

## ORIGINAL ARTICLE

# Dexmedetomidine preconditioning ameliorates kidney ischemia-reperfusion injury

Juha Lempiäinen<sup>1</sup>, Piet Finckenberg<sup>1</sup>, Elina E. Mervaala<sup>1</sup>, Markus Storvik<sup>2</sup>, Juha Kaivola<sup>3</sup>, Ken Lindstedt<sup>3</sup>, Jouko Levijoki<sup>3</sup> & Eero M. Mervaala<sup>1</sup>

<sup>1</sup>Institute of Biomedicine, Pharmacology, University of Helsinki, Helsinki, Finland

<sup>2</sup>School of Pharmacy, University of Eastern Finland, Kuopio, Finland

<sup>3</sup>Orion Pharma Ltd., Espoo, Finland

## Keywords

Acute kidney injury, AHR, CD44, dexmedetomidine, fadolmidine, ischemia/reperfusion injury, NRF2, p38, Rac

## Correspondence

Eero Mervaala, Institute of Biomedicine, Pharmacology, University of Helsinki, P. O. BOX 63, FI-00014, Finland. Tel: +358-9-191-25-355; Fax: +358-9-191-25-364; E-mail: eero.mervaala@helsinki.fi

## Funding Information

The present study was supported by the Sigrid Juselius Foundation and the Academy of Finland.

Received: 30 October 2013; Revised: 18 February 2014; Accepted: 13 March 2014

*Pharma Res Per*, 2 (3), 2014, e00045, doi: 10.1002/prp2.45

doi: 10.1002/prp2.45

## Abstract

Kidney ischemia-reperfusion (I/R) injury is a common cause of acute kidney injury. We tested whether dexmedetomidine (Dex), an  $\alpha_2$ -AR agonist, protects against kidney I/R injury. Sprague–Dawley rats were divided into four groups: (1) Sham-operated group; (2) I/R group (40 min ischemia followed by 24 h reperfusion); (3) I/R group + Dex (1  $\mu\text{g}/\text{kg}$  i.v. 60 min before the surgery), (4) I/R group + Dex (10  $\mu\text{g}/\text{kg}$ ). The effects of Dex postconditioning (Dex 1 or 10  $\mu\text{g}/\text{kg}$  i.v. after reperfusion) as well as the effects of peripheral  $\alpha_2$ -AR agonism with fadolmidine were also examined. Hemodynamic effects were monitored, renal function measured, and acute tubular damage along with monocyte/macrophage infiltration scored. Kidney protein kinase B, toll like receptor 4, light chain 3B, p38 mitogen-activated protein kinase (p38 MAPK), sirtuin 1, adenosine monophosphate kinase (AMPK), and endothelial nitric oxide synthase (eNOS) expressions were measured, and kidney transcriptome profiles analyzed. Dex preconditioning, but not postconditioning, attenuated I/R injury-induced renal dysfunction, acute tubular necrosis and inflammatory response. Neither pre- nor postconditioning with fadolmidine protected kidneys. Dex decreased blood pressure more than fadolmidine, ameliorated I/R-induced impairment of autophagy and increased renal p38 and eNOS expressions. Dex downregulated 245 and upregulated 61 genes representing 17 enriched Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways, in particular, integrin pathway and CD44. Ingenuity analysis revealed inhibition of Rac and nuclear factor (erythroid-derived 2)-like 2 pathways, whereas aryl hydrocarbon receptor (AHR) pathway was activated. Dex preconditioning ameliorates kidney I/R injury and inflammatory response, at least in part, through p38-CD44-pathway and possibly also through ischemic preconditioning.

## Abbreviation

AHR, aryl hydrocarbon receptor; AMPK, adenosine monophosphate kinase; ANOVA, analysis of variance; ATN, acute tubular necrosis; BP, blood pressure; Dex, dexmedetomidine; eNOS, endothelial nitric oxide synthase; HR, heart rate; I/R, ischemia-reperfusion; IGF-1, insulin-like growth factor 1; IPC, ischemic preconditioning; KEGG, Kyoto Encyclopedia of Genes and Genomes; MAPK, mitogen activated protein kinases; MAP, mean arterial blood pressure; Nec-1, necrostatin-1; PK, pharmacokinetic; RAS, renin–angiotensin system; RIPK1, receptor-interacting protein kinase 1; SD, Sprague–Dawley; SNS, sympathetic nervous system; TNF, tumor necrosis factor;  $\alpha_1$ -AR, alpha1 adrenoceptors;  $\alpha_2$ -AR, alpha2 adrenoceptor.

## Introduction

Kidney ischemia-reperfusion (I/R) injury is a common cause of acute kidney injury which often occurs in the context of trauma, sepsis, and multiorgan failure. Despite advances in preventive strategies and support measures, acute kidney injury is associated with high morbidity and mortality. Causes that present I/R – injury are numerous. It is known that hypotension, hypoperfusion, hypoxia, oxidative stress, and renal vasoconstriction contribute to its pathogenesis. (Kunzendorf et al. 2010) This leads to tubular and endothelial cell injury, mitochondrial damage, and further renal vasoconstriction due to activation of the sympathetic nervous system (SNS) and renin–angiotensin–aldosterone axis (Noiri et al. 2001; Devarajan 2006; Hasegawa et al. 2010). There is no specific medication for I/R-induced acute kidney injury (AKI) in clinical use.

The alpha2 adrenoceptor ( $\alpha$ 2-AR) agonist dexmedetomidine (Dex) has sedative, analgesic, and sympatholytic properties (Mantz et al. 2011). There is accumulating evidence to suggest that Dex protects tissues, including kidneys, against ischemic injuries in animal models by affecting various intracellular kinases, oxidant status, and adhesion molecules (Maier et al. 1993; Kuhmonen et al. 1997; Jolkkonen et al. 1999; Gu et al. 2011b; Ibacache et al. 2012; Kilic et al. 2012; Tufek et al. 2013). Dex is already in clinical use as a sedative for intensive care unit patients who require only mild sedation and it seems to enhance renal function also in some clinical cases (Kulka et al. 1996; Frumento et al. 2006). The mechanism(s) behind the possible renoprotective action remains still elusive, especially as Dex has been shown to exert some  $\alpha$ 2-receptor-independent effects (Gu et al. 2011b). The hypotensive effect of Dex might be an asset or a limitation depending on the clinical setting (Coursin et al. 2001; Richa et al. 2004).

We have recently shown in a murine model of renal I/R, that caloric restriction, and thus metabolic preconditioning, attenuates acute kidney injury through peroxisome proliferator-activated receptor gamma coactivator 1 $\alpha$ -eNOS pathway and enhanced autophagy (Lempiäinen et al. 2013). Furthermore, we also demonstrated that metabolic preconditioning with 5-aminoimidazole-4-carboxamide-1- $\beta$ -D-ribofuranoside, an adenosine monophosphate kinase (AMPK) activator, also ameliorated I/R-induced kidney injury (Lempiäinen et al. 2012).

The aim of the present study was to further explore the mechanisms behind the renoprotective effects of Dex. In particular, we investigated whether the time-course of drug administration (preconditioning vs. postconditioning) impacts on the therapeutic effect of Dex, and whether metabolic reprogramming is involved. Furthermore, we compared the therapeutic effects of Dex with

those of peripherally acting  $\alpha$ 2-AR agonist fadolmidine (Fado) in our renal I/R injury model.

## Materials and Methods

### Experimental animals, renal ischemia/reperfusion, drug treatments and sample preparation

All procedures were approved by the Provincial State Office of Southern Finland (approval number STH059A). Every effort was made to ensure that animals experienced minimal discomfort. We used 6–7-week-old male Sprague–Dawley (SD) rats for this study, and results are recorded in accordance with the Animal Research: Reporting In Vivo Experiments guidelines for reporting experiments involving animals. Rats were kept under 12-h light/12-h dark cycle and they had free access to food and water.

An established murine model of acute kidney I/R injury was used (Forbes et al. 2000). Experimental protocols and sample preparation are described elsewhere (Lempiäinen et al. 2012). Briefly, rats were anesthetized with isoflurane, intubated, abdominal incisions were made, and bilateral renal ischemia was induced by clamping renal pedicles for 40 min with microvascular clamps. Control animals were subjected to sham operation without renal pedicle clamping. The rats were hydrated with warm saline during the operation and the body temperature was maintained constantly at 37°C by using a heating pad until awake. The wounds were sutured after removing the clips, and the animals were allowed to recover. Buprenorphine (0.1 mg/kg s.c.) was used as postoperative analgesia.

### Protocol 1: Effects of Dex preconditioning on kidney I/R injury

Six to seven-week-old SD rats were divided into four groups ( $n = 8–11$  per group): (1) Sham-operated group; (2) I/R group (40 min bilateral ischemia followed by 24 h of reperfusion); (3) I/R group + Dex (1  $\mu$ g/kg i.v. given 60 min before the surgery), (4) I/R group + Dex (10  $\mu$ g/kg i.v.). Dex was given as an intravenous bolus 60 min before the surgery. In additional experiments, the effects of Dex infusions (0.1 or 0.3  $\mu$ g/kg/min started 60 min before surgery and continued until 30 min reperfusion period) ( $n = 10$  in both groups) on kidney I/R injury were also examined. Blood samples were taken during the follow-up period for pharmacokinetic (PK) analyses. To further investigate the molecular mechanisms of Dex, we conducted an additional study with the following four groups ( $n = 10$  in each group): (1) I/R group, (2) I/R group + Dex (10  $\mu$ g/kg i.v.), (3) I/R group + necroptosis inhibitor necrostatin-1 (Nec-1) (1.65 mg/kg i.p. given 15 min before operation),

(4) I/R group + Dex + Nec-1. The drug dosage of Nec-1 was selected from previous *in vivo* studies performed in mice (Linkermann et al. 2012, 2013).

### Protocol 2: Effects of Dex postconditioning on kidney I/R injury

Six to seven-week-old SD rats were divided into four groups ( $n = 5-10$  per group): (1) Sham-operated group; (2) I/R group (40 min bilateral ischemia followed by 24 h of reperfusion); (3) I/R group + Dex (1  $\mu\text{g}/\text{kg}$  i.v. given immediately after the 40 min ischemic period), (4) I/R group + Dex (10  $\mu\text{g}/\text{kg}$  i.v.).

### Protocol 3: Effects of fadolmidine pre- and postconditioning on kidney I/R injury

In these studies, the effects pre- and postconditioning with a peripheral  $\alpha_2$ -AR agonist fadolmidine, on kidney I/R injury were examined as outlined in protocols 1 and 2. Fadolmidine preconditioning was given as an intravenous bolus 60 min before the surgery at the dosage of 1 or 10  $\mu\text{g}/\text{kg}$  ( $n = 7-18$  per group). Fadolmidine postconditioning ( $n = 5-10$  per group) was given as an intravenous bolus (1 and 10  $\mu\text{g}/\text{kg}$ ) given immediately after the 40 min ischemic period.

### Protocol 4: Effects of Dex and fadolmidine preconditioning and infusions on hemodynamics in kidney I/R injury

In a separate experiment ( $n = 5-6$  per group), the hemodynamic effects of Dex and fadolmidine, 1  $\mu\text{g}/\text{kg}$  and 10  $\mu\text{g}/\text{kg}$  preconditioning bolus groups, and 0.1  $\mu\text{g}/\text{kg}/\text{min}$  and 0.3  $\mu\text{g}/\text{kg}/\text{min}$  infusion groups, were continuously recorded by using intra-arterial catheter inserted into carotid artery and a Powerlab system (AD Instruments, Germany). Twenty four hours after the operation, the rats were anesthetized with isoflurane and blood samples were collected from inferior vena cava with 5 mL syringe and 22G needle for biochemical measurements. The kidneys were excised, washed with ice-cold saline, blotted dry, and weighed. Tissue samples for histology were fixated in 10% formalin and processed to paraffin with routine methodology. Samples for immunohistochemistry were snap-frozen in  $-38^\circ\text{C}$  isopentane. For protein and gene expression studies, kidney samples were snap-frozen in liquid nitrogen and stored at  $-80^\circ\text{C}$  until assayed. All samples were stored at  $-80^\circ\text{C}$  until assayed.

### Kidney histology

For histological examination, 4- $\mu\text{m}$  thick paraffin sections were cut and stained with hematoxylin-eosin.

Morphological changes from the whole cross-sectional area of cortex and medulla were assessed ( $n = 5-11$ ) by pathologist (P. F.) according to acute tubular necrosis (ATN) – scoring system (magnification 200 $\times$ ,  $\geq 20$  fields per kidney section quantified using the ATN scoring system) as described elsewhere (Lempiäinen et al. 2012). Evaluation of histopathological changes included the loss of tubular brush border, tubular dilatation, cast formation, and cell lysis. Tissue damage was quantified in a blinded manner and scored according to the percentage of damaged tubules in the sample: 0, no damage; 1, <25% damage; 2, 25–50% damage; 3, 50–75% damage; and 4, >75% damage.

### Immunohistochemistry

For immunohistochemistry, frozen kidneys were processed and semiquantitative scoring of inflammatory cells was performed ( $n = 10$  per group) as described elsewhere (Lempiäinen et al. 2012). The relative amount of antibody-positive signal in cortical and medullary areas per sample was determined with computerized densitometry (Leica IM500 and Leica QWIN software; Leica Microsystems AG, Heerbrugg, Switzerland). Primary monoclonal antibody against rat monocyte/macrophage ED-1 (1/300; Serotec Ltd, Oslo, Norway) as well as peroxidase-conjugated rabbit anti-mouse and biotinylated anti-rabbit (Vector Laboratories Inc., Burlingame, CA) secondary antibodies (Dako A/S, Glostrup, Denmark) were used.

### Western blotting

Western blotting of the target proteins ( $n = 5-11$  per group) was performed as described elsewhere (Lempiäinen et al. 2013). Sirtuin 1 (SIRT1) expression was assessed from nuclear proteins. The following antibodies were used: protein kinase B (Akt) (Akt, 1/1000; Cell Signaling, Beverly, MA), pAkt (Phospho-Akt, 1/750; Cell Signaling), AMPK (AMPKalpha, 1/750; Cell Signaling), pAMPK (pAMPKalpha, 1/1000; Cell Signaling), endothelial nitric oxide synthase (eNOS) (NOS3, 1/500; Santa Cruz Biotechnologies, Santa Cruz, CA), eNOS-P (p-NOS3, 1/500; Santa Cruz Biotechnologies), light chain 3B (LC-3B) (LC3B, 1/500; Cell Signaling), p38 (p38 MAP Kinase, 1/1000; Cell Signaling), phospho-p38 (Phospho-p38 MAPK, 1/1000; Cell Signaling), toll like receptor 4 (TLR4) (Anti-TLR4, 1/1000; Abcam, Cambridge, MA), and SIRT1 (Anti-Sir2, 1/1000; Upstate, Millipore, Temecula, CA). Tubulin (Antialpha tubulin, 1/3000; Abcam) and beta-actin (beta-Actin, 1/3000; Cell Signaling) were used as the loading controls.

## Microarray

Kidney samples from I/R injury group with and without Dex preconditioning ( $n = 3$  in both groups) were powdered in liquid nitrogen. Total RNA was isolated with TRIzol Reagent (Invitrogen, Carlsbad, CA) and purified with the RNeasy mini kit (Qiagen, Valencia, CA) according to the manufacturers' instructions. The concentration and integrity of total RNA were analyzed with a spectrophotometer and 2100 Bionalyzer (Agilent Technologies, Santa Clara, CA). Further, sample processing and hybridization to Sureprint G3 Rat GE 8x60K microarrays (Agilent Technologies) representing over 30,000 rat transcripts was performed by Biomedicum Functional Genomics Unit ([www.helsinki.fi/fugu/](http://www.helsinki.fi/fugu/)). Data were normalized to the median with the RMA reprocessing algorithm and analyzed with GeneSpring 11 software (Agilent Technologies). Differentially expressed probe sets were selected based on filtering by the parametric statistical analysis not assuming equal variances (Welch-type *t*-test) with  $P < 0.05$  as a threshold for significance, followed by filtering for fold change ( $\pm 1.2$ -fold) between the compared groups. The functional annotation describing the categorical data for gene ontology and pathway information for the lists of genes were performed with DAVID (Database for Annotation, Visualization and Integrated Discovery, 2008) (Dennis *et al.* 2003). Data were also analyzed through the use of IPA (Ingenuity<sup>®</sup> Systems, [www.ingenuity.com](http://www.ingenuity.com)).

## Biochemical determinations

Serum creatinine, electrolytes, lipids, and liver enzymes were measured by routine laboratory techniques (ADVIA 1650 Chemistry System; Siemens Healthcare Diagnostics Inc., Deerfield, IL).

## Measurement of plasma Dex concentration

In the PK study, blood samples were taken at 0.25, 0.5, 2, and 3 h from the tail vein of conscious rats. Dex concentrations were determined by a validated liquid chromatography–mass spectrometry/mass spectrometry method. The estimate for plasma concentration at 1 h was performed using nonlinear fit in GraphPad Prism software (v6.02, San Diego, CA).

Drug concentrations after different Dex infusion rates were measured from plasma samples taken just before the reperfusion phase (100 min after beginning of infusion) ( $n = 7$  in each infusion group and  $n = 2–3$  for each time point after *i.v.* bolus). For bolus and infusion PK studies, the lower limits of quantification were 0.0200 and 0.100 ng/mL for Dex.

## Statistical analysis

Data are presented as the mean + SEM. Statistically significant differences in mean values were tested by analysis of variance (ANOVA) and the Bonferroni's post hoc test. The differences were considered significant when  $P < 0.05$ .

## Results

### Effects of Dex preconditioning on kidney I/R injury

Acute kidney I/R injury was associated with a 7.1-fold increase in serum creatinine (S-creatinine) concentration and a 7.0-fold increase in serum urea (S-urea) level as compared to sham-operated controls. Histopathological analysis of the kidneys harvested 24 h after I/R injury showed marked loss of brush border, tubular dilatation, cast formation, and cell lysis indicating severe ATN. Dex preconditioning with higher 10  $\mu\text{g}/\text{kg}$  bolus dose decreased S-creatinine concentration 30% and S-urea concentration 23% (Fig. 1A and B), while the smaller dose (1  $\mu\text{g}/\text{kg}$ ) did not influence AKI-induced kidney dysfunction. Interestingly, both Dex doses ameliorated I/R-injury-induced ATN (Fig. 1C–F).

Continuous Dex infusions (0.1 or 0.3  $\mu\text{g}/\text{kg}/\text{min}$ ) did not have any effect on serum creatinine or urea levels. However, I/R-injury-induced ATN was ameliorated by the infusion with 0.3  $\mu\text{g}/\text{kg}/\text{min}$  (Fig. S1A–C).

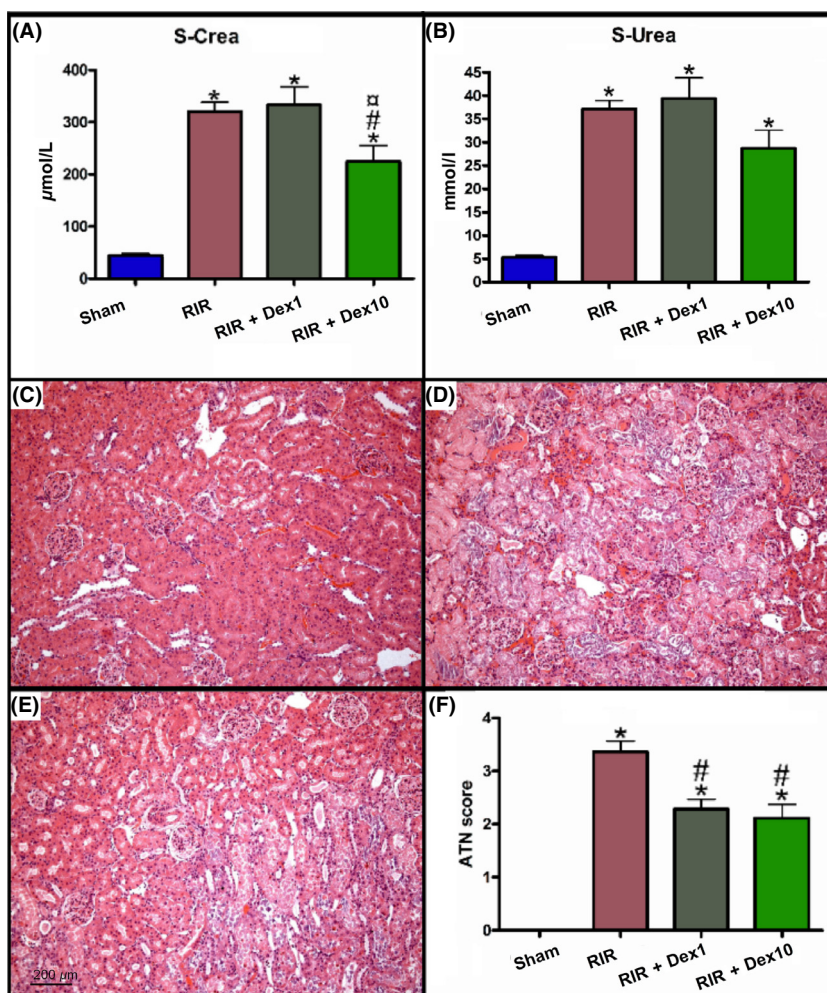
In an additional study where we investigated the effects of Dex and Nec-1 alone and in combination, Dex decreased the serum creatinine level by 44% ( $P < 0.05$ ) and urea level by 22% in rats with I/R injury. Treatment with necroptosis inhibitor Nec-1 alone, slightly but not significantly, decreased serum creatinine level by 12% and urea level by 6%. Cotreatment with Dex and Nec-1 decreased serum creatinine by 50% ( $P < 0.05$ ) and urea by 36%. There were no statistically significant differences between Dex and Dex+ Nec-1 groups.

### Effects of Dex postconditioning on kidney I/R injury

Dex postconditioning (1 or 10 mg/kg *i.v.* bolus after 40 min ischemic period and prior reperfusion phase), did not influence kidney function or the degree of ATN. (Fig. 2A–C).

### Effects of fadolmidine preconditioning on kidney I/R injury

As compared to untreated RIR group, fadolmidine preconditioning with 10  $\mu\text{g}/\text{kg}$  bolus decreased S-creatinine



**Figure 1.** Effects of dexmedetomidine (Dex) preconditioning on serum creatinine and urea levels (A and B) and on kidney histology (C–F) in rats with renal ischemia-reperfusion injury. Representative photomicrographs from sham-operated rats (C), untreated rats with I/R injury (D), and rats with I/R injury treated with Dex 10 μg/kg bolus (E) are given. Magnification 100×, scale bar 200 μm. Quantification of kidney acute tubular necrosis (ATN) score assessed by percentage of damaged tubules is given in (F). Sham denotes sham-operated rats; RIR, rats with renal I/R injury; RIR + Dex1, rats with I/R injury treated with Dex 1 μg/kg bolus; RIR + Dex10, rats with I/R injury treated with Dex 10 μg/kg bolus. Means ± SEM are given,  $n = 5–11$  in each group. \* $P < 0.05$  vs. Sham; # $P < 0.05$  vs. RIR. □ $P < 0.05$  vs. Dex 1 μg/kg bolus.

and S-urea by and 19% and 17%, respectively, however, the differences did not reach statistical significance. Smaller (1 mg/kg) fadolmidine bolus did not have any effect on S-creatinine or S-urea. Neither of the fadolmidine doses affected the I/R-injury-induced ATN. (Fig. 2A–F).

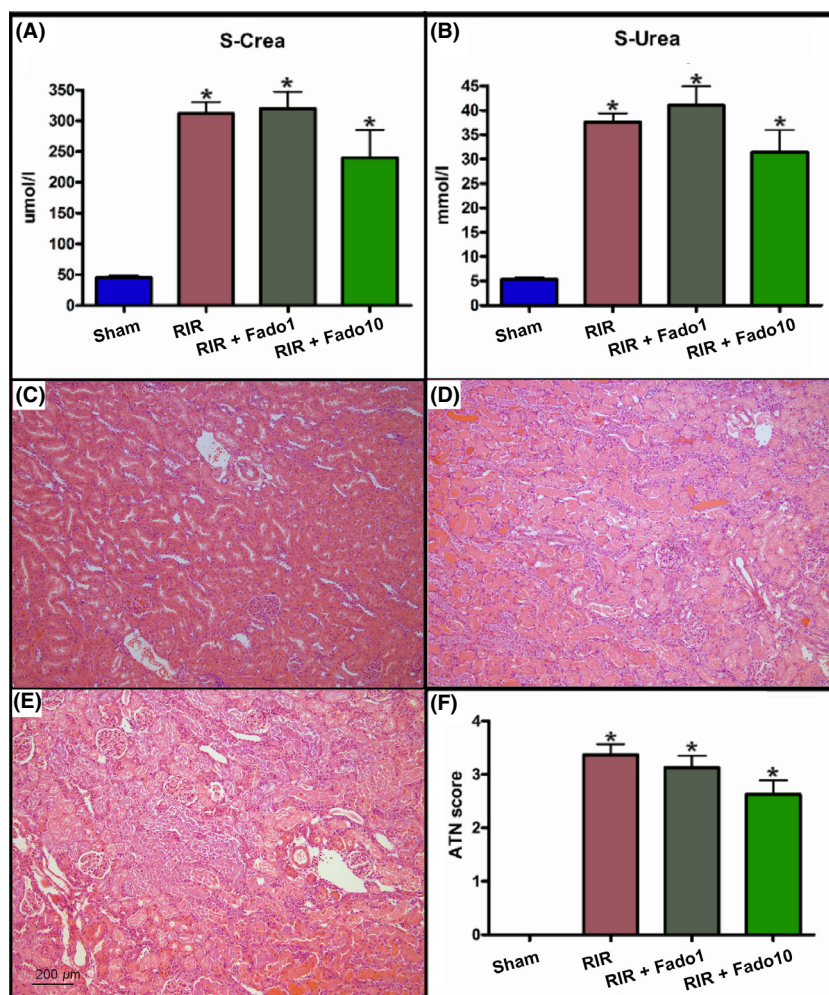
### Effects of fadolmidine postconditioning on kidney I/R injury

Fadolmidine postconditioning (1 or 10 mg/kg) bolus administered after 40 min of ischemia, did not have any effect on S-creatinine, S-urea, or to the degree of tubular necrosis. (Fig. S3A–C).

### Hemodynamic effects of Dex and fadolmidine

Mean arterial blood pressure (MAP) and heart rate (HR) at baseline before administration of drugs averaged  $107.1 \pm 11.3$  mmHg and  $405 \pm 35$  bpm, respectively, and did not differ between the treatment groups (ANOVA  $P = 0.09$  and  $0.32$ , respectively).

Dex decreased blood pressure (BP) more prominently than fadolmidine (Figs. 3 and 4). In bolus groups, the percentage change in MAP was dose-dependent with both drugs (Fig. 3A). Both Dex infusion rates (0.1 μg/kg/min and 0.3 μg/kg/min) decreased BP to a similar extent



**Figure 2.** Effects of fadolmidine preconditioning on serum creatinine and urea levels (A and B) and on kidney histology (C–F) in rats with renal ischemia-reperfusion injury. Representative photomicrographs from sham-operated rats (C), untreated rats with I/R injury (D), and rats with I/R injury treated with fadolmidine 10  $\mu\text{g}/\text{kg}$  bolus (E) are given. Magnification 100 $\times$ , scale bar 200  $\mu\text{m}$ . Quantification of kidney acute tubular necrosis (ATN) score assessed by percentage of damaged tubules is given in (F). RIR + Fado1 denotes rats with I/R injury treated with fadolmidine (Fado) 1  $\mu\text{g}/\text{kg}$  bolus; RIR + Fado10, rats with I/R injury treated with Fado 10  $\mu\text{g}/\text{kg}$  bolus. For other abbreviations, see Figure 1. Means  $\pm$  SEM are given,  $n = 5\text{--}18$  in each group. \* $P < 0.05$  vs. Sham.

compared to higher Dex bolus, whereas neither Fado infusion influenced BP (Figs. 3B and 4B).

Percentage change in MAP 0–50 min after administration of drugs with Dex 10  $\mu\text{g}/\text{kg}$  bolus differed from all other bolus groups (Fig. 4A).

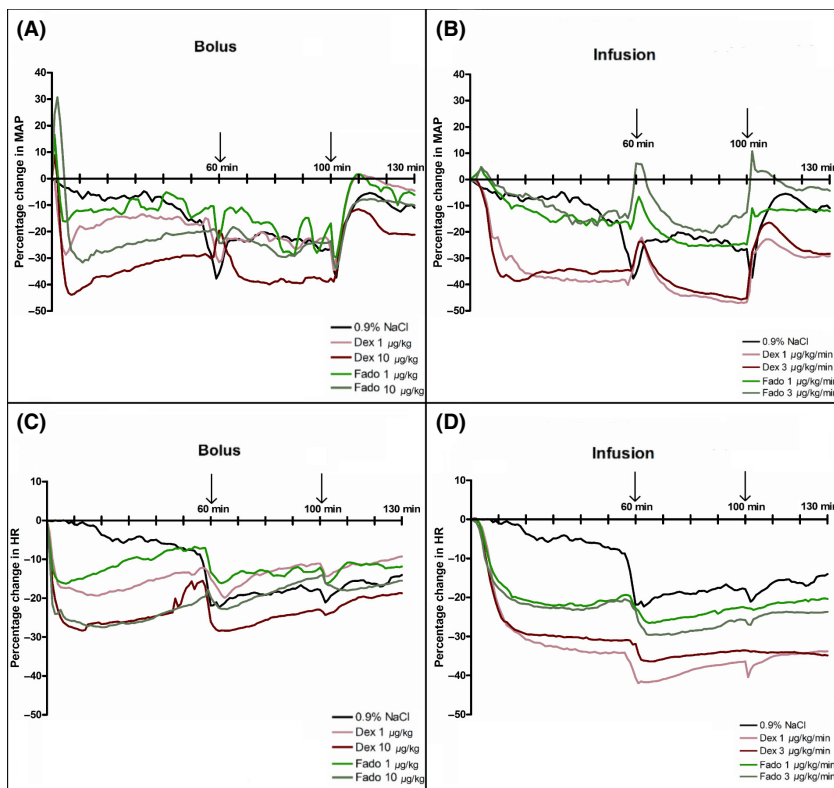
After a transient increase in BP, fadolmidine-treated animals receiving lower dose showed a similar decrease in the BP than the group receiving saline, while the higher dose had a slight hypotensive effect (Fig. 4A). The transient increase in BP occurred after 2 min with bolus dosing and after four with infusions. The biggest percentage change in MAP within bolus groups was caused by Dex 10  $\mu\text{g}/\text{kg}$  bolus 8 min after administration of drugs. Within infusions, the biggest decrease

occurred in Dex 0.3  $\mu\text{g}/\text{kg}/\text{min}$  group after 18 min (Fig. 3A and B).

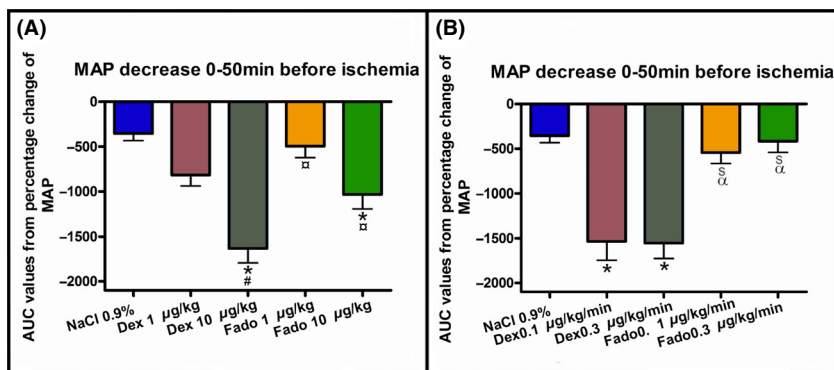
Dex decreased also HR more profoundly than fadolmidine. Dex 0.1  $\mu\text{g}/\text{kg}/\text{min}$  infusion decreased HR more than Dex 0.3  $\mu\text{g}/\text{kg}/\text{min}$  infusion. With boluses and with Fado infusions, decrease in HR was dose-dependent (Fig. 3C and D).

### Plasma Dex concentration

Plasma Dex concentrations 15, 30, 120, and 180 min after the 10  $\mu\text{g}/\text{kg}$  bolus administration were 2.8, 0.80, 0.28, and 0.16 ng/mL, respectively. Calculated from the measured time points, the plasma dex concentration at time point 60 min (corresponding the time point when



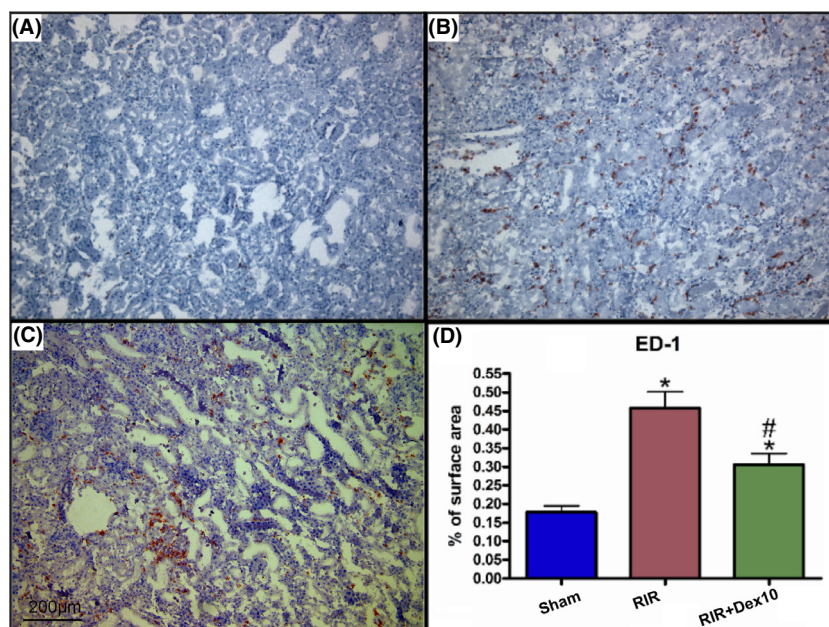
**Figure 3.** Effects of dexmedetomidine and fadolmidine on hemodynamics during the experimental procedure in rats with renal ischemia-reperfusion injury. Percentage change from baseline MAP is given in (A and B). Percentage change in HR is given in (C and D). Timescale on x-axis begins when drugs were administered. Ischemic period begins 60 min after administration of drugs and lasts for 40 min. Maximum decreases in blood pressure and heart rate are given in (C and D).  $n = 5-6$  in each group. MAP, mean arterial blood pressure; HR, heart rate. For other abbreviations, see Figures 1 and 4.



**Figure 4.** Effects of dexmedetomidine (Dex) and fadolmidine on MAP before ischemic period. Percentage change in MAP from baseline, represented with AUC values, during 0–50 min time period after administration of drugs for bolus groups are given in (A) and for infusion groups in (B).  $n = 5-6$  in each group. \* $P < 0.05$  vs. NaCl 0.9%; # $P < 0.05$  vs. Dex 1  $\mu\text{g}/\text{kg}$  bolus;  $\square P < 0.05$  vs. Dex 10  $\mu\text{g}/\text{kg}$  bolus;  $\square P < 0.05$  vs. Dex 0.1  $\mu\text{g}/\text{kg}/\text{min}$  infusion;  $\alpha P < 0.05$  vs. Dex 0.3  $\mu\text{g}/\text{kg}/\text{min}$  infusion. AUC, area under curve; MAP, mean arterial blood pressure; min, minutes, NaCl 0.9%, Sodium chloride 0.9%. For other abbreviations see Figures 1 and 4.

ischemic period started) and time point 40 min (corresponding time point when reperfusion was initiated) were 0.38 and 0.33 ng/ml, respectively.

Plasma Dex concentrations at time point 90 min after continuous infusion with infusion rates 0.1 and 0.3  $\mu\text{g}/\text{kg}/\text{min}$  were 0.58 and 1.3 ng/mL, respectively.



**Figure 5.** Effects of dexmedetomidine on monocyte/macrophage infiltration (ED1-immunopositive cells) (A–D) in rats with renal ischemia-reperfusion injury. Representative photomicrographs from sham-operated rats (A) and untreated rats with I/R injury (B) and rats with I/R injury treated with dexmedetomidine 10  $\mu\text{g}/\text{kg}$  bolus (C) are given. Magnification 200 $\times$ , scale bar 100  $\mu\text{m}$ . Quantification of ED1-positive cells in the kidney is given in (D). Means  $\pm$  SEM are given,  $n = 10$ –11 in each group. \* $P < 0.05$  vs. Sham. # $P < 0.05$  vs. RIR. For abbreviations see Figure 1.

### Effects of Dex on renal inflammation, autophagy, p38 and eNOS

Renal I/R injury was associated with a 2.6-fold increase in the number of ED1-positive inflammatory cells in the kidney as compared to sham-operated controls. The higher dose of Dex suppressed the I/R-injury-induced monocyte/macrophage infiltration in the renal parenchyma (Fig. 5).

Dex prevented the I/R-induced decrements in the total (14 kDa/ $\beta$ -actin) and relative (14 kDa/16 kDa ratio) expression levels of the active short form of autophagy marker LC-3B (Fig. 6A and B).

The total expression of p38 MAPK was elevated to 1.6-fold in I/R animals while expression of its phosphorylated form was increased by 1.4-fold. Dex further increased the expression of both the total and phosphorylated forms of p38 MAPK (Fig. 6C and D).

I/R injury increased renal eNOS expression, both in the presence and in the absence of Dex.

I/R injury did not have any effect on phosphorylated eNOS expression, whereas Dex treatment increased it significantly (Fig. 6E and F).

### Dex-induced changes in renal transcriptomics

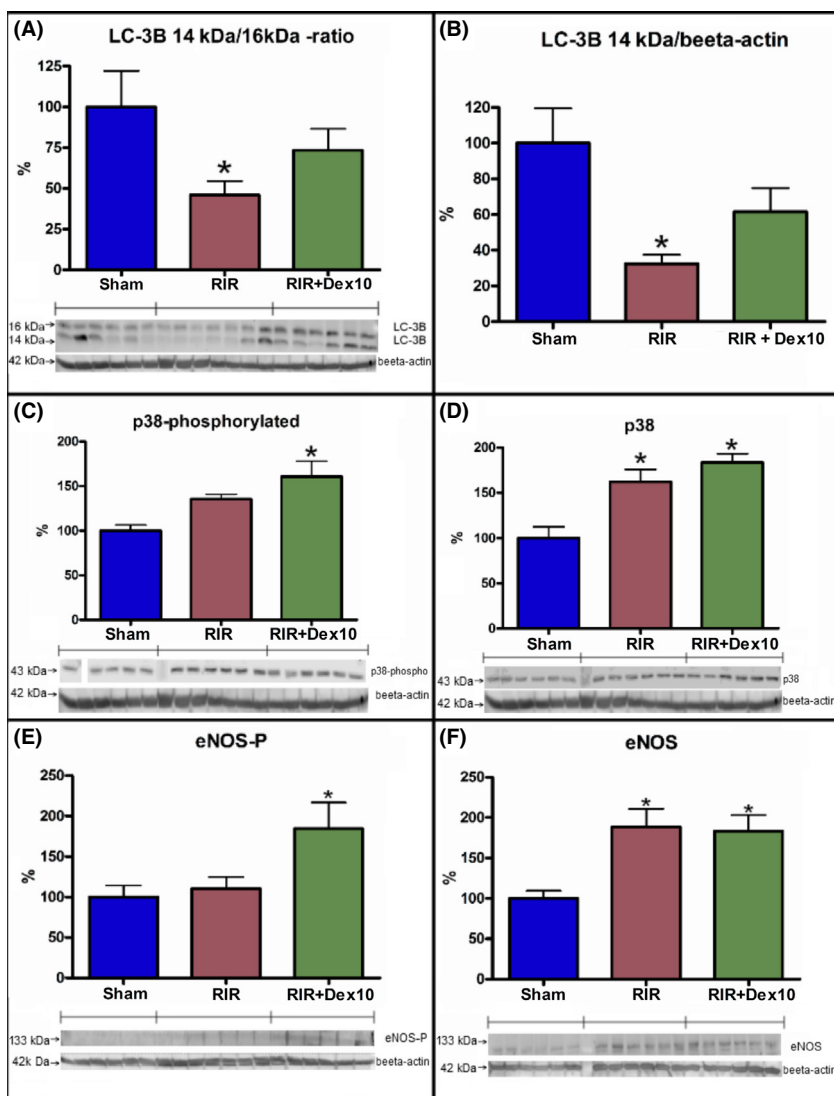
Dex treatment altered the expression of 306 genes as compared to untreated I/R group. 245 of these genes

were downregulated with Dex and 61 genes were upregulated. There were 17 enriched “Kyoto Encyclopedia of Genes and Genomes” (KEGG) pathways among all regulated genes, of which extracellular matrix (ECM)-receptor interaction, hypertrophic cardiomyopathy, (transforming growth factor) TGF- $\beta$  signaling, dilated cardiomyopathy, focal adhesion, regulation of actin cytoskeleton, chemokine signaling, and pathways in cancer pathways were most significantly enriched (Table 2.). Ingenuity analysis revealed inhibition of Rac and nuclear factor (erythroid-derived 2)-like 2 pathways, whereas “aryl hydrocarbon receptor” (AHR) pathway was activated. Dex decreased the expression of Rab27b, Rasa2 and CDC42-binding protein kinase alpha isoform B, which are all associated to RAC signaling. Also expression of two RAB GTPases (also members of RAS superfamily) associated genes, Rabgap1 and Rabep1, decreased with Dex treatment. Moreover, Ingenuity analysis revealed also, that Dex treatment decreased the expression of inflammatory marker CD44 mRNA levels. (Fig. 7).

### Effects of Dex on TLR-4, Akt, SIRT1, and AMPK expressions

Acute kidney I/R injury was associated with a 1.6-fold increase in TLR-4 expression. Dex did not influence renal TLR-4 expression. (Fig. 8A).





**Figure 6.** Effects of dexmedetomidine on kidney LC-3B, p38 MAPK and eNOS expressions in rats with renal ischemia-reperfusion injury. The expression of LC-3B 14 kDa/16 kDa -ratio, measured by Western blot (20  $\mu$ g total protein of the whole cell lysate per lane), is given in (A). The expression level of LC-3B 14 kDa is given in (B). ( $n = 10-11$ ). The expression of phosphorylated p38, measured by Western blot (20  $\mu$ g total protein of the whole cell lysate per lane), is given in (C). The expression level of total p38 is given in (D). ( $n = 6-7$ ). The expression of phosphorylated eNOS, measured by Western blot (20  $\mu$ g total protein of the whole cell lysate per lane), is given in (E). The expression level of total eNOS is given in (F) ( $n = 6$ ). Means  $\pm$  SEM are given.  $n = 6-11$  in each group. \* $P < 0.05$  vs. Sham. For abbreviations, see Figure 1.

The total Akt expression was increased by 2.5-fold and the level of phosphorylated-Akt by 6.2-fold in I/R animals as compared to sham group while Dex did not have any significant effect on the Akt expressions (Fig. 8B–D).

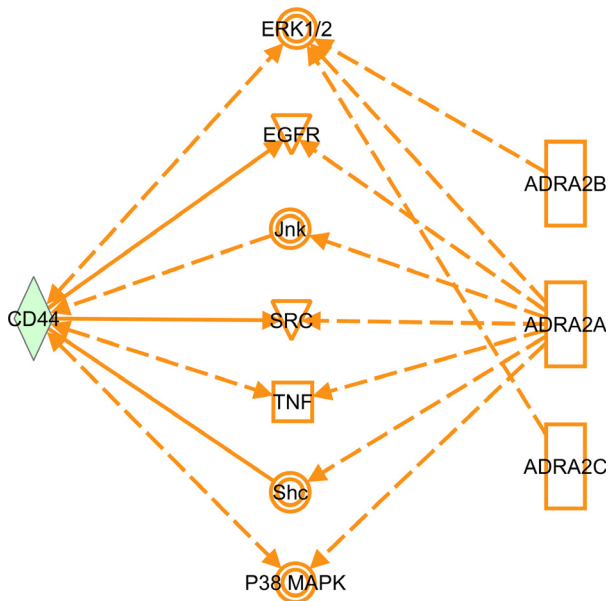
The nuclear SIRT1 expression was increased by 26.2-fold in I/R animals, whereas Dex treatment resulted to a 17.5-fold expression of SIRT1 compared to sham group (Fig. S4A).

The I/R injury led to a 70% decrease in the ratio of phosphorylated/total AMPK expression when compared to sham-operated rats, albeit the total expression of

AMPK increased by 150%. Dex did not affect the phosphorylation level, nor the total expression of AMPK (Fig. S4B–D).

### Effects of Dex on serum biochemistry

Acute kidney I/R injury was associated with 84% and 340% increases in the serum concentrations of alanine aminotransferase and aspartate aminotransferase, respectively, as compared to sham-operated controls, indicating an accompanying AKI-induced hepatic injury (Table 1).



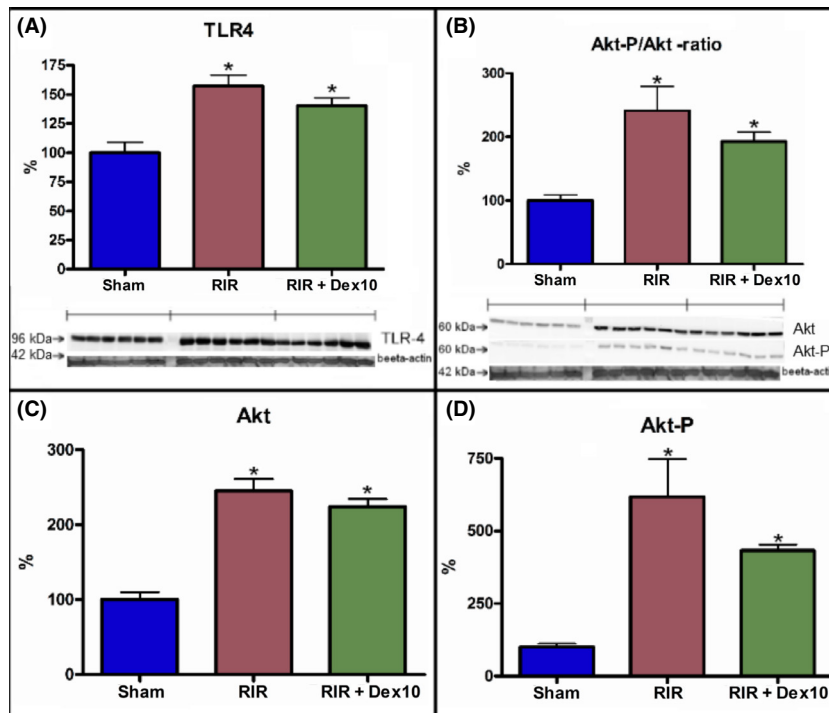
**Figure 7.**  $\alpha$ 2-adrenergic receptors regulate CD 44. Ingenuity analysis shows that  $\alpha$ 2A-ARs regulate CD44 through p38 MAPK. Also other  $\alpha$ 2-adrenergic receptor subtypes and related pathways are shown. The networks were generated through the use of IPA (Ingenuity® Systems, www.ingenuity.com). ADRA2,  $\alpha$ 2-adrenergic receptor.

Serum levels of gamma-glutamyl transferase were also markedly elevated reflecting injury to the proximal tubules (Table 1). Pretreatment with Dex 10  $\mu$ g/kg decreased serum alanine aminotransferase, aspartate aminotransferase, and gamma-glutamyl transferase levels (Table 1).

The I/R-induced alterations in the serum potassium, chloride, and phosphate concentrations were prevented by Dex (Table 1). Neither the I/R injury nor Dex influenced serum sodium, calcium, albumin, or protein concentrations. Nor did the serum glucose, cholesterol, or triglyceride levels differ between the groups. ( $n = 6-11$  per group).

### Discussion

This study demonstrated the renoprotective effects of Dex preconditioning in a murine model of I/R injury. We also demonstrated that peripheral  $\alpha$ 2-AR agonist fadolmidine did not protect kidneys, thus suggesting that the protective effects of Dex are, to some extent, mediated via central  $\alpha$ 2-ARs. Finally, we performed kidney transcriptomics profiling combined with the “Ingenuity Pathways Analysis”



**Figure 8.** Effects of dexmedetomidine on kidney TLR-4 and Akt expressions in rats with renal ischemia-reperfusion injury. The expression of TLR-4 and housekeeping protein beta-actin, measured by Western blot (20  $\mu$ g nuclear protein fraction per lane), are given in (A). The phosphorylation level of Akt measured as Akt-P/Akt ratio and expressions of total Akt, phosphorylated-Akt and housekeeping protein beta-actin are given in (B–D). Means  $\pm$  SEM from three different runs are given,  $n = 5-7$  in each group. \* $P < 0.05$  vs. Sham. For abbreviations see Figure 1.

**Table 1.** Effects of dexmedetomidine treatment on serum biochemistry in rats with kidney I/R injury.

Variable	Sham (n = 7–10)	RIR (n = 8–11)	RIR + Dex (n = 6–10)	ANOVA P-value
s-ALAT (U/L)	46.2 ± 3.0	85.2 ± 5.0*	56.6 ± 3.2 <sup>#</sup>	<0.0001
s-AFOS (U/L)	262 ± 16.2	293 ± 23.3	259 ± 22.7	0.4576
s-ASAT (U/L)	129 ± 7.2	571 ± 44.4*	316.1 ± 39.5* <sup>#</sup>	<0.0001
s-GGT (U/l)	<1.0	29.3 ± 3.6*	16.1 ± 3.6* <sup>#</sup>	<0.0001
s-Protein(g/L)	52.7 ± 1.0	53.1 ± 1.2	54.4 ± 1.1	0.5429
s-Albumin (g/L)	28.2 ± 0.5	28.1 ± 0.7	28.9 ± 0.6	0.6143
s-K (mmol/L)	4.6 ± 0.1	6.6 ± 0.3*	5.0 ± 0.1 <sup>#</sup>	<0.0001
s-Na (mmol/L)	138 ± 1.3	136 ± 2.3	139 ± 1.1	0.5285
s-Cl (mmol/L)	97.3 ± 0.9	89.0 ± 1.8*	98.2 ± 1.6 <sup>#</sup>	0.0005
s-Calsium (mmol/L)	2.4 ± 0.0	2.4 ± 0.1	2.4 ± 0.0	0.8251
s-Pi (mmol/L)	2.9 ± 0.1	4.9 ± 0.3*	3.6 ± 0.3 <sup>#</sup>	<0.0001
s-Chol (mmol/L)	1.74 ± 0.1	1.65 ± 0.1	1.63 ± 0.1	0.6517
s-Trigly (mmol/L)	0.82 ± 0.1	0.85 ± 0.1	0.63 ± 0.1	0.1492
s-Gluk (mmol/L)	7.9 ± 0.2	7.9 ± 0.5	7.4 ± 0.2	0.5623

Sham denotes sham-operated rats; RIR, rats with renal I/R injury; Dex, rats with I/R injury treated with dexmedetomidine at dose 10 µg/kg i.v. Means ± SEM are given, n = 6–11 in each group. \*P < 0.05 vs. Sham; #P < 0.05 vs. RIR.

**Table 2.** The enriched KEGG pathways among all regulated genes.

Term	Genes	P-value	Benjamini	Downregulated genes		Upregulated genes			
ECM-receptor interaction	5	8.0E-3	5.2E-1	Cd44	Golga4	Itgb1	Itga2	Itgb6	
Hypertrophic cardiomyopathy (HCM)	5	9.0E-3	3.4E-1	Igf1	Golga4	Itgb1	Itga2	Itgb6	
TGF-β signaling pathway	5	9.8E-3	2.6E-1	Bmpr1b	Bmpr2	Cul1	Dcn	Inhbb	
Dilated cardiomyopathy	5	1.1E-2	2.3E-1	Igf1	Golga4	Itgb1	Itga2	Itgb6	
Focal adhesion	7	1.2E-2	1.9E-1	Igf1	Golga4	Itgb1	Itga2	Itgb6	Pgf
Regulation of actin cytoskeleton	7	1.6E-2	2.2E-1	Wasf1	Golga4	Itgam	Itgb1	Itga2	Itgb6
Chemokine signaling pathway	6	2.6E-2	2.9E-1	Fgr	Ccl12	Ccl2	Ccl3	Ccl7	Pik3ca
Arrhythmogenic right ventricular cardiomyopathy (ARVC)	4	3.6E-2	3.4E-1	Golga4	Itgb1	Itga2	Itgb6		
Aminoacyl-tRNA biosynthesis	3	5.8E-2	4.6E-1	Rars	Nars	Eprs			
Arginine and proline metabolism	3	9.5E-2	6.0E-1	Aldh1a7	Glul	Gamt			
mTOR signaling pathway	3	9.5E-2	6.0E-1	Igf1	Pgf	Pik3ca			
Leukocyte transendothelial migration	4	1.0E-1	5.9E-1	Txk	Itgam	Itgb1	Pik3ca		
Spliceosome	4	1.2E-1	6.1E-1	Ddx46	Thoc1	Plrg1	Snrpf		
NOD-like receptor signaling pathway	3	1.2E-1	6.0E-1	Ccl12	Ccl2	Ccl7			
Hematopoietic cell lineage	3	1.8E-1	7.2E-1	Cd44	Itgam	Itga2			
Small cell lung cancer	3	2.0E-1	7.3E-1	Itgb1	Itga2	Pik3ca			
Pathways in cancer	6	2.0E-1	7.2E-1	Igf1	Itgb1	Itga2	Msh2	Pgf	Pik3ca

to identify the pathways involved in the renoprotective effects of Dex.

Dex attenuates myocardial infarcts and protects against the ischemic brain, intestine, liver, and kidney injuries, as well as I/R-induced AKI-related lung injuries in animal models (Maier et al. 1993; Kuhmonen et al. 1997; Jolkkonen et al. 1999; Ma et al. 2004; Gu et al. 2011a,b; Ibacache et al. 2012; Kilic et al. 2012; Tufek et al. 2013). In our study, Dex preconditioning bolus and, to a lesser extent, infusion protected against I/R-induced acute kidney injury reflected as decrements in the S-creatinine and histopathological damage while postconditioning did not provide

any tissue protection. In a majority of studies where Dex has been shown to protect against tissue damage, Dex has been administered before or during the ischemic period by a bolus, infusion, or the combination of both (Maier et al. 1993; Kuhmonen et al. 1997; Jolkkonen et al. 1999; Ma et al. 2004; Gu et al. 2011b; Ibacache et al. 2012; Tufek et al. 2013). In contrast, data on the effects of Dex postconditioning are scarce. In some of these studies, the effects of Dex have been beneficial (Dahmani et al. 2010; Gu et al. 2011b; Kilic et al. 2012). Gu et al. (2011b) combined various in vivo and in vitro I/R models and were able to demonstrate that the renoprotective effects of Dex

were linked to suppression of the HMBG1-TLR4 proinflammatory signaling pathway. In the same study, Dex postconditioning protected against I/R-induced kidney damage. However, while I/R injury increased kidney TLR4 expression in the present study, Dex did not seem to have an effect on it, apparently depicting the differences in experimental settings between the aforementioned studies.

$\alpha$ 2-AR agonists act through binding to G-protein-coupled  $\alpha$ 2-AR, of which there are three subtypes in humans ( $\alpha$ 2A,  $\alpha$ 2B, and  $\alpha$ 2C). These receptors are found ubiquitously in the central, peripheral, and autonomic nervous systems, as well as in vital organs and blood vessels. (Kaur and Singh 2011). Human and rat kidneys contain all three types of  $\alpha$ 2-ARs (Cussac *et al.* 2002) and rats have also  $\alpha$ 2D ARs, which is a rodent species variant of human  $\alpha$ 2A-AR (Lehtimäki *et al.* 2008). Beside  $\alpha$ 2-ARs, alpha1 adrenoceptors ( $\alpha$ 1-AR) are also widely distributed and can be found in the kidneys with a ratio of ~1:3 compared to  $\alpha$ 2-ARs (Schmitz *et al.* 1981).

Dex is highly selective  $\alpha$ 2-AR agonist (Hamasaki *et al.* 2002) and its sedative actions come mainly through  $\alpha$ 2A-ARs of locus ceruleus of the brain stem and analgesic actions through  $\alpha$ 2A-ARs in the spinal cord. In the heart,  $\alpha$ 2-AR agonists induce bradycardia and in peripheral blood vessels,  $\alpha$ 2-AR agonists cause sympatholysis-mediated long-term vasodilatation while in the smooth muscle cells a transient  $\alpha$ 2 and  $\alpha$ 1 receptor-mediated vasoconstriction may be noted (Lehtimäki *et al.* 2008; Kaur and Singh 2011). Fadolmidine is another  $\alpha$ 2-AR agonist having antinociceptive effects when administered intrathecally (Lehtimäki *et al.* 2008). It is a full agonist at all three subtypes of human  $\alpha$ 2-ARs ( $\alpha$ 2A,  $\alpha$ 2B, and  $\alpha$ 2C) and activates also rodent  $\alpha$ 2D-ARs, as well as human  $\alpha$ 1A- and  $\alpha$ 1B-ARs. (Lehtimäki *et al.* 2008; Leino *et al.* 2009).

In the current study, Dex provided protection against the I/R-induced renal dysfunction and histopathological damage while fadolmidine did not. Both substances lowered BP slightly when the preconditioning bolus was given but significant drop was only reached with a continuous Dex infusion. In contrast to Dex, Fado is also a full agonist at the  $\alpha$ 1-ARs, thus explaining the observed transient rise in BP following the bolus (Lehtimäki *et al.* 2008). The BP-dependent effects seemed to play a minor role in this study as the Dex infusion causing the most pronounced hypotensive effect did not affect the serum creatinine levels. However, the involvement of ischemic preconditioning (IPC) in this study cannot be completely ruled out (Sharfuddin and Molitoris 2011).

The aim of the present study was also to investigate whether the renoprotective effects of Dex were mediated via central and/or peripheral  $\alpha$ 2-ARs. Dex exerts potent sympatholytic and analgesic properties through central and peripheral  $\alpha$ 2-ARs, while fadolmidine, due to its

polarity and less lipophilic properties, acts mainly peripherally (Lehtimäki *et al.* 2008; Leino *et al.* 2009). Renal SNS plays an important role in the development of ischemic acute renal failure and nociceptive signaling is known to activate SNS (Fujii *et al.* 2003). Dex reduces renal sympathetic nerve activity and catecholamine release by central sympatho-inhibitory effect. It also decreases the release of lactate from the tissues and thus relieves the recovery from ischemic events (Xu *et al.* 1998; Taoda *et al.* 2001; Willigers *et al.* 2003).

Along with the previous studies suggesting that Dex might protect against kidney I/R injury through its anti-inflammatory properties (Gu *et al.* 2011b), we noted a marked protective effect of Dex against the I/R-induced infiltration of monocyte/macrophage cells (ED1).

Autophagy is a cell protecting mechanism which is activated in response to stress signals from endoplasmic reticulum (Ogata *et al.* 2006). In the current study, autophagy measured as the presence of autophagy marker Light Chain 3 (LC-3B), was markedly downregulated by I/R. This, while revealing a disturbance in cellular survival signaling in this model, also proposes a novel organoprotective mechanism for Dex, as Dex prevented the impairment of autophagic response hence maintaining the crucial degradation and recycling of various cellular components. This finding also incites further studies revealing the more detailed mechanisms of Dex in affecting the individual steps of autophagic flux, that is, the total autophagic process starting with the sequestration of p62 and autophagosome synthesis and ending to the degradation of cellular materials in autolysosomes.

$\alpha$ 2-receptors activate mitogen activated protein kinases (MAPK) and activation of p38 MAPK is known to protect against I/R injury in the rat heart and to contribute to the commencement of autophagic response (Mocanu *et al.* 2000; Nakano *et al.* 2000; Bonventre 2002; Cussac *et al.* 2002; Tang *et al.* 2008). In our study, the I/R induced the total level of renal p38 expression. However, the phosphorylated, active form, of p38 was upregulated only with Dex indicating enhancements in the activities of prosurvival kinase pathways. In addition, the active form of eNOS, a downstream mediator of p38, was significantly induced by Dex.

As to further investigate the cytoprotective mechanisms of Dex in this model, we studied the effect of a death receptor-interacting protein kinase 1 (RIPK1) inhibitor, Nec-1. Necroptosis, a form of programmed necrosis, involves a tumor necrosis factor (TNF)-induced cell death pathway participating in the experimental models of brain, cardiac, and renal ischemia-reperfusion injury (Linkermann *et al.* 2012). In line with the previous studies, we also noticed minor but nonsignificant renoprotective effects by Nec-1 as reflected by decrements in S-creatinine and S-urea levels.

The renoprotective effects of Dex, when given as monotherapy, clearly exceeded those achieved with Nec-1. A minor additive protective effect was found when Dex and Nec-1 were given as combination, thus suggesting that the prevention of necroptosis was not a major cell-protective mechanism of Dex.

Dex treatment altered the expression of 306 genes of which 245 were downregulated and 61 upregulated. These genes represented 17 enriched KEGG pathways. Dex downregulated TGF $\beta$ -pathway which is a known proinflammatory mediator in renal injury (Spurgeon et al. 2005). Dex also suppressed “Hypertrophic Cardiomyopathy”-pathway (broadly overlapping the “Dilated Cardiomyopathy”-pathway) which promotes inflammatory signaling, apoptosis, cellular senescence, activation of the renin-angiotensin system (RAS) and induction of the insulin-like growth factor 1 (IGF-1). Interestingly, the inhibition of RAS is a key therapeutic approach to prevent the development of renal damage among hypertensives and diabetics. In addition, Dex has been shown to suppress the (RAS inducible) janus kinase/signal transducer and activator of transcription pathway in a murine model of renal I/R (Si et al. 2013). Reduced IGF-1 signaling, in turn, is suggested to contribute to the “antiaging” effects achieved with caloric restriction (Barzilai and Bartke 2009).

Dex downregulated the expression of “ECM-receptor interaction”-pathway in which the CD44 gene is included. The CD44 receptor has a crucial role in cell adhesion and promotes the transendothelial migration of leukocytes to injured tissue (Chase et al. 2012). Therefore, Dex-induced decrease in CD44 expression is likely to contribute to the decreased leukocyte extravasation after I/R injury. While several proinflammatory pathways were inhibited by Dex, there was a concomitant activation of the organoprotective AHR pathway. AHR pathway consists of a family of transcriptional regulators which are activated in response to hypoxia and are capable of inhibiting the proinflammatory mediator NF- $\kappa$ B.

In conclusion, Dex preconditioning, but not Dex postconditioning or treatment with a peripheral  $\alpha$ 2-AR agonist fadolmidine, ameliorates kidney I/R injury and inflammatory response. The renoprotective effects of Dex were mediated, at least in part, through central mechanisms and associated with increased renal p38 MAPK, maintenance of autophagy and suppression of several pathways promoting hypertrophy and inflammatory signaling.

## Acknowledgements

The present study was supported by the Sigrid Juselius Foundation and the Academy of Finland. We are grateful to Päivi Leinikka, Nada Bechara-Hirvonen and Anneli von Behr for excellent technical assistance.

## Disclosure

None declared.

## References

- Barzilai N, Bartke A (2009). Biological approaches to mechanistically understand the healthy life span extension achieved by calorie restriction and modulation of hormones. *J Gerontol A Biol Sci Med Sci* 64: 187–191.
- Bonventre JV (2002). Kidney ischemic preconditioning. *Curr Opin Nephrol Hypertens* 11: 43–48.
- Chase SD, Magnani JL, Simon SI (2012). E-selectin ligands as mechanosensitive receptors on neutrophils in health and disease. *Ann Biomed Eng* 40: 849–859.
- Coursin DB, Coursin DB, Maccioli GA (2001). Dexmedetomidine. *Curr Opin Crit Care* 7: 221–226.
- Cussac D, Schaak S, Gales C, Flordellis C, Denis C, Paris H (2002).  $\alpha$ (2B)-Adrenergic receptors activate MAPK and modulate proliferation of primary cultured proximal tubule cells. *Am J Physiol Renal Physiol* 282: 943–952.
- Dahmani S, Rouelle D, Gressens P, Mantz J (2010). Characterization of the postconditioning effect of dexmedetomidine in mouse organotypic hippocampal slice cultures exposed to oxygen and glucose deprivation. *Anesthesiology* 112: 373–383.
- Dennis G Jr, Sherman BT, Hosack DA, Yang J, Gao W, Lane HC, et al. (2003). DAVID: database for annotation, visualization, and integrated discovery. *Genome Biol* 4: 3.
- Devarajan P. (2006). Update on mechanisms of ischemic acute kidney injury. *J Am Soc Nephrol* 17:1503–1520.
- Forbes JM, Hewitson TD, Becker GJ, Jones CL (2000). Ischemic acute renal failure: Long-term histology of cell and matrix changes in the rat. *Kidney Int* 57: 2375–2385.
- Frumento RJ, Logginidou HG, Wahlander S, Wagener G, Playford HR, Sladen RN (2006). Dexmedetomidine infusion is associated with enhanced renal function after thoracic surgery. *J Clin Anesth* 18: 422–426.
- Fujii T, Kurata H, Takaoka M, Muraoka T, Fujisawa Y, Shokoji T, et al. (2003). The role of renal sympathetic nervous system in the pathogenesis of ischemic acute renal failure. *Eur J Pharmacol* 481: 241–248.
- Gu J, Chen J, Xia P, Tao G, Zhao H, Ma D (2011a). Dexmedetomidine attenuates remote lung injury induced by renal ischemia-reperfusion in mice. *Acta Anaesthesiol Scand* 55: 1272–1278.
- Gu J, Sun P, Zhao H, Watts HR, Sanders RD, Terrando N, et al. (2011b). Dexmedetomidine provides renoprotection against ischemia-reperfusion injury in mice. *Crit Care* 15: 153.
- Hamasaki J, Tsuneyoshi I, Katai R, Hidaka T, Boyle WA, Kanmura Y (2002). Dual  $\alpha$ (2)-adrenergic agonist and

- alpha(1)-adrenergic antagonist actions of dexmedetomidine on human isolated endothelium-denuded gastroepiploic arteries. *Anesth Analg* 94: 1434–1440.
- Hasegawa K, Wakino S, Yoshioka K, Tatematsu S, Hara Y, Minakuchi H, et al. (2010). Kidney-specific overexpression of Sirt1 protects against acute kidney injury by retaining peroxisome function. *J Biol Chem* 285: 13045–13056.
- Ibacahe M, Sanchez G, Pedrozo Z, Galvez F, Humeres C, Echevarria G, et al. (2012). Dexmedetomidine preconditioning activates pro-survival kinases and attenuates regional ischemia/reperfusion injury in rat heart. *Biochim Biophys Acta* 1822: 537–545.
- Jolkkonen J, Puurunen K, Koistinaho J, Kauppinen R, Haapalinna A, Nieminen L, et al. (1999). Neuroprotection by the alpha2-adrenoceptor agonist, dexmedetomidine, in rat focal cerebral ischemia. *Eur J Pharmacol* 372: 31–36.
- Kaur M, Singh PM. (2011). Current role of dexmedetomidine in clinical anesthesia and intensive care. *Anesth Essays and Res* 5: 128–133.
- Kilic K, Hanci V, Seleik S, Sozmen M, Kilic N, Citil M, et al. (2012). The effects of dexmedetomidine on mesenteric arterial occlusion-associated gut ischemia and reperfusion-induced gut and kidney injury in rabbits. *J Surg Res* 178: 223–232.
- Kuhmonen J, Pokorny J, Miettinen R, Haapalinna A, Jolkkonen J, Riekkinen PS, et al. (1997). Neuroprotective effects of dexmedetomidine in the gerbil hippocampus after transient global ischemia. *Anesthesiology* 87: 371–377.
- Kulka PJ, Tryba M, Zenz M (1996). Preoperative alpha2-adrenergic receptor agonists prevent the deterioration of renal function after cardiac surgery: results of a randomized, controlled trial. *Crit Care Med* 24: 947–952.
- Kunzendorf U, Haase M, Rolver L, Haase-Fielitz A (2010). Novel aspects of pharmacological therapies for acute renal failure. *Drugs* 70: 1099–1114.
- Lehtimäki J, Leino T, Koivisto A, Viitamaa T, Lehtimäki T, Haapalinna A, et al. (2008). In vitro and in vivo profiling of fadolmidine, a novel potent alpha(2)-adrenoceptor agonist with local mode of action. *Eur J Pharmacol* 599: 65–71.
- Leino T, Viitamaa T, Haapalinna A, Lehtimäki J, Virtanen R (2009). Pharmacological profile of intrathecal fadolmidine, a alpha2-adrenoceptor agonist, in rodent models. *Naunyn Schmiedebergs Arch Pharmacol* 380: 539–550.
- Lempiäinen J, Finckenberg P, Levijoki J, Mervaala E (2012). AMPK activator AICAR ameliorates ischemia reperfusion injury in the rat kidney. *Br J Pharmacol* 166: 1905–1915.
- Lempiäinen J, Finckenberg P, Mervaala E, Sankari S, Levijoki J, Mervaala E (2013). Caloric restriction ameliorates kidney ischemia reperfusion injury through PGC-1alpha-eNOS-pathway and enhanced autophagy. *Acta Physiol (Oxf)* 208: 410–421.
- Linkermann A, Bräsen JH, Himmerkus N, Liu S, Huber TB, Kunzendorf U, et al. (2012). Rip1 (receptor-interacting protein kinase 1) mediates necroptosis and contributes to renal ischemia/reperfusion injury. *Kidney Int* 81: 751–761.
- Linkermann A, Heller JO, Prókai A, Weinberg JM, De Zen F, Himmerkus N, et al. (2013). The RIP1-kinase inhibitor necrostatin-1 prevents osmotic nephrosis and contrast-induced AKI in mice. *J Am Soc Nephrol* 24: 1545–1557.
- Ma D, Hossain M, Rajakumaraswamy N, Arshad M, Sanders RD, Franks NP, et al. (2004). Dexmedetomidine produces its neuroprotective effect via the alpha 2A-adrenoceptor subtype. *Eur J Pharmacol* 502: 87–97.
- Maier C, Steinberg GK, Sun GH, Zhi GT, Maze M (1993). Neuroprotection by the alpha 2-adrenoceptor agonist dexmedetomidine in a focal model of cerebral ischemia. *Anesthesiology* 79: 306–312.
- Mantz J, Jossierand J, Hamada S (2011). Dexmedetomidine: new insights. *Eur J Anaesthesiol* 28: 3–6.
- Mocanu MM, Baxter GF, Yue Y, Critz SD, Yellon DM (2000). The p38 MAPK inhibitor, SB203580, abrogates ischaemic preconditioning in rat heart but timing of administration is critical. *Basic Res Cardiol* 95: 472–478.
- Nakano A, Cohen MV, Critz S, Downey JM (2000). SB 203580, an inhibitor of p38 MAPK, abolishes infarct-limiting effect of ischemic preconditioning in isolated rabbit hearts. *Basic Res Cardiol* 95: 466–471.
- Noiri E, Nakao A, Uchida K, Tsukahara H, Ohno M, Fujita T, et al. (2001). Oxidative and nitrosative stress in acute renal ischemia. *Am J Physiol Renal Physiol* 281: 948–957.
- Ogata M, Hino S, Saito A, Morikawa K, Kondo S, Kanemoto S, et al. (2006). Autophagy is activated for cell survival after endoplasmic reticulum stress. *Mol Cell Biol* 26: 9220–9231.
- Richa F, Yazigi A, El Hage C, Jebara S, Hokayem N, Antakly MC (2004). Demedetomidine: an agent for controlled hypotension in maxillo-facial surgery. *Eur J Anaesthesiol* 21: 60.
- Schmitz JM, Graham RM, Sagalowsky A, Pettinger WA (1981). Renal alpha-1 and alpha-2 adrenergic receptors: biochemical and pharmacological correlations. *J Pharmacol Exp Ther* 219: 400–406.
- Sharfuddin AA, Molitoris BA (2011). Pathophysiology of ischemic acute kidney injury. *Nat Rev Nephrol* 7: 189–200.
- Si Y, Bao H, Han L, Shi H, Zhang Y, Xu L, et al. (2013). Dexmedetomidine protects against renal ischemia and reperfusion injury by inhibiting the JAK/STAT signaling activation. *J Transl Med* 11: 141.
- Spurgeon KR, Donohoe DL, Basile DP (2005). Transforming growth factor-beta in acute renal failure: receptor expression, effects on proliferation, cellularity, and vascularization after

recovery from injury. *Am J Physiol Renal Physiol* 288: 568–577.

Tang G, Yue Z, Tallozy Z, Hagemann T, Cho W, Messing A, et al. (2008). Autophagy induced by Alexander disease-mutant GFAP accumulation is regulated by p38/MAPK and mTOR signaling pathways. *Hum Mol Genet* 17: 1540–1555.

Taoda M, Adachi YU, Uchihashi Y, Watanabe K, Satoh T, Vizi ES (2001). Effect of dexmedetomidine on the release of [3H]-noradrenaline from rat kidney cortex slices: characterization of alpha2-adrenoceptor. *Neurochem Int* 38: 317–322.

Tufek A, Tokgoz O, Aliosmanoglu I, Alabalik U, Evliyaoglu O, Ciftci T, et al. (2013). The protective effects of dexmedetomidine on the liver and remote organs against hepatic ischemia reperfusion injury in rats. *Int J Surg* 11: 96–100.

Willigers HM, Prinzen FW, Roekaerts PM, de Lange S, Durieux ME (2003). Dexmedetomidine decreases perioperative myocardial lactate release in dogs. *Anesth Analg* 96: 657–664.

Xu H, Aibiki M, Seki K, Ogura S, Ogli K (1998). Effects of dexmedetomidine, an alpha2-adrenoceptor agonist, on renal sympathetic nerve activity, blood pressure, heart rate and central venous pressure in urethane-anesthetized rabbits. *J Auton Nerv Syst* 71: 48–54.

## Supporting Information

Additional Supporting Information may be found in the online version of this article:

**Figure S1.** Effects of dexmedetomidine infusions on serum creatinine and urea levels (A and B) and on kidney histology (C) in rats with renal ischemia-reperfusion (I/R) injury. Quantification of kidney acute tubular necrosis (ATN) score assessed by percentage of damaged tubules is given in (C). RIR + Dex0.1 denotes rats with I/R injury treated with Dex 0.1  $\mu\text{g}/\text{kg}/\text{min}$  infusion; RIR + Dex0.3, rats with I/R injury treated with Dex 0.3  $\mu\text{g}/\text{kg}/\text{min}$

infusion. For other abbreviations see Figure 1. Means  $\pm$  SEM are given,  $n = 5–11$  in each group. \* $P < 0.05$  vs. Sham; # $P < 0.05$  vs. RIR.

**Figure S2.** Effects of dexmedetomidine postconditioning on serum creatinine and urea levels (A and B) and on kidney histology (C) in rats with renal ischemia-reperfusion (I/R) injury. Quantification of kidney acute tubular necrosis (ATN) score assessed by percentage of damaged tubules is given in (C). RIR + Dex1 denotes rats with I/R injury treated with Dex 1  $\mu\text{g}/\text{kg}$  postconditioning bolus; RIR + Dex10, rats with I/R injury treated with Dex 10  $\mu\text{g}/\text{kg}$  postconditioning bolus. For other abbreviations see Figure 1. Means  $\pm$  SEM are given,  $n = 5–11$  in each group. \* $P < 0.05$  vs. Sham.

**Figure S3.** Effects of fadolmidine postconditioning on serum creatinine and urea levels (A and B) and on kidney histology (C) in rats with renal ischemia-reperfusion injury. Quantification of kidney acute tubular necrosis (ATN) score assessed by percentage of damaged tubules is given in (C). RIR + Fado1 denotes rats with I/R injury treated with fadolmidine (Fado) 1  $\mu\text{g}/\text{kg}$  postconditioning bolus; RIR + Fado10, rats with I/R injury treated with Fado 10  $\mu\text{g}/\text{kg}$  postconditioning bolus. For other abbreviations see Figure 1. Means  $\pm$  SEM are given,  $n = 5–18$  in each group. \* $P < 0.05$  vs. Sham.

**Figure S4.** Effects of dexmedetomidine on kidney SIRT1 and AMPK expressions in rats with renal ischemia-reperfusion (I/R) injury. The expression of SIRT1 and housekeeping protein beta-actin, measured by Western blot (20  $\mu\text{g}$  nuclear protein fraction per lane), are given in (A). The phosphorylation level of AMPK measured as AMPK-P/AMPK ratio and expressions of total AMPK, phosphorylated-AMPK and housekeeping protein beta-actin are given in (B–D). Means  $\pm$  SEM from three different runs are given,  $n = 5–7$  in each group. \* $P < 0.05$  vs. Sham. For abbreviations see Figure 1.