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Original Research

Dexmedetomidine Alleviates Ischemia/Reperfusion-Associated Acute Kidney Injury by Enhancing Autophagic Activity *via* the α2-AR/AMPK/mTOR Pathway

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Abstract

Background: Dexmedetomidine (DEX) reportedly protects against ischemia-reperfusion (I/R) injury and associated damage to the kidneys, but the underlying mechanisms have yet to be established. **Methods**: Unilateral nephrectomy was performed in Wistar rats, and the remaining kidney was clamped for 1 h prior to reperfusion to establish an experimental model system. These animals were then randomized into Sham, DEX + Sham, DEX + I/R, ATI (Altepamizole, α2-adrenergic receptor inhibitor) + DEX + I/R, and 3-MA (3-methyladenine, autophagy inhibitor) + DEX + I/R groups. Serum renal function biomarkers, acute kidney injury (AKI) histopathological scores, serum inflammatory factors, redox biomarkers, markers of autophagic flux, and autophagosome numbers were assessed. Levels of proteins related to the autophagic pathway, including mTOR and AMPK, were also analyzed. **Results**: Serum creatinine and urea nitrogen levels in the I/R group were significantly elevated over those in sham control rats, as were AKI scores, serum inflammatory cytokine concentrations (IL-6, IL-1β, and TNF-α), and serum levels of the oxidative stress biomarker malondialdehyde (MDA). All of these parameters were significantly reduced in the DEX + I/R group relative to I/R model rats. I/R group rats also exhibited significant decreases in renal levels of autophagic flux-related biomarkers and autophagosome numbers relative to sham controls, while DEX administration partially restored normal autophagic flux in these rats. Acute I/R also suppress the expression of AMPK in the kidney while increasing mTOR expression, and DEX reversed these effects. The beneficial impact of DEX on I/R-associated AKI was ablated by ATI or 3-MA administration. **Conclusions**: These analyses provide strong evidence for the ability of DEX to protect against I/R-associated AKI *via* the α2-AR/AMPK/mTOR pathway-mediated enhancement of autophagic activity.

Keywords: acute kidney injury; renal ischemia and reperfusion; dexmedetomidine; autophagy; α2-AR/AMPK/mTOR pathway

1. Introduction

Acute kidney injury (AKI) is a frequently diagnosed disease in the clinic that can develop for a variety of reasons, resulting in a rapid drop in normal renal function, rising serum creatinine levels, decreased urine output, and the accumulation of metabolic waste and toxic compounds that ultimately contribute to widespread organ failure [1]. According to the KDIGO (Kidney Disease: Improving Global Outcomes) guidelines, AKI is defined by an elevated serum creatinine level $\geq$0.3 mg/dL for 48 h or $\geq$1.5-fold above baseline for 7 days, or by reduced urine output to $<$0.5 mL/kg/h for 6 h [2]. AKI is a common condition that affects 10–15% of patients in the hospital and over 50% of individuals in intensive care settings, contributing to poor prognostic outcomes [1]. An estimated 2 million individuals per year die as a result of AKI throughout the world, with a 90-day mortality rate of approximately 28% [3]. In addition to adverse short-term outcomes including fluid overload, bleeding-related complications, acid-base or electrolyte imbalances, and immune dysfunction that can result in prolonged hospitalization or death, AKI can also contribute to a long-term risk of chronic kidney disease or end-stage renal disease, imposing high medical costs on affected patients and society as a whole [4].

AKI is a complex, multifactorial condition that is often driven in large part by ischemia-reperfusion injury (IRI) [5]. IRI can result from myocardial infarction, trauma, hypovolemic shock, vascular or cardiac surgery, renal transplantation, and other conditions that cause pronounced hypoxia, increased reactive oxygen species biogenesis, aberrant antioxidant activity, inflammatory cytokine production, and activation of the complement system through the alternative pathway, culminating in renal tubular cell damage and death [6–8]. The necrotic debris released from these cells in

the local environment can serve as a danger-related signal that can provoke further inflammation in a damaging positive feedback loop [9]. At present, no effective treatments for IRI-induced AKI (IRI-AKI) have been established.

The α2 adrenergic receptor agonist dexmedetomidine (DEX) exhibits a high degree of selectivity and has repeatedly been demonstrated to protect against IRI [10–12]. Renal α2 adrenergic receptor expression is primarily confined to the proximal and distal tubules as well as the peritubular capillaries. Despite evidence for the ability of DEX to alleviate IRI-AKI, the underlying mechanisms have yet to be established [13]. However, autophagic activity has been reported to be protective in the context of AKI [14], and DEX is capable of alleviating sepsis-related AKI *via* autophagic induction [15]. Autophagy is a process through which cells degrade proteins and organelles in autophagosomes, recycling their components in a manner essential for the maintenance of cellular homeostasis [16]. 5′-adenosine monophosphate-activated protein kinase (AMPK) and mammalian target of rapamycin (mTOR) are core autophagic regulators [17]. Here, the ability of DEX to control autophagy *via* the α2-AR/AMPK/mTOR pathway was investigated in a rat model of IRI-AKI.

2. Materials and Methods

2.1 Animals

In total, 30 healthy Wistar rats (males, 180–220 g) were obtained from the Animal Experiment Center of Soochow University and housed in SPF-grade cages with free food and water access under controlled conditions (24–26 °C, 40–60% relative humidity, 12 h light-dark cycle). All animal studies were in accordance with the NIH Guide for the Care of Laboratory Animals and approved by the Institutional Animal Care and Use Committee of Soochow University (Suzhou, China).

After intraperitoneal anesthesia using pentobarbital sodium (60 mg/kg), the skin and muscle were cut 1 cm away from the spin and 1 cm away from the lower rib edge such that the bilateral kidneys were visible. After ligating the right kidney at the pedicle with a silk thread, the pedicle was cut and the right kidney was excised. After separating the left renal artery, a vascular clip was applied to arrest blood flow for 1 h. After this ischemic period, the clip was removed to permit reperfusion such that the kidney changed in color from dark to bright red. The wound site was sutured and rats were injected (i.p.) with 0.5 mL of warm normal saline and returned to the incubator until awake, at which time they were transferred into clean cages. At 24 h post-surgery, rates received a pentobarbital sodium (60 mg/kg, i.p.) for anesthesia and were sacrificed, after which samples of blood and renal tissue were harvested.

For this study, rats were randomly assigned to six groups (n = 5/group): (1) A sham group in which all procedures other than kidney excision and left renal artery clamping were performed; (2) A DEX + sham group in which sham rats received an i.p. injection of DEX (30 ug/kg; Yangzijiang Pharmaceutical Company); (3) An ischemia-reperfusion (I/R) model group that underwent the surgical procedure detailed above; (4) A DEX + I/R group in which DEX (30 ug/kg, i.p.) was injected 30 min prior to the clamping of the left renal artery; (5) An ATI + DEX + I/R group in which the α2-AR inhibitor atipamezole (ATI; 250 ug/kg; HY-12380, MCE) was intraperitoneally injected 30 min prior to DEX injection as in the DEX + I/R group [18]; and (6) A 3-MA + DEX + I/R group wherein the autophagy inhibitor 3-methyladenine (3-MA; 15 mg/kg; HY-19312, MCE) was intraperitoneally injected 30 min prior to DEX injection as in the DEX + I/R group [19].

2.2 Renal Function Analyses

Blood samples were rapidly collected *via* opening the ventricle apex, and were stored for 30 min at room temperature followed by centrifugation (10 min, 3500 cm, 4 °C) to collect the serum samples. Serum creatinine (Cre) and blood urea nitrogen (BUN) levels were analyzed with a fully automatic biochemical analyzer (C8000, ARCHITECH, Kyoto, Japan).

2.3 Renal Injury Scoring

Kidney samples were fixed using 4% paraformaldehyde, paraffin-embedded, prepared into 5 µm sections, and subjected to hematoxylin and eosin (H&E) staining followed by imaging under light microscopy (BX-FM; Olympus Corporation, Tokyo, Japan). The percentages of tubules exhibiting brush border shedding and dilation were evaluated in 10 renal cortical regions to score the magnitude of renal injury as follows: 0 (0%), 1 (1–19%), 2 (20–49%), 3 (50–69%), and 4 ($\geq$70%) [20]. Two observers independently scored all samples.

2.4 Inflammatory Cytokine Analyses

Commercial ELISA kits were used to assess serum IL-1β (E-EL-R0012, Elabscience, Wuhan, China), IL-6 (E-EL-R0015, Elabscience), and TNF-α (Multisciences, Hangzhou, China) concentrations based on provided directions.

2.5 Oxidative Stress Analyses

Serum malondialdehyde (MDA) levels were detected using a lipid oxidation detection kit (S0131S, Beyotime, Shanghai, China). This assay reflects the extent of lipid peroxidation based on the production of a red product from the reaction between MDA and thiobarbituric acid (TBA). Levels of the reduction marker glutathione (GSH) were assessed in serum with GSH and GSSG test kits (S0053, Beyotime).

2.6 Autophagic Marker Detection

Beclin1, LC3, and p62 expression in kidney tissue samples were detected *via* qPCR, Western immunoblotting, and immunohistochemical (IHC) staining.

Table 1. primer sequence in the study.

Genes	Sequences (5′-3′)
Beclin1	(F)TCAAGATCCTGGACCGAGTGACC
	(R)CTCCTCTCCTGAGTTAGCCTCTTCC
LC3	(F)GAGCGAGTTGGTCAAGATCATCCG
	(R)GATGTCAGCGATGGGTGTGGATAC
P62	(F)CCAGCACAGGCACAGAAGATAAGAG
	(R)TCCCACCGACTCCAAGGCTATC
GAPDH	(F)ACGGCAAGTTCAACGGCACAG
	(R)CGACATACTCAGCACCAGCATCAC

Trizol (Invitrogen, Waltham, MA, USA) was used to isolate total RNA from renal tissue samples based on provided directions, after which the RrimeScriptRT kit (RR036A, Takara, Kyoto, Japan) was used to prepare cDNA. Then, a TB Green Premix Ex TaqII kit (RR820A, Takara) and a 480 II Detection System (Roche) were used to assess relative gene expression *via* qPCR using primers compiled in Table 1. GAPDH served as a normalization control, and relative expression was quantified *via* the $2^{-\Delta\Delta Ct}$ method.

For Western immunoblotting, minced kidney tissue samples were suspended in RIPA buffer (P0013C, Beyotime, China) containing PMSF (P0100, Solarbio, Beijing, China), protease inhibitors (05892791001, Roche, Basel, Switzerland), and phosphatase inhibitors (04906845001, Roche) and homogenized in a tissue grinder (F6/10 F013200021, JingXin, Xincang, China). After centrifugation (10 min, 3000 rpm, 4 °C) supernatant protein levels were detected *via* BCA assay (P0012, Beyotime, China). Equal protein amounts were separated *via* SDS-PAGE and transferred onto PVDF blots that were blocked for 2 h at room temperature with 5% skim milk followed by overnight incubation with primary antibodies (diluted 1:1000 unless otherwise noted) specific for the following proteins: Beclin 1 (1:2000; ab207612, abcam, Cambridge, UK); LC3B (2775S, Cell Signaling Technology, Danvers, MA, USA); p62 (5114S, Cell Signaling Technology); GAPDH (60004-1-Ig, Proteintech Group, Wuhan, China); β-Actin (3700, Cell Signaling Technology). Blots were then incubated for 2 h at room temperature with goat anti-rabbit IgG (GAR0072, Multisciences, China) or goat anti-mouse IgG antibody (GAM0072, Multisciences, China), and signal was subsequently detected with a chemiluminescence system (FluorChem M, USA) with high-sensitivity ECL substrate (180501, Tanon, Shanghai, China). Image J (V1.51, Bethesda, Maryland, USA) was then used to analyze protein band intensity.

For IHC staining, xylene was used to deparaffinize 3 µm thick kidney sections that were dehydrated with an ethanol gradient and treated with 3% H_2O_2 for endogenous peroxidase inactivation. After microwaving samples for 15 min in citric acid, these sections were blocked at room temperature for 15 min with goat serum and probed overnight at 4 °C with appropriate primary antibodies specific for Beclin1 (1:100; Servicebio, Wuhan, China), LC3 (1:100; Servicebio), p62 (1:100; Servicebio). After rinsing thrice with PBS, sections were stained using a DAB horseradish peroxidase chromogen kit (Beyotime) and counterstained with hematoxylin, followed by visualization with a PD37 microscope (Olympus). Five random fields per section were analyzed at 200× magnification.

2.7 Ultrastructural Analyses

AKI-related ultrastructural changes and autophagosome numbers were evaluated *via* transmission electron microscopy (TEM). Briefly, kidney tissue samples were washed using chilled PBS (pH 7.4), cut into 2 mm^3 cubes, and fixed using 2.5% glutaraldehyde (Servicebio). Ultrathin 70 nm sections were then cut, stained using uranyl acetate and lead citrate, and visualized *via* TEM (HT7700; Japan).

2.8 Autophagic Pathway Detection

Western immunoblotting was used to detect AMPK and mTOR levels in renal tissue samples as above using the following primary antibodies (diluted 1:1000): anti-AMPK (5831S, Cell Signaling Technology), anti-mTOR (CY5306, Abways, Shanghai, China). Proteins were detected with the same secondary antibodies used above.

2.9 Statistical Analysis

Data are reported as means ± SEM and were analyzed using Student's *t*-tests and ANOVAs in GraphPad Prism 9.20 (GraphPad Inc, San Diego, CA, USA). $p < 0.05$ served as the cut-off for statistical significance.

3. Results

3.1 DEX Abrogates Deteriorating Renal Function in IRI-AKI Model Rats

To explore the impact of DEX on renal function in IRI-AKI model rats, serum creatinine (Cre) and urea nitrogen (BUN) levels were analyzed as functional biomarkers. Both Cre and BUN levels in the I/R group were significantly elevated relative to sham control rats, while they were significantly reduced in the DEX + I/R group as compared to the I/R model group. This suggests that DEX is capable of reversing IRI-AKI-associated reductions in kidney function. Strikingly, both Cre and BUN levels were significantly elevated in the ATI + DEX + I/R and 3-MA + DEX + I/R groups as compared to the DEX + I/R group, suggesting that α2-AR signaling is responsible for the protective benefits of DEX in this experimental system, and that autophagic activity plays a role in IRI-AKI pathogenesis. No differences were observed between the sham and DEX + sham groups, indicating that DEX alone had no significant effects in animals exhibiting healthy renal function (Fig. 1A,B).

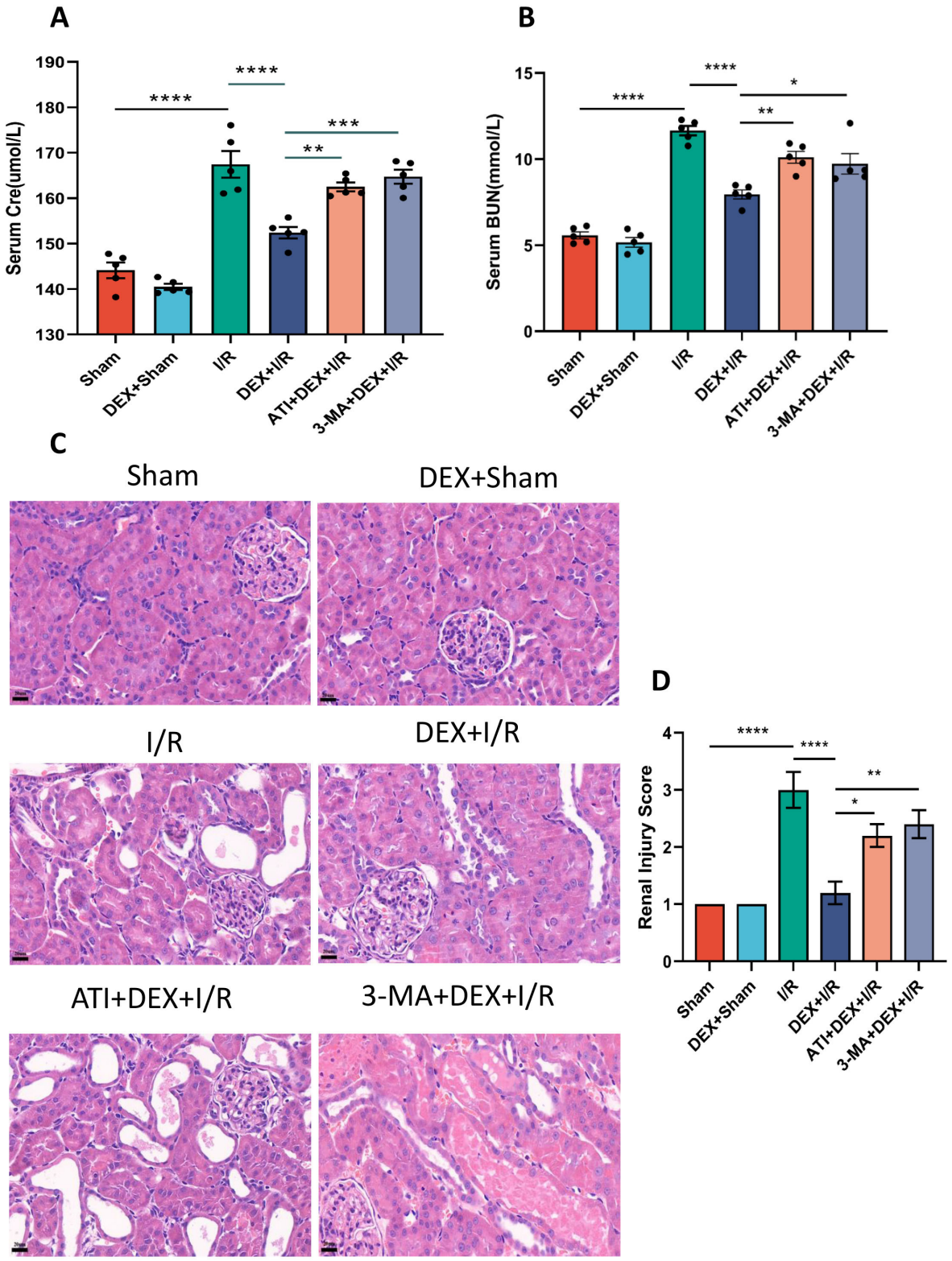

Fig. 1. DEX alleviates renal functional impairment in IRI-AKI model rats. (A,B) Serum Cre (A) and BUN (B) levels. (C) H&E staining of renal cortical tissues (400×, Scale bar: 20 μm). (D) Renal injury scores based on histopathological findings. Data are means ± SEM (n = 5). *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, ****$p < 0.0001$. DEX, dexmedetomidine; IRI, ischemia-reperfusion injury; AKI, acute kidney injury; ATI, atipamezole; 3-MA, autophagy inhibitor.

3.2 DEX Alleviates Renal Pathology in IRI-AKI Model Rats

To test the effects of DEX in IRI-AKI model rats, H&E staining and associated scores were used to evaluate the extent of renal pathology. Significantly higher scores were evident in I/R model rats relative to sham controls, while these scores were significantly reduced in the DEX + I/R group, supporting the ability of DEX to reverse I/R-associated kidney damage. Renal scores for ATI + DEX + I/R and 3-MA + DEX + I/R rats were also significantly elevated as compared to the DEX + I/R group, supporting the ability of ATI or 3-MA to eliminate the protective benefits of DEX against IRI-AKI (Fig. 1C,D).

3.3 DEX Suppresses IRI-AKI-Associated Inflammation

To better understand the effects of DEX on IRI-AKI pathogenesis, inflammatory cytokine production was next evaluated at serum. Significant increases in serum IL-6, IL-1β, and TNF-α levels were evident in the I/R group relative to sham control rats, with all three being found at significantly lower levels in DEX + I/R rats relative to those in the I/R model group, consistent with the ability of DEX to suppress inflammatory activity. Both 3-MA and ATI reversed these DEX-mediated reductions in inflammatory cytokine levels (Fig. 2A–C).

3.4 DEX Protects Against I/R-Associated Oxidative Stress

The impact of DEX on IRI-AKI-associated oxidative stress was next evaluated. Serum MDA levels in I/R model rats were significantly elevated over sham controls, while these levels were significantly reduced in DEX + I/R rats, demonstrating the ability of DEX to abrogate oxidative stress induced in response to I/R. However, significantly increased MDA levels were evident in both the ATI + DEX + I/R and 3-MA + DEX + I/R groups relative to the DEX + I/R group. Changes in serum GSH levels were the inverse of those observed for serum MDA in these rats. Overall, these data are consistent with the ability of DEX to regulate oxidative stress *via* α2-AR and autophagic signaling (Fig. 3).

3.5 DEX Increases Autophagic Flux in Renal Tissues

The role that autophagy plays in the protective benefits of DEX in this experimental system was next assessed by evaluating key autophagic biomarkers (Beclin1, LC3, and p62) and quantifying autophagosome numbers in renal tissue samples. Significantly lower Beclin1 levels and LC3-II/LC3-I ratio values were observed in I/R model rats relative to sham controls, with significantly higher levels of expression of these biomarkers in DEX + I/R rats as compared to the I/R model group. These changes were reversed in rats treated with ATI or 3-MA, which ablated the beneficial effects of DEX. Changes in relative p62 expression were the inverse of those for Beclin1 and the LC3-II/LC3-I ratio, suggesting that DEX can reverse the I/R-associated

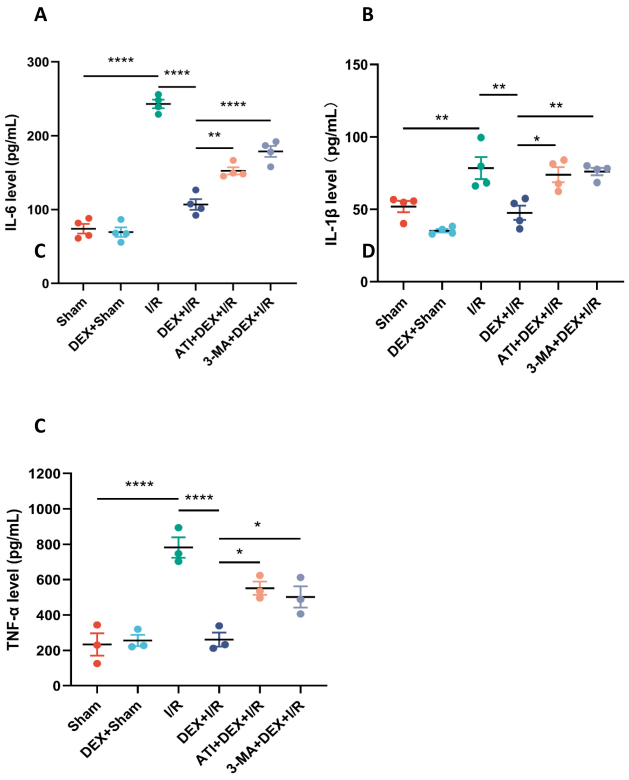

Fig. 2. DEX suppresses renal and systemic inflammation in IRI-AKI rats. Serum levels of IL-6 (A, n = 4), IL-1β (B, n = 4), TNF-α (C, n = 3). Data are means ± SEM. *$p < 0.05$, **$p < 0.01$, ****$p < 0.0001$. DEX, dexmedetomidine; IRI, ischemia-reperfusion injury; AKI, acute kidney injury; ATI, atipamezole; 3-MA, autophagy inhibitor.

suppression of autophagy in a manner that can be overcome by ATI or 3-MA treatment (Fig. 4).

IHC staining revealed Beclin1, LC3, and p62 expression levels consistent with the qPCR and Western immunoblotting data shown above (Fig. 5).

Next, electron microscopy analyses were conducted that revealed a significant drop in autophagosome numbers in the I/R group as compared to sham control rats, whereas DEX + I/R rats exhibited significantly elevated autophagosome counts as compared to I/R model animals consistent with the ability of DEX to remediate I/R-associated autophagic inhibition. As compared to the DEX + I/R group, both ATI and 3-MA were able to reduce autophagosome numbers, thereby abrogating the protective benefits of DEX administration (Fig. 6).

3.6 DEX Promotes Enhanced AMPK/mTOR Pathway Activity

To determine the extent to which DEX influences AMPK/mTOR pathway activity, protein levels of both AMPK and mTOR were assessed *via* Western immunoblotting. Significant reductions in AMPK levels were detected in I/R model rats relative to sham controls, while AMPK levels in the DEX + I/R group were significantly elevated

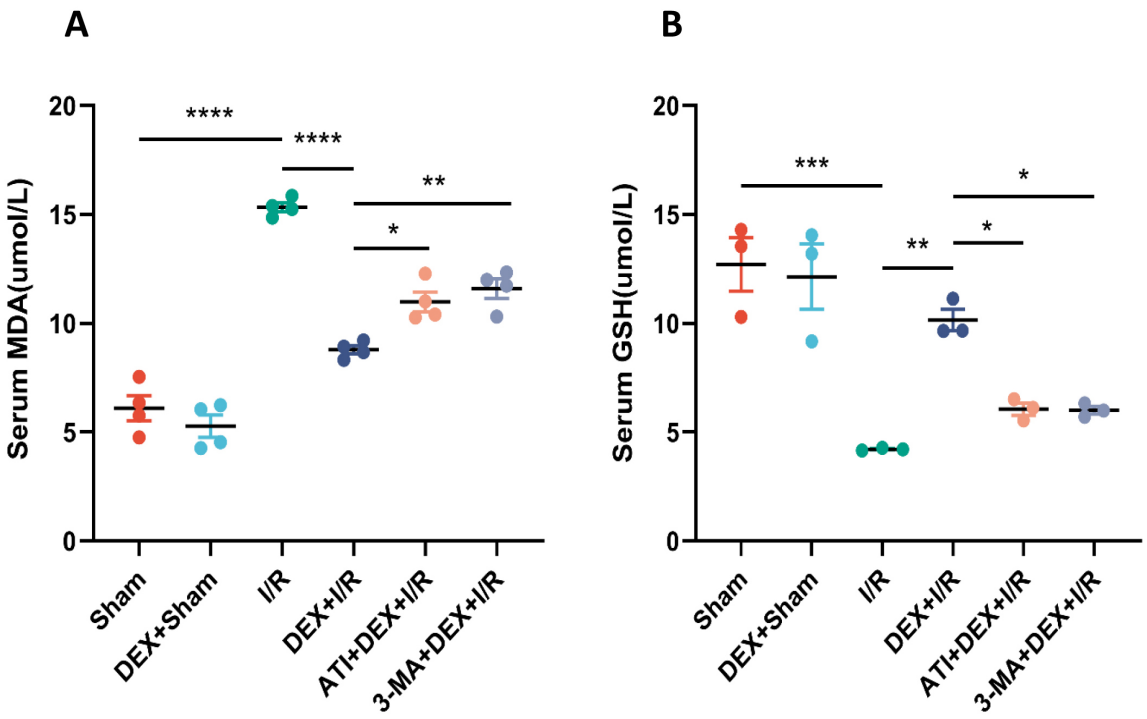

Fig. 3. DEX alleviates oxidative stress in IRI-AKI model rats. (A,B) Serum MDA (A) and GSH (B) levels (n = 4). Data are means ± SEM. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, ****$p < 0.0001$. DEX, dexmedetomidine; IRI, ischemia-reperfusion injury; AKI, acute kidney injury; ATI, atipamezole; 3-MA, autophagy inhibitor.

as compared to the I/R group. Compared with the DEX + I/R group, however, ATI or 3-MA administration resulted in significant decreases in AMPK expression, suggesting that IRI-AKI is associated with the suppression of AMPK/mTOR pathway activity, whereas DEX can facilitate the activation of this pathway in an α2-AR-dependent manner to control autophagic flux (Fig. 7).

4. Discussion

IRI-AKI is an extremely debilitating clinical syndrome that can cause high rates of patient morbidity and mortality owing to a lack of effective treatment options. In an effort to support the design of novel IRI-AKI treatments, a rat IRI-AKI model system was herein established and leveraged to confirm the renoprotective effects of DEX administration. In this system, DEX was found to enhance autophagic activity and to thereby alleviate IRI-AKI severity, decreasing both oxidative stress and inflammatory cytokine biogenesis. Mechanistically, DEX was found to promote autophagy through a process dependent on the α2-AR/AMPK/mTOR pathway.

After first establishing a rat IRI-AKI model system, pathological changes associated with renal damage were evaluated. Serum biochemical analyses revealed markedly elevated Cre and BUN levels in IRI-AKI model rats, and histological analyses revealed clear tubule dilation and brush border shedding in these animals, thus confirming successful model generation.

These rats were next used to explore the mechanisms whereby DEX is able to exert its protective benefits. DEX is known to function as an α2 adrenergic receptor agonist with a high degree of selectivity, whereas ATI is an α2-AR inhibitor [21,22]. Pretreatment with DEX was sufficient to prevent the onset of IRI-AKI as detected based on both biochemical and histological approaches, while ATI was able to reverse these protective benefits, confirming the ability of DEX to abrogate IRI-AKI development. IRI-AKI model rats also exhibited higher levels of both inflammatory cytokines (IL-6, IL-1β, TNF-α) and redox markers indicative of oxidative stress (MDA, GSH), together with fewer autophagosomes and lower levels of autophagic markers (Beclin1, LC3, p62), while DEX reversed these changes. As an extremely dynamic process, autophagy provides cells with a means of degrading and recycling unnecessary or dysfunctional proteins and organelles [23]. The initiation of autophagic activity is characterized by the initial formation of a small vesicle-like phagocytic bubble enclosing cytosolic components to be degraded. Isolated membrane then encloses these contents in a double-membrane structure known as an autophagosome. This autophagosome, in turn, fuses with lysosomes to establish autolysosomes wherein lysosomal enzymes can degrade the enclosed cytosolic components. Autophagic activity can be assessed both directly *via* electron microscopy and indirectly through the IHC or Western immunoblotting-based detection of autophagy-related proteins [24]. The au-

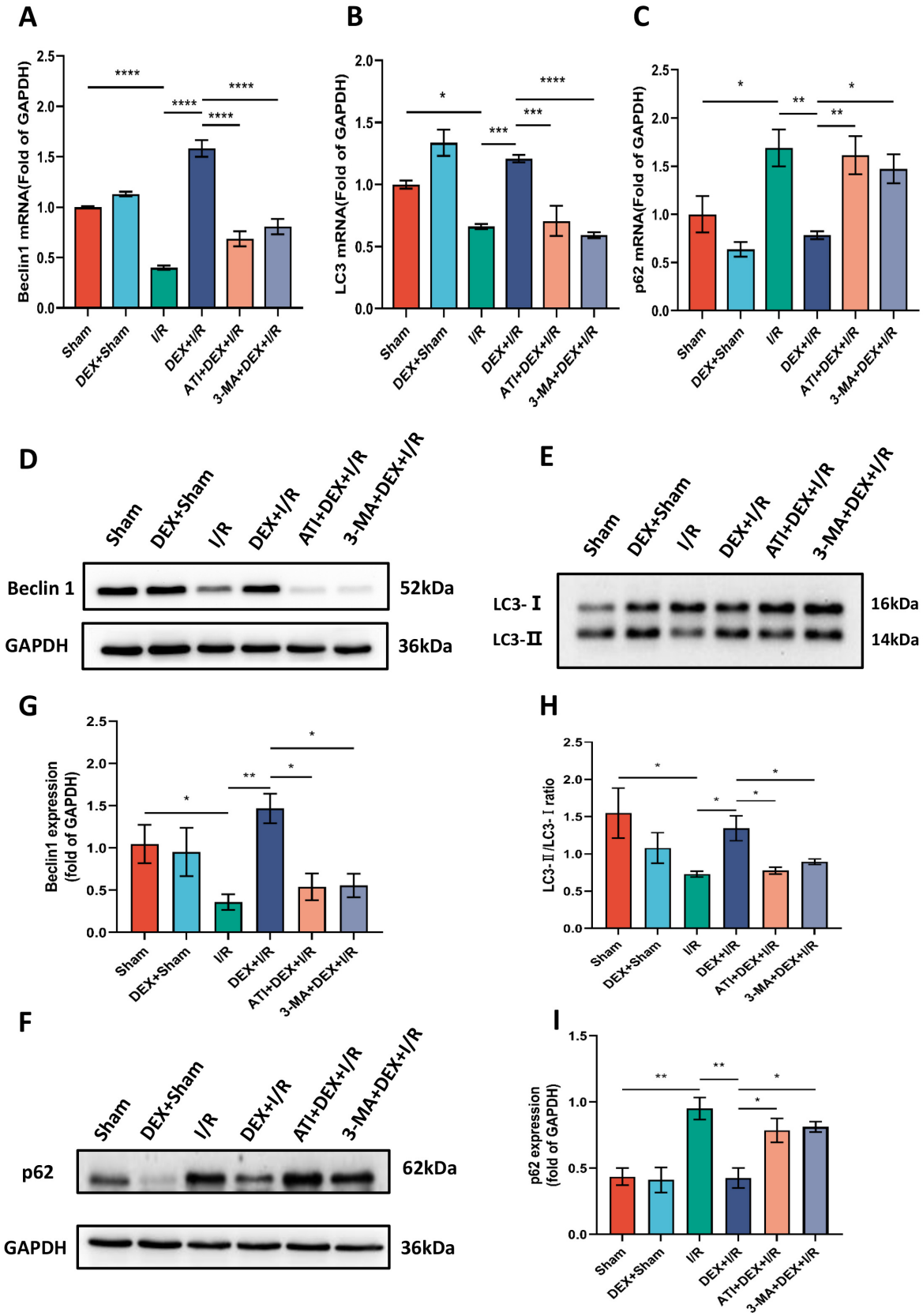

Fig. 4. DEX enhances autophagy in a manner reversed by ATI or 3-MA treatment. (A–C) Beclin1 (n = 4), LC3 (n = 4), and p62 (n = 5) mRNA levels. (D–F) Beclin1, LC3, and p62 protein levels, with (G–I) corresponding quantification, using GAPDH as a normalization control (n = 4). Data are means ± SEM. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, ****$p < 0.0001$. DEX, dexmedetomidine; ATI, atipamezole; 3-MA, autophagy inhibitor.

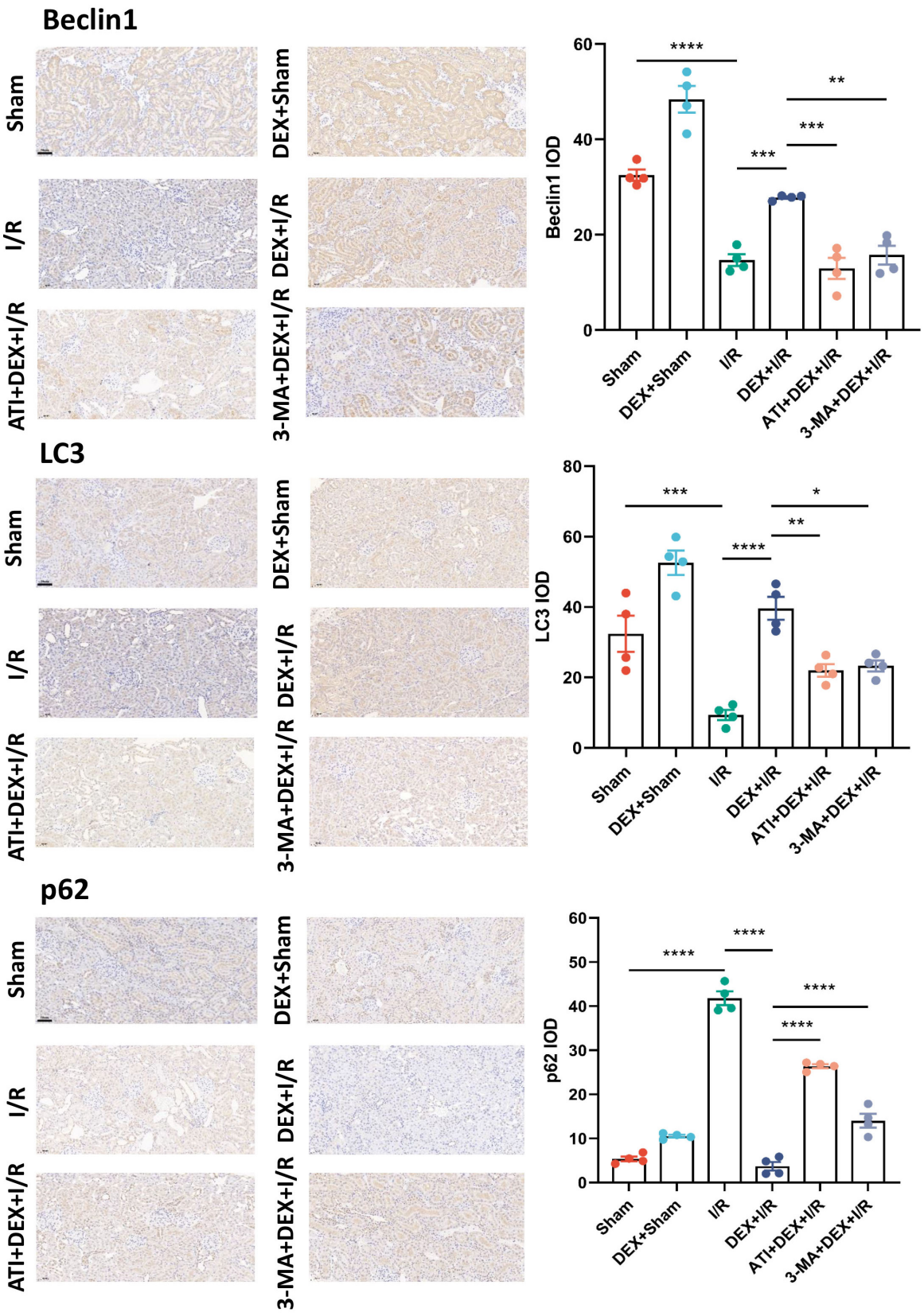

Fig. 5. DEX induces enhanced autophagic activity. Beclin1, LC3, and p62 levels were detected *via* IHC staining with corresponding quantification (n = 4). Scale bar: 50 μm. Data are means ± SEM. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, ****$p < 0.0001$. DEX, dexmedetomidine; ATI, atipamezole; 3-MA, autophagy inhibitor.

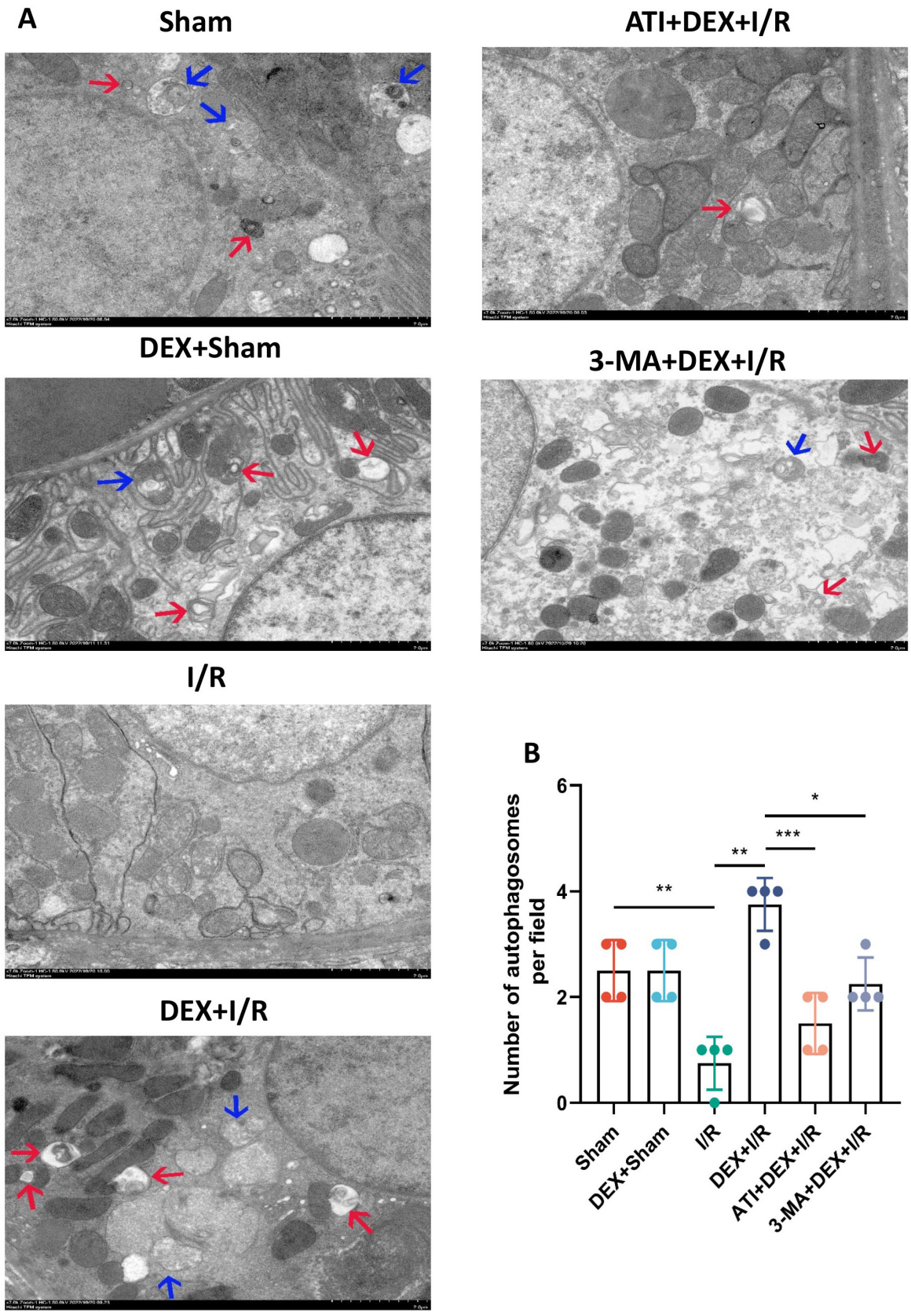

Fig. 6. DEX promotes enhanced autophagic activity. (A) Transmission electron microscopy images of renal tissue samples. Autolysosomes and autophagosomes are respectively indicated with blue and red arrows. Scale Bar: 2.0 µm. (B) Quantitative analyses of autophagosome numbers from 10 regions per group (n = 4). Data are means ± SEM. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, ****$p < 0.0001$. DEX, dexmedetomidine; ATI, atipamezole; 3-MA, autophagy inhibitor.

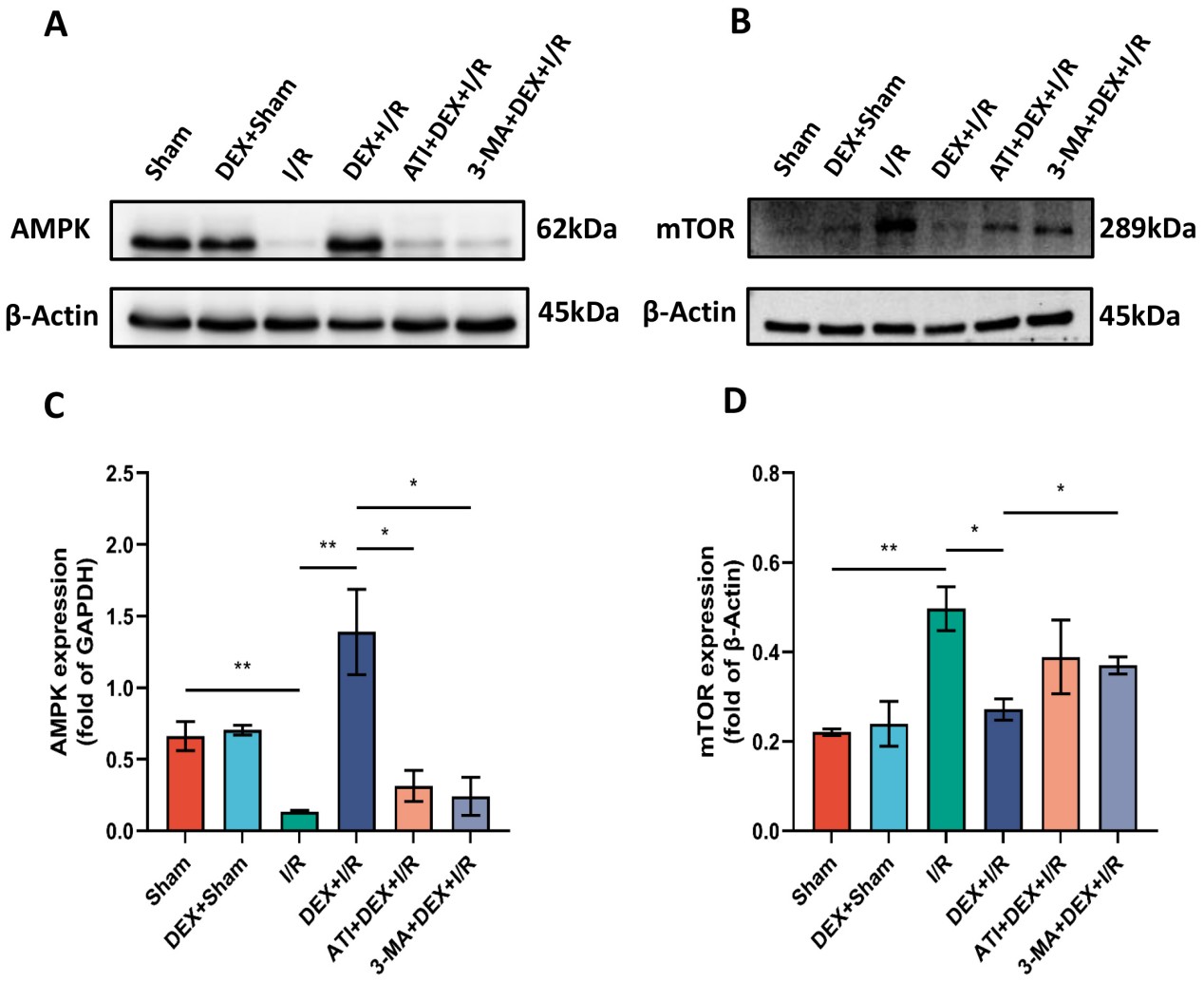

Fig. 7. DEX promotes enhanced autophagic activity *via* the AMPK/mTOR pathway. (A,B) AMPK (A) and mTOR (B) protein levels. (C,D) The expression of AMPK (n = 4) (C) and mTOR (n = 3) (D) was normalized to β-Actin. Data are means $\pm$ SEM. *$p < 0.05$, **$p < 0.01$. DEX, dexmedetomidine; ATI, atipamezole; 3-MA, autophagy inhibitor.

tophagosome membrane protein LC3 serves as a marker of autophagosome formation. At baseline, LC3 exists in the LC3-I isoform that has undergone Atg4 protease-mediated C-terminal cleavage in the cytosol, whereas this protein couples with phosphatidylethanolamine upon autophagosome formation to produce LC3-II [25], which localizes to the inner and outer autophagosome membrane. Analyzing this LC3-II/LC3-I ratio can thus provide direct insight into the magnitude of autophagic activity [26]. The autophagic adaptor protein p62 is capable of binding to LC3-II and ubiquitylated protein aggregates [27], with levels of this protein changed inversely with those of Beclin1 and LC3. Here, IRI-AKI model rats exhibited reduced renal Beclin1 levels, LC3-II/LC3-I ratio values, elevated p62 expression, and fewer autophagosomes, while DEX reversed these effects consistent with its ability to overcome IRI-related suppression of autophagic activity. However, the class III phosphoinositide 3-kinase inhibitor 3-MA, which inhibits autophagy [15,19], was sufficient to reverse the effects of DEX. 3-MA also overcame the impact of DEX administration on oxidative stress and inflammation, supporting a key role for autophagy in the onset and treatment of IRI-AKI, with initial reductions in autophagy contributing to the subsequent enhancement of oxidative stress and inflammation.

Lastly, the ability of DEX to enhance autophagic activity *via* the α2-AR/AMPK/mTOR pathway was explored. Both AMPK and mTOR serve as central regulators of autophagy. AMPK, which was first identified in 1973 [28], regulates defense-related molecules in a range of pathological contexts through its function as a serine/threonine kinase that exists in a heterotrimeric form composed of α, β, and γ subunits [29]. The binding of AMP to an allosteric site on the γ subunit promotes AMPK activation through catalytic α subunit phosphorylation [30,31]. When energy levels are in short supply, AMPK is activated by a rising AMP/ATP ratio such that it can function to restore appropriate energetic homeostasis in these cells. AMPK is

capable of activating a large range of catabolic processes within multicellular organisms and it can function as an autophagic inducer [32]. In line with its important roles, AMPK activation can protect against a range of diseases such as AKI [33–36], heart disease [37], liver disease [38], and lung disease [39]. mTOR is another serine/threonine kinase that functions to coordinate the proliferation, growth, and survival of cells [14]. A growing body of evidence suggests that mTOR functionality is tied to autophagy and renal cell homeostasis such that it can regulate the pathogenesis of AKI [40]. AMPK can also reportedly inhibit mTOR, thus inducing autophagy [41,42]. Here, IRI was found to suppress AMPK/mTOR pathway activity, whereas DEX reversed these effects in a manner that could be reduced by ATI or 3-MA. These findings thus strongly suggest that DEX enhanced autophagic activity *via* the α2-AR/AMPK/mTOR axis.

5. Conclusions

In conclusion, the present data provide evidence for the ability of DEX to enhance autophagic activity and to thereby protect against renal damage in a rat IRI-AKI model system *via* the modulation of α2-AR/AMPK/mTOR pathway signaling, ultimately suppressing oxidative stress and inflammation. As such, DEX represents an attractive therapeutic target with great potential for use in the management of IRI-AKI.

Availability of Data and Materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Author Contributions

BZ, JY, and RL designed the work, analyzed the data and interpreted the data, also drafted the work. YS and HZ interpreted the data and participated in the writing. AY interpreted the data, designed, drafted the work and wrote the manuscript. GZ analyzed data, reviewed the work for important intellectual content, made significant revisions and proofread the manuscript, and supervised the program. All authors read and approved the final manuscript. All authors have participated in sufficiently in the work and agreed to be accountable for all aspects of the work.

Ethics Approval and Consent to Participate

The experiments were performed in according with the National Institutes of Health Guidelines for the Use of Laboratory Animals (NIH, publication number 85-23, revised 1996.), which were approved by and performed according to guidelines for the care and use of animals established by ethics licensing committee of Soochow University (No. 202201A0303). The present study is reported in accordance with ARRIVE guidelines.

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Conflict of Interest

The authors declare no conflict of interest.

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