β2-Adrenoceptor Activation Attenuates Endotoxin-Induced Acute Renal Failure

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Abstract. Abnormalities in the β2-adrenergic control of organ function have been implicated in the pathogenesis of several disease states, such as septic shock. The objectives of the present study were to define the contribution of β2-adrenoceptors (β2-AR) to normal renal physiology and to investigate whether overexpression of renal β2-AR might be potentially beneficial in preventing progressive renal damage associated with endotoxemia. Adenoviral transgenes containing the human β2-AR (Adeno-β2-AR) were constructed and delivered into the rat kidney by means of intraparenchymal injections. Administration of 10⁹ total viral particles of Adeno-β2-AR induced an approximately threefold increase in β2-AR density in the renal tissue, which 2 wk after delivery, enhanced GFR and sodium reabsorption compared with control rats. The enhanced GFR was abolished by the addition of the β1-AR antagonist, ICI 118,551. Administration of lipopolysaccharide (LPS) caused a reduction in GFR, β2-AR density, and cAMP together with enhanced TNF-α mRNA in the kidney. In rats overexpressing β2-AR, the reduction in baseline GFR and elevation of TNF-α mRNA and leukocyte infiltration into the kidney associated with the endotoxin were blocked. These findings suggested the possibility that a renal-specific overexpression of β2-AR preserves basal renal function in response to a ligand-independent β2-AR activation and that the delivery of Adeno-β2-AR gene is a potential novel therapeutic strategy for treatment of acute renal failure associated with sepsis.

Fundamental alterations in the tissue content of β2-adrenoceptors (β2-AR) and their activity may contribute to the deterioration of the immune system and accompany the development of failure in organ function. An altered expression and/or function of β2-AR has been considered to be a pathogenic factor in some disease states; for example, allergy (1,2), heart failure (3,4), and sepsis (5,6). It has been demonstrated that patients with septic shock suffer from hypotension and cardiac dysfunction that is refractory to high doses of intravenous catecholamines (5). Bernardin et al. (6) showed that the tissue density of β-AR was significantly reduced and adenylyl cyclase stimulation was heterogeneously desensitized in peripheral blood mononuclear cells freshly isolated from septic patients. There is a growing body of evidence that the β2-AR system has an antiinflammatory influence on the cytokine network during the course of immunologic responses (7–9). Importantly, the administration of β2-AR agonists was found to attenuate the stimulation of TNF-α (10,11) associated with lipopolysaccharide (LPS) (12) and Shiga toxin-2 of hemolytic uremic syndrome (13,14), which is considered to be a central mediator of the pathophysiologic changes. These observations would suggest that disturbances of the β2-AR system in an organ may be involved in the uncontrolled inflammatory responses that occurs during sepsis (15–17).

Endotoxemia caused by Gram-negative bacteria can result in sepsis and organ dysfunction, which includes kidney damage and renal failure (18,19). Septic shock after surgery, trauma, burns, or severe infection is a common cause of acute renal failure, resulting in a high mortality rate (20). Pathologic examination of the failing kidneys has revealed that there is an occurrence of focal necrosis of the proximal tubular epithelium, eosinophilic casts within proximal and distal tubules, and microthrombi in the glomerular capillaries (21). Recently presented data from the Madrid Acute Renal Failure Study Group (22) reported that sepsis caused acute tubular necrosis in 35% of patients in intensive care units (ICU) and 27% of non-ICU patients. If there is an impaired regulation of β2-AR function during sepsis in the failing kidney, it may be that restoration of β2-AR function might be able to prevent renal inflammation and injury associated with sepsis.

For the purpose of activation and restoration of β2-AR function, drugs targeting β2-AR signaling, including β2-AR agonists, may be used as a first-line approach for therapy. However, administration of β2-AR agonists to regulate β2-AR function has an inherently limited efficacy, partly because of the downregulation and desensitization of β2-AR (23). On the other hand, 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 organ, as has been reported in the myocardium (24). Therefore, the application of adenoviral mediated β2-AR gene delivery to elevate β2-AR density and prevent desensitization would be an attractive option whereby β2-AR could be active over a prolonged period. Importantly, adenoviral vector (25) as well as adeno- associated virus (26) delivered in vivo by intraparenchymal injection has been found to result in viral transduction within renal tubular epithelial cells (25, 26), which is one of the major targets of endotoxin within the kidney (21, 22). With this in mind, we used adenoviral mediated β2-AR gene delivery to investigate whether overexpression of β2-AR could alter both biochemical and in vivo renal function and to test the hypothesis that the β2-AR gene delivery affects the kidney protection against endotoxin-induced acute renal failure.

Materials and Methods

Reagents

Adenovirus Expression Vector Kit was obtained from Takara Bio- medicals (Shiga, Japan). Rabbit polyclonal anti-β2-AR antibody (H-20) was obtained from Santa Cruz Biotechnology Inc. (Santa Cruz, CA). ICI 118,551 was from Funakoshi Co. (Tokyo, Japan), and glass slides precoated with siran was from Matsunami Glass Ltd. (Tokyo, Japan). [125I] cyanopindolol ([125I]CYP) was obtained from Perkin Elmer Life Science (Tokyo, Japan). [α-33P]dCTP and cAMP ELISA kit were supplied by Amersham International Plc. (Little Chalfont, Buckinghamshire, UK). GF/C filter was from Whatman Japan Ltd. (Tokyo, Japan). Unless stated, reagents were from Sigma Chemical Co. (St. Louis, MO).

Construction of Recombinant Adenovirus

The human β2-adrenoceptor–expressing adenovirus (Adeno-β2-AR) and the cytoplasmic β-galactosidase expressing adenovirus (Adeno-LacZ) were a kind gift from Drs. Walter J. Koch and Robert J. Lefkowitz (Departments of Surgery and of Medicine and 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 (27). Virus isolates of Adeno-β2-AR, Adeno-LacZ, and no transgene (empty adenovirus: EV) were plaque-purified and propagated in 293 cells given by Dr. I. Wada (Department of Biochemistry, Sapporo Medical University, Japan), isolated, concentrated, and titered using 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-d acclimatization period, rats were anesthetized with pentobarbitone (50 mg/kg, intraperitoneally), and the right kidney was exposed via a retroperitoneal incision. A 50-μl sample of the virus was injected into the right kidney using a 25-gauge needle attached to a 1-ml syringe. Briefly, the needle was inserted at the lower pole of the right kidney just under the capsule and parallel to the renal surface and was carefully pushed upward to the upper pole. As the needle was removed slowly, injections were made along the straight line within the capsule. The flank incision was then closed by suturing the muscles and skin layers in stages. The Adeno-β2-AR–treated, Adeno- LacZ–treated, and EV-treated rats ranged in weight from 130 to 150 g, and there was no significant difference between groups. One to five weeks later, animals were housed in metabolic cages for 24 h to collect urine samples. After animals were given an overdose of pentobarbitone, blood samples were collected and the kidneys were removed and weighed. Systolic blood pressure (BP) and heart rate (HR) were monitored weekly by means of a tail cuff sphygmomanometer, using an automated system with a photoelectric sensor (KN-201-1; Natsume Seiskakusho Co., Tokyo, Japan).

To induce acute renal failure in the rats, LPS (Escherichia coli O127:B8, 5 mg/kg) was injected intraperitoneally into rats 25 d after the administration of the adenoviral vectors. Control rats were injected intraperitoneally with an equal volume of physiologic saline (PBS). The β2-AR antagonist ICI 118,551 was given intraperitoneally 2 h before an injection of the LPS or PBS. The rats were housed in metabolic cages for urine collection and 24 h later were killed after an overdose of sodium pentobarbitone. Blood, urine, and kidneys were collected for assay.

Biochemical Measurements

Serum and urine creatinine levels were determined using a creatinine assay kit, according to the protocols specified by the manufacturer. GFR (ml/min) was expressed as a creatinine clearance rate. Serum and urinary sodium (Na+) or potassium (K+) concentrations were measured using spectrophotometer (7170; Hitachi, Japan).

Renal Morphologic Analyses

Tissue for light microscopy was fixed using 3% formalin-PBS and embedded in paraffin. After sectioning, the tissues were stained with hematoxylin and eosin (H&E) for assessment of cellular filtration and inflammation. For the β-galactosidase (β-gal) assay, frozen kidneys were mounted on a freezing microtome, and 10-μm sections were transferred to glass slides precoated with siran. Sections were fixed in 10% formalin for 2 min at room temperature and washed twice in PBS. β-gal staining was carried out in 2 mmol/L K4Fe(CN)6, 2 mmol/L MgCl2, 0.5 mg/ml X-gal (5-bromo-4-chloro-3-indoyl-β-D-galactopyranoside) in PBS, pH 7.4. After being stained for 2 h at 37°C, the sections were rinsed in PBS solution and taken for histologic evaluation.

β-AR Binding Assay

Membrane fractions were extracted following the method described by Lefkowitz et al. (27,28) with minor modifications. Membrane preparations (25 μg) were incubated with [125I] CYP (15 to 315 pmol/L) in binding buffer either alone or 20 μmol/L alpranolol, which was used for determination of nonspecific 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 (Bmax) was determined using linear regression analysis of saturation isotherm data linearly transformed to give a Scatchard plot. Receptor density (measured in femtomoles) was normalized to milligrams of membrane protein. The protein concentration was assayed using a micro protein determination kit.

β2-AR Immunohistochemistry

Frozen kidney sections were cut 10-μm-thick for the immunofluorescence studies. Sections were rinsed in PBS, then with PBS containing 0.05% Triton X-100 (triton-PBS), blocked with serum diluent (10% goat serum in PBS with BSA and 0.1% sodium azide), and then
rinsed for 15 min in Triton-PBS before overnight incubation at 4°C with a primary rabbit antihuman β2-AR antiserum (1:500 dilution in serum diluent). The sections were then washed four times for 10 min in Triton-PBS at room temperature and incubated for 1 h in FITC-conjugated goat anti-rabbit IgG (1:50 dilution in serum diluent). After five 3-min rinses in PBS, the sections were mounted with sodium iodide (25 g/L) in 1:1 PBS/glycerol solution and photographed using a confocal laser-scanning microscope (CLSM; BIO-RAD, Hemel Hempstead, UK).

**Analysis of TNF-α mRNA and cAMP Activity**

We estimated mRNA levels using Northern blot hybridization analysis as described in our previous study (29). For Northern blot hybridization, the 546-bp cDNA for TNF-α (30) and the 420-bp Hinf I fragment of human β-actin (National Children’s Research Centre, Japan, Tokyo) were labeled using the oligo-labeling method in the presence of [α-33P] dCTP and used as a hybridization probe. All mRNA samples (10 μg) were applied to a Biodyne A membrane, hybridized simultaneously, and exposed for the same time. The β-actin cDNA probe was used as a loading control after the TNF-α probe was stripped from the membrane. Urinary and renal cAMP levels were estimated using a commercially available ELISA kit in which the assay was based on the competition between unlabelled cAMP and a fixed quantity of peroxidase-labeled cAMP for a limited number of binding sites on a cAMP-specific antibody. For measurement of renal cAMP levels, the frozen kidney samples were lysed using a liquid phase extraction method (31), and the supernatants were taken for the analysis of renal cAMP levels according to the manufacturer’s manual. Renal cAMP levels were expressed as pmol/kidneys weight (g).

**Statistical Analyses**

Statistical analyses were undertaken using ANOVA followed by a Bonferroni and Dunnett test for multiple comparisons. The unpaired t test was used for comparisons of GFR, β-AR density, and urinary or renal cAMP between LPS-treated and untreated rats. Results were expressed as mean ± SE.

**Results**

**Adenoviral-Mediated β2-AR and β-gal Expression**

The Adeno-LacZ was delivered to assess the efficacy of adenovirus-mediated transfer of the marker gene LacZ. Figure 1 shows the β-gal staining of a cross-section of a right kidney taken 1 to 5 wk after intraparenchymal delivery of 10⁹ total viral particles (t.v.p) of Adeno-LacZ. β-galactosidase expression was measurable in the renal proximal and distal tubules at 1–3 wks but could not be detected at 5 wk. The gene was also expressed in the liver but not in other organs, such as left kidney (data not shown), and was probably transferred via the blood stream. Once the in vivo global renal transgene delivery had been demonstrated with Adeno-LacZ, the delivery of the therapeutic transgene Adeno-β2-AR was assessed. Figure 2A shows the immunofluorescence staining of β2-AR expressed in the right kidney 4 wk after intraparenchymal injection (10⁹ t.v.p) of Adeno-β2-AR. Diffuse β2-AR overexpression was evident throughout the kidney, in the proximal and distal tubules and glomeruli. By contrast, control sections of the right kidney treated with equivalent doses of EV revealed a low level of β2-AR expression in the renal tubules (Figure 2B).

![Figure 1. β-gal staining in a cross-section of the right kidney injected with 10⁹ total viral particles (t.v.p.) of Adeno-LacZ. Expression of β-galactosidase in the right kidney taken at 1 wk (A), 3 wk (B), 5 wk (C) was measured after in vivo intraparenchymal delivery of adenovirus. A part of the section (A) was magnified and is shown in the panel.](image-url)
Doses of Adenovirus-H9252 resulted in overexpression of β2-AR in tissues such as lung and liver, with FEK levels returning to basal levels by 3 weeks. On the other hand, β2-AR was not expressed in tubules but also in glomeruli as indicated by the circle (A). Scale bars: 100 μm in A and B.

### β2-AR Density in the Kidneys

To test how long β2-AR transgene overexpression was supported in the renal tissue, β2-AR density levels were measured in the right and left kidneys during a 5-week period after intraparenchymal gene delivery (Figure 3A). There was a sharp increase in β2-AR density level 2 weeks after intraparenchymal gene delivery (10^9 t.v.p. of Adeno-β2-AR) in the right kidney, which was sustained until the 4-week time point. Furthermore, measurable β2-AR overexpression was also observed in the contralateral left kidney, which was elevated at 2 weeks after the gene delivery. Normal endogenous β2-AR density in the right and left kidneys (control group) was unaltered over this timeframe. It was evident from Figure 3B that the degree of β2-AR overexpression depended on the adenoviral dose injected into the right kidney. Endogenous β2-AR density in the right kidney (control group) was unaltered by delivery of EV without the β2-AR gene. The administration of virus to the right kidney resulted in overexpression of β2-AR in nonrenal tissue, for example liver and lung. Although the time course of change in lung β2-AR density was similar to that in the left kidney, liver β2-AR density reached a peak level at 1 to 2 weeks and had returned to basal levels by 3 weeks. On the other hand, β2-AR density in the heart was not significantly increased at any time point (data not shown).

### Effects of β2-AR Overexpression on Renal Function

Figure 4A shows the time course of GFR (ml/min per 100 g body wt) after delivery of various doses of Adeno-β2-AR. Although there was a significant increase (P < 0.05) in GFR 2 weeks after intraparenchymal delivery of Adeno-β2-AR (10^9 t.v.p.), GFR levels at 1, 3, and 4 weeks after delivery of Adeno-β2-AR (10^8-9 t.v.p.) were not changed compared with those in control rats. By contrast, the higher dose of 10^10 t.v.p. Adeno-β2-AR produced a diminished GFR with advancing age. The changes in time course of FENa and FEK (%) after various doses of Adeno-β2-AR are shown in Figures 4B and 4C. There was a significant decrease in FENa (P < 0.05) 1 to 2 weeks after intraparenchymal delivery of Adeno-β2-AR (10^8-9 t.v.p.); whereas, at 3 and 4 weeks after delivery of Adeno-β2-AR (10^8-9 t.v.p.), it was not different from that in control rats (Figure 4B). FEK levels (Figure 4C) after delivery of Adeno-β2-AR (10^8-9 t.v.p.) were unchanged compared with those in control rats, while there was a significant increase in FEK 3 to 4 weeks after intraparenchymal delivery of the higher dose of Adeno-β2-AR (10^10 t.v.p.). These results indicate that GFR, FENa, and FEK became stable approximately 3 weeks after the delivery of Adeno-β2-AR (10^8-9 t.v.p.). Furthermore, Adeno-β2-AR (10^9 t.v.p.) did not change weight, BP, or HR compared with those of control rats or EV-treated rats (Table 1). Thus, because 10^9 t.v.p. of Adeno-β2-AR was the highest dose able to provide stability to renal function and physiologic function, this dose was chosen as the “therapeutic dose” to estimate an effect of β2-AR on renal dysfunction induced by endotoxin.

### Role of β2-AR in the Regulation of GFR

The mechanisms underlying the enhanced GFR 2 weeks after delivery of 10^9 t.v.p. of Adeno-β2-AR were investigated. Figure 5 shows that the β2-AR antagonist (ICI 118,551) was able to block the increased GFR in the Adeno-β2-AR–treated rats in a
dose-dependent manner. This would be compatible with Adeno-ß2-AR–treated rats producing an increase in GFR via a ß2-AR ligand–independent mechanism and that ß2-AR constitutively active receptors in the kidney were able to elevate GFR. Importantly, there was no difference in GFR levels between control rats and EV-treated rats, suggesting that 10^9 t.v.p adenovirus did not of itself affect glomerular filtration.

Renal Function in Adeno-ß2-AR Treated Rats after Sepsis

A study was undertaken to examine whether ß2-AR overexpression in the kidney prevented the renal failure induced by endotoxin. This was done using intraperitoneal injection of LPS (5 mg/kg) to induce renal failure in control and Adeno-ß2-AR treated rats. The experiment was performed on the 25th day after the delivery of 10^9 t.v.p of Adeno-ß2-AR, which was chosen as the therapeutic dose. All rats in this study survived, but the LPS (5 mg/kg) decreased weight gain in both control and the Adeno-ß2-AR–treated rats (a weight loss of 20 to 30 g). It can be seen in Figure 6 that GFR in the Adeno-ß2-AR treated rats was not changed by the injection of LPS while in the control rats it was significantly (P < 0.05) depressed by the LPS challenge. The addition of the antagonist, ICI 118,551 blocked the ability of the Adeno-ß2-AR–treated rats to maintain GFR, suggesting that constitutive ß2-AR activity plays an important role in preserving renal function against the endotoxin. The changes in the BP and HR after LPS injection in Adeno-ß2-AR–treated rat were not different from those in control rats (Table 2), suggesting that these parameters were not involved in the regulation of GFR in Adeno-ß2-AR–treated rats. In addition, GFR, FENa, and FEK were monitored over a

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Table 1. In vivo measurements in rats with 10^9 t.v.p. of Adeno-ß2-AR 25 d after ß2-AR gene delivery

<table>
<thead>
<tr>
<th></th>
<th>Control (n = 6)</th>
<th>EV (n = 5)</th>
<th>Adeno-ß2-AR (n = 6)</th>
</tr>
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<tr>
<td>Weight (g)</td>
<td>338 ± 8</td>
<td>322 ± 10</td>
<td>337 ± 5</td>
</tr>
<tr>
<td>BP (mmHg)</td>
<td>124 ± 2</td>
<td>118 ± 3</td>
<td>123 ± 4</td>
</tr>
<tr>
<td>Heart rate (beats/min)</td>
<td>395 ± 18</td>
<td>358 ± 21</td>
<td>381 ± 14</td>
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* Data are the mean ± SE.

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Figure 4. In vivo assessment of renal function in rats treated with Adeno-ß2-AR. (A) Time course of GFR (ml/min per 100 g body wt) after delivery of various doses of Adeno-ß2-AR. (B) Time course of FENa (%) after delivery of various doses of Adeno-ß2-AR. (C) Time course of FEK (%) after delivery of various doses of Adeno-ß2-AR. Data are mean ± SE. *P < 0.02 versus control level at the corresponding period; n = 6 to 8.

Figure 5. Changes in GFR (ml/min per 100 g body wt) of control (cont), EV, and Adeno-ß2-AR–treated rats 2 wk after delivery of 10^9 t.v.p of Adeno-ß2-AR. Intraperitoneal injection of increasing doses of the ß2-AR antagonist (ICI 118,551) given 2 h before measurement of GFR. Doses of ICI 118,551 from 31.4 µg/kg to 31.4 ng/kg were given. Data are the mean ± SE. *P < 0.05 versus control. †P < 0.05 versus Adeno-ß2-AR rats without ICI 118,551 injection; n = 5 to 8.
by the injection of LPS ($P < 0.05$). Furthermore, the LPS challenge suppressed $\beta$-AR densities in the liver and lung of both groups, but $\beta$-AR density in the heart was unchanged by the LPS challenge. Urinary cAMP levels were not altered by any of the treatments (Figure 7B). On the other hand, renal cAMP content (of both right and left kidneys) was depressed ($P < 0.05$) by the LPS challenge in the control rats but not in the Adeno-$\beta_2$-AR–treated rats (Figure 7C). The responses in renal cAMP level induced by the LPS in both groups correlated with the changes in renal $\beta$-AR density.

### Histologic Findings in Kidneys Exposed to LPS

Figure 8 shows a cross-section (H&E staining) through the right kidney 24 h after PBS injection in a control rat (A), after LPS (5 mg/kg) injection into an Adeno-$\beta_2$-AR–treated rat (B) and into an untreated rat (C). Although the control rats exposed to LPS demonstrated a minor inflammatory response in the renal interstitium, signified by cellular infiltration (C), the Adeno-$\beta_2$-AR–treated rat had little, if any, cellular infiltration in the kidney (B).

### Renal TNF-α mRNA Levels in Adeno-$\beta_2$-AR–Treated Rats Challenged with LPS

Figure 9 presents the levels of TNF-α mRNA in both left and right kidneys 24 h after PBS or LPS (5 mg/kg) injection into untreated control rats or Adeno-$\beta_2$-AR–treated rats. The LPS increased the level of TNF-α mRNA in control rats, but this was significantly depressed (by some 39%, $P < 0.05$) by prior treatment with Adeno-$\beta_2$-AR. Importantly, the suppression of TNF-α mRNA in Adeno-$\beta_2$-AR–treated rats could be prevented by the addition of the antagonist, ICI 118,551 in a dose-dependent manner. There was no difference in TNF-α mRNA levels between control rats and Adeno-$\beta_2$-AR–treated rats in the absence of exposure to LPS.

### Discussion

The model presented herein is of in vivo gene transfer of Adeno-$\beta_2$-AR into the kidney, which has been demonstrated to be an efficient and reproducible global delivery of transgene to the renal glomeruli and tubular epithelial cells of the rat. In this model, we present novel findings indicating that constitutive $\beta_2$-AR activation, independent of the receptor ligand, was

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**Table 3. Long-term effects of $\beta_2$-AR overexpression on kidney function**

<table>
<thead>
<tr>
<th></th>
<th>GFR (ml/min/100 g bw)</th>
<th>FENa (%)</th>
<th>FEK (%)</th>
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<tbody>
<tr>
<td>Control rats</td>
<td></td>
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<tr>
<td>PBS ($n = 4$)</td>
<td>0.75 ± 0.07</td>
<td>1.28 ± 0.09</td>
<td>11.52 ± 1.02</td>
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<tr>
<td>LPS ($n = 5$)</td>
<td>0.70 ± 0.05</td>
<td>1.39 ± 0.10</td>
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<td>$\beta_2$-AR rats</td>
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<tr>
<td>PBS ($n = 4$)</td>
<td>0.73 ± 0.04</td>
<td>1.09 ± 0.08</td>
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<tr>
<td>LPS ($n = 5$)</td>
<td>0.72 ± 0.03</td>
<td>1.07 ± 0.07</td>
<td>11.72 ± 1.08</td>
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*Results were expressed as mean ± SE. bw, body weight.

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**Figure 6. Rescue of endotoxin-induced renal dysfunction in the Adeno-$\beta_2$-AR rats.** LPS (5 mg/kg intraperitoneally) was injected into control and Adeno-$\beta_2$-AR–treated rats on the 25th day after delivery of the Adeno-$\beta_2$-AR (10⁷ t.v.p.). Intraperitoneal injection of various doses of the Adeno-$\beta_2$-AR antagonist (ICI 188,551) was given 2 h before the LPS challenge. Dose of 10⁻¹ or 10⁻² or ICI 118,551 represents administration of 3.14 μg/kg or 0.314 μg/kg, respectively. Data are mean ± SE. *$P < 0.05$ versus control without any treatment; †$P < 0.05$ versus Adeno-$\beta_2$-AR rats without any treatment. $n = 5$ to 8.

**Table 2. BP and heart rate at baseline and during follow-up monitoring after LPS**

<table>
<thead>
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<th>Parameter</th>
<th>BP (mmHg)</th>
<th>Heart Rate (beats/min)</th>
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<td>Control ($n = 6$)</td>
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<tr>
<td>Baseline</td>
<td>120 ± 6</td>
<td>119 ± 3</td>
</tr>
<tr>
<td>3 h</td>
<td>74 ± 5b</td>
<td>72 ± 12b</td>
</tr>
<tr>
<td>6 h</td>
<td>72 ± 12b</td>
<td>74 ± 75b</td>
</tr>
<tr>
<td>12 h</td>
<td>76 ± 6b</td>
<td>79 ± 2b</td>
</tr>
<tr>
<td>24 h</td>
<td>94 ± 14</td>
<td>93 ± 5</td>
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*Results are expressed as mean ± SE.

b $P < 0.05$ vs. base line.

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**Figure 7.** (A) Histologic findings in kidneys exposed to LPS. Rats challenged with LPS demonstrated a minor inflammatory response in the renal interstitium, signified by cellular infiltration (C), the Adeno-$\beta_2$-AR–treated rat had little, if any, cellular infiltration in the kidney (B).

**GFR (ml/min/100g body weight)**

![Graph showing GFR](image)

Figure 6. Rescue of endotoxin-induced renal dysfunction in the Adeno-$\beta_2$-AR rats. LPS (5 mg/kg intraperitoneally) was injected into control and Adeno-$\beta_2$-AR–treated rats on the 25th day after delivery of the Adeno-$\beta_2$-AR (10⁷ t.v.p.). Intraperitoneal injection of various doses of the Adeno-$\beta_2$-AR antagonist (ICI 188,551) was given 2 h before the LPS challenge. Dose of 10⁻¹ or 10⁻² or ICI 118,551 represents administration of 3.14 μg/kg or 0.314 μg/kg, respectively. Data are mean ± SE. *$P < 0.05$ versus control without any treatment; †$P < 0.05$ versus Adeno-$\beta_2$-AR rats without any treatment. $n = 5$ to 8.

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<td>72 ± 12b</td>
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<td>6 h</td>
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**Discussion**

The model presented herein is of in vivo gene transfer of Adeno-$\beta_2$-AR into the kidney, which has been demonstrated to be an efficient and reproducible global delivery of transgene to the renal glomeruli and tubular epithelial cells of the rat. In this model, we present novel findings indicating that constitutive $\beta_2$-AR activation, independent of the receptor ligand, was...
implicated in the regulation of glomerular filtration and tubular sodium reabsorption. In addition, we found that renal overexpression of \( \beta_2 \)-AR using gene transfer with Adeno-\( \beta_2 \)-AR was effective in preventing endotoxin-induced renal injury. This finding was intriguing in that the sepsis-induced renal failure occurred with the decreases in \( \beta_2 \)-AR density and cAMP activity, which suggested an impaired renal \( \beta_2 \)-AR signaling system.

The method of in vivo \( \beta_2 \)-AR gene transfer using intraparenchymal injection utilized herein had an enhanced effectiveness compared with other delivery methods, that is intravenous or percutaneous injections, which were utilized in preliminary studies (unpublished data). Intraparenchymal injection of \( \beta_2 \)-AR gene elevated \( \beta_2 \)-AR density in the kidney at a more rapid rate and achieved higher densities compared with the other methods. In addition, the technique of direct intraparenchymal injection was the most effective way to ensure consistent uptake of the \( \beta_2 \)-AR gene into the kidney. The elevation of \( \beta_2 \)-AR density in the right kidney began quickly after the

Figure 7. Changes in \( \beta_2 \)-AR density (A), urinary cAMP (B), and renal cAMP (C) after injection of LPS (5 mg/kg intraperitoneally). \( \beta_2 \)-AR density and renal cAMP levels were recorded from samples of combined right and left kidneys. LPS (5 mg/kg intraperitoneally) was injected into control and Adeno-\( \beta_2 \)-AR rats on the 25th day after delivery of Adeno-\( \beta_2 \)-AR (10⁹ t.v.p.), and samples were collected after 24 h. Data are the mean ± SE. *P < 0.05 versus control without any treatment. †P < 0.05 versus Adeno-\( \beta_2 \)-AR rats without any treatment. n = 6 to 8.

Figure 8. Cross-section (H&E staining) of the right kidney 24 h after saline injection to control rates (A) and LPS (5 mg/kg) injection to Adeno-\( \beta_2 \)-AR–treated rats (B) or untreated rats (C). Adeno-\( \beta_2 \)-AR–treated rats were used on the 25th day after delivery of 10⁹ t.v.p. of Adeno-\( \beta_2 \)-AR. Cellular infiltration (arrow) was found in the renal interstitium in the LPS-injected control rat. Original magnifications: ×200 in A and B, ×100 in C.
intraparenchymal injection and reached peak levels 3 to 4 wk after the delivery. Interestingly, β-AR density in the left non-injected kidney was also increased, but this took place slowly over 1 to 2 wk. This implied that the adenovirus from the right kidney had spread to other organs, including the left kidney. In fact, an increased β₂-AR expression was also found in the liver and lung. Whereas β₂-AR gene delivery increased expression in the glomeruli, β-galactosidase was not expressed in glomeruli after gene delivery. The discrepancy may be due to a difference in transfer efficiency or transduction into the kidney via the blood stream. This would help explain to some degree the observation that β₂-AR was overexpressed in the contralateral left kidney while β-gal expression was not detected. It is likely that the adenovirus encoding β₂-AR passed into the systemic circulation after intraparenchymal injection, which would have resulted in deposition in the contralateral kidney glomeruli and caused expression in that area.

It was also evident that BP, HR, and growth rate in Adeno-β₂-AR rats were not influenced by the 10⁹ t.v.p. dose of Adeno-β₂-AR, as GFR and FENa became stable 3 wk after the administration. Furthermore, histologic examination of the kidney indicated that the 10⁹ t.v.p. dose of the Adeno-β₂-AR produced no evidence of inflammation within the tissue as had been reported with higher doses of adenovirus (32). Interestingly, although it is well known that β₂-AR agonists given acutely will cause hypokalemia (33), the adenoviral treatment (10⁹ t.v.p.) in the present study had no influence on serum potassium levels or the level of FEK (%). In addition, as shown in Table 3, there was no long-term effect on renal function in the Adeno-β₂-AR–treated rats. Thus, although further evaluation will be required, intraparenchymal injection of 10⁹ t.v.p. of Adeno-β₂-AR into the kidney was taken to be efficient, comparatively nonpathogenic, and potentially therapeutic.

Previous reports (34,35) demonstrated that renal β₂-AR in normal rats were predominantly localized to the apical and sub-apical compartments of proximal and, to a lesser extent, distal tubular epithelia and the membranes of smooth muscle cells from renal arteries. From this morphologic evidence, it was proposed that β₂-AR may regulate glomerular function and thereby sodium and water balance in the different nephron segments. This hypothesis was supported by the present observations using Adeno-β₂-AR–treated rats that overexpressed β₂-AR in the proximal and distal tubules and in the glomeruli as evaluated using an immunohistologic approach. The important finding arising from the present study was that constitutive β₂-AR activation in the Adeno-β₂-AR rats played an important physiologic role in causing an increase in GFR and sodium absorption in the kidney. Elevation in β₂-AR activity may contribute to an increase in tissue blood flow (36), which in the kidney might lead to a subsequent increase in GFR and decrease in FENa. However, the β-AR density in the Adeno-β₂-AR rats could not be correlated with the elevation in GFR and sodium absorption, suggesting that the mechanisms whereby renal function was modulated through intracellular signals via β₂-AR were complex (37).

The additional important finding arising from this study was that transfection with Adeno-β₂-AR to a large degree blocked the renal dysfunction associated with endotoxemia. Administration of LPS on the 25th day after the Adeno-β₂-AR gene delivery was found to protect renal function from the LPS-induced insult. Because GFR and FENa were stable from 3 wk after the delivery and the peak level of renal β₂-AR expression was observed between 3 and 4 wk after the delivery, we chose day 25 as the experimental day for LPS injection. The question arose as to what mechanisms were involved in the β₂-AR–mediated protective effect. β₂-AR activation causes the generation of the second-messenger cAMP via the activation of adenylate cyclase (38). Indeed, it was observed that there was
an increase in the renal content of cAMP in the Adeno-β₂-AR–treated rats. Importantly, renal cAMP content levels in the Adeno-β₂-AR–treated rat were not depressed after the exposure to LPS unlike those in the control rat which were significantly decreased. The fall in renal cAMP level was correlated with the depression in GFR caused by the LPS challenge, suggesting the possibility that the protective effect of β₂-AR activation was exerted through the intracellular cAMP/cAMP-dependent protein kinase (PKA) pathway. This would be compatible with previous reports that cAMP-PKA activation plays an important role in the protection against acute renal failure (39,40).

Another mechanism that may be implicated after β₂-AR activation is an antiinflammatory action. On the basis of the histologic findings, it was apparent that the LPS-induced leukocyte infiltration was absent in the kidney sections of the Adeno-β₂-AR–treated rats. Moreover, it was possible to demonstrate that the LPS-induced TNF-α mRNA in the kidney was suppressed in the Adeno-β₂-AR–treated rats. Another possibility is that the kidney could be influenced indirectly by β₂-AR overexpression in nonrenal tissue. There was no evidence that the β₂-AR system was overexpressed in the heart, so it could not have mediated the reduction in GFR after the LPS challenge. However, the possibility remains that overexpression in other organs could modify renal function. It is also recognized that activation of β₂-AR can inhibit perimicrovesel edema formation (41,42) as it has been reported that the β₂-AR agonist, dopexamine, attenuated endotoxin-induced vascular permeability in rat mesentery (41). Indeed, a similar mechanism may operate as a consequence of β₂-AR activation in the present study, conferring some protection from the LPS-induced reduction in renal function. Taken together, these findings suggest that constitutive β₂-AR activation is able to protect renal function through several mechanisms, including the cAMP-PKA pathway. The replacement of lost receptors as a consequence of sepsis may represent a novel therapeutic approach.

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References


