ANG II promotes autophagy in podocytes

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Yadav A, Vallabu S, Arora S, Tandon P, Slahan D, Teichberg S, Singhal PC. ANG II promotes autophagy in podocytes. Am J Physiol Cell Physiol 299: C488–C496, 2010. First published May 19, 2010; doi:10.1152/ajpcell.00424.2009.—Podocytes are an integral and important constituent of the glomerular filtration barrier (GFB) and are exposed to a higher concentrations of ANG II in diseased states; consequently, podocytes may accumulate oxidized proteins and damaged mitochondria. In the present study, we evaluated the effect of ANG II on the podocyte autophagic process, which is likely to be triggered in order to degrade unwanted proteins and damaged organelles. To quantitate the occurrence of autophagy, electron microscopic studies were carried out on control and ANG II–treated conditionally immortalized mouse podocytes (CIMPs). ANG II–treated cells showed a fivefold greater number of autophagosomes/field compared with control cells. This proautophagic effect of ANG II was inhibited by pretreatment with 3-methyladenine, an inhibitor of autophagy. ANG II also enhanced podocyte expression of autophagic genes such as LC3-2 and beclin-1. Since oxidative stress is often associated with the induction of autophagy, we examined the effect of ANG II on podocyte reactive oxygen species (ROS) generation. ANG II enhanced podocyte ROS generation in a time-dependent manner. To determine whether there is a causal relationship between ANG II–induced oxidative stress and induction of autophagy, we evaluated the effect of antioxidants on ANG II–induced autophagy. As expected, the proautophagic effect of ANG II was inhibited by antioxidants. We conclude that ANG II promotes podocyte autophagy through the generation of ROS.

oxidative stress; angiotensin II

GLOMERULAR VISCERAL EPITHELIAL cells or podocytes are highly specialized cells that play a key role in the pathogenesis of focal segmental glomerulosclerosis (FSGS)—a common pathway for the progression of renal failure (3, 16). The podocyte, strategically positioned along the outer aspect of the glomerular basement membrane, is a critical component of the glomerular filtration barrier (GFB). It functions in tandem with its associated slit diaphragm to limit passage of albumin and plasma proteins into the urinary space (18). The absence of podocyte regeneration following cell injury or apoptosis is a major limitation in the approach to glomerular healing. Accordingly, interventions that increase the resistance of this terminally differentiated cell population to death signals offer a novel approach to preserve the integrity and permselectivity of the GFB.

Podocytopenia, either as a result of podocyte injury or due to a defect in embryogenesis, has been considered to be an important factor for the development of glomerulosclerotic both in congenital and acquired renal diseases (6, 37). Podocytes, being an important component of the GFB, are often exposed to cytotoxic and phlogogenic macromolecules, which have the potential to induce oxidative stress and/or DNA damage (5, 30). Normally, if a cell is exposed to oxidative stress and/or has sustained DNA damage, its machinery is geared to form autophagosomes—to get rid of damaged proteins and organelles; in addition, DNA injury is often associated with the stimulation of DNA synthesis—either to repair DNA molecules or to provide material for cell division (25, 36). Since the podocyte is a terminally differentiated cell and cannot get rid of an excessive load of synthesized DNA (in the form of cellular division), it is dependent for its survival on efficient machinery to sequester the unwanted or damaged proteins/organelles into autophagosomes (1). Nevertheless, if an insult is overwhelming it may directly tilt the balance toward apoptosis without any attempt for autophagy induction (10).

Autophagy and apoptosis constitute the two processes through which injured/aged cells or organelles are eliminated (2, 11, 17, 21, 22). Autophagy is a highly regulated process that is involved in the turnover of long-lived proteins and whole organelles or distinct organelles (11, 21, 22). On the other hand, apoptosis removes damaged or unwanted cells. In several settings, autophagy is a mode of stress adaptation that suppresses apoptosis, whereas in other scenarios, autophagy provides an alternative pathway to cell death and is described as autophagic cell death, or programmed cell death (PCD) type II. It appears that the same stimuli can induce either autophagy or apoptosis depending on the threshold of the stimuli (2). Autophagy involves sequestration of proteins and cell organelles in autophagosomes (double-membrane structures), which directs them to lysosomes (24). The formation of autophagosomes is dependent on the induction of several genes including LC3, phosphatidylinositol 3-kinase, Beclin 1, and Atgs (34).

Angiotensin II (ANG II) has long been demonstrated to contribute to the progression of renal injury through its hemodynamic effects (9, 20). These effects are confirmed by diminishing its production and blocking its binding to its receptors (15, 31). However, apart from its hemodynamic effects, the direct effects of ANG II on kidney cells are being increasingly recognized (4, 19). Moreover, tissue (intrarenal) generation of ANG II has also been demonstrated to be important for its net effect (23). In the past, the production of ANG II in the kidney was attributed only to specialized cells; nevertheless, during the last decade the majority of renal cells including podocytes, mesangial cells, and tubular epithelial cells have been demonstrated to carry machinery to generate ANG II (8, 29, 32). On the basis of...
these findings, it has been suggested that local concentration of ANG II in the kidney is likely to be many folds higher than systemic blood ANG II levels (32). In that scenario, direct biological effects of ANG II on renal cells are perhaps more critical for the net outcome of renal injury.

The effect of ANG II on the growth of podocytes has been evaluated previously in in vitro studies (7). In those studies, we delineated the ANG II-induced downstream signal transduction pathway (7). However, the role of oxidative stress was not evaluated in those studies (7). Recent in vivo studies carried out in our laboratory further confirmed the effect of ANG II on podocyte injury (14). Besides podocyte apoptosis, rats receiving ANG II also showed attenuated podocyte nephrin expression (14).

In the present study, we evaluated the effect of ANG II on the induction of autophagy in mouse podocytes. In addition, we studied the mechanism involved in ANG II-induced podocyte autophagy.

MATERIALS AND METHODS

Conditionally immortalized mouse podocytes. Conditionally immortalized mouse podocytes (CIMPs) were a gift from Dr. Mohammad Husain (Renal Molecular Laboratory, Long Island Jewish Medical Center, New Hyde Park, NY). The cells were maintained in RPMI medium supplemented with 10% fetal bovine serum (FBS), 1× Pen Strep, and 2 mM L-glutamine (Life Technologies, Rockville, MD) at 33°C (permissive temperature, PT) in the presence of 5% CO2. To permit immortalized growth, the medium was supplemented with 10 U/ml murine recombinant interferon-γ (rIFN-γ; Life Technologies) to induce the H-2Kb promoter driving synthesis of the temperature-sensitive (tsA58) SV-40 T antigen (TAg). At 37°C (nonpermissive temperature, NPT), the TAg is inactivated and podocytes show differentiated morphology with multiple foot processes (12). All experiments were performed in differentiated podocytes.

Determination of reactive oxygen species kinetics in CIMPs. The kinetics of reactive oxygen species (ROS) metabolism in CIMPs was determined by measuring the fluorescent signal from the redox-sensitive fluorprobe 2’,7’-dichlorodihydrofluorescein diacetate (DCFDA) at multiple time points. DCFDA is converted by intracellular esterases to 2’,7’-dichlorodihydrofluorescein, which in turn is oxidized by H2O2 to the fluorescent 2’,7’-dichlorohydrofluorescein (DCF). Briefly, CIMPs were grown in 24-well plates in phenol red-free DMEM. Cells were washed, and DCFDA (10 mM) in serum-free medium was added for 30 min at 37°C. Subsequently, cells were washed with phenol red-free DMEM, followed by addition of either vehicle or ANG II (10−8 M), and DCF fluorescence was detected at the indicated time points by a Fluorescence Multi-Well Plate Reader CytoFluor 4000 (PerSeptive Biosystems) set for excitation of 485 nm and emission of 530 nm. The intensity of the fluorescent signal was calculated with Microsoft Excel and the equation (Ft − F0)/F0 × 100 (32), where Ft is measured fluorescence and F0 is baseline fluorescence.

Immunofluorescence detection of oxidative stress associated with podocyte ANG II treatment. The trafficking of 2,3,4- and 5,6-pentafluorodihydrotetramethylrosamine (PF-HTMRos or Redox Sensor Red CC-1, Molecular Probes, Eugene, OR) was used to detect reactive oxygen intermediates in control and ANG II-treated cells. Redox Sensor Red CC-1 is oxidized in the presence of O2 and H2O2. In brief, CIMPs grown on culture slides were treated with either vehicle or ANG II for either 15 min or 24 h. Cells were washed twice with PBS and then loaded at 37°C for 20 min with Redox Sensor Red CC-1 (1 μM) and a mitochondria-specific dye, MitoTracker Green FM (50 nM; Molecular Probes). Culture slides

![Image](http://ajpcell.physiology.org/)
were washed and mounted with PBS and visualized with a Nikon fluorescence microscope (Nikon Eclipse E800) equipped with a triple filter cube and a charge-coupled device (CCD) camera (Nikon DXM1200). The staining was performed in quadruplicate for each group, and 10 random fields were studied in replicate. Images were captured with Nikon ACT-1 (version 1.12) software and combined for publishing format with Adobe Photoshop 6.0 software.

Detection of apoptosis by cell nuclear staining. Morphological evaluation of podocyte apoptosis was carried out by staining the cell nucleus with H-33342 (Invitrogen, Carlsbad, CA) and propidium iodide (Sigma, St. Louis, MO). H-33342 stains the nuclei of live cells and identifies apoptotic cells by increased fluorescence, whereas propidium iodide costains the necrotic cells (pink). Double staining with these two dyes provides the percentage of live, apoptotic, and necrotic cells under control and experimental conditions. Briefly, at the end of the incubation period the cells were stained with H-33342 (1 μg/ml) for 7 min at 37°C. The cells were then placed on ice with the addition of propidium iodide (1 μg/ml). The cells were incubated with both dyes for 10 min, and the incubation was protected from light. The stained cells were then examined under ultraviolet light with a Hoechst filter (Nikon, Melville, NY). The percentage of live, apoptotic, and necrotic cells was recorded in eight random fields by two observers who were unaware of the experimental conditions.

Evaluation of autophagy by electron microscopic studies. Control and ANG II-treated CIMPs were washed and fixed with 2% glutaraldehyde, buffered with 0.05 M Na cacodylate (pH 7.3), and prepared for electron microscopy (EM). During EM studies, 10 cytoplasmic fields in a grid were captured randomly in each cell. The autophagosomes were labeled, and measurements were applied by using the ruler provided. The numbers of autophagic vacuoles were counted by two observers unaware of the experimental conditions, and data were recorded. In estimating the size of the autophagic vacuoles, the measurement along the largest diameter was taken and recorded.

GFP-LC3 overexpression and autophagy detection. CIMPs were grown to subconfluence on glass coverslips and then transfected with GFP-LC3 plasmid DNA (a gift from Dr. T. Yoshimori, Osaka University, Osaka, Japan) for 36 h. Transfection was carried out with Lipofectamine 2000 (Invitrogen) per the manufacturer’s recommendation, and 2 μg/ml GFP-LC3 plasmid DNA was used for each coverslip. The GFP-LC3-transfected cells were treated under control and experimental conditions. Microphotographs of GFP-LC3 fluorescence were taken under confocal microscopy.

Evaluation of autophagic vacuoles by acridine orange. CIMPs were grown to subconfluence in six-well plates and incubated in medium containing either buffer or variable concentrations of ANG II for variable time periods. In parallel series of experiments, CIMPs were treated under control or experimental conditions for 24 h. At the end of the incubation period, cells were treated with 5 μg/ml acridine orange (Sigma) in serum-free medium for 15 min. Subsequently, the cells were washed three times in PBS (acridine orange was removed) and examined under an inverted fluorescence microscope. Occurrence of autophagy was graded semiquantitatively (1+ to 4+; number of orange vacuoles and intensity of orange fluorescence) by two observers unaware of the experimental conditions. A total of 200 cells were counted for each variable.

Monodansylcadaverine staining of autophagosomes. CIMPs were subcultured in 35-mm coverslip-bottomed dishes and were grown to 40% confluence. The cells were then treated with either buffer or ANG II (10^-8 M) for a period of 36 h, and a control was maintained. The cells were then treated with monodansylcadaverine (MDC) at a concentration of 0.05 mmol/l for a period of 10 min. Subsequently, cells were washed with PBS (4 times) and immediately visualized with a Zeiss Apotome microscope set for excitation at 330 nm and emission at 515 nm. The images were captured and processed per standard criteria.

Quantitation of autophagy with monodansylcadaverine assay. CIMPs were subcultured in 96-well plates after treatment with ANG II (10^-8 M) or buffer for 36 h; subsequently, cells were

![Fig. 2. ANG II enhances autophagy.](image-url)

A: representative microphotographs of GFP-LC3-transfected CIMPs incubated in medium containing either buffer (control) or ANG II (10^-8 M) for 36 h. ANG II-treated cells show bright fluorescence, indicating the presence of GFP-LC3-labeled autophagosomes. B: representative microphotographs of CIMPs incubated in medium containing either buffer (control) or ANG II (10^-8 M) for 36 h, followed by treatment with monodansylcadaverine (MDC) for 10 min. Cells were washed and examined under confocal microscope. C: equal numbers of CIMPs were incubated in medium containing either buffer or ANG II (10^-8 M) for 72 h, followed by treatment with MDC for 10 min. Subsequently, cells were washed and fluorescence assay was carried out. Results (means ± SD) are from 3 sets of experiments.
incubated with MDC for 10 min at 37°C. After four washings with PBS, the fluorescence was read with microplate fluorometer at excitation of 335/40 nm and emission of 530/30 nm. The fluorescence was then normalized to the number of cells by trypsinizing and performing a cell count. A total of 200 cells were counted for each variable. The mean fluorescence per cell was then calculated and plotted as a graph.

**Western blotting studies.** Control and experimental CIMPs were washed at the end of the incubation period, and cells were harvested and lysed in RIPA buffer containing 50 mM Tris-Cl (pH 7.5), 150 mM NaCl, 1 mM EDTA, 1% NP-40, 0.25% deoxycholate, 0.1% SDS, 1X protease inhibitor cocktail (Calbiochem, Cocktail Set 1), 1 mM PMSF, and 0.2 mM sodium orthovanadate. Protein concentration was measured with the BCA Protein Assay Kit (Pierce, Rockford, IL). Total protein extracts (20 μg/lane) were separated on a 15% polyacrylamide (PAGE) premade gel (Bio-Rad, Hercules, CA) and transferred onto a nitrocellulose membrane with a Bio-Rad miniblot apparatus. Nitrocellulose membranes were then processed further for immunostaining with primary antibodies against beclin-1 (1:500, Cell Signaling Technology, Beverly, MA) and LC3-2 (1:600, Cell Signaling Technology) and subsequently with horseradish peroxidase-labeled appropriate secondary antibodies. The blots were developed with a chemiluminescence detection kit (Pierce) and exposed to X-ray film (Eastman Kodak, Rochester, NY). Equal protein loading and the protein transfer were confirmed by immunoblotting for determination of actin protein using a polyclonal α-actin antibody (I-19, Santa Cruz Biotechnology) on the same Western blots.

**Statistical analysis.** For comparison of mean values between groups, the unpaired \( t \)-test was used. To compare values between multiple groups, analysis of variance (ANOVA) was applied and a Newman-Keuls multiple-range test was used to calculate a \( P \) value. All values are means ± SD except where otherwise indicated. Statistical significance was defined as \( P < 0.05 \).

**RESULTS**

**ANG II promotes autophagy.** To determine whether ANG II promotes autophagy, CIMPs were incubated in medium containing either vehicle (control) or ANG II (10^{-8} M) for 36 h \( (n = 3) \). Subsequently, cells were prepared for EM studies. Representative electron microphotographs of control and ANG II-treated cells are shown in Fig. 1, A (control) and B (ANG II). The cumulative data of three sets of experiments are shown in Fig. 1C. ANG II-treated cells not only showed larger autophagosomes but also displayed a higher \( (P < 0.008) \) number of autophagosomes.

To confirm the effect of ANG II on podocyte autophagy, CIMPs were transfected with GFP-LC3 and then incubated in medium containing either buffer or ANG II (10^{-8} M) for 36 h. At the end of the incubation period, cells were examined under a confocal microscope. Representative microphotographs are shown in Fig. 2A. ANG II-treated cells showed bright fluorescence indicating the presence of GFP-LC3-labeled autophagosomes.

To quantitate the occurrence of autophagy under control and experimental conditions, CIMPs were incubated in medium containing either buffer or ANG II (10^{-8} M) for 36 h, followed by treatment with MDC for 10 min. Subsequently, cells were stained with acridine orange and examined under fluorescence microscope. Results (means ± SD) are from 3 sets of experiments, each carried out in triplicate. *\( P < 0.001 \) compared with control; **\( P < 0.01 \) compared with ANG II alone; ***\( P < 0.001 \) compared with ANG II alone and rapamycin alone.

![Fig. 3. Effect of rapamycin on ANG II-induced autophagosome formation. A: representative microphotographs of control, ANG II-treated, and rapamycin + ANG II-treated cells. Both ANG II and rapamycin promoted vacuoles formation (indicated by red staining). This effect of ANG II was further enhanced by rapamycin. B: equal numbers of CIMPs were incubated in medium containing vehicle (control), ANG II (10^{-8} M), rapamycin (1 ng/ml), or ANG II + rapamycin for 36 h \( (n = 3) \). At the end of the incubation period, cells were stained with acridine orange and examined under fluorescence microscope. Results (means ± SD) are from 3 sets of experiments, each carried out in triplicate. *\( P < 0.001 \) compared with control; **\( P < 0.01 \) compared with ANG II alone; ***\( P < 0.001 \) compared with ANG II alone and rapamycin alone.](http://ajpcell.physiology.org/).
were washed and fluorescence assay was carried out. Representative microphotographs are shown in Fig. 2B. Cumulative data are shown in the bar diagram in Fig. 2B. These findings indicate that ANG II promotes autophagy in CIMPs.

**Rapamycin enhances ANG II-induced autophagosome formation.** Rapamycin has been reported to enhance autophagosome formation in a variety of cells (33). We asked whether rapamycin has potential to induce autophagy in podocytes and, if so, whether it can modulate ANG II-induced autophagy. CIMPs were incubated in medium containing either vehicle (control), ANG II (10^{-8} M), rapamycin (1 ng/ml), or ANG II + rapamycin for 36 h (n = 3). Subsequently, cells were stained with acridine orange and examined under a fluorescence microscope. Representative micrographs of control, ANG II-treated, rapamycin-treated, and rapamycin + ANG II-treated cells are shown in Fig. 3A. Both ANG II and rapamycin enhanced autophagosome formation in podocytes. However, rapamycin further enhanced ANG II-induced autophagosome formation. Cumulative data of three sets of experiments are shown in Fig. 3B.

**3-Methyladenine inhibits ANG II-induced autophagy.** To determine whether 3-methyladenine (3-MA), a known inhibitor of autophagy, can also inhibit ANG II-induced autophagy, CIMPs were incubated in medium containing vehicle (control), ANG II (10^{-8} M), 3-MA (2 mM), or 3-MA + ANG II for 36 h. Subsequently, cells were stained with acridine orange. Representative microphotographs showing the effect of 3-MA on ANG II-induced enhanced vacuoles formation are seen in Fig. 4A. Cumulative data of three sets of experiments are shown in Fig. 4B. ANG II-treated cells showed an enhanced (P < 0.001) number of vacuoles compared with control cells. However, this proautophagic effect of ANG II was inhibited by 3-MA. In parallel sets of experiments, cells were treated under similar conditions and then prepared for apoptotic assay. There was no difference in number of apoptosed cells between control and 3-MA-treated podocytes (data not shown). These findings indicated that at this concentration 3-MA did not induce podocyte apoptosis.

**Inhibition of autophagy facilitates ANG II-induced CIMP apoptosis.** Since stimuli that induce apoptosis are often demonstrated to invoke autophagy (11), we studied the role of autophagy in ANG II-induced CIMP apoptosis. CIMPs were treated with either buffer or ANG II (10^{-8}–10^{-6} M) in the presence or absence of 3-MA (2 mM; Sigma) for variable time periods. Subsequently, cells were evaluated for the occurrence of apoptosis. As shown in Fig. 5, CIMPs treated with 3-MA + ANG II (at 24 h) showed comparable percentage of apoptosed cells (12 h earlier) compared with CIMPs treated with ANG II (alone) at 36 h. These findings suggest that inhibition of CIMP autophagy by 3-MA facilitated the proapoptotic effect of ANG II.

**ANG II enhances podocyte LC3-2 expression.** To determine the effect of ANG II on LC3-2 expression by podocytes, CIMPs were incubated in medium containing vehicle (control), ANG II (10^{-8} M and 10^{-6} M), or H2O2 (100 μM, positive control for autophagy) for 24 h. As shown in Fig. 6, H2O2 enhanced expression of LC3-2 by CIMPs. Similarly, ANG II promoted expression of LC3-2 by podocytes.
Rapamycin enhances ANG II-induced LC3-2 expression by podocytes. Since rapamycin promoted ANG II-induced autophagy, we asked whether this effect of rapamycin is associated with enhanced LC3-2 expression by CIMPs. CIMPs were incubated in medium, containing either vehicle (control) or ANG II (10^{-8} M), rapamycin (1 ng/ml), or rapamycin + ANG II for 24 h, followed by preparation of Western blots and probing for LC3-2. As shown in Fig. 7, both ANG II and rapamycin enhanced expression of LC3-2 by CIMPs. However, rapamycin further enhanced expression of LC3-2 in ANG II-treated cells.

ANG II induces beclin-1 expression in CIMPs. To evaluate the effect of ANG II on the induction of beclin-1 (an autophagy regulatory gene), CIMPs were treated with either buffer or ANG II (10^{-8} – 10^{-6} M) for 24 h. Subsequently, cell proteins were extracted, and Western blots were prepared and probed for beclin-1. ANG II enhanced CIMP expression of beclin-1 (Fig. 8A).

Role of oxidative stress in induction of CIMP autophagy. To evaluate whether oxidative stress plays a role in autophagy induction, CIMPs were treated with either buffer or ANG II (10^{-6} M) in the presence or absence of N-acetylcysteine (NAC, 50 μM) for 24 h. Subsequently, Western blots were prepared and probed for beclin-1 and actin. ANG II enhanced beclin-1 expression by CIMPs. However, this effect of ANG II was partly inhibited by NAC (Fig. 8B). These findings indicate that oxidative stress plays a role in ANG II-induced autophagy-associated gene expression.

ANG II enhances ROS generation by podocytes. To determine the effect of ANG II on podocyte ROS generation, CIMPs were incubated in serum-free medium containing DCFDA for 30 min, followed by incubation in medium containing either vehicle (control) or ANG II (10^{-8} M) for 60 min, and ROS generation was recorded at the indicated time periods. As shown in Fig. 9, ANG II enhanced ROS generation by CIMPs in a time-dependent manner.

To determine the site of ROS generation in response to ANG II we labeled control and ANG II-treated cells (15 min and 24 h) with Mito Tracker Green (labeling of mitochondria) and Redox Sensor Red (ROS generation). As shown in Fig. 10, ANG II-treated cells showed enhanced mitochondrial ROS generation (orange color) compared with control cells.

ANG II enhances podocyte expression of MnSOD and catalase. To confirm whether ANG II induces oxidative stress on podocytes, we evaluated the effect of ANG II on podocyte expression of MnSOD and catalase. CIMPs were treated under the same conditions.
incubated in serum-free medium containing either vehicle (control) or ANG II (10^{-8} M and 10^{-7} M) for 24 h, followed by preparation of cells for Western blotting and probing for MnSOD and catalase. As shown in Fig. 11, ANG II-treated CIMPs showed enhanced expression of both MnSOD and catalase.

DISCUSSION

The present study demonstrates that ANG II enhances autophagy. Rapamycin further enhances ANG II-induced autophagy, whereas 3-MA inhibits ANG II-induced autophagy. The proautophagic effect of ANG II was associated with podocyte expression of LC3-2 and beclin-1. Interestingly, antioxidants such as NAC inhibit ANG II-induced autophagy. ANG II also induced ROS generation by podocytes in a time-dependent manner. Moreover, ANG II enhanced podocyte expression of MnSOD and catalase. These findings indicate that ANG II-induced podocyte autophagy is mediated through ROS generation.

Several reports indicate that podocytes have both machinery and capability to synthesize ANG II (32). Various agents including glucose have been reported to stimulate ANG II production by podocytes (8). In the present study we did not evaluate the role of endogenous ANG II in the induction of autophagy. However, it is possible that endogenous production of ANG II in podocyte-stimulated states may also be contributing to ongoing oxidative stress and autophagy.

ANG II is known to induce oxidative stress in a variety of renal cells (4, 19). Interestingly, oxidative stress has also been reported to induce autophagy (2, 11, 21, 22). In this scenario, induction of autophagy may be required for the removal of damaged protein and organelles (oxidized protein aggregates and damaged mitochondria). For example, enhancement of autophagy can cause a decrease in mitochondrial mass by 50% while reducing the susceptibility of cells to mitochondrial outer...
membrane permeabilization-dependent apoptotic stimuli (27). It appears that the induction of autophagy may be a default mechanism to prevent ANG II-induced podocyte injury.

Beclin-1 is an autophagic gene and is also considered to be a tumor suppressor gene (26, 35). Its tumor-suppressive effect is linked to the induction of autophagy in providing the cell protection from DNA damage. Reduced autophagy not only leads to chromosomal aberrations and mutations but also compromises the mitochondrial quality control—ROS-mediated DNA damage. Therefore, oncogenic activation by downregulation or inactivation of an autophagic gene may be considered the breakdown of this cellular protection machinery.

In the present study, in which 3-MA enhanced ANG II-induced apoptosis, it appears that ANG II-mediated autophagy may be providing partial protection against ANG II-induced podocyte apoptosis. Since both autophagy and apoptosis may be the outcome of the same insult, it appears that cross-inhibitory interactions between apoptosis and autophagy determine the net result. In addition, the type as well as severity of initial stimulus will modulate the outcome. For example, during nutrient deprivation the default pathway would be autophagy—removal of the damaged organelles followed by repair and adaptation, which would allow survival, whereas, if autophagy was not able to remove unwanted proteins or damaged organelles, it would result in delayed apoptosis. Nevertheless, if the default pathway is triggered by specific signals such as DNA damage or death receptor activation, then it would lead to direct apoptosis without invoking a preceding or parallel autophagic response (33).

We conclude that ANG II promotes autophagy in podocytes. This effect of ANG II is mediated through mitochondrial ROS generation.

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**DISCLOSURES**

No conflicts of interest, financial or otherwise, are declared by the author(s).
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