Am J Transl Res 2019;11(5):2925-2939
www.ajtr.org /ISSN:1943-8141/AJTR0091450

Original Article

Renal and cerebral RAS interaction contributes to diabetic kidney disease

Yufeng Liu[1,2], Lanying Li[1], Minzi Qiu[1], Lishan Tan[1], Mengbi Zhang[1], Jiawen Li[1], Hongguo Zhu[1], Shaoling Jiang[1], Xiaoyan Su[2], Aiqing Li[1]

[1]*State Key Laboratory of Organ Failure Research, National Clinical Research Center for Kidney Disease, Nanfang Hospital, Southern Medical University, Guangzhou, China;* [2]*Nephropathy Department, Tungwah Hospital of Sun Yat-sen University, Dongguan 523110, China*

Received January 17, 2019; Accepted March 15, 2019; Epub May 15, 2019; Published May 30, 2019

Abstract: The diabetes mellitus has posed a grave threat on human health, and is bound to result in renal trauma by uncertain mechanisms. Increasing evidences indicated that the activation of the renin-angiotensin system plays a pivotal role during the progression of diabetic kidney disease. In streptozotocin (STZ)-induced type 1 diabetic rat model, the losartan (a selective angiotensin II type 1 (AT1) receptor antagonist) and tempol (4-Hydroxy-TEMPO, reactive oxygen species scavenger) were administrated through intracerebroventricular injection or intragastric gavage. Intracerebroventricular administration of clonidine or renal denervation was carried out to block sympathetic nerve traffic. Compared with non-diabetic rats, the reno-cerebral axis was over-activated, including activity of renin-angiotensin system (RAS), oxidative stress, and sympathetic activity in diabetic rats. Central blockade of RAS inhibited the central oxidative stress and sympathetic activity, which led to decrease of intrarenal RAS activity and oxidative stress. Meanwhile, central administration of tempol reduced brain RAS, thus downregulated renal RAS activity and oxidative stress. Importantly, oral administration by intragastric gavage of high dose of losartan and tempol achieved the same effect. The results suggested that there is a cross-talk between renal and cerebral RAS/reactive oxygen species, contributing to the progression of diabetic kidney disease. The subfornical organ, paraventricular nucleus, and supraoptic nucleus in the forebrain also play a key role in development and progression of renal trauma through reno-cerebral reflex axis.

Keywords: Diabetic kidney disease, renin-angiotensin system, oxidative stress, sympathetic nervous system

Introduction

Diabetes mellitus (DM) is becoming an increasingly serious public health problem worldwide, that resulted in more than 30% morbidity of diabetic kidney disease (DKD) patients [1]. DKD is a leading cause of chronic renal failure and end-stage renal disease worldwide [2]. A great effort has been dedicated to decode pathogenic mechanism of DKD aiming to develop novel therapeutic strategies [3]. However, at present, the mechanism underlying DKD remains elusive, and there are no effective interventions to prevent the decline in the renal function in DKD patients.

Several factors are implicated in the kidney damage, such as oxidative stress, hemodynamic dysregulations, inflammation, and metabolic toxins [4, 5]. The mechanism of oxidative stress-induced renal injury has been well recognized, including the accumulation of advanced glycation end products [6]. However, another potentially important factor, the activation of renin-angiotensin system (RAS) [7], needs to be fully perceived during the progression of diabetes. Increasing evidences indicated that the overactive RAS contributes to the pathologies of atherosclerosis, hypertension, and diabetic end-organ damage [2]. A previous study reported that increasing activity of RAS in local tissue resulted in insulin resistance, that promoted the progression of DKD [8]. Overexpression of RAS may induce the oxidative stress, that is taken as the main reason for organ damage in diabetes into account [9, 10]. The majority of organs possess a local RAS that is compartmentalized from the circulation, and respond to

the change of internal environment independently [11]. Our previous study revealed the vital role of the central RAS activation in the loss of renal function in salt-loaded CKD rats [12].

In the present study, we used streptozotocin (STZ)-induced type 1 diabetic rat model to examine our hypothesis that the renal and cerebral RAS axes interact via changes in sympathetic nerve activity, contributing to the progression of DKD. In this study, we demonstrated that central blockade of RAS or oxidative stress prevented renal RAS activity. Interrupting the sympathetic outflow blocked interaction between renal and cerebral RAS/reactive oxygen species (ROS). These results revealed a new mechanism underlying DKD.

Materials and methods

Animals

Male Sprague Dawley rats (body weight, 250-300 g) which were fed in a pathogen-free facility at a constant temperature (24±2°C) and humidity (55%±5%) under a 12-h light/dark cycle were supplied by Animal Experiment Center of Nanfang Hospital (Guangzhou, China). All animal experiments were approved by the Animal Ethics Committee of Nanfang Hospital.

Treatments

Here, STZ was used to induce type 1 diabetic rat model as previously described [13]. All normal rats were randomly divided into two groups, including diabetes mellitus (DM) group and non-diabetes mellitus (non-DM) group. Rats in DM group were injected with STZ abdominally, and the Non-DM rats were injected with the same volume of vehicle (sodium citrate solution, pH 4.2). At the 3rd, 5th, and 7th days after the injection treatment, the tail vein blood was collected to test blood glucose, and the rats with the mean value of the three time points blood glucose level >16.8 mmol/L were taken as successful DM models into account.

Before conducting different interventions, we compared the DM and Non-DM rats in different aspects at the end of 6 weeks. Consecutively, another part of the experiment was undertaken. The newly qualified type 1 diabetic rats were randomly divided into 11 groups based on body weight and blood glucose level (n=6 in each group). They were administrated with the following drugs or vehicle for 6 consecutive weeks as follows: intragastric gavage (IG) of vehicle (phosphate-buffered saline (PBS), pH 7.4) or losartan (a selective angiotensin II type 1 (AT1) receptor antagonist) (Sigma-Aldrich, St. Louis, MO, USA) at 1, 50, or 500 mg/kg/day (groups 1-4); intracerebroventricular (ICV) injection of vehicle (artificial cerebrospinal fluid) or losartan at 1 mg/kg/day using an ALZET Osmotic pump (DURECT Corp., Cupertino, CA, USA) (group 5 and 6); ICV clonidine (Sigma-Aldrich, St. Louis, MO, USA) at 5.76 mg/kg/day using an ALZET Osmotic pump (DURECT Corp., Cupertino, CA, USA) (group 7); renal denervation (RDX) (group 8); ICV tempol (4-Hydroxy-TEMPO) at 4.5 mg/kg/day using an ALZET Osmotic pump (DURECT Corp., Cupertino, CA, USA) (group 9); IG tempol at 30 mg/kg/day (group 10); and IG hydralazine (Sigma-Aldrich, St. Louis, MO, USA) at 15 mg/kg/day (group 11).

Measurement of basic physiological parameters and evaluation of RAS components, oxidative stress and sympathetic activity

Basic physiological parameters: Body weight was measured by electronic analytical balance (Mettler Toledo, Columbus, OH, USA), the blood glucose level in rats was detected by Accu-chek Sensor Comfort Advantage Blood Glucose Test Strips X50 (Roche, Basel, Switzerland) and the blood pressures were measured indirectly by tail arteries [14].

Serum creatinine concentrations were measured with an automated chemistry analyzer (AU480; Beckman Coulter, Brea, CA, USA) and urinary albumin was measured with an ELISA kit (ImTec Diagnostics, Antwerpen, Belgium).

Renal inflammatory response and glomerular sclerosis: Both kidneys of the rats were harvested and the periodic acid-Schiff staining and monocyte chemoattractant protein-1 (MCP-1) staining were carried out as previously described [12, 15].

Evaluation of blood-brain barrier (BBB) permeability

The BBB permeability of rats were assessed by concentration of Evans blue in forebrain as previously described [16].

Evaluation of RAS activity

RAS activity was illustrated by angiotensinogen (AGT) and angiotensin II receptor type 1 (AT1) expressions.

RAS activity in kidneys: The renal cortex tissue was dissected into four-micrometer-thick sections, and immunohistochemical staining was carried out using anti-rat AGT (1:200; ABclonal Technology, Woburn, MA, USA), anti-rat AT1 receptors (1:100; Abcam, Cambridge, UK) antibodies. The intrarenal expression was semiquantitated as described previously [17].

The protein levels of RAS in homogenates of renal cortex were measured using anti-AGT (1:500; ABclonal Technology, Woburn, MA, USA), anti-AT1 receptors (1:500; Abcam, Cambridge, UK) antibodies by Western blotting as mentioned previously [18].

RAS in circulation: Plasma concentrations of angiotensin II (Ang II) were assessed by a competitive ELISA kit (Peninsula Laboratories International Inc., San Carlos, CA, USA) according to the manufacturer's instructions.

RAS activity in brain: Cerebral localization of AGT and AT1 receptors was determined by double-staining immunofluorescence using anti-AGT or anti-AT1 receptors as the first primary antibody, and anti-neuron-specific enolase (Boster Biological Technology, Pleasanton, CA, USA) or anti-glial fibrillary acidic protein (Boster Biological Technology, Pleasanton, CA, USA) as the second primary antibody.

The protein level of AGT and AT1 receptors was measured in homogenates of brain nucleus [12]. The cerebral expression of AGT and AT1 receptors was confirmed by immunohistochemistry using anti-rat AGT (1:200; ABclonal Technology, Woburn, MA, USA), anti-rat AT1 receptors (1:100; Abcam, Cambridge, UK) antibodies.

Sympathetic activity

Norepinephrine concentrations: Norepinephrine concentrations in plasma were measured with an ELISA kit (ALPCO Diagnostics, Salem, NH, USA) according to the manufacturer's protocol.

Tyrosine hydroxylase (TH) in brain: Expression of TH in homogenates of brain nuclei was determined by Western blotting using an anti-TH antibody (1:200; Boster Biological Technology, Pleasanton, CA, USA).

The number of c-fos-positive and TH-expressing neurons in the rostral ventrolateral medulla (RVLM) was determined as previously described [19]. Brain stem sections were double stained with antibodies against TH (Boster Biological Technology, Pleasanton, CA, USA) and c-fos (1:100; Santa Cruz Biotechnology, Dallas, TX, USA).

Evaluation of oxidative stress

The levels of nicotinamide adenine dinucleotide phosphate (NADPH) oxidase subunits Nox2 and Nox4 in homogenates of renal cortex and brain were determined using anti-Nox2 and anti-Nox4 antibodies (Boster Biological Technology, Pleasanton, CA, USA) by Western blotting.

Statistical analysis

All data were expressed as the mean ± standard deviation (SD). Continuous variables were compared using one-way analysis of variance (ANOVA), followed by the least significant difference (LSD) test. Statistical analysis was undertaken by using SPSS 17.0 software (IBM, Armonk, NY, USA). *P*-value <0.05 was considered statistically significant.

Results

Renal RAS and oxidative stress were excessively activated in type 1 diabetic rats

As expected, compared with the non-DM group, the DM group showed increased concentrations of urinary albumin excretion, peripheral Ang II, norepinephrine and urinary 8-iso prostaglandin E2 (**Table 1**).

Additionally, overexpression of renal RAS was observed by immunohistochemistry and Western blot analysis in renal cortex (**Figure 1A**). There were higher inflammatory response and glomerulosclerosis index in DM group compared with non-DM group as presented with higher MCP-1 expression and periodic acid-Schiff staining (**Figure 1A**, **1B**). NADPH oxidase subunits (Nox2 and Nox4) were upregulated in the renal cortex of DM group (**Figure 1C**).

Table 1. Changes in metabolic and biochemical parameters between Non-DM and DM (n=15, x ± s)

Groups	Non-DM	DM	*p* value
Body weight (g)	378.7±19.3	252.5±23.1#	8.8842E-16
Blood glucose (mmol/L)	5.5±0.8	26.2±4.3#	1.2021E-11
Blood pressure (mmHg)	120.3± 7.2	118.4±7.5	0.478
Albumin/creatinine (ug/mg)	77.6±21.6	267.6±51.8#	7.0126E-11
Plasma Angiotensin II (pg/ml)	58.0±12.6	145.3±21.9#	1.1049E-13
Plasma norepinephrine (ng/ml)	0.2±0.06	0.5±0.07#	6.4037E-13
Urinary 8-epi-isoprostane PGF2α (pg/ml)	205.7±49.4	494.3±60.8#	2.2524E-14

Data are expressed as the mean ± SD (n=15 in each group), #P<0.05 versus Non-DM.

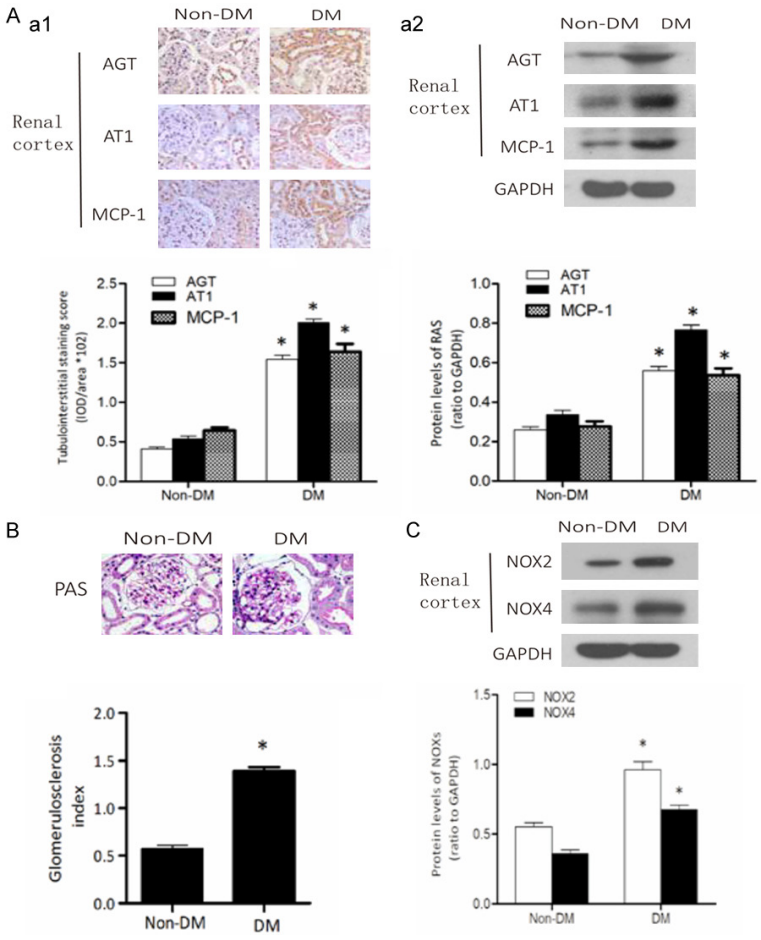


Figure 1. Renal RAS, oxidative stress, inflammation and glomerulosclerosis were up-regulated in DM rats. A. Representative photographs and semiquantitative data of AGT, AT1 and MCP-1 expression detected by immunohistochemistry (a1) and Western blot (a2). B. Glomerulosclerosis index measured by PAS. C. Protein level of Noxs in renal cortex measured by Western blot. Data are expressed as the mean ± SD (n=15 in each group). **P*<0.05 versus non-DM rats. PAS, periodic acid-Schiff.

The central RAS was mainly located in the cardiovascular regions of the forebrain, such as subfornical organ (SFO), paraventricular nucleus (PVN), and supraoptic nucleus (SON) [20]. The brain RAS components (AGT and AT1) were upregulated at the protein level in SFO (exposed to cerebrospinal fluid), PVN, and SON (within BBB) in DM group compared with non-DM group (**Figure 2A**, **2B**). Double immunofluorescence with antibodies recognizing the neuron-specific enolase or glial fibrillary acidic protein demonstrated that DM group showed overexpression of AGT and AT1 receptors in neurons, while glial cells were excluded (**Figure 3A**).

The BBB is taken as an important bridge between the central and peripheral environment into account, and the integrity and permeability of BBB become critical. The BBB permeability in DM group was increased compared with non-DM group, however, there were no changes in BBB permeability among the intervention groups (**Figure 3B**).

Central RAS, oxidative stress, and sympathetic outflow were upregulated in type I diabetic rats

At the same time, we attempted to concentrate on changes in central nervous system (CNS). Similar to the renal NADPH oxidase subunits, central NADPH oxidase subunits were also upregulated in these brain regions in DM group (**Figure 2C**). The expression of tyrosine hydroxylase (TH), the rate-limiting enzyme for cerebral

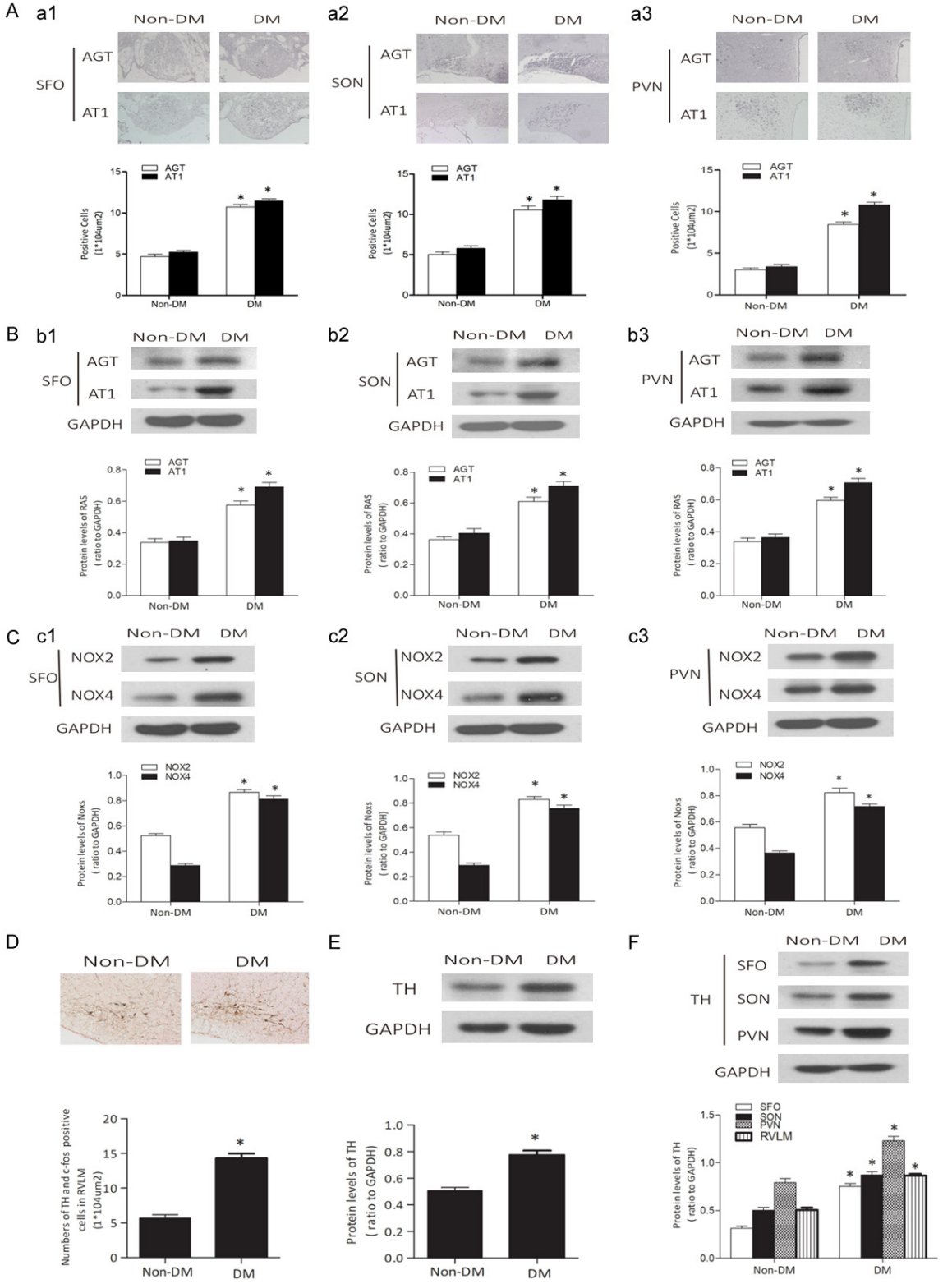
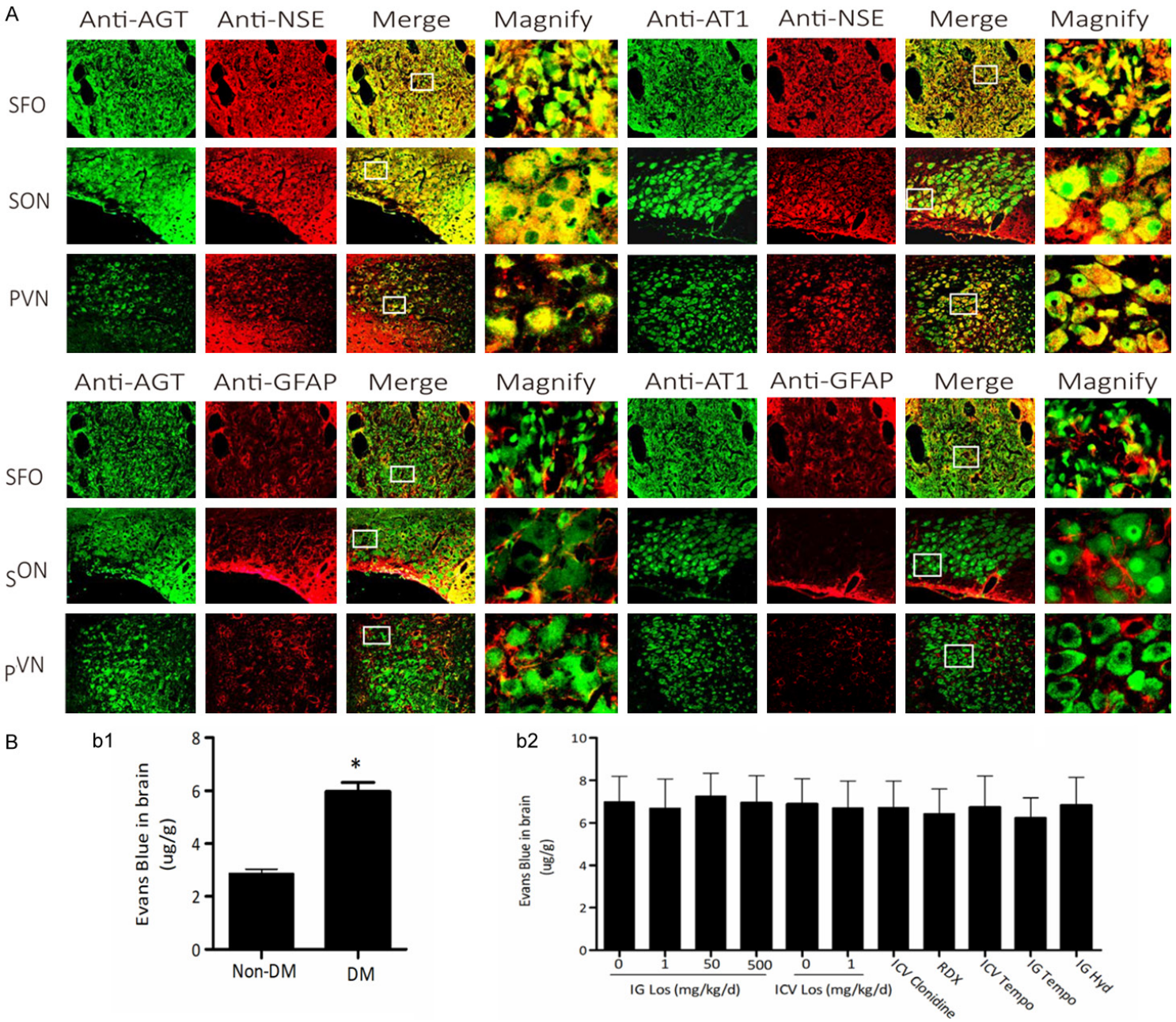


Figure 2. Brain RAS, oxidative stress and sympathetic activity were up-regulated in DM rats. A. AGT and AT1 receptors in SFO (a1), SON (a2) and PVN (a3) measured by immunohistochemistry. B. AGT and AT1 receptors in SFO (b1), SON (b2) and PVN (b3) measured by Western blot. C. Protein levels of NOX2 and NOX4 in SFO (c1), SON (c2) and PVN (c3) measured by Western-blot. D. Representative photographs of TH+c-fos positive cells in RVLM measured by immunohistochemistry. E. Protein levels of TH in RVLM measured by Western-blot. F. Protein levels of TH in SFO, SON, PVN measured by Western-blot. Data are expressed as the mean ± SD (n=15 in each group). *P<0.05 versus Non-DM.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ICV Los (mg/kg/d)
ICV Clonidine
RDX
ICV Tempo
IG Tempo
IG Hyd

Figure 3. Localization of central AGT and AT1 receptors and Blood brain barrier permeability. A. Localization of central AGT and AT1 receptors determined by doublestaining with the antibodies against AGT or AT1 receptors (green) and the antibodies-recognized NSE or GFAP (red). NSE, neuron-specific enolase; GFAP, glial fibrillary acidic protein. B. Blood brain barrier permeability was up-regulated in DM rats (b1), but there was no significant difference in all intervention groups (b2).

norepinephrine synthesis, was upregulated in the SFO, PVN, SON, and RVLM in DM group (**Figure 2D-F**).

Central oxidative stress and tyrosine hydroxylase expression were downregulated by blockade of central AT1 receptors or oxidative stress in type 1 diabetic rats

In order to examine the relationship among RAS, oxidative stress, and sympathetic excitability in central nervous system, we found that blockade of oxidative stress by ICV tempol or IG tempol significantly decreased the overexpression of brain RAS components (**Figures 4A**, **6A**). In contrast, ICV administration of clonidine or RDX that blocked sympathetic outflow did not change the central RAS activity. Decreased central sympathetic activity was noted by reduced remarkable reduction of the number of double stained c-fos positive and TH-expressing neurons in RVLM in group of ICV losartan (**Figure 4B**). Blockade of sympathetic nerve traffic by ICV administration of clonidine or renal denervation did not affect the central oxidative stress. These results indicated that the central RAS may be in the upstream of sympathetic nervous system.

Expectedly, overexpression of brain NADPH oxidase subunits was downregulated by ICV administration of losartan or a high dose of IG losartan (500 mg/kg) (**Figure 6B**).

The activation of the central RAS, ROS, and sympathetic nerve activity was independent of hypertension as their overexpression persisted after normalization of blood pressure with hydralazine.

The activation of the renal RAS, oxidative stress, inflammation, and glomerular sclerosis index were alleviated by blockade of central AT1 receptors or oxidative stress in type 1 diabetic rats

We examined the hypothesis that the renal and cerebral RAS interaction via changes in sympathetic nerve activity contributed to the DKD. Central blockade of RAS inhibited the central oxidative stress and sympathetic activity, that led to decrease of renal RAS activity and oxidative stress. Peripheral concentrations of urinary albumin excretion, peripheral Ang II, norepinephrine, and urinary 8-iso prostaglandin E2 were decreased in the IG losartan (50 and 500 mg/kg) and IG tempol in DM group (**Table 2**).

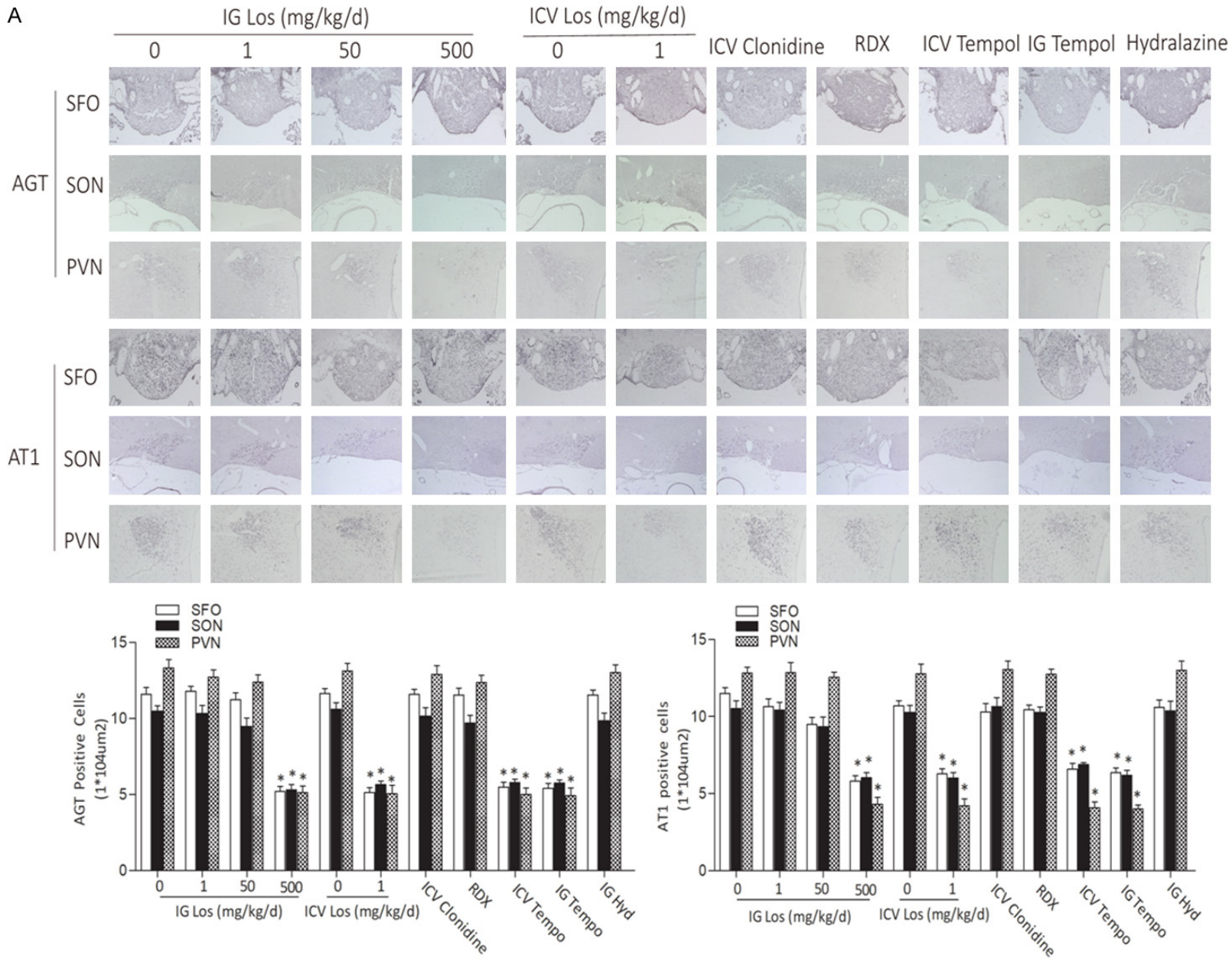
Overexpression of renal RAS components and oxidase subunits was prevented by IG losartan (50 and 500 mg/kg) or ICV tempol to block peripheral RAS or oxidative stress. Blockade of sympathetic traffic by ICV clonidine or RDX also significantly inhibited overexpression of renal RAS components (**Figures 5A**, **6D**).

The inflammatory response presented by MCP-1 expression and glomerulosclerosis index detected by periodic acid-Schiff was attenuated by the peripheral administration of losartan or tempol ICV (**Figure 5A**, **5B**). In contrast, neither blockade of central RAS or oxidative stress by ICV losartan or ICV tempol alleviated either renal inflammation, nor the blockade of sympathetic outflow.

Discussion

The goal of this study was to understand the mechanism underlying diabetes-induced kidney damage and the role of intrarenal and cerebral RAS interaction in progression of the damage. Based on type 1 diabetic rat model, this study revealed that the over-activation of brain-renal RAS/ROS axis contributes to DKD via changes in sympathetic nerve activity.

It has been reported that dysregulation of the renin-angiotensin system plays a pivotal role in the development of chronic renal failure in DM [21]. Serum concentrations of norepinephrine [22], plasma Ang II [23], urinary albumin [24], and urinary 8-iso prostaglandin E2 were increased in type 1 diabetic rat model [25]. Furthermore, local RAS and oxidative stress were upregulated in both kidney and brain. Numerous studies demonstrated an increase in RAS activity especially in renal tissue or nephrons,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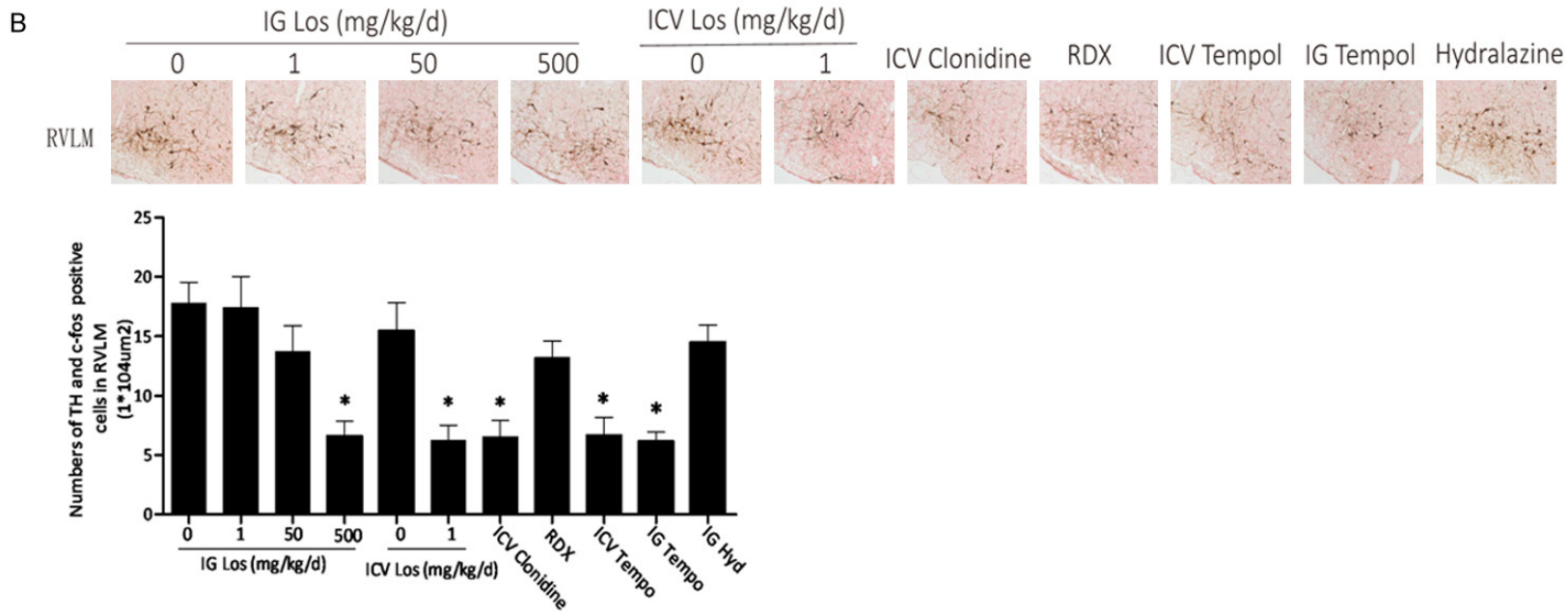

Figure 4. Central administration of tempol, but not clonidine, downregulate overexpression of brain RAS and sympathetic nerve activity in DM rats. A. Central administration of tempol downregulate overexpression of brain RAS in DM rats measured by immunohistochemistry. B. Central administration of losartan or tempol decreased sympathetic nerve activity in DM rats. Representative photographs of TH- and c-fos-positive neurons in RVLM measured by double immunohistochemical staining. Data are expressed as the mean ± SD (n=6 in each group). *P<0.05 versus IG 0 mg/kg/d Los.

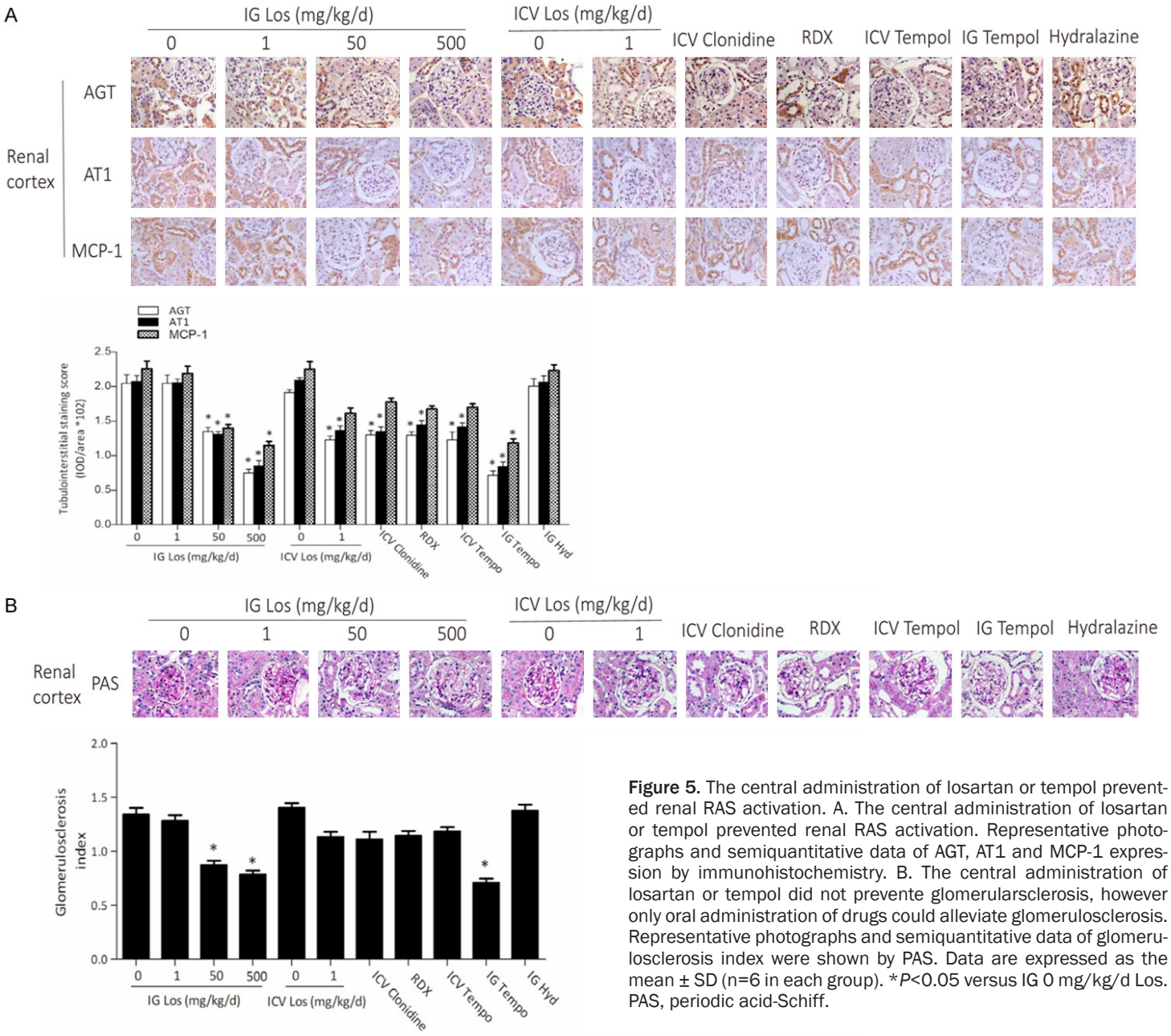

Figure 5. The central administration of losartan or tempol prevented renal RAS activation. A. The central administration of losartan or tempol prevented renal RAS activation. Representative photographs and semiquantitative data of AGT, AT1 and MCP-1 expression by immunohistochemistry. B. The central administration of losartan or tempol did not prevente glomerularsclerosis, however only oral administration of drugs could alleviate glomerulosclerosis. Representative photographs and semiquantitative data of glomerulosclerosis index were shown by PAS. Data are expressed as the mean ± SD (n=6 in each group). **P*<0.05 versus IG 0 mg/kg/d Los. PAS, periodic acid-Schiff.

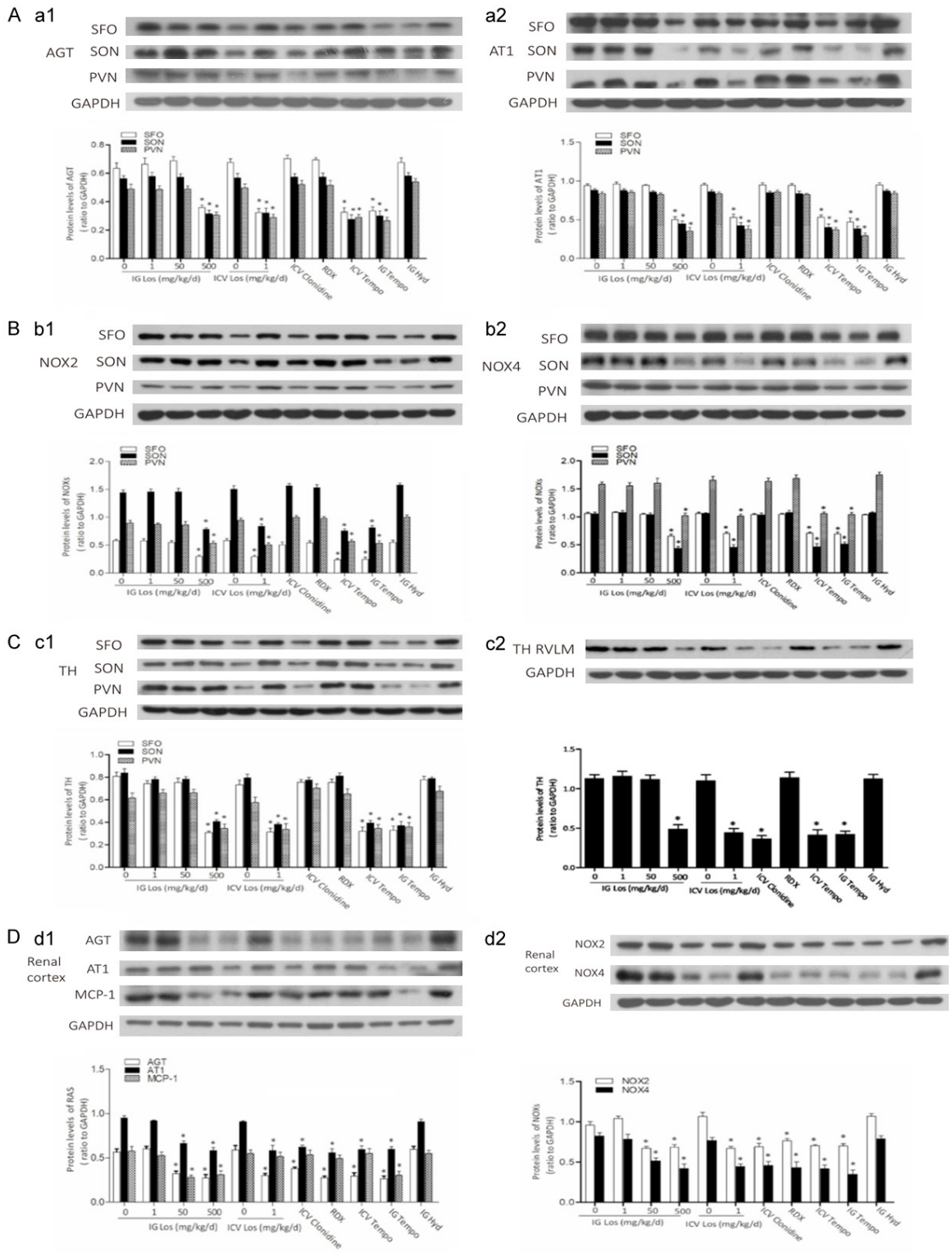

Figure 6. Expression of RAS components, NOXs and TH in brain nuclei and kidney measured by western-blot. A. Protein levels of AGT (a1) and AT1 receptors (a2) in brain nuclei measured by Western-blot. B. Protein levels of NOX2 (b1) and NOX4 (b2) in brain nuclei measured by Western-blot. C. Protein expression of TH in SFO, SON PVN (c1) and RVLM (c2) measured by western-blot. D. Protein levels of AGT, AT1, MCP-1 (d1) and Noxs (d2) in renal cortex homogenates measured by Western-blot. Data are expressed as the mean ± SD (n=6 in each group). **P*<0.05 versus IG 0 mg/kg/d Los.

Table 2. Changes in metabolic and biochemical parameters among the groups (n=6, x ± s)

Groups	Body weight (g)	Blood glucose (mmol/L)	Blood pressure (mmHg)	Albumin/ creatinine (ug/mg)	Plasma Angiotensin II (pg/ml)	Plasma norepinephrine (ng/ml)	Urinary 8-epi-isoprostane PGF2α (pg/ml)
IG 0 mg/kg/d Los	262.8±18.4	26.8±3.8	121.2±7.0	293.5±49.0	176.0±21.0	0.11±0.032	549.0±83.8
IG 1 mg/kg/d Los	265.8±30.4	25.4±5.9	120.9±8.7	276.1±47.0	189.0±28.5	0.12±0.032	554.5±77.5
IG 50 mg/kg/d Los	267.7±17.6	27.7±3.4	117.8±9.3	144.2±21.6#	114.9±14.2#	0.07±0.023#	276.0±57.3#
IG 500 mg/kg/d Los	262.9±27.9	24.3±4.5	119.8±6.2	163.1±30.8#	121.4±19.1#	0.06±0.020#	212.9±67.0#
ICV 0 mg/kg/d Los	251.0±26.6	25.6±3.4	120.4±8.4	269.2±47.6	182.5±23.9	0.11±0.027	699.0±63.3
ICV 1 mg/kg/d Los	258.5±22.2	27.9±3.3	118.6±9.6	275.4±58.6	176.7±28.9	0.11±0.030	580.8±68.3
ICV 5.76 μg/kg/d Clo	263.1±25.9	27.4±3.6	118.5±5.9	286.7±66.5	166.9±22.1	0.07±0.021	565.8±88.6
RDX	252.6±29.8	28.3±3.4	116.8±8.9	253.3±45.8	170.6±21.3	0.13±0.019	568.2±81.1
ICV 4.5 μg/kg/d Tem	257.9±23.1	26.5±4.3	119.7±9.3	297.9±73.3	182.1±19.6	0.12±0.030	569.6±82.9
IG 30 mg/kg/d Tem	246.5±22.6	27.8±3.0	119.3±9.3	157.2±43.2#	110.6±19.2#	0.06±0.020#	242.5±68.5#
IG 15 mg/kg/d Hyd	255.6±27.2	27.4±2.4	119.2±8.6	290.3±37.8	174.6±27.5	0.11±0.022	562.3±87.4
F	0.703	1.094	0.249	14.735	16.875	18.976	10.403
P	0.719	0.375	0.990	6.4023E-25	5.4248E-30	3.4345E-30	1.2583E-19

Data are expressed as the mean ± SD (*n*=6 in each group), #*P*<0.05 versus IG 0 mg/kg/d Los. IG, Intragastric administration; ICV, Intracerebroventricular injection.

including overexpression of renin, AGT, and AT1 [26-28], in which their biological effects may be enhanced. It also has been reported that treatment by suppression of systemic RAS activity is helpful to improve renal function [26]. The present study revealed that brain RAS is over-activated in STZ-induced diabetic rat models. Immunofluorescence double staining on neurons in brain nuclei (SFO, SON, and PVN) showed that the function of RAS activity may be depended on these neurons.

As an important factor mediating renal injury [29], together with RAS, oxidative stress plays a major role in the progression of DKD, however, the underlying mechanism still needs to be further studied [30, 31]. The NOXs were expressed in several tissues, including vascular smooth muscle cells and renal tubular epithelial cells, mediating diverse biological functions [32]. A number of scholars suggested that accumulation of advanced oxidation protein products, promoting renal inflammation through activation of renal oxidative stress, was involved in development and/or progression of DKD [33]. Previous studies also suggested that oxidative stress and RAS could mutually regulate each other by multiple mechanisms and contribute to the development of diabetes mellitus as well [34-36]. Consistent with a previous study [37], neuronal activity and oxidative stress have been upregulated in brain.

Various nuclei/areas in the brain were found to be involved in the regulation of sympathetic outflow, and may specifically affect the heart and kidney [38]. We previously found that increased central sympathetic activity in RVLM is the gateway for activation of the sympathetic nervous system as previously described [19]. Sympathetic nerve hyperactivity, which is associated with the incidence of metabolic diseases (e.g., diabetes mellitus), was found to be frequently resulted in renal trauma [39, 40]. The cardiac and renal sympathetic nerve activities were impaired and a sympathetic afferent-mediated reflex elevation was evoked in the diabetic rats [41, 42], and it seemed difficult to prevent the deterioration of the neuropathology [43]. Evidence indicated that neural activity in the PVN was markedly increased, which was involved in autonomic dysfunction during type 1 diabetes [44]. Thus, RAS and oxidative stress both participated in the central sympathetic abnormalities during STZ-induced diabetic rats.

Increased BBB permeability may account for decreased central RAS activity and oxidative stress in IG administration of high dose of losartan (500 mg/kg) or IG tempol, which further demonstrated the association between RAS and oxidative stress. It has been demonstrated that BBB permeability was altered in diabetes [45]. In the present study, BBB permeability was increased in DM rats, while there was no obvious improvement after various drug or vehicle treatments. This may explain that drug infiltrated from peripheral circulation into the cerebrospinal fluid, resulting in the inhibition of RAS and oxidative stress [46].

The present study also revealed that decrease of cerebral RAS activity could downregulate the expression of renal RAS and oxidative stress, that is consistent with our previous study [12]. Some biomarkers were used to predict the progression of diabetic nephropathy, such as RAS components, ROS, inflammatory cytokine, and other protein molecules [47, 48]. In the present study, the renal expression of RAS and oxidative stress was decreased by antagonist of RAS or oxidase stress peripherally or cerebrally, however, the trend regarding changes in the MCP-1 was not fully consistent with RAS activity or oxidative stress [49]. It is well-known that DM is characterized as a kind of systemic inflammatory response syndrome, and inflammatory response has already been upregulated before organ injury [50]. The level of MCP-1 was increased before decrease of the estimated glomerular filtration rate [51], which was associated with RAS activity in some extent [52].

There are some limitations in this study. After STZ was induced, duration of 6 weeks may not be long enough to observe the significant renal fibrosis. The expression of RAS components were upregulated and positively correlated with proteinuria before renal injury [53, 54]. However, a significant glomerular mesangial cell proliferation was observed in the DM rat model, that was closely associated with glomerular sclerosis [55]. In addition, drug concentration in cerebrospinal fluid was not detected. Thus, the detailed mechanism underlying the attenuation of oxidative stress by peripheral administration of losartan in high dose has not been clarified yet, as peripheral losartan may filtrate into the cerebrospinal fluid because of increased BBB permeability [56].

In conclusion, this study demonstrated that the increased RAS activity, oxidative stress, and sympathetic activity play a pivotal role as renocerebral RAS axis in the progression of diabetic nephropathy in DM rat models. This reveals a new regulatory mechanism of DKD, thereby presenting insights into novel therapeutic strategies for prevention and management of DKD, such as central blockade of RAS/ROS, or interruption of renal nerve.

Acknowledgements

This study was financially supported by the National Nature and Science Foundation of China (Grant Nos. 81770727 and 81270825), GDUPS (2017), Key Project of Science and Technology Planning of Guangzhou (Grant No. 201804020054) and Technology Planning Project of Guangdong Province (Grant No. 2017A010103041).

Disclosure of conflict of interest

None.

Address correspondence to: Aiqing Li, State Key Laboratory of Organ Failure Research, Nanfang Hospital, Southern Medical University, Guangzhou 510515, China. E-mail: liaiqing@smu.edu.cn; Xiaoyan Su, Nephropathy Department, Tungwah Hospital of Sun Yat-Sen University, Dongguan 523110, China. E-mail: suxiaoyan769@hotmail.com

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