Modulation of 11β-hydroxysteroid dehydrogenase 1 by β2-adrenoceptor in the ischaemia-reperfused rat kidney

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Abstract

Background. 11β-Hydroxysteroid dehydrogenase Type 1 (11βHSD-1) amplifies intracellular levels of active glucocorticoids which possess protective effects against organ ischaemia and reperfusion (I/R). However, the mechanisms by which 11βHSD-1 is modified after a renal I/R challenge remain unclear. This study investigated the effect of β2-adrenoceptor (β2-AR) activation and the subsequent signalling pathways on renal 11βHSD-1 gene expression following renal I/R.

Methods. Renal I/R was induced using 25 min of bilateral renal artery occlusion in 4-week-old Wistar rats followed by an intraperitoneal injection of various doses of adeno-β2-AR gene. Following renal I/R, kidneys, plasma and urine were collected to assay 11βHSD messenger RNA (mRNA) levels, β2-AR signalling cascades and renal function.

Results. On the second day after the renal I/R challenge, there was a reduction in renal 11βHSD-1 mRNA levels associated with a decrease in stimulatory G protein α (Gsα) and adenylyl cyclase-1 (ACY-1) in the kidney. The addition of the adeno-β2-AR gene resulted in greater increases in 11βHSD-1 mRNA and β2-AR–Gsα–ACY–cyclic adenosine monophosphate (cAMP)–protein kinase A (PKA) activity in the kidney but had no effect on 11βHSD-2 mRNA or protein kinase C levels in the kidney.

Conclusions. Over-expression of β2-AR resulting from the gene delivery improved renal function and 11βHSD-1 production following renal I/R, which were actions exerted through the cAMP–PKA pathway. The stimulatory effect of functional β2-AR activation on renal 11βHSD-1 expression may offer a means of protection from renal I/R injury.

Keywords: adenovirus; cAMP–PKA; glucocorticoid; kidney; rat

Introduction

11β-Hydroxysteroid dehydrogenase (11βHSD) enzymes are expressed in two isoforms that reversibly interconvert glucocorticoids (cortisol and corticosterone) and inactive 11-oxoestroid precursors (cortisone and 11 hydrocortisone) [1]. 11βHSD-1 is widely distributed and acts as a pre-receptor mechanism in the local activation of glucocorticoid receptors (GRs), whereas, 11βHSD-2 enables selective access of aldosterone to the mineralocorticoid receptor. Importantly, 11βHSD-1 functions as a determinant of glucocorticoid action in several organs [2–6]. In the kidney, 11βHSD-1 has been reported to be expressed in the renal proximal tubule and the medullary interstitial cells [5, 6] and is likely to be a factor that permits activation of circulating glucocorticoids to act on this segment during nephron maturation. Indeed, the activity of 11βHSD-1 increases during development [7] and with exposure to a high salt diet [8]. While the physiological role of 11βHSD-1 in the kidney has been previously investigated by Brem et al. [7–9], the mechanism by which 11βHSD-1 in the kidney is modified in pathophysiological states such as acute kidney injury (AKI) remains uncertain at present.

The classical pathways of β2-AR agonist and glucocorticoid action suggest multiple ways by which drugs may interact at a cellular level [10]. It has been demonstrated that glucocorticoids interact with β2-adrenoceptor (β2-AR) agonists through the cyclic adenosine monophosphate (cAMP)–protein kinase A (PKA) pathway [11]. For example, glucocorticoids increase the number of β2-ARs and their coupling with stimulatory G protein α (Gsα), while β2-ARs induce GR nuclear translocation, activate transcription factor/enhancer-binding protein C/EBPα together with glucocorticoids or phosphorylate corticosteroid receptors [12]. However, it is likely that there are many interactions between β2-AR signalling cascades and glucocorticoid metabolism that have not been covered. Recently, Tetsuka et al. [13] demonstrated that pharmacological activators of PKA (forskolin and dibutyryl cAMP) stimulated 11βHSD-1 messenger RNA (mRNA) expression in functionally mature granulosa cells. This may imply that PCA activation via stimulation of β2-ARs modulates renal 11βHSD-1 gene expression. In particular, it has been found that both renal β2-AR and 11βHSD-1 co-localize in human kidney proximal tubules [9, 14]. β2-ARs in normal rats are also predominantly localized to the proximal tubule and, to a lesser extent, distal tubular epithelia and the membranes of smooth muscle cells from renal arteries [5, 6]. Thus, these findings suggest the possibility of a direct functional and physical association...
between PKA activation via β2-AR and 11βHSD-1 expression in the kidney.

Activation of cAMP–PKA is potentially beneficial in preventing the renal dysfunction associated with AKI [15–17]. Recently, we showed that constitutive β2-AR activation through the cAMP–PKA pathway in the kidney was a critical effector in the host defense against the deterioration in cellular responses to AKI [18]. On the other hand, there is increasing evidence that glucocorticoids play an important role in organ protection against renal ischaemia/reperfusion (I/R) injury [19, 20]. From this evidence, we hypothesized that stimulation of the cAMP–PKA signalling pathway via functional β2-AR activation, during renal I/R, would increase kidney tissue 11βHSD-1 levels and glucocorticoid action, resulting in kidney protection. With this in mind, the current study was undertaken to explore whether constitutive β2-AR activation by means of β2-AR gene delivery might be able to up-regulate 11βHSD-1 expression in the kidney following renal I/R injury and to prevent the progressive renal damage associated with renal I/R.

Materials and methods

Reagents

AST (aspartate aminotransferase) and ALT (alanine aminotransferase) were assayed with SpotchemTMII (Arakay, Kyoto, Japan). Rabbit or mouse polyclonal antibodies against Gsα, inhibitory G protein α (Gia), β-actin and adenylate cyclase 1 (ACY-1) were purchased from Santa Cruz Biotech Inc. (Santa Cruz, CA), Peprotech (London, UK) and Fab-Gennix Inc. (Fisco, TX). The BioMatcher homogenizer was manufactured by Funakoshi Co. (Tokyo, Japan). Mem-PER Eukaryotic Membrane Protein Extraction Reagent kit, T-PER Tissue Protein Extraction Reagent kit, Halt Protease Inhibitor Cocktail and Micro BCA Protein Assay Reagent kit were purchased from PIERCE Inc. (Rockford, IL). ECF Western Blotting Reagent Packs were obtained from Amershams Biosciences UK (Little Chalfont, UK). Protein kinase C (PKC), PKA and cAMP ELISA kits were obtained from Stressgen Bioreagents Co. (Ann Arbor, MI), Cayman Chemical Co. and Assay Designs Inc., respectively. High Pure RNA Tissue Kit and Transcripter first-strand cDNA synthesis kits were obtained from Roche Diagnostics Co. (Tokyo, Japan). β-Galactosidase Enzyme Assay kits were purchased from Promega Co. (Madison, WI). Unless stated, reagents were from Sigma Chemical Co. (St. Louis, MO).

Construction of recombinant adenovirus

The adenov-β2-AR and the cytoplasmic β-galactosidase-expressing adenovirus (adeno-Lac Z) were a kind gift from Dr Walter J. Koch and Dr I. Wada (Department of Biochemistry in Duke University Medical Center, Durham, NC). These adenoviruses were a replication-deficient first-generation type V adenovirus with deletions of the E1 and E3 genes as described previously [21]. Virus isolates of adenov-β2-AR and adenov-Lac Z were plaque purified and propagated in 293 cells, gifted by Dr I. Wada (Department of Biochemistry, Sapporo Medical University, Sapporo, Japan), isolated, concentrated and titred using a Adenovirus Expression Vector kit.

Rat preparation and protocols

All procedures and protocols were approved by the Teikyo University Guide for the Care and Use of Laboratory Animals. Four-week-old Wistar rats were fed a standard laboratory diet (126 mEq of Na+/kg and 118 mEq of K+/kg food) and had free access to water. After a 7-day acclimatization period, the rats were anaesthetized with pentobarbitone (50 mg/kg) intraperitoneally (i.p.). The ischaemic rat model of I/R was induced by occluding both renal arteries as described in detail previously [22]. Briefly, a midline abdominal incision was made and both renal arteries were occluded using non-traumatic vascular clamps, after which the clamps were removed from the renal arteries and the abdominal incision was sutured (AKI group). The control group (Cont.) group of rats was subjected to a sham operation. The AKI+β2 groups received 1 mL of various doses of adenov-β2-AR gene [AKI+β2 (L) group: 109, AKI+β2 (M) group: 5 × 108 or AKI+β2 (H) group: 107 total adenoviral particles (t.v.p.)], i.p. On the other hand, the Cont. and AKI groups of rats received 1 mL of adenov-Lac Z (109 t.v.p.) i.p. All groups of rats were then allowed to recover from the anaesthesia without further interventions. From 1 to 3 days later, animals were housed in metabolic cages for 24 h to collect urine samples. Thereafter, the animals were given an overdose of pentobarbital, blood samples were collected and the kidneys were removed, weighed and immediately frozen at −70°C.

Evaluation of gene transfer

To estimate whether β2-AR density and PKA activity in the kidney were changed dependent on the duration of the ischaemic insult, adenov-β2-AR (109 t.v.p)–treated rats were killed after 0, 25 and 45 min of renal ischaemia (each n = 4–5). Furthermore, to assess the efficacy of adenovirus-mediated transfer after differing ischaemia times, β-galactosidase activity levels in the adenov-Lac Z (109 t.v.p.)–treated rats were measured after 0, 25 and 45 min of renal ischaemia (each n = 6).

Biochemical measurements

Creatinine, sodium and potassium levels were measured according to the protocols specified by the manufacturer (SRL, Tachikawa, Japan). Glomerular filtration rate was measured as creatinine clearance (CrCl: mL/min/100 g body weight). Serum and urinary sodium (Na) or potassium (K) concentrations were measured using a spectrophotometer (SRL). Fractional excretions of sodium (FENA) or potassium (FEK) were calculated from the serum and urine sodium, potassium and creatinine using the equation: 

\[
\text{FENA (K)} = \frac{(\text{Serum Na}) - (\text{Serum Na})}{(\text{Serum Na}) - (\text{Serum Na})} \times 100. 
\]

A comparable equation was used to calculate FEK. The analysis of renal tissue protein was performed on the homogenized samples extracted from the frozen kidney using the T-PER Tissue Protein Extraction Reagent. The frozen kidney samples were lysed using a liquid-phase extraction method [23], and the supernatants were taken for the analysis of cAMP, PKA and PKC activities using commercially available ELISA kits according to the manufacturer’s manual. PKA and PKC were expressed as the ratio to milligram of kidney protein. On the other hand, cAMP was normalized to gram of kidney weight.

Western blot analyses

Western blots for Gsα, Gia and ACY-1 were performed on membrane proteins or cytosolic proteins prepared from whole kidneys. Freshly isolated kidneys from rats were rapidly dissected and homogenized on ice in homogenization buffer (TBS + Halactone Protease Inhibitor Cocktail), using a BioMatcher homogenizer. The membrane proteins were extracted using Mem-PER Eukaryotic Membrane Protein Extraction Reagent kit and the cytosolic proteins were extracted with T-PER Tissue Protein Extraction Reagent and stored at ~20°C. Samples of 20–30 μg of these proteins were electrophoresed on 7.5 or 10% sodium dodecyl sulphate-polyacrylamide gels and blotted onto the polyvinylidene difluoride membrane. Membranes were blocked with ATTO blocking system (ATTO Co., Tokyo, Japan) for 1 h at room temperature. Thereafter, the membranes were incubated for 1 h with rabbit or mouse polyclonal antibody using ECF Western Blotting Reagent Packs and then were scanned by means of a fluorescent scanning instrument (Fluoro-imager S; Molecular Dynamics, Tokyo, Japan), which detected immunoreactive bands at 45–48 kDa (Gsa), 40 kDa (Gia), 120–125 kDa (ACY-1) or 42 kDa (β-actin). Densitometry analysis was performed using Image QuaNT software (Molecular Dynamics). Levels of each protein were expressed as the ratio to β-actin for each sample.

β2-AR-binding assay

Membrane fractions from all tissues were extracted following the method described by Lefkowitz et al. [21, 24] with minor modifications. Membrane preparations (25 μg) were incubated with 125I-cyanopindolol (15–315 nmol/L) in binding buffer [75 mMol/L Tris/HCl (pH 7.4), 12.5 mMol/L MgCl2 and 2 mMol/L ethylenediaminetetraacetic acid] either alone or with 20 μmol/L alprenolol, which was used for the determination of non-specific binding. The incubation was carried out at 37°C for 1 h in a total volume of 500 μL, followed by rapid filtration on GF/C filters and three washings with 750 μL of ice-cold binding buffer. β-AR density (B_max) was determined using linear regression analysis of
gene delivery was effective in providing sustained levels after the adeno-
levels. Importantly, on the 11β levels was normalized to milligram of membrane protein. The protein concentration was assayed using a Micro BCA Protein Assay kit.

β-Galactosidase assay

Kidneys were immersed in 2 mL ice-cold phosphate-buffered saline containing the protease inhibitors phenylmethylsulphonyl fluoride (1 U/mL) and aprotonin (0.2 U/mL). The kidney was then cut into small pieces and homogenized for 30 s in a BioMasher homogenizer. Homogenates were then stored at -80°C before further processing or directly lysed by five cycles of freeze–thawing. Tissue lysates were then centrifuged at 11,000 g for 5 min at 4°C, and the supernatant transferred to a new tube. Enzymatic activity was measured using commercially available β-Galactosidase Enzyme Assay kits according to the manufacturer’s manual. The optical density of each sample was then read at 420 nm with a spectrophotometer to determine activity. Activity was normalized for protein concentration, which was determined using a Micro BCA Protein Assay kit.

Quantitative real-time polymerase chain reaction

For real-time polymerase chain reaction (PCR) analysis, total RNA was extracted using the High Pure RNA Tissue Kit, reverse transcribed to first-strand cDNA synthesis kit and subjected to real-time PCR using the LightCycler 480 system (Roche Diagnostics). Real-time PCR primers and universal probes (Roche), which are listed in Table 1, were designed according to the Universal Probe Assay Design Center (http://www.roche-applied-science.com/sisrtpcr/up/adc.jsp). Relative expression compared with the internal control β-actin was determined according to a previous report [25].

Histology

To estimate immunological reactions and organ injury in response to I/R and/or adenoviral vector delivery, we performed a morphological analysis of haematoxylin and cosin (H&E)-stained kidney and liver tissue. Liver and kidney tissues were removed on the third day after the renal I/R challenge and fixed for 5–7 days in 4% paraformaldehyde in 0.1 M phosphate buffer, pH 7.4, then embedded into paraffin wax and cut to a thickness of 2-3 μm. Sections were stained with H&E using routine procedures.

Statistics

The results were expressed as mean ± SE. Statistical analysis was undertaken using the analysis of variance followed by a Bonferroni/Dunnett test for multiple comparisons.

Results

Figure 1A and B shows that the adeno-β2-AR (10⁹ t.v.p) gene delivery was effective in providing sustained levels of β2-AR density and PKA activity in the kidney for at least 25 min of renal ischaemia. However, in the rats subjected to 45 min of renal ischaemia, there was a significant reduction (P < 0.05) in β2-AR density and PKA levels. Importantly, on the first, second and third days after the adeno-β2-AR gene delivery, a similar pattern of changes in β2-AR density and PKA levels was observed dependent on the duration of the ischaemic insult. To quantify the transfection efficiency of adenoviral vector, kidneys were assessed for β-galactosidase activity. As seen in Figure 1C, while β-galactosidase levels in the kidney were sustained in the rats subjected to 25 min of ischaemia, the rats subjected to 45 min of ischaemia showed a significant reduction (P < 0.05) in β-galactosidase levels. From these preliminary studies, a time of 25 min of renal ischaemia was chosen as the optimal conditions for transfection efficiency for adeno-β2-AR gene in the AKI + β2(L–H) group rats to evaluate the effect of β2-AR gene delivery on 11βHSD-1 gene expression in the I/R kidney.

Figure 2 presents the time course of β2-AR mRNA, 11βHSD-1 mRNA and Ccr in the Cont., AKI and AKI + β2(H) group of rats during AKI over the 3 days post-I/R. In the AKI group of rats, β2-AR and 11βHSD-1 mRNA levels in the kidney remained at unchanged levels over the 1–3 days after the renal I/R challenge (Figure 2A and B). In contrast, in the AKI + β2(H) group of rats, the time course of changes in renal β2-AR mRNA was elevated (P < 0.05) by the adeno-β2-AR gene delivery and reached a peak level on the second day (Figure 2A). Moreover, administration of 10⁸–⁹ t.v.p of the AKI + β2(L, M, H) groups caused a concentration-dependent stimulation of β2-AR mRNA levels over the three days (data not shown). On the other hand, there was no statistical difference between the time course of changes in renal 11βHSD-1 mRNA levels in any of the groups (Figure 2B). However, administration of adeno-β2-AR of the AKI + β2(L, M, H) groups increased 11βHSD-1 mRNA levels on the first and second day in a concentration-dependent manner (data not shown). Ccr in both the AKI group and AKI + β2(H) group rats was depressed (P < 0.05) by the I/R challenge (Figure 2C). However, the magnitude of reduction in Ccr in the AKI + β2(H) group rats over the 3-day period was attenuated (P < 0.05) by the administration of the adeno-β2-AR gene.

Figure 3 shows the effect of β2-AR activation with adeno-β2-AR gene delivery on renal 11βHSD-1 and -2 mRNA levels on the second day after the I/R challenge. While I/R had no effect on renal 11βHSD-2 mRNA (Figure 3B), renal 11βHSD-1 mRNA levels were significantly (P < 0.01) depressed by the I/R challenge (Cont. versus AKI group; Figure 3A). Importantly, the suppression of 11βHSD-1 mRNA in the AKI group rats could be prevented by the addition of the adeno-β2-AR gene in a dose-dependent manner (AKI versus AKI + β2 group; Figure 3A). Figure 4 shows the changes in renal β2-AR mRNA and density in all groups of rats 2 days after the onset of AKI. It was

<table>
<thead>
<tr>
<th>Gene</th>
<th>Probe number</th>
<th>Forward primer (5‘−3’)</th>
<th>Reverse primer (5‘−3’)</th>
</tr>
</thead>
<tbody>
<tr>
<td>β1-AR</td>
<td>105</td>
<td>AGA GCA GAA GGC GCT CAA G</td>
<td>AGC CAG CAG AGC GTG AAC</td>
</tr>
<tr>
<td>β2-AR</td>
<td>40</td>
<td>TGC TAT CAC ATC GCC CTTC</td>
<td>ACC ACT CGG GCC TTAT TTC</td>
</tr>
<tr>
<td>11βHSD-1</td>
<td>1</td>
<td>AGA GAG TGC TTC AGA CCA G</td>
<td>GCC CCA GTG ACA ATC ACT TT</td>
</tr>
<tr>
<td>11βHSD-2</td>
<td>26</td>
<td>CGT CAC TCA AGG GGA CGT AT</td>
<td>AGG GGT ATG GCA TGT CTC C</td>
</tr>
</tbody>
</table>
evident from Figure 4A and B that the degree of β2-AR mRNA and density levels depended on the adeno-viral dose injected into the AKI + β2 groups of rats (AKI versus AKI + β2 group). On the other hand, β1-AR mRNA levels were not changed by the addition of the adeno-β2-AR gene (data not shown). Table 2 shows the changes in the tissue concentrations of different signalling proteins (Gsα, Giα, ACY-1, cAMP, PKA and PKC) involved in β2-AR activation in the kidney on the second day after the I/R challenge. In the AKI group rats, Gsα and ACY-1 levels in the kidney were significantly (P < 0.01) depressed on the second day after the onset of AKI. However, in the AKI + β2 group, the addition of adeno-β2-AR gene increased Gsα, ACY-1, cAMP and PKA levels in the kidney but did not change the levels of Giα and PKC (Table 2). Table 3 presents the changes of renal function (Ccr, FENa and FEK), body weight and liver function (AST and ALT) in all groups of rats. Ccr was depressed (by some 51%, P < 0.01) after the onset of AKI, but the levels in the AKI group were restored by the administration of the adeno-β2-AR gene. It can also be seen in Table 3 that in all groups, there were no differences between the body weights, FENa, FEK, AST or ALT levels over the course of the study.

Figure 5 showed that no significant difference in the liver and kidney histology was observed between the AKI group of rats (A and C) and the AKI + β2(H) group of rats (B and D). A 10^7 t.v.p dose of adeno-β2-AR [AKI + β2(H) group] did not produce any evidence of cellular deterioration or toxicity in the liver (B) and kidney (D) using this approach. On the other hand, renal histological findings in the ischaemic rat model showed a relative intact tubular epithelium, only small amounts of intraluminal cellular debris and an absence of cast formation (C and D).
The key finding arising from the present study was that over-activation of the β2-ARs in the kidney was potentially beneficial in preventing the renal dysfunction and the reduction in renal 11βHSD-1 mRNA following the I/R challenge. In response to the renal I/R injury, there was a down-regulation of renal 11βHSD-1 mRNA which was associated with a decrease in renal Gsα and ACY-1. Moreover, the up-regulation of renal 11βHSD-1 mRNA was correlated with the increase in β2-AR and its downstream cascades resulting from the delivery of the β2-AR gene, suggesting the possibility that β2-AR activation might be involved in the regulation of renal 11βHSD-1 production through the Gsα–ACY–cAMP–PKA signalling pathway.

The physiological and pathological mechanisms responsible for 11βHSD-1 production may involve a number of mediators. Some pro-inflammatory cytokines [interleukin (IL)-1β and tumour necrosis factor (TNF-α)], long-term hypoxia and salt-induced hypertension cause an increase in 11βHSD-1 gene expression [26–28], whereas growth hormone (acting via insulin-like growth factor I) and liver X-receptor agonists inhibit the expression [29, 30]. In the kidney, 11βHSD-1 expression in cultured rat glomerular mesangial cells is up-regulated by IL-1β and TNF-α, suggesting that 11βHSD-1 may modulate the anti-inflammatory effects of glucocorticoids at this site [31]. Previously, we have investigated in vivo the time course of changes in TNF-α, IL-6, IL-10 and nitric oxide (NO) in the systemic circulation and kidney after an I/R challenge and found that the ischaemic AKI challenge had no effect on either cytokine or NO levels [32]. Therefore, in the present study, it is unlikely that the inflammatory cytokines are a major key mediator of renal 11βHSD-1 gene expression. On the other hand, it was found that the effects of PKA vary from tissue to tissue and between species. In rat granulosa cells, forskolin increases 11βHSD-1 activity [13]. Conversely, forskolin inhibits the activity in 2S FAZA rat hepatoma cell [33]. The present study showed that renal 11βHSD-1 gene expression after the renal I/R challenge was stimulated by PKA activation via β2-AR signalling cascades. Importantly, the down-regulation of 11βHSD-1 mRNA was associated with a reduction in Gsα and ACY-1 after the renal I/R challenge. One interpretation of these findings is that there is a close relationship between the β2-AR signalling cascades and the 11βHSD-1 gene expression after the I/R challenge.

The present study provides evidence that renal 11βHSD-1 mRNA levels were decreased following the renal I/R injury. Down-regulation of renal 11βHSD-1 may be involved in the suppression of local glucocorticoid metabolism that occurs during AKI after renal I/R injury. Clinically, it has been considered that glucocorticoids are not effective as a therapeutic agent in critically ill subjects at high risk and who already have sepsis-induced AKI.

**Fig. 3.** Changes in renal 11βHSD-1 mRNA (A) and 11βHSD-2 mRNA (B) levels (units) on the second day after the onset of I/R-induced AKI. n = 5–9 (11βHSD-1 mRNA), n = 5–6 (11βHSD-2 mRNA). Data are means ± SE (units). *P < 0.01 versus Cont. group rats. **P < 0.01 versus AKI group rats.

**Fig. 4.** Changes in renal β2-AR mRNA (A: units) and β2-AR density (B: fmol/mg protein) levels on the second day after the onset of I/R-induced AKI. n = 5–6 (β2-AR mRNA), n = 4–6 (β2-AR density). Data are means ± SE. *P < 0.05 versus AKI group rats. **P < 0.01 versus AKI group rats.
Therefore, the findings would suggest that glucocorticoid action in the kidney is depressed after renal I/R injury. Similarly, an altered expression and/or function of $\beta_2$-ARs has been observed in renal I/R injury [32]. This was supported by evidence that fundamental alterations in the activity of $\beta_2$-ARs contribute to the deterioration of the immune system and accompany the development of failure in organ function [35–37].

Table 2. Changes in intracellular signalling cascades 2 days after the onset of AKI by the I/R challenge

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>Gs$\alpha$ (units)</th>
<th>Go$\alpha$ (units)</th>
<th>ACY-1 (units)</th>
<th>cAMP (pM/g kidney)</th>
<th>PKA (ng/mg protein)</th>
<th>PKC (ng/mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cont.</td>
<td>6</td>
<td>1.33 ± 0.2</td>
<td>2.45 ± 0.32</td>
<td>1.13 ± 0.08</td>
<td>91.2 ± 4.1</td>
<td>5.53 ± 0.77</td>
<td>1.56 ± 0.39</td>
</tr>
<tr>
<td>AKI</td>
<td>6</td>
<td>0.7 ± 0.21</td>
<td>2.21 ± 0.39</td>
<td>0.75 ± 0.12</td>
<td>74.0 ± 2.5</td>
<td>5.01 ± 0.52</td>
<td>1.96 ± 0.31</td>
</tr>
<tr>
<td>AKI + $\beta_2$(L)</td>
<td>4–6</td>
<td>1.07 ± 0.13</td>
<td>2.16 ± 0.18</td>
<td>0.79 ± 0.06</td>
<td>76.0 ± 9.2</td>
<td>5.43 ± 0.45</td>
<td>1.72 ± 0.07</td>
</tr>
<tr>
<td>AKI + $\beta_2$(M)</td>
<td>4–6</td>
<td>1.19 ± 0.14</td>
<td>2.07 ± 0.47</td>
<td>0.97 ± 0.04</td>
<td>81.5 ± 9.5</td>
<td>8.2 ± 0.4</td>
<td>1.80 ± 0.10</td>
</tr>
<tr>
<td>AKI + $\beta_2$(H)</td>
<td>4–6</td>
<td>1.41 ± 0.23</td>
<td>2.19 ± 0.43</td>
<td>1.19 ± 0.09</td>
<td>94.5 ± 13.2</td>
<td>13.21 ± 1.03</td>
<td>1.57 ± 0.15</td>
</tr>
</tbody>
</table>

Results are expressed as mean ± SE.

* $P < 0.01$ versus Cont.

$^{b}$ $P < 0.05$ versus AKI.

$^{c}$ $P < 0.01$ versus AKI.

Table 3. Changes in body weight, renal function and hepatic function 2 days after the onset of AKI by the I/R challenge

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>Weight (g)</th>
<th>FENa (%)</th>
<th>FEK (%)</th>
<th>Ccr (100 g weight)</th>
<th>AST (IU/L)</th>
<th>ALT (IU/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cont.</td>
<td>5–6</td>
<td>113 ± 2</td>
<td>0.44 ± 0.02</td>
<td>12.7 ± 0.5</td>
<td>1.08 ± 0.23</td>
<td>50 ± 7</td>
<td>15 ± 3</td>
</tr>
<tr>
<td>AKI</td>
<td>5–10</td>
<td>120 ± 9</td>
<td>0.37 ± 0.05</td>
<td>15.2 ± 1.0</td>
<td>0.53 ± 0.07$^{b}$</td>
<td>58 ± 5</td>
<td>25 ± 2</td>
</tr>
<tr>
<td>AKI + $\beta_2$(L)</td>
<td>5</td>
<td>121 ± 9</td>
<td>0.59 ± 0.12</td>
<td>18.9 ± 2.3</td>
<td>0.56 ± 0.07$^{b}$</td>
<td>64 ± 5</td>
<td>28 ± 3</td>
</tr>
<tr>
<td>AKI + $\beta_2$(M)</td>
<td>5–6</td>
<td>134 ± 9</td>
<td>0.57 ± 0.10</td>
<td>17.9 ± 3.0</td>
<td>0.65 ± 0.10</td>
<td>59 ± 5</td>
<td>26 ± 5</td>
</tr>
<tr>
<td>AKI + $\beta_2$(H)</td>
<td>5–6</td>
<td>129 ± 8</td>
<td>0.46 ± 0.03</td>
<td>14.3 ± 0.4</td>
<td>0.72 ± 0.09</td>
<td>62 ± 2</td>
<td>29 ± 6</td>
</tr>
</tbody>
</table>

Results are expressed as mean ± SE.

* $P < 0.05$ versus Cont.

$^{b}$ $P < 0.01$ versus Cont.

Fig. 5. Cross-section (H&E staining) of the liver and kidney from AKI group rats (A and C) compared with AKI + $\beta_2$(H) group rats (B and D) 3 days after the onset of I/R-induced AKI.

[34]. Therefore, the findings would suggest that glucocorticoid action in the kidney is depressed after renal I/R injury. Similarly, an altered expression and/or function of $\beta_2$-ARs has been observed in renal I/R injury [32]. This was supported by evidence that fundamental alterations in the activity of $\beta_2$-ARs contribute to the deterioration of the immune system and accompany the development of failure in organ function [35–37]. The present study
indicated that β2-AR activation had the ability to modulate renal 11βHSD-1 gene expression. Taken together, it is suggested that β2-AR dysfunction in renal I/R injury may lead to the reduction of renal glucocorticoid metabolism through the suppression of 11βHSD-1.

**In vivo** gene therapy using adenoviral constructs containing the β2-AR gene has been demonstrated to be an efficient and reproducible global transgene delivery system that results in long-term expression in the kidney. It was evident that treatment of the rat model with adeno-β2-AR resulted in a greater increase in renal β-AR density and mRNA levels in a dose-dependent manner. Because adeno-β2-AR did not change the levels of renal β1-AR mRNA, cAMP–PKA activation in the kidney was mainly modulated by over-expression of renal β2-AR via the gene delivery. Importantly, Gisz and PKC expressions and activity in the kidney were not changed by the β2-AR gene delivery, indicating that the β2-AR gene delivery led to activation of constitutive β2-AR and the classical linear Gsα–AC–cAMP–PKA signalling cascade. On the other hand, the viral vectors have some negative characteristics, in that they can trigger inflammatory and immune responses with the possibility that adenovirus gene therapy may worsen the organ functions. In the present study, intraperitoneal administration of the adeno-β2-AR via gene delivery had no effect on hepatotoxicity compared with Cont. rats. Furthermore, plasma biochemistry (AST and ALT) levels after delivery of adeno-β2-AR were also unchanged compared with Cont. rats. Moreover, there was no elevation in cytokines (TNF-α, IL-6 and IL-10) in the circulation or tissue TNF-α expression in the kidney as a consequence of adeno-β2-AR administration (data not shown). Together, these findings provide support for the view that the adeno-β2-AR administration utilized in the present study did not initiate any inflammatory responses in the liver and kidney at least over the 3-day observation period.

Renal I/R produced severe tubular damage characterized by a loss of brush border, lumen dilatation or collapse and cellular detachment from tubular basement membranes. Moreover, after renal artery occlusion, glomerular filtration rate was markedly reduced and FENa and inflammatory cytokines were elevated at the same time point [38, 39]. However, the present study showed that renal tissue and FENa did not significantly differ between AKI group and Cont. group rats. This is supported by the report of Zager [22] that the ischaemic rat model showed a relative intact tubular epithelium, only small amounts of intraluminal cellular debris and an absence of cast formation. Importantly, the degree of I/R injury correlated with the duration of the ischaemic insult in this model [38]. Jablonski et al. [40] demonstrated that ischaemic warm ischaemia time reduced Na reabsorption in the renal tubule. Kidneys with moderate cell damage as seen after the shorter period (>25 min) of ischaemia showed early recovery of FENa, cytokines and NO in the ischaemic rat groups.

The question arose as to why the longer ischaemia time (45 min) was not used in the present study. β2-AR density and PKA activity levels in the adeno-β2-AR-treated rats were significantly decreased after 45 min of renal ischaemia. This suggests that the longer period of ischaemia may have resulted in an acute stress inducing a reduction in renal β2-AR density level and cAMP–PKA activity [41, 42]. Moreover, the optimal transfection time for β-galactosidase is up to 25 min of renal ischaemia, after which a decrease in efficiency is seen. Importantly, a total time of 45 min of renal artery occlusion produced severe tubular damage in the kidney as seen after the longer period of ischaemia. Adenovirus delivered into the kidney has been found to result in viral transduction within renal tubular epithelial cells [18]. These findings suggest that the delivery of adeno-β2-AR into the damaged tubular cells is not effective in providing sustained and therapeutic levels of transgene in the target cells.

Interactions between β2-AR and glucocorticoid could occur at almost any step of these intracellular pathways, and several interactions have been identified in vitro [43, 44]. 11βHSD-1 acts as a pre-receptor mechanism in the local activation of the GR. The present in vivo study showed that an increase in PKA activation by the β2-AR gene delivery was correlated with an elevation of 11βHSD-1 mRNA level in the kidneys, suggesting a close relationship between functional β2-AR activation and glucocorticoid metabolism through the cAMP–PKA pathway. Glucocorticoids have been successfully used in the treatment of numerous clinical conditions for their anti-inflammatory and immunosuppressive effects [45]. β2-AR agonists also act to suppress the cytokine cascade during the course of the immunological response [46]. Figure 6 depicts these findings and summarizes the
known and discussed effects of $\beta_2$-AR and the possible interaction with 11$\beta$HSD-1 in the cells. Crosstalk between $\beta_2$-AR and 11$\beta$HSD-1 via cAMP–PKA may produce additive or even synergistic anti-inflammatory effects, resulting in kidney protection against renal I/R injury.

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High salt intake causes adverse fetal programming—vascular effects beyond blood pressure

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Abstract

Background. High salt intake causes hypertension, adverse cardiovascular outcomes and potentially also blood pressure (BP)-independent target organ damage. Excess salt intake in pregnancy is known to affect BP in the offspring. The present study was designed to assess whether high salt intake in pregnancy affects BP and vascular morphology in the offspring.

Methods. Sprague-Dawley rats were fed a standard rodent diet with low-normal (0.15%) or high (8.0%) salt content during pregnancy and lactation. After weaning at 4 weeks of age, offspring were maintained on the same diet or switched to a high- or low-salt diet, respectively. Vascular geometry was assessed in male offspring at 7 and 12 weeks postnatally.

Results. Up to 12 weeks of age, there was no significant difference in telemetrically measured BP between the groups of offspring. At 12 weeks of age, wall thickness of central (aorta, carotid), muscular (mesenteric) and intrapulmonary arteries was significantly higher in offspring of mothers on a high-salt diet irrespective of the post-weaning diet. This correlated with increased fibrosis of the aortic wall, more intense nitrotyrosine staining as well as elevated levels of marinobufagenin (MBG) and asymmetric dimethyl arginine (ADMA).

Conclusions. High salt intake in pregnant rats has long-lasting effects on the modeling of central and muscular arteries in the offspring independent of postnatal salt intake and BP. Circulating MBG and ADMA and local oxidative stress correlate with the adverse vascular modeling.

Keywords: blood pressure; fetal programming; nitric oxide; salt; vessel development

Introduction

There is evidence from observational epidemiological studies [1], small intervention studies [2] and controlled intervention trials [3, 4] as well as from studies in primates [5] that dietary salt intake is one causal factor responsible for elevated blood pressure (BP) in adults. This has also been shown in children [6].

In grown experimental animals, high salt intake causes cardiac fibrosis and vascular remodeling [7, 8] partially...