Uninephrectomy reduces 11\textbeta\text{-hydroxy}steroid dehydrogenase type 1 and type 2 concomitantly with an increase in blood pressure in rats

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Abstract

Renal allograft donors are at risk of developing hypertension. Here, we hypothesized that this risk is at least in part explained by an enhanced intracellular availability of 11\textbeta\text{-hydroxy}glucocorticoids due to an increased 11\textbeta\text{-hydroxy}steroid dehydrogenase type 1 enzyme (11\textbeta-HSD1), an intracellular prereceptor activator of biologically inactive 11-ketocorticoids in the liver, and/or a diminished 11\textbeta\text{-hydroxy}steroid dehydrogenase type 2 (11\textbeta-HSD2), an inactivator of 11\textbeta\text{-hydroxy}glucocorticoids in the kidney. To test this hypothesis, uninephrectomized (UNX) (n = 9) and sham-operated (n = 10) adult Sprague–Dawley rats were investigated. Mean arterial blood pressure and heart rate were measured continuously by telemetry for 6 days in week 5 after UNX. The mRNA of 11\textbeta-Hsd1 and 11\textbeta-Hsd2 in liver and kidney tissues were assessed by RT-PCR and the 11\textbeta-HSD activities were directly quantified in their corresponding tissues by determining the ratios of (tetrahydrodehydrocorticosterone / 5\alpha\text{-tetrahydrocorticosterone})/ and of corticosterone/dehydrocorticosterone (B/A) by gas chromatography–mass spectrometry. The apparent total body activities of 11\textbeta-HSD1 and 11\textbeta-HSD2 were estimated using the urinary and plasma ratios of (THB + 5\alpha-THB)/THA and B/A. Mean arterial blood pressure was increased after UNX when compared with sham operation. Hepatic mRNA content of 11\textbeta-Hsd1 and hepatic, plasma, and urinary ratios of (THB + 5\alpha-THB)/THA were decreased after UNX, indicating diminished access of glucocorticoids to its receptors. In renal tissue, 11\textbeta-Hsd2 mRNA was reduced and B/A ratios measured in kidney, plasma, and urine were increased, indicating reduced 11\textbeta-HSD2 activity and enhanced access of glucocorticoids to mineralocorticoid receptors. Both 11\textbeta-Hsd1 and 11\textbeta-Hsd2 are downregulated after UNX in rats, a constellation considered to induce hypertension. Journal of Endocrinology (2012) 214, 373–380

Introduction

The kidneys regulate body fluid volume and electrolyte balance and therefore play a key role in blood pressure control. This homeostasis is disturbed by removing one kidney. Thus, living kidney donors place themselves at risk for the benefit of someone else, and for that they deserve special attention with respect to potential complications following kidney donation. Even though uninephrectomy (UNX) is considered to have minimal adverse effects on overall health status, a meta-analysis revealed an increase in blood pressure of 5 mmHg over 5–10 years beyond that associated with normal aging and an increased risk for hypertension (Boudville et al. 2006, Davis 2009, Ibrahim et al. 2009). The mechanisms underlying arterial hypertension after UNX are poorly understood and investigations trying to identify these mechanisms after UNX are scarce. Talseth et al. (1986) observed a correlation between the decrease in glomerular filtration rate (GFR) and the increment in arterial pressure after UNX, suggesting a renal mechanism for this type of hypertension. Interestingly, Mimran et al. (1993) reported a decline in plasma renin activity in individuals with an increase in blood pressure after UNX, a finding tentatively explained by volume expansion as a consequence of a decreased GFR. The molecular mechanism of the presumed volume expansion is unclear as plasma aldosterone concentrations did not increase following UNX in kidney donors with hypertension. Furthermore, these aldosterone concentrations were not different from those in kidney donors without hypertension after UNX, exhibiting a similar degree of renal function impairment. Thus, a mechanism other than increased aldosterone concentrations might account for arterial hypertension after UNX.

Cellular cortisol availability is modulated by the two 11\textbeta\text{-hydroxy}steroid dehydrogenase enzymes, 11\textbeta-HSD1 and 11\textbeta-HSD2 (Lakshmi & Monder 1988, Albiston et al. 1994, Draper & Stewart 2005). 11\textbeta-HSD1 is strongly expressed in liver tissue and converts inactive cortisone into active cortisol. Overexpression of 11\textbeta-HSD1 in the liver induced hypertension by an unknown mechanism (Paterson et al. 2004). 11\textbeta-HSD2 on the contrary is expressed in the renal cortical collecting duct where it catalyzes the reaction of cortisol into cortisone and by this mechanism protects the mineralocorticoid receptor (MR) from promiscuous activation by...
endogenous glucocorticoids (Edwards et al. 1988, Funder et al. 1988). A decreased 11β-HSD2 activity was shown to induce hypertension in both rodents and humans (Kotelevtsev et al. 1999, Atanasov et al. 2007, Ferrari 2010). Here we hypothesize that UNX induces a dysregulation of 11β-HSD1 and/or 11β-HSD2, which might explain at least in part the changes in blood pressure after UNX.

Materials and Methods

Experimental animals

Experiments were performed in male Sprague–Dawley rats (Charles River Laboratories, Sulzfeld, Germany). The experiments were approved by the Ethical Committee for Animal Experiments of the Veterinary Department of the University of Bern. Eight-week-old animals were uninephrectomized (UNX) or sham-operated and all animals were implanted with a blood pressure telemetry device, TA11PA-C40 (Data Sciences International, St Paul, MN, USA). After the surgery, they were fed ad libitum with ordinary rat chow containing 0.4% sodium for 5 weeks (Provimi Kliba AG, Kaiseraugst, Switzerland). For blood pressure recording and 24 h urine collections, the rats were housed individually in metabolic cages during week 5 after surgery. On day 6 of the metabolic cage study period, the rats were killed and both kidneys and the liver were immediately frozen in liquid nitrogen and kept at −70°C. Plasma was separated by centrifugation and stored at −20°C. For quantification of activity and mRNA of 11β-HSD1 and 11β-HSD2, tissues were powdered with a mixture of dry ice and acetone using mortar and pestle.

Surgery and blood pressure measurements

UNX was performed by removing the right kidney. The renal vessels and the ureter were isolated and a single ligature was placed around them and tied tightly. The distal portions were then cut and the kidney removed. During the same surgery, the implantation of the telemetry device was performed as described previously (Schumacher et al. 2002). Mean arterial pressure was measured continuously for 10 s every 15 min during 6 consecutive days.

Measurement of corticosterone metabolites in urine, plasma, and tissue

The apparent activities of 11β-HSD2 and 11β-HSD1 were assessed in vivo by measuring the ratios of Table 1

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Sham (n=10)</th>
<th>UNX (n=9)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body weight (g)</td>
<td>441 ± 11</td>
<td>434 ± 8</td>
</tr>
<tr>
<td>Weight per kidney (g)</td>
<td>2.0 ± 0.1</td>
<td>2.5 ± 0.1*</td>
</tr>
<tr>
<td>Plasma</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sodium (mmol/l)</td>
<td>129.6 ± 1.4</td>
<td>133.8 ± 2.2</td>
</tr>
<tr>
<td>Potassium (mmol/l)</td>
<td>4.8 ± 0.6</td>
<td>4.5 ± 0.2</td>
</tr>
<tr>
<td>Urea (mmol/l)</td>
<td>6.3 ± 0.4</td>
<td>8.4 ± 0.2</td>
</tr>
<tr>
<td>Creatinine (µmol/l)</td>
<td>31 ± 2</td>
<td>37 ± 2</td>
</tr>
<tr>
<td>Urine</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Urine volume (ml)</td>
<td>18 ± 1</td>
<td>15 ± 1</td>
</tr>
<tr>
<td>Protein (mg/24 h urine)</td>
<td>8.8 ± 0.7</td>
<td>9.6 ± 0.7</td>
</tr>
<tr>
<td>Sodium (mmol/24 h urine)</td>
<td>1.49 ± 0.06</td>
<td>1.41 ± 0.05</td>
</tr>
<tr>
<td>Potassium (mmol/24 h urine)</td>
<td>1.48 ± 0.06</td>
<td>1.47 ± 0.04</td>
</tr>
<tr>
<td>Chloride (mmol/24 h urine)</td>
<td>3.3 ± 0.2</td>
<td>3.2 ± 0.2</td>
</tr>
<tr>
<td>Creatinine (µmol/24 h urine)</td>
<td>126 ± 3</td>
<td>112 ± 3</td>
</tr>
<tr>
<td>Clearance</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Creatinine clearance (ml/min)</td>
<td>3.0 ± 0.3</td>
<td>2.2 ± 0.1*</td>
</tr>
<tr>
<td>Creatinine clearance/g of kidney tissue</td>
<td>0.72 ± 0.06</td>
<td>0.91 ± 0.08</td>
</tr>
</tbody>
</table>

*P<0.012 vs sham; †P=0.003 vs sham; ††P=0.006 vs sham.
corticosterone/dehydrocorticosterone (B/A) and (tetrahydrocorticosterone + 5α-tetrahydrocorticosterone)/tetrahydrodehydrocorticosterone (THB + 5α-THB)/THA) respectively. The metabolites were analyzed by gas chromatography–mass spectrometry according to the method described by Shackleton (1993).

**Measurement of protein content**

For 11b-HSD1, 50 μg liver homogenates were separated by PAGE. Proteins were electroblotted onto nitrocellulose membranes, blocked with 5% dry milk, and incubated with an 11b-HSD1 antibody at a dilution of 1:200 in 2% dry milk (Cayman, Ann Arbor, MI, USA). For 11b-HSD2, 300 μg kidney homogenates were separated by PAGE. Proteins were electroblotted onto PVDF membranes, blocked with 5% dry milk, and incubated with an 11b-HSD2 antibody at a dilution of 1:3000 in 5% dry milk (Chemicon, Billerica, MA, USA). For internal standard, GAPDH was used on the same membranes at a dilution of 1:10 000 in 2% dry milk. Bands were visualized using the enhanced chemiluminescence kit (Amersham) and quantified by densitometry using Image J.

**Measurement of aldosterone**

Aldosterone concentrations in plasma were quantified by a commercially available ELISA Kit (Uscn Life Science, Inc., Wuhan, China).

**Extraction of RNA and quantification by RT-PCR**

Total RNA was isolated from powdered tissue samples using Trizol Reagent (Invitrogen). Reverse transcription was performed with ImProm-II Reverse Transcriptase (Promega). For real-time PCR, the following primers and probes from Applied Biosystems were used: 11b-HSD1 (Rn00567167_m1), 11b-HSD2 (Rn00492539_m1), and GAPDH as an internal standard (4352338E).

**Statistical analysis**

Values are expressed as mean±S.E.M. Statistical differences between the groups were assessed by unpaired t-test or two-way ANOVA followed by Bonferroni post-tests. Statistical significance was indicated by a value of P<0.05 or better.

**Results**

At the beginning of the study, rats used for UNX or sham-operation had similar body weights (data not shown). Five weeks after surgery the body weight was slightly, albeit not significantly, decreased in the UNX group (Table 1). The weight of the remaining kidney after UNX was increased when compared with the weight of the corresponding kidney of sham-operated rats. The GFR, as assessed by creatinine was lower in UNX than sham-operated rats. The creatinine clearance divided by the total kidney weight increased after UNX, suggesting glomerular hyperfiltration. The decreased GFR was also reflected by an increased plasma urea.
concentration after UNX (Table 1). The urinary protein excretion was slightly, but not significantly, increased after UNX, whereas the urinary excretion of sodium, potassium, and chloride was the same in both groups.

Telemetric measurements

Blood pressure was measured continuously (96 measurements/day/rat) for 6 days in week 5 after surgery. Blood pressure was significantly higher in UNX than in sham-operated animals on all 6 days of blood pressure recording (Fig. 1A). Heart rate declined slightly during the stay in the metabolic cages in both groups (Fig. 1B). The heart rate was higher every day in the UNX when compared with the sham-operated rats.

Assessment of 11β-HSD1

In order to analyze the contribution of 11β-HSD to the observed changes in blood pressure, the apparent activity of 11β-HSD1 in sham-operated and UNX rats was measured in urine on day 6 (Fig. 2C). An increased (THB+5α–THB)/THA ratio indicates an enhanced 11β-HSD1 activity. Simultaneous measurements of the corticosterone metabolites in liver tissue and plasma showed that UNX induced a decline in the 11β-HSD1 activity (Fig. 2A and B). In line with these observations, the mRNA levels of 11β-HSD1 were decreased in liver tissue of UNX rats (Fig. 3A). The relative amount of 11β-HSD1 protein in liver tissue was not affected by UNX (Fig. 3B and C). 11β-HSD1 activity and mRNA levels were also assessed in kidney tissue extracts. Both parameters remained unchanged after UNX (data not shown). The corticosterone concentrations in urine (1305±339 vs 1383±433 ng/24 h) and plasma (187±19 vs 181±19 ng/ml) were not different when measured in sham-operated and UNX rats. Similarly, the aldosterone concentrations in plasma were of the same magnitude in both groups (417±86 vs 444±122 pg/ml), an observation in line with a previous investigation with a decreased 11β-HSD2 activity in rats (Tang et al. 2011). Thus, a change in systemic or urinary concentrations of mineralocorticoids cannot explain the difference in mineralocorticoid action after UNX as shown in Fig. 1.

Assessment of 11β-HSD2 and corticosterone and aldosterone concentrations

As the kidney expresses preferentially 11β-HSD2, the effect of UNX on the apparent 11β-HSD2 activity was determined by measuring B/A in kidney tissue as well as in plasma and urine (Fig. 4). The 11β-HSD2 enzyme converts corticosterone (B) to dehydrocorticosterone (A). Thus, an increased B/A ratio reflects a decreased 11β-HSD2 activity. The B/A ratio increased in kidney tissue, plasma, and urine after UNX (Fig. 4A, B and C). These observations suggest a decreased 11β-HSD2 activity, a finding corroborated by the decline of 11β-Hsd2 mRNA expression after UNX (Fig. 5A). The relative amount of renal 11β-HSD2 protein was not affected by UNX (Fig. 5B and C). 11β-HSD2 activity and mRNA levels were also assessed in liver tissue extracts. Both parameters remained unchanged after UNX (data not shown).

Discussion

The present investigation was designed to assess the effect of UNX on the activities of 11β-HSD enzymes and blood pressure. The results indicate a decrease in 11β-HSD1 and 11β-HSD2 activities and an increase in blood pressure following UNX. The urinary (THB+5α–THB)/THA ratio is considered, at least by some authors, to be a measure of the activity of 11β-HSD1 (Palermo et al. 1996, Best & Walker 1997). This value was decreased after UNX, suggesting a decreased overall activity of 11β-HSD1 in the body. The urinary decline of (THB+5α–THB)/THA was paralleled by a similar change in plasma. The (THB+5α–THB)/THA ratios in urine and plasma might not only depend on the activity of 11β-HSD1 but also on 11β-HSD2 expression in cortical collecting ducts and possibly other enzymes. Therefore, we assessed this steroid metabolite ratio.
directly in liver tissue where no 11β-HSD2 is expressed. The analysis indicated again a decreased 11β-HSD1 activity, a conclusion in line with the decreased 11β-Hsd1 mRNA expression in liver tissue.

11β-HSD1 is a bidirectional enzyme in tissue homogenates that converts 11β-hydroxysteroids into 11-ketosteroids and vice versa. In intact cells, including hepatocytes, 11β-HSD1 acts predominately as an 11β-reductase (Low et al. 1994, Jamieson et al. 1995). Thus, the present finding of a decreased 11β-HSD1 activity indicates a decreased intracellular availability of biologically active 11β-hydroxyglucocorticoids in the liver of UNX rats. The evidence of the biological relevance of a diminished 11β-Hsd1 gene expression for glucocorticoid action was best demonstrated in 11β-Hsd1 null mice (Kotelevtsev et al. 1997). These mice were unable to regenerate B (corticosterone) from inert A (dehydrocorticosterone), proving the unique reductase activity of this enzyme in vivo. As a consequence of the 11β-HSD1 deficiency, an attenuated gluconeogenic response on stress, a resistance to hyperglycemia induced by chronic high fat feeding, an increased insulin sensitivity, lower plasma triglyceride levels, and increased high-density lipoprotein cholesterol concentrations have been observed (Kotelevtsev et al. 1997, Morton et al. 2001). Our finding of a decreased 11β-HSD1 activity following UNX might therefore be considered beneficial and potentially protective for the development of the metabolic syndrome, a disease state recently tentatively treated by specific inhibitors of 11β-HSD1 (Ge et al. 2010).

To protect the MR from glucocorticoid occupancy in mineralocorticoid target tissues, the 11β-HSD2 enzyme converts biologically active 11β-hydroxyglucocorticoids to inactive 11-ketocorticosteroids. In this study, this enzyme appears to be decreased after UNX, as evidenced by increased B/A ratios in urine, plasma, and kidney tissue and a diminished 11β-Hsd2 mRNA expression in kidney tissue, observations in line with the previously reported increased renal tissue ratio of the exogenous prednisolone to prednisone 1 day after UNX in rats (Escher et al. 1998b). The protein content and activity of 11β-HSD2 expressed per microgram of kidney tissue remained unchanged after UNX. These results have to be interpreted by considering the total mass of kidney tissue in the body of the two groups of rats investigated. After UNX, the weight of the remaining kidney increased from 2 to 2.5 g, whereas the total body weight of the animals remained constant. Thus, the total amount of kidney tissue available for converting B to A was 4 g in sham and 2.5 g in UNX rats. Therefore, the total amount of renal 11β-HSD2 protein and activity per animal were increased by a factor of 1.6 in sham when compared with UNX rats. This might explain the higher urinary B/A ratio in UNX than in

Figure 4 11β-HSD2 in sham-operated or uninephrectomized rats 4 weeks after surgery. An estimate of 11β-HSD2 obtained by measuring the steroid metabolite ratio B/A by gas chromatography-mass spectrometry in kidney tissue (A), plasma (B), and 24 h urine (C). An increased ratio indicates a decreased 11β-HSD2 activity. B indicates corticosterone; A, dehydrocorticosterone. n=7 per group. Values are expressed as mean±s.e.m. *P=0.002 vs sham; **P<0.0001 vs sham.
sham-operated rats. As the kidney is the principal organ for converting B to A, similarly the B/A ratio was increased after UNX in plasma. A decreased 11β-HSD2 activity enhances activation of the MR with consecutive renal sodium retention and potassium excretion. In line with such an effect, sodium concentrations in plasma tended to increase, those of potassium to decrease (Table 1), and blood pressure to increase after UNX (Fig. 1).

Both changes of 11β-HSD1 and 11β-HSD2 activity have been linked to arterial hypertension. Transgenic mice expressing increased 11β-HSD1 activity selectively in the liver under the transcriptional control of the hepatic regulatory sequence derived from the human APOE gene exhibited increased arterial pressure by a mechanism that has not been elucidated so far (Paterson et al. 2004). Since in our animals with UNX we observed increased blood pressure and 11β-HSD1 downregulation in the liver, this enzyme is unlikely to account for the increase in blood pressure after UNX. More likely the blood pressure increase is explained by a diminished activity and expression of 11β-HSD2, the enzyme that protects the renal MR from activation by endogenous glucocorticoids (Edwards et al. 1988, Funder et al. 1988). One might finally hypothesize that another 11β-steroid dehydrogenase, possibly 11β-HSD3, or another steroid metabolizing enzyme could additionally be involved in blood pressure regulation. In a review considering the phenotype descriptions of all genetically modified animals with specific over- or under-expression in the kidney after UNX modulates the 11β-HSD activity in the remaining kidney (Recordati et al. 2000).

The increased heart rate as a consequence of a down-regulation of 11β-HSD2 is an expected finding. Inhibition of 11β-HSD2 by carbenoxolone was shown to enhance the heart rate in rats (Zhang et al. 2006). Furthermore, urinary catecholamine levels were double in 11β-Hsd2−/− mice (Bailey et al. 2008). Thus, the increased blood pressure following UNX might be the consequence of both an enhanced renal sodium retention and an increased sympathetic drive, a hypothesis of interest as it is conceivable that the dampened renal efferent nerve activity to the contralateral kidney after UNX modulates the 11β-HSD activity in the remaining kidney (Recordati et al. 2000).

The gene expression in the remaining kidney after UNX is complex and was first investigated by Kujubu et al. (1991) 20 years ago. These authors identified transient (up to 1 h) changes in the expression of 12-O-tetradecanoylphorbol-13-acetate-inducible sequences genes and concluded that compensatory renal hypertrophy is a distinct form of cell growth that does not share the pattern of early gene expression observed in several examples of mitogenesis or differentiation. Thus, time-course studies after UNX in the remaining kidney must be performed in order to identify relevant regulatory factors for the expression of 11β-HSD1 and 11β-HSD2. A number of factors regulating the expression of 11β-HSD enzymes have previously been identified including TNF-α, Erg1, NF-κB, NF1, C/EBPs, GR, Sp1/Sp3, Arnt, PPAR-α/PPAR-γ, IL1, glucocorticoids, sex steroids, and thyroid hormones (Alikhani-Koupaai et al. 2004, Tomlinson et al. 2004, Kostadinova et al. 2005, Alikhani-Koupaai et al. 2007, Wake et al. 2007, Ignatova et al. 2009). The ultimate proof for a pivotal relevance of one or a combination of these factors would require the investigation of animals with specific over- or under-expression in the cortical collecting duct. Another approach might be to

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**Figure 5** In line with a decreased activity of 11β-HSD2, the corresponding mRNA expression in kidney tissue declined ***P=0.011 vs sham (A). The amount of 11β-HSD2 protein in kidney tissue did not change following UNX (B and C).
perform microarray studies in UNX animals in order to identify yet unknown regulatory factors of 11β-HSD1 and 11β-HSD2.

Five weeks after uninephrectomy, blood pressure was higher in UNX than in sham-operated animals. UNX performed in immature rats was associated with an increase in blood pressure in virtually all studies (Woods 1999, Carlstrom et al. 2007). On the other hand, UNX in adult rats without concomitant administration of a high salt diet and/or an excess of a mineralocorticoid exhibits a variable effect on blood pressure (Deen et al. 1974, Pupilli et al. 1992). The variable effect of UNX is poorly understood, as data of the time course of changes following UNX are scarce – a deficiency also true for this study. As a corollary, the association between changes of 11β-HSD and blood pressure might only be tentatively interpreted as being causal at the end of the study.

In the past, ablation of renal mass has been performed in order to analyze within a short time the mechanisms explaining progressive glomerular injury and/or to induce severe hypertension (Hostetter et al. 1981, Anderson et al. 1985). By contrast, UNX is a model causing only modest changes that were not of interest for the purpose of the investigations in the past. It is also conceivable that some modest changes, such as a slight increase in blood pressure, were missed with the methodology available in those days or appeared only after a prolonged time of observation. In humans, the observation time after UNX is extremely long as life expectancy of healthy organ donors before donation is by definition even longer than that of the general population. Therefore, subtle changes in renal function and/or blood pressure might become clinically relevant only over time. As a corollary, the modest but steady increase in blood pressure and the changes of enzymes determining endogenous glucocorticoid metabolites at a cellular level deserve consideration in human kidney donors in the future.

Based on this study, it appears that a reduced 11β-HSD2 activity acts as a contributing factor for the development of hypertension in UNX animals by activating the MR pathway in the cortical collecting duct. Therefore, the inhibition of the MR or downstream effectors such as the epithelial sodium channel using agents such as eplerenone/spironolactone or amiloride/benzamil, respectively, might lower blood pressure in UNX animals. Such an effect would not be completely specific for glucocorticoid-mediated MR activation, as other MR-activating agents including aldosterone are still present after UNX. Nevertheless, if the observation of a decreased 11β-HSD2 activity following UNX is also true in humans, it is conceivable that the treatment with MR antagonists might be clinically beneficial. Even though this therapeutic concept appears to be rational, it would need confirmation in a clinical study.

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**References**


**Declaration of interest**

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.


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