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# Expression and regulation of adrenomedullin in renal glomerular podocytes

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**Abstract**

Adrenomedullin (AM) is postulated to exert organ-protective effects. It is expressed in the renal glomeruli, but its roles in the glomerular podocytes have been poorly elucidated. In the present study, we investigated the expression and regulation of AM in recently established conditionally immortalized mouse podocyte cell line in vitro and podocyte injury model in vivo. The cultured differentiated podocytes expressed AM mRNA and secreted measurable amount of AM. AM secretion from the podocytes was increased by $H_2O_2$, hypoxia, puromycin aminonucleoside (PAN), albumin overload, and TNF-α. Real-time RT-PCR analysis revealed that AM mRNA expression in the podocytes was enhanced by PAN and TNF-α, both of which were suppressed by mitochondrial antioxidants. Furthermore, AM expression was upregulated in the glomerular podocytes of PAN nephrosis rats. These results indicated that AM expression in the podocytes was upregulated by stimuli or condition relevant to podocyte injury, suggesting its potential role in podocyte pathophysiology.


*Keywords:* Adrenomedullin; Cultured murine podocyte cells; Glomerular epithelial cells; Podocyte injury; Oxidative stress; Puromycin aminonucleoside; TNF-α; Hypoxia; Mitochondrial antioxidants; Nephrosis rats

Glomerular visceral epithelial cells, also termed podocytes, line the outer aspect of the glomerular basement membrane, and play a pivotal role as a barrier to protein loss in the process of primary urine formation [1,2]. Podocyte dysfunction is often associated with marked proteinuria. Podocytes are terminally differentiated cells and do not generally proliferate [3], thus appropriate in vitro cell culture system had long been unavailable. Although methods had previously been devised to culture glomerular epithelial cells, there is controversy as to the visceral or parietal origin, the uniformity, and the differentiation status of the cells isolated by these conventional methods [4–6]. Recently, Mundel et al. [7] established a novel podocyte cell line, which is conditionally immortalized. Their cells proliferate at lower temperature (permissive condition) and become fully differentiated at higher temperature (non-permissive condition), thus overcoming the above-mentioned problems. The establishment of this podocyte cell line as well as the discovery of a number of podocyte-specific proteins [2] have greatly facilitated podocyte

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researches, and podocytes are now becoming a focus of interest among researchers. Podocyte injury has been shown to be involved in the etiology of proteinuria in diabetic and hypertensive nephropathy, triggering the progression of glomerulosclerosis [8,9]. Moreover, podocytes were demonstrated to express a variety of vasoactive substances and their receptors [10–13], which may regulate podocyte function.

Adrenomedullin (AM), a potent hypotensive peptide discovered from pheochromocytoma, is postulated to counteract cardiovascular injury [14,15]. AM is found in many organs including the kidney [16], where it exerts vasodilatory, natriuretic, and growth-modulatory effects, and is considered to regulate renal function in an autocrine/paracrine fashion [17]. AM level is increased in the plasma of patients with chronic renal failure [18] and also in the kidneys of renal injury models [19], possibly by a compensatory mechanism under such pathophysiologic conditions. The renoprotective role of AM was indicated in ischemic renal injury model [20]. AM is reported to be expressed in the glomeruli, cortical distal tubules and medullary collecting ducts in the kidney [21,22], and also in cultured mesangial cells [22,23]. On the other hand, AM expression in glomerular podocytes has been poorly investigated. Although Lai et al. [24] examined AM expression in the conventional primary cultured podocytes, there has been no report examining AM expression in the conditionally immortalized cell line established by Mundel et al. Here we investigated the expression and modulation of AM using Mundel's podocyte cell line. AM was actually synthesized in and secreted from the podocytes under differentiated condition. AM expression in the podocytes was increased in response to podocyte injurious stimuli in vitro, as well as in a rat model of podocyte injury induced by puromycin aminonucleoside (PAN). Our results suggest a potential role for AM in podocyte pathophysiology.

## Materials and methods

*Cell culture of mouse podocytes.* We used conditionally immortalized mouse podocyte cell line established by Mundel et al. [7]. In brief, this cell line was generated from renal glomeruli of transgenic mice harboring thermo-sensitive mutant of the SV-40 large T antigen, which is under the control of the H-2K$^b$-promoter whose activity is inducible by γ-interferon. The podocyte lineage was selected as WT-1-positive cells. To propagate, podocytes between passage 14 and 20 were cultivated in RPMI 1640 (Sigma) supplemented with 10% heat-inactivated fetal bovine serum (FBS), 100 U/ml penicillin, and 100 μg/ml streptomycin in the presence of 10 U/ml recombinant murine γ-interferon (Peprotech) at 33 °C in 5% $CO_2$/95% air (permissive condition). To differentiate, podocytes were plated on type I collagen at a density of $1 \times 10^4$ cells/cm$^2$ and cultured with 1% FBS in the absence of γ-interferon at 37 °C (nonpermissive condition). Two days later, concentration of FBS was reduced to 0.5%.

*Stimulation of podocytes.* Podocytes maintained under nonpermissive condition for 10–14 days were exposed to the following drugs or stimuli for 24 h: PAN (stock 50 mg/ml in normal saline, final 100 μg/ml; Sigma), recombinant mouse tumor necrosis factor-α (TNF-α; stock 20 mg/ml in medium containing 1% FBS, final 20 ng/ml, Sigma), human serum albumin (HSA; final 10 mg/ml; Sigma), $H_2O_2$ (stock 100 mM in water, final 10 μM), and hypoxia (1% $O_2$). Hypoxic condition was created in air-tight chambers flushed with pre-analyzed gas mixtures, as previously reported [25]. Time dependency was analyzed by harvesting cells at 6–48 h. For antioxidant experiments, podocytes were pretreated with rotenone (stock 50 mM in ethanol, final 100 μM; Sigma), antimycin A (stock 10 mM in ethanol, final 1 μM; Sigma), diphenyleneiodonium chloride (DPI; stock 10 mM in DMSO, final 5 μM; Sigma) or 4′-hydroxy-3′-methoxyacetophenone (apocynin; stock 30 mM in water, final 60 μM; Tokyo kasei) 40 min prior to stimulation with PAN or TNF-α.

*Animals.* Male Sprague–Dawley rats (150 g) were purchased from Tokyo Laboratory Animals Science and were fed a normal rat chow. PAN nephrosis was induced by a single intravenous injection of PAN (100 mg/kg body weight; Sigma). Control rats received intravenous injection of 4 ml/kg saline. At day 6, urine was collected using a metabolic cage for 24 h. On the following day, the rats were euthanized under ether anesthesia. Kidneys were harvested and glomeruli were isolated by the sieving method [26]. We confirmed that the glomerular fraction recovered by our sieving method mainly contained the glomeruli (data not shown). Samples for RNA extraction were immersed in RNA later solution (Qiagen). Those for immunostaining were fixed in 4% paraformaldehyde solution. All animal procedures conducted were in accordance with the Guideline for the Care and Use of Laboratory Animals approved by University of Tokyo Graduate School of Medicine.

*Reverse transcription-polymerase chain reaction (RT-PCR).* Total RNA was extracted using an RNeasy kit (Qiagen). Total RNA (2.5 μg) was reverse-transcribed to cDNA with Superscript II reverse transcriptase (Invitrogen) and random hexamers (Promega). The resultant cDNA (1/10 of total) was subjected to semi-quantitative PCR with *Taq* DNA polymerase (Takara) and primer sets for AM or GAPDH. Primers used were: 5′-TCAGAGCATCGCCACAGAAT-3′ (sense) and 5′-TAGCTGCTGGATGCTTGTAG-3′ (antisense) for mouse AM; 5′-TCGGAGCATCGCTACAGAAT-3′ (sense) and 5′-TAGCT GCTGGACGCTTGTAG-3′ (antisense) for rat AM; and 5′-CTC TACCCACGGCAAGTTCAA-3′ (sense) and 5′-GGATGACCTTGC CCACAGC-3′ (antisense) for mouse and rat GAPDH. The amplification of AM was carried out for 40 cycles of 30 s at 94 °C, 30 s at 62 °C, and 30 s at 72 °C. The amplification of GAPDH was carried out for 22 cycles of 30 s at 94 °C, 30 s at 60 °C, and 40 s at 72 °C. To confirm the authenticity of PCR, one sample was subcloned into pBluescript II and sequenced using an ABI PRISM 310 Genetic Analyzer (Applied Biosystems). The PCR products were electrophoresed on 2% agarose gels and stained with ethidium bromide.

*Radioimmunoassay for AM.* Podocytes under nonpermissive condition were subjected to the above-mentioned stimuli for 24 h. Then the culture medium was collected, acidified with trifluoroacetic acid, and applied to a Sep-Pak C18 cartridge (Millipore). AM concentration in the culture medium was measured using AM (1-50) (mouse) radioimmunoassay kit (Phoenix Pharmaceuticals). Samples or standards were incubated with rabbit antiserum against mouse AM (1-50) for 16 h and then $^{125}$I-AM (8000–10,000 cpm) was added. After incubation for 16 h, goat anti-rabbit IgG serum was added. The mixture was incubated for 90 min and centrifuged at 1700*g* for 20 min. The radioactivity in the pellet was counted in a gamma counter.

*Real-time quantitative RT-PCR.* Total RNA was extracted using an RNeasy kit (Qiagen) and treated with DNase I (Qiagen) when required to remove contaminating genomic DNA. The cDNA was synthesized from 1 μg of total RNA with random primers and Superscript II reverse transcriptase.

Gene expression was quantitatively analyzed by real-time RT-PCR using an ABI PRISM 7000 (Applied Biosystems). TaqMan chemistry and assay by design primers and probe sets were used for the mouse and rat AM, β-actin, and mouse B7-1. PCR was carried out on ABI PRISM 7000 with an initial activation of AmpliTaq Gold DNA

polymerase at 95 °C for 10 min, then 40 cycles of denaturation at 95 °C for 15 s, and annealing and extension at 60 °C for 1 min. Relative quantification was accomplished with measurement of the threshold cycle and use of the standard curve. Gene expression of the target sequence was normalized to that of β-actin. Transcript level in control group was arbitrarily expressed as 1.

*F-actin staining.* Podocytes were grown on collagen I-coated glass coverslips under nonpermissive condition and incubated in the presence or absence of PAN (100 μg/ml) for 48 h. Cells on coverslips were fixed in 4% paraformaldehyde for 30 min and permeabilized with 0.1% Triton X-100 for 5 min on ice. Nonspecific binding sites were saturated by 1% bovine serum albumin for 30 min. F-actin was visualized by incubating with Texas red-X phalloidin for 30 min (1:40 dilution; Molecular Probes). Coverslips were mounted on glass slides using Vectashield (Vector Laboratories). Confocal images were obtained with an inverted microscope (DMIRE2, Leica) and a confocal laser scanning unit (TCS-SP2, Leica). Multiple (13–18) frames of 0.6 μm intervals were integrated for fluorescent images.

*Immunohistochemistry.* Immunostaining was performed as described previously [26] with some modifications. Briefly, cryosections (8 μm thick) were pretreated with 3% $H_2O_2$, avidin–biotin blocking system (DakoCytomation) and 0.5% blocking solution (Roche), and incubated overnight at 4 °C with rabbit anti-rat AM (1:3000) [27] or mouse anti-human desmin (1:500; DakoCytomation). Subsequently, samples were reacted with biotinylated anti-rabbit or anti-mouse IgG, and immunoreactivity was detected using an ABC kit (Vector Laboratories) and a metal enhanced diaminobenzidine kit (Pierce).

*Statistics.* Data are expressed as means ± SEM. Statistical analyses were performed by unpaired *t* test or analysis of variance and subsequent Tukey's simultaneous multiple comparison. A *p* value less than 0.05 was considered to be statistically significant.

## Results

### *AM mRNA was expressed in cultured podocytes*

The podocyte cell line, when cultured under permissive condition, proliferated with undifferentiated cobblestone-like appearance (Fig. 1A). Under nonpermissive condition, on the other hand, most of the cells became growth arrested and acquired characteristics of differentiated podocytes, such as cell process elongation by day 7 (Fig. 1B).

We first examined whether AM mRNA was expressed in our podocyte cell line cultivated under nonpermissive condition for 10–14 days. RT-PCR analysis showed that AM transcripts were detected in cultured differentiated podocytes as well as in rat glomeruli and whole kidneys (Fig. 1C). The specific band was not amplified in the mock reaction without reverse transcriptase. The authenticity of the PCR product was confirmed by direct sequencing.

### *AM secretion from podocytes was increased by various stimuli*

We next determined AM levels secreted into the culture media from podocytes under basal condition and in response to various stimuli (Fig. 2). Measurable amount of AM was released from differentiated podocytes under

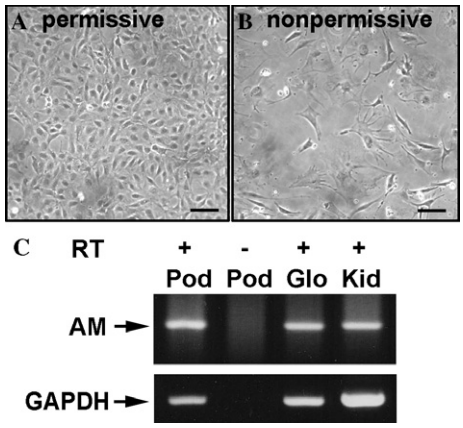


Fig. 1. Gene expression of AM in cultured mouse podocyte cell line. (A,B) Representative micrographs of mouse podocytes cultivated under permissive (33 °C in the presence of γ-interferon; A) and nonpermissive (37 °C in the absence of γ-interferon; B) conditions. Magnification, 100×. Bars represent 100 μm. (C) Expression of AM mRNA in cultured mouse podocytes, rat glomeruli, and whole kidneys. RT-PCR was carried out with primers specific for AM and GAPDH. RT-PCR was also performed without the addition of reverse transcriptase (RT-) using total RNA from cultured podocytes. PCR products (173 bp for AM; 515 bp for GAPDH) were electrophoresed on 2% agarose gel.

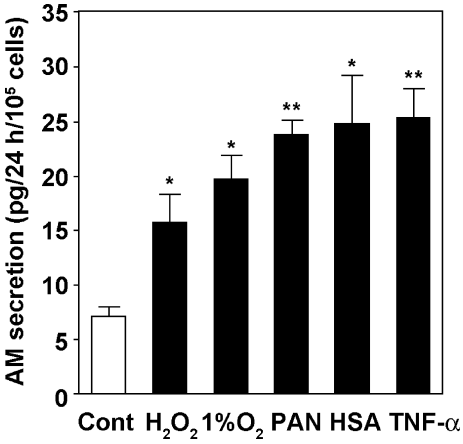


Fig. 2. Secretion of AM from cultured podocytes determined by radioimmunoassay. AM was detectable in the medium of podocytes cultured for 24 h under basal condition (Cont). AM secretion was increased by $H_2O_2$ (10 μM), hypoxia (1% $O_2$), PAN (100 μg/ml), human serum albumin (10 mg/ml), and TNF-α (20 ng/ml). Data are means ± SEM. Statistical analysis was performed by unpaired *t* test. $^*p < 0.05$; $^{**}p < 0.01$ vs. Cont.

resting condition. AM secretion from podocytes was significantly increased in response to $H_2O_2$ (10 μM) and hypoxia (1% $O_2$). AM production was also enhanced by PAN (100 μg/ml), protein overload (HSA 10 mg/ml), and inflammatory cytokine TNF-α (20 ng/ml). Increased AM secretion from podocytes by oxidative stress and hypoxia is compatible with our previous observations that AM expression was induced by these stimuli in cultured vascular endothelial and smooth

muscle cells [25,28]. PAN, protein overload, and inflammatory cytokine are considered to be important mediators of podocyte injury in vitro and in vivo [2], suggesting that AM in the podocytes is upregulated in response to podocyte injury.

*AM gene regulation by PAN and TNF-α, and effects of antioxidants*

We also examined the changes in AM expression at mRNA level using real-time quantitative RT-PCR (Fig. 3). Incubation of the podocytes with PAN (100 μg/ml) for 6–48 h increased AM mRNA expression in a time-dependent manner (8.0-fold after 48 h) (Fig. 3A). Exposure of podocytes to TNF-α (20 ng/ml) for 6–24 h also caused a time-dependent increase in AM transcripts (2.3-fold after 24 h) (Fig. 3B).

There is accumulating evidence to suggest that reactive oxygen species (ROS) contribute to the podocyte damage caused by PAN or TNF-α [2,29,30]. The oxidants may be derived from mitochondrial electron transfer system or plasma membrane NADPH oxidase system. To evaluate the influence of oxidative stress in the AM gene modulation, cells were pretreated with mitochondrial antioxidants or NADPH oxidase inhibitors 40 min prior to stimulation with PAN or TNF-α (Figs. 3C and D). The enhanced expression of AM mRNA by PAN was significantly blocked by mitochondrial antioxidants rotenone (−98%, $p < 0.01$) or antimycin A (−69%, $p < 0.05$) but not by NADPH oxidase inhibitors DPI or apocynin. Likewise, TNF-α-evoked elevation of AM transcripts was suppressed after pretreatment with rotenone (−91%, $p < 0.01$) but not with apocynin (Fig. 3D). These results suggest that mitochondria-derived oxidative stress plays an important role in the AM gene regulation.

*PAN caused functional and morphological changes in cultured podocytes*

In order to ascertain that PAN really leads to podocyte damage, we analyzed PAN-induced functional and morphological alterations in our podocyte cell line. As a functional change, we examined the mRNA expression of B7-1, a recently identified sensitive marker for podocyte injury [31]. It was reported that B7-1 expression was upregulated in the podocytes from nephrotic patients or animals as well as in the cultured podocytes stimulated with PAN. Furthermore, enhanced B7-1 signaling in podocytes is proposed to result in cytoskeletal reorganization and proteinuria. We found that incubation of podocytes with PAN (100 μg/ml) for 6–48 h caused a time-dependent increase in B7-1 mRNA expression with its peak at 12 h (2.7-fold) (Fig. 4A). In regard to a morphological change, confocal images of phalloidin labeling showed that control cells contained arrays of

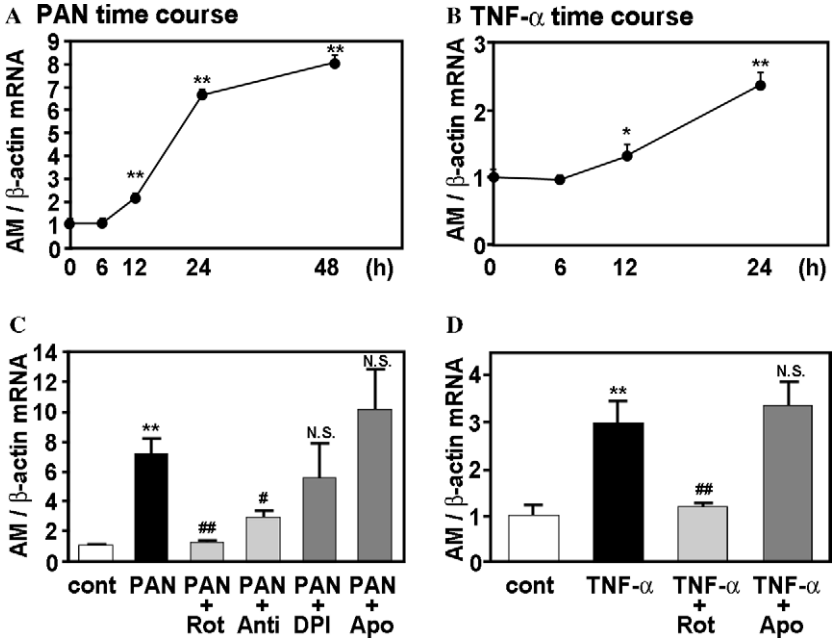


Fig. 3. Gene regulation of AM by PAN and TNF-α, and effects of antioxidants in cultured podocytes. (A) Podocytes were incubated with PAN (100 μg/ml) for various periods of time (6–48 h). (B) Podocytes were exposed to TNF-α (20 ng/ml) for 6–24 h. (C) Podocytes were incubated in control medium or in the presence of PAN (100 μg/ml) for 24 h. The effect of PAN was also examined after pretreatment with rotenone (Rot; 100 μM), antimycin A (Anti; 1 μM), DPI (5 μM), or apocynin (Apo; 60 μM) for 40 min. (D) The effect of TNF-α was examined after pretreatment with rotenone (Rot; 100 μM) or apocynin (Apo; 60 μM) for 40 min. Quantification of AM mRNA was performed using real-time RT-PCR and standardized with β-actin. Data represent means ± SEM. The mean value in control group was arbitrarily expressed as 1. Statistical analysis was by analysis of variance followed by Tukey–Kramer's test. $^{*}p < 0.05$; $^{**}p < 0.01$ vs. Cont. $^{\#}p < 0.05$; $^{\#\#}p < 0.01$; NS not significant vs. PAN.

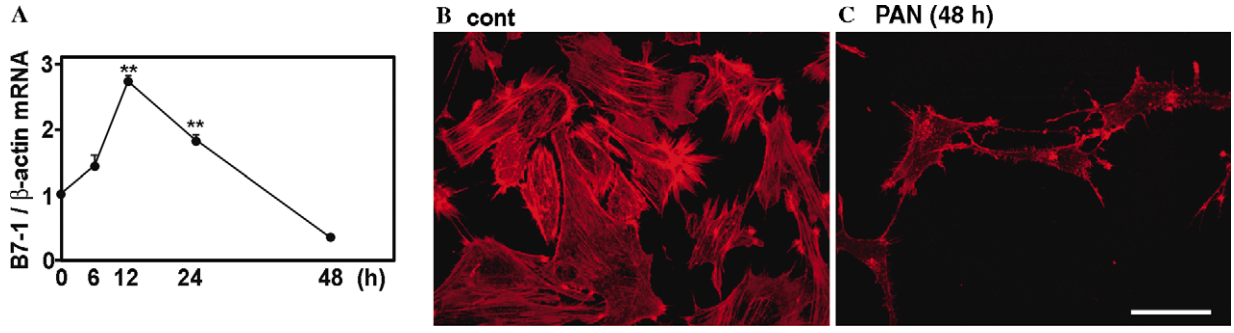


Fig. 4. Functional and morphological changes of cultured podocytes in response to PAN treatment. (A) Gene regulation of B7-1 in cultured podocytes. Podocytes were incubated with PAN (100 μg/ml) for various periods of time (6–48 h). Quantification of B7-1 mRNA was performed as described in Fig. 3. $^{**}p < 0.01$ vs. Cont. (B,C) Representative confocal images of F-actin by Texas red-X phalloidin binding in the mouse podocytes cultured in the absence or presence of PAN (100 μg/ml) for 48 h. Bar represents 40 μm.

F-actin fibers that extended across the entire cell body (Fig. 4B). On the other hand, intracellular organization of actin fibers was severely disrupted in the podocytes exposed to PAN for 48 h (Fig. 4C). Taken together, we confirmed that treatment with PAN indeed caused podocyte injury in our culture system.

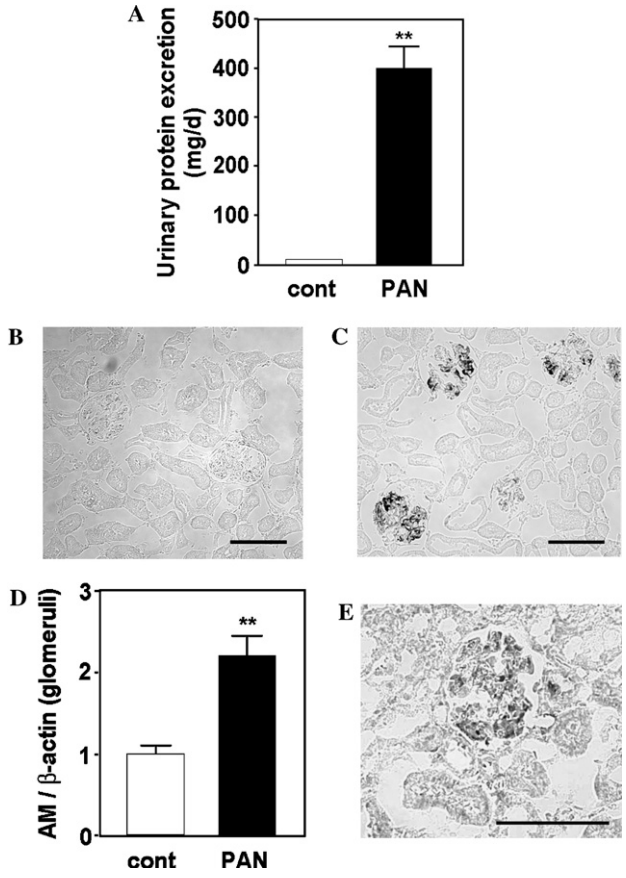


Fig. 5. Enhancement of AM expression in PAN nephrosis rats. (A) Urinary protein excretion in control (Cont) and PAN-injected (PAN) rats at day 7. (B,C) Immunostaining for desmin, a podocyte injury marker, in the kidneys of control (B) and PAN-injected rats (C) at day 7. Signals were detected with diaminobenzidine. Scale bar, 100 μm. (D) Gene expression of AM in the glomeruli at day 7 in Cont and PAN rats. Quantification of AM mRNA was performed using real-time RT-PCR and standardized with β-actin. Values are means ± SEM. Statistical analysis was by unpaired *t* test. $^{**}p < 0.01$. (E) Immunohistochemical localization of AM in the glomeruli of PAN-injected rats at day 7. Scale bar, 100 μm.

### *AM expression was upregulated in the glomerular podocytes of PAN nephrosis rats*

We further investigated whether AM expression is actually regulated in the in vivo setting of podocyte injury. We created PAN nephrosis rats, a well-established animal model of podocyte injury mimicking human minimal change nephrotic syndrome, and examined the AM modulation in the glomerular fraction (Fig. 5). Urinary protein excretion was markedly increased in PAN-injected rats as compared with sham-operated rats (Fig. 5A). The presence of podocyte injury was confirmed by the induction of desmin expression in PAN-treated rats (Figs. 5B and C). Real-time RT-PCR analysis revealed that AM mRNA expression level was significantly augmented in the glomeruli of PAN nephrosis rats compared with those of control rats (Fig. 5D). Glomerular fraction in PAN-injected rats is composed of intrinsic renal cells, i.e., the glomerular endothelial cells, mesangial cells, and glomerular epithelial cells, as well as infiltrating cells such as macrophages. In order to determine which cells were responsible for the AM upregulation in the glomeruli of PAN nephrosis model, we performed immunohistochemical analysis. Immunostaining for AM in the glomeruli was detected principally in the podocytes (Fig. 5E). These findings further support the viewpoint that AM upregulation is intimately associated with podocyte injury.

## Discussion

In the present study, we demonstrated that AM is synthesized in the glomerular podocyte cell line established by Mundel et al. AM expression is upregulated in response to oxidative stress, hypoxia, PAN, protein

overload, and TNF-α in the cultured podocytes in vitro, and in the glomerular podocytes of PAN nephrosis rats in vivo. These modulatory patterns of AM in response to various stimuli suggest that AM is involved in the podocyte damage.

This is the first paper indicating that AM is expressed in the cultured podocyte cell line established by Mundel. AM is reported to be expressed in the kidney and localized to the glomeruli, distal and collecting ducts [21,22]. In vitro analyses were generally performed using cultured glomerular mesangial cells and tubular epithelial cells to examine the expression and regulation of AM [23,32]. On the other hand, few studies addressed its expression in the glomerular podocytes, because of the lack of proper cell culture system. Lai et al. [24] reported the presence of AM in primary cultured glomerular epithelial cells. However, there is a concern that the cells isolated by their conventional method do not represent pure glomerular visceral epithelial cells (podocytes) but contain parietal epithelial cells [4,6]. It is also suggested that primary cultured podocytes undergo de-differentiation associated with loss of podocyte-specific gene expressions [5] and lack of processes [6]. It was reported that the podocyte cell line we used retain differentiation characteristics [7]. In this sense, we believe that the authenticity of cells as podocytes is more guaranteed by using Mundel's cell line.

It has recently been reported that podocytes express a variety of vasoactive substances and their receptors, including the renin–angiotensin system, catecholamines, endothelins, natriuretic peptide family, and nitric oxide, suggesting novel biological activities of these substances in podocyte functions [10–13]. Indeed, recent attention has been focused on angiotensin II (Ang II) and its receptors in the podocytes and on their causative roles in proteinuria of diabetic nephropathy [33,34]. Endothelin was reported to be involved in the podocyte damage associated with aging [35]. Similarly, AM may play a regulatory role in the podocytes in an autocrine manner. Indeed, cAMP, one of the main second messengers of the AM signaling cascade, was indicated to regulate podocyte function [36]. Alternatively, AM secreted from the podocytes may regulate glomerular function by acting on glomerular endothelial or mesangial cells in a paracrine manner.

We observed that $H_2O_2$, hypoxia, PAN, HSA, and TNF-α all upregulated AM expression. Stimulation of AM secretion by TNF-α, oxidative stress, and hypoxia has been reported in other cell types such as vascular smooth muscle cells and endothelial cells [25,28,37]. TNF-α also enhanced AM production in cultured renal tubular epithelial cells and glomerular mesangial cells [23,24,32]. However, the extent of upregulation was much greater in our glomerular podocytes as compared with renal tubular epithelial cells and mesangial cells. In contrast, cultured glomerular epithelial cells by Lai et al. [24] were demonstrated to be downregulated by TNF-α. The discrepancy in the reactivity to TNF-α between their podocytes and ours may be explained by the different characteristic of the two podocytes. As stated previously, the conventional culture method used in the paper by Lai et al. has uncertainty about the purity and differentiation status of the cultured cells. In addition, our in vitro data that PAN caused a marked upregulation of AM expression in cultured podocytes are compatible with the in vivo data that AM expression was enhanced in the glomerular podocytes of PAN nephrosis rats. Therefore, our data of positive regulation of AM may be more plausible from the viewpoint of cellular authenticity used in the analysis.

We found that AM expression was enhanced by $H_2O_2$. In addition, AM upregulation by PAN and TNF-α was suppressed by antioxidants, specifically mitochondrial antioxidants. These findings suggest the involvement of mitochondrial ROS in AM gene regulation. Oxidative stress has been implicated in the pathogenesis of podocyte injury induced by PAN [2,29,30]. It can be assumed that PAN generates ROS, which activates injurious signaling on one hand, and stimulates AM secretion to counteract the injury at the same time. As for mitochondrial oxidative stress, one report indicated that PAN-injected rats displayed enhanced mitochondrial oxidative stress [30].

What is the biological significance of AM upregulated in injured podocytes? There are two possibilities. AM may be involved in the promotion of podocyte injury. Alternatively, AM may counteract podocyte dysfunction in a protective fashion. The temporal profile that injurious B7-1 upregulation preceded that of AM might implicate that induction of AM is the sequel, rather than the initial trigger, of podocyte injury. Previous studies revealed that PAN nephrosis rats manifest podocyte damage characterized by cytoskeletal reorganization, reduction, and disarrangement of slit diaphragm-associated molecules nephrin and podocin, foot process effacement, impaired filtration barrier function, podocyte apoptosis, and in some situations, podocytopenia and glomerulosclerosis [1,2,38]. Enhanced oxidative stress and inflammatory reactions are suggested to be important mediators. Furthermore, recent studies suggest a pivotal role for Ang II in the pathogenesis of podocyte injury by pressure-independent mechanisms [39]. Ang II blockade was shown to improve podocyte injury in PAN nephrosis as well as in diabetic glomerulopathy [33,34,40]. On the other hand, AM has been shown to possess anti-oxidant, anti-inflammatory, and anti-apoptotic properties, in addition to vasodilatory and natriuretic actions [41,42]. cAMP, the main second messenger of AM, is suggested to modify the assembly of actin filaments and affect the ultrafiltration coefficient $K_f$ [43]. Moreover, AM has been reported to counteract the deleteri-

ous effects of Ang II [15]. We previously reported that AM exerts protective effects against Ang II- or cuff-induced vascular injury through the inhibition of oxidative stress using AM knockout mice [15,44]. Therefore, it may be plausible to speculate that upregulated AM in the podocytes of PAN nephrosis rats modifies podocyte function, possibly in a protective way, directly or via the inhibition of Ang II action. Phenotypic analyses of the podocyte injury model using AM knockout mice may prove the precise pathophysiologic roles of AM in the podocytes, which is to be investigated in the future studies.

In summary, our in vitro and in vivo data demonstrate the presence of AM expression in the glomerular podocytes as well as its upregulation by podocyte injurious stimuli in a redox-sensitive mechanism. Our study suggests that AM may participate in the regulation of podocyte function, especially in the setting of podocyte injury and proteinuria, although its exact pathophysiologic role should await further studies.

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