 81–95.

23. Pohlová I, Mikšík I, Kuneš J, Pácha J: 11β-Hydroxysteroid dehydrogenase activity in spontaneously hypertensive and Dahl rats. *Am J Hypertens* 2000; **13**: 927–933.
24. Riddle MC, McDaniel PA: Renal 11β-hydroxysteroid dehydrogenase activity is enhanced by ramipril and captopril. *J Clin Endocrinol Metab* 1994; **78**: 830–834.
25. Vylitová M, Mikšík I, Pácha J: Metabolism of corticosterone in mammalian and avian intestine. *Gen Comp Endocrinol* 1998; **109**: 315–324.
26. Ruschitzka F, Quaschning T, Noll G, *et al*: Endothelin 1 type A receptor antagonism prevents vascular dysfunction and hypertension induced by 11β-hydroxysteroid dehydrogenase inhibition: role of nitric oxide. *Circulation* 2001; **103**: 3129–3135.
27. Ashton N, Balment RJ: Sexual dimorphism in renal function and hormonal status of New Zealand genetically hypertensive rats. *Acta Endocrinol* 1991; **124**: 91–97.
28. Roman RJ: Abnormal renal hemodynamics and pressure-natriuresis relationship in Dahl salt-sensitive rats. *Am J Physiol* 1986; **251**: F57–F65.
29. Dietz R, Schömig A, Haebra H, Pascher W, Lüth JB, Gross F: Studies on the pathogenesis of spontaneous hypertension of rats. *Circ Res* 1978; **43** (Suppl 1): S98–S106.
30. Franco-Saenz R, Shen P, Lee SJ, Cicila GT, Henrich WL: Regulation of the genes for 11β-hydroxysteroid dehydrogenase type 1 and type 2 in the kidney of the Dahl rat. *J Hypertens* 1999; **17**: 1089–1093.
31. Hadoke PWF, Christy C, Kotelevtsev YV, *et al*: Endothelial cell dysfunction in mice after transgenic knockout of type 2, but not type1, 11β-hydroxysteroid dehydrogenase. *Circulation* 2001; **104**: 2832–2837.
32. Hardy DB, Pereria LE, Yang K: Prostaglandins and leukotriene B_4 are potent inhibitors of 11β-hydroxysteroid dehydrogenase type 2 activity in human choriocarcinoma JEG-3 cells. *Biol Reprod* 1999; **61**: 40–45.
33. Doi M, Shichiri M, Yoshida M, Marumo F, Hirata Y: Suppression of integrin α_v expression by endothelin-1 in vascular smooth muscle cells. *Hypertens Res* 2000; **23**: 643–649.
34. Tan DY, Meng S, Cason GW, Manning RD: Mechanisms of salt-sensitive hypertension: role of inducible nitric oxide synthase. *Am J Physiol* 2000; **279**: R2297–R2303.
35. Ni Z, Oveisi F, Vaziri ND: Nitric oxide synthase isotype expression in salt-sensitive and salt-resistant Dahl rats. *Hypertension* 1999; **34**: 552–557.
36. Sun K, Yang K, Challis JRG: Differential regulation of 11β-hydroxysteroid dehydrogenase type 1 and 2 by nitric oxide in cultured human placental trophoblast and chorionic cell preparation. *Endocrinology* 1997; **138**: 4912–4920.
37. Hatakeyama H, Inaba S, Miyamori I: 11β-Hydroxysteroid dehydrogenase activity in human aortic smooth muscle cells. *Hypertens Res* 2001; 24: 33–37.
38. Brem AS, Bina RB, King TC, Morris DJ: Localization of 2 11β-OH steroid dehydrogenase isoforms in aortic endothelial cells. *Hypertension* 1998; **31**:459–462.