

Aquaporin Changes during Diabetic Retinopathy in Rats Are Accelerated by Systemic Hypertension and Are Linked to the Renin-Angiotensin System

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PURPOSE. We explored the relationship between the renin-angiotensin system (RAS) and aquaporins (AQP1 and AQP4 in Müller glia and astrocytes) in diabetic retinopathy (DR) with and without systemic hypertension.

METHODS. Diabetes was induced in spontaneously hypertensive rats (SHR) and normotensive control Wistar Kyoto (WKY) rats by intraperitoneal injections of streptozotocin. The diabetic and control non-diabetic rats were assigned randomly to receive no anti-hypertension treatment, or to be treated with the angiotensin II receptor blocker (ARB), valsartan (40 mg/kg/d) or the beta-blocker, metoprolol (50 mg/kg/day). Eight weeks later, retinas were evaluated by immunohistochemistry and Western blot to detect changes in the expression of AQP1, AQP4, and glial fibrillary acidic protein (GFAP).

RESULTS. Hypertension increased expression of glial GFAP and AQP4 ($P < 0.01$), but not AQP1 ($P > 0.05$) in diabetic rats. Valsartan and metoprolol decreased GFAP, AQP1, and AQP4 expression in diabetic SHR rats ($P < 0.01$). Valsartan decreased GFAP and AQP1 expression in diabetic WKY rats ($P < 0.01$), while metoprolol did not.

CONCLUSIONS. Activation of Müller glia and astrocytes was involved in the mechanism by which systemic hypertension affects DR. AQPs and macroglia were linked to changes in the RAS in DR. Changes in aquaporin expression in DR were increased by hypertension. This provides additional support for the early use of an ARB in the treatment of DR, especially in cases with retinal edema. (*Invest Ophthalmol Vis Sci.* 2012; 53:3047–3053) DOI:10.1167/iovs.11-9154

Diabetic retinopathy (DR) remains a major cause of blindness in patients aged 20–64 years.¹ In addition to hyperglycemia, epidemiologic studies identify clearly hypertension as the most important independent risk factor for DR.^{2,3} The incidence of diabetic macular edema in patients with hypertension is three times higher than that in

normotensive patients.⁴ Accordingly, it has been demonstrated that lowering blood pressure in diabetic patients with an angiotensin-converting enzyme (ACE) inhibitor, beta blocker, or even a calcium channel blocker has beneficial effects on retinopathy.^{5,6} However, the mechanism by which systemic hypertension affects DR remains largely unknown.

Results from the Controlled Trial of Lisinopril in Insulin-Dependent Diabetes (EURODIAB EUCLID)⁷ and U.K. Prospective Diabetes Study⁸ suggest that controlling blood pressure and, specifically, targeting the renin-angiotensin system (RAS) are important strategies for treating DR. More recently, the Diabetic Retinopathy Candesartan Trial (DIRECT), a randomized double-blind placebo-controlled study with types 1 and 2 diabetics, showed the importance of the RAS in DR.^{9,10} These data showed that the potential benefits of angiotensin II (Ang II) type 1 (AT1) receptor blockers (ARB) might be seen in the early stages of DR. Previous studies demonstrated the presence of all RAS components in the retina.^{11,12} Funatsu et al. showed increased levels of Ang II in vitreous specimens of diabetic patients with retinopathy, demonstrating that the RAS is activated in DR.¹³ Studies have shown that ARBs prevent accumulation of advanced glycation end-products (AGE),¹⁴ changes in the level of vascular endothelial growth factor (VEGF),¹⁵ inflammation,¹⁶ damage to the blood-retina barrier,¹⁷ oxidative stress, and neuron apoptosis¹⁸ in DR. These effects may be responsible partially for the benefits of ARB in the treatment of DR. Despite the evidence that retinal macroglia cells (including Müller cells and astrocytes) express AT1R,¹⁹ and changes in macroglia have a vital role in the progression of DR,^{14,20} very little is known regarding the role of Ang II in regulating macroglia function in the diabetic retina. Ang II may have a role in the interaction between hyperglycemia and hypertension that can worsen DR.

Aquaporins (AQPs) located on macroglia have an important role in the balance of water-electrolyte metabolism in the nervous system.^{21–23} In our previous reports, we showed that changes in the expression and distribution of AQP1,4 occur during the early stages of DR.^{24,25} Few studies have focused on the mechanism of how AQP1,4 changes as DR progresses. The RAS is involved deeply in the regulation of AQP1 expression in renal disorders,^{26,27} and treatment with an ARB slows the progression of chronic kidney disease in patients with diabetes by regulating medullary transport proteins, including AQP2.²⁸ However, to our knowledge the relationship between the RAS and AQP1,4 in the central nervous system has not been investigated. In our study, we evaluated: 1) if systemic hypertension increases the macroglia cell reaction in DR, and changes the expression and distribution of AQP1,4; 2) if lowering blood pressure, either with an ARB or a beta blocker, has effects on AQP1,4 expression in DR, and 3) if ARB affects AQP1,4 expression and distribution in early DR in the absence of systemic hypertension.

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MATERIALS AND METHODS

Experimental Animals

All animal experiments were conducted in accordance with the ARVO (Association for Research in Vision and Ophthalmology) Statement for the Use of Animals in Ophthalmic and Vision Research, and were approved by the local committee for ethics in animal research.

Spontaneously hypertensive rats (SHR) and normotensive control Wistar Kyoto rats (WKY; Slac Laboratory Animal Company, Shanghai, China) were used in our experiment. Rats were housed at a constant temperature (22°C) on a 12-hour light/dark cycle with ad libitum access to food and tap water. Experimental diabetes was induced in 8-week-old male SHRs and male WKY rats with a single intraperitoneal injection of streptozotocin (60 mg/kg in sodium citrate buffer, pH 4.5; Sigma, St. Louis, MO). One week after streptozotocin injection, 15 animals in each group with blood glucose levels >15 mmol/L were included in the study. Another 5 animals in each group received citrate buffer alone. Blood glucose levels were measured using the colorimetric GOD-PAP assay (Merck, Darmstadt, Germany).

Oral Administration of Anti-Hypertension Drugs

Beginning the day after diabetes induction, the diabetic rats (including SHRs and WKY rats) were assigned randomly to one of three groups: no anti-hypertension treatment; treatment with the ARB, valsartan (40 mg/kg/d; Merck, Sharp & Dohme Farmaceutica, Sao Paulo, Brazil), or treatment with the beta blocker, metoprolol (50 mg/kg/d, AstraZeneca, UK Limited).

Drugs were placed in the drinking water. Systolic blood pressure (SBP) was obtained by tail-cuff plethysmography (Physiograph MK-III-S; Narco Bio-System, Houston, TX). Body weight, blood glucose, and SBP were measured 0, 4, and 8 weeks after inducing diabetes. Eight weeks after inducing diabetes, the rats were killed, and the retina of the right eye was detached from the retinal pigmented epithelium cell layer and used for protein extraction. The retina of the left eye was used for immunohistochemical assays.

Animals were arranged into eight groups: WKY rats without diabetes (WKY-control), WKY rats with diabetes (WKY-diabetes), WKY rats with diabetes treated with valsartan (WKY-DARB), WKY rats with diabetes treated with metoprolol (WKY-DBB), SHR without diabetes (SHR-control), SHR with diabetes (SHR-diabetes), SHR with diabetes treated with valsartan (SHR-DARB), and SHR with diabetes treated with metoprolol (SHR-DBB). Each group was comprised of five animals.

Immunohistochemistry

Eyes were enucleated and fixed with 4% paraformaldehyde, cryoprotected in 30% sucrose in phosphate buffer, frozen in an embedding medium (OCT; Sakura Finetek, Torrance, CA), and cut perpendicular to the vitreal surface. Cryosections with a thickness of 14 µm were mounted onto slides, rinsed in PBS, and then blocked for 1 hour at room temperature in blocker liquid with 10% normal goat serum, 0.3% (weight/volume) Triton X-100 in PBS. Slides were incubated for 24 hours at 4°C with the primary antibody diluted in blocker liquid. Primary antibodies also were used to detect astrocytes/Müller glia (polyclonal anti-gial fibrillary acidic protein, GFAP, 1:100; Santa Cruz, CA), and membrane channels known to be important for retinal water and ion transport (AQP-1,4, 1:100; Abcam, Cambridge, MA). Following removal of the primary antibody, slides were incubated at room temperature in PBS with an appropriate fluorescent conjugated secondary antibody (Alexa Fluor488 or Alexa Fluor568; Invitrogen, Eugene, OR) for 1 hour. Negative controls were performed in parallel by omitting the primary antibody.

Fluorescence was visualized with a confocal system (Olympus Fluoview FV1000, Tokyo, Japan). Images were obtained from the inferior central retina (0–1 mm below the optic nerve head). When measuring immunofluorescence, the sample with the brightest

fluorescence was measured first. Confocal settings were held constant across all recordings. Images were processed and analyzed using Image J software (NIH, Bethesda, MD). Images were imported, color-separated into their red and green components, and converted into 8-bit grayscale images. Immunofluorescence was measured by determining the intensity of the pixels above a threshold defined as 4 SD above the mean background fluorescence intensity measured from regions devoid of retinal tissue. A user-defined frame was drawn around individual or multiple layers of the retina, and the retina was divided into a ganglion cell layer (GCL) and from the top of the inner plexiform layer to the bottom of the outer plexiform layer (IPL-OPL). The average fluorescence intensity above the threshold was calculated. The number of GFAP-positive fibers (per 100 µm of retinal cross-section) was counted in the IPL. A total of three tissue sections was imaged per retina and an overall average intensity value was computed.

Western Blot Analysis

Isolated retinas were placed into a lysis buffer (10 mmol/L Tris-HCl at pH 7.6, 100 mmol/L NaCl, 1 mmol/L EDTA, 1% Triton X-100, protease inhibitors). Each sample was separated using sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) and electro-blotted onto polyvinylidene fluoride (PVDF) membranes (Millipore, Bedford, MA). After nonspecific binding was blocked using 4% skim milk, membranes were incubated at 4°C overnight with a rabbit polyclonal antibody against AQP1,4 (1:200; Sigma-Aldrich, Santa Cruz, CA), or a mouse monoclonal antibody against GFAP (1:500; Sigma-Aldrich) or β-actin (1:2000; Sigma). Membranes then were incubated with a horseradish peroxidase-conjugated goat antibody against rabbit or mouse immunoglobulins, or with a biotinylated secondary antibody followed by avidin-biotin horseradish peroxidase complexes (Vectastain Elite ABC Kit; Vector, Burlingame, CA). Signals were visualized with chemiluminescence (ECL Blotting Analysis System; Amersham, Arlington Heights, IL), measured with Image J software, and normalized to β-actin. This process was repeated three times.

Statistical Analysis

Data are presented as mean ± SE of the mean. Statistical analyses were performed using Prism V4.02 (GraphPad, San Diego, CA). All data sets were tested to verify that they met assumptions for parametric testing. Two-way ANOVA was conducted to compare overall treatment differences. A significance level of $\alpha = 0.05$ was used for all tests. When a statistically significant difference was detected, post-hoc multiple pair-wise comparisons were performed using Bonferroni's multiple range tests.

RESULTS

Animal Characteristics

Body weight decreased after streptozotocin injection in SHR and WKY rats ($P < 0.01$). Treatment with an ARB or beta blocker had no effect on body weight. As expected, SBP was significantly higher in SHRs than in WKY rats. SBP was reduced in the treated SHRs ($P < 0.01$), but not in the treated WKY ($P > 0.05$) rats. Blood glucose was higher in diabetic rats compared to non-diabetic rats ($P < 0.01$), and was unaffected by treatment with the ARB or beta blocker ($P > 0.05$, Table).

Retinal Glial Reaction

The retinal glial reaction, demonstrated by a local increase in GFAP expression, is an early reaction in the pathogenesis of DR. Low levels of GFAP were detected in the innermost portion of the retina of WKY-control rats. In the short-term following induction of diabetes in WKY-diabetes rats, there was a prominent increase in GFAP ($P < 0.01$). Similar patterns were

TABLE. Measurement of Blood Glucose, Body Weight, and Blood Pressure

Groups N = 10	Blood Glucose (mmol/L)		Body Weight (g)		SBP (mmHg)	
	Day 0	Week 8	Day 0	Week 8	Day 0	Week 8
WKY-control	4.2 ± 0.2	4.3 ± 0.3	140 ± 3	280 ± 12	102 ± 7	105 ± 3
WKY-diabetes	18.6 ± 0.5	25.5 ± 2.5	145 ± 2	184 ± 5	98 ± 7	105 ± 4
WKY-DARB	25.3 ± 2.8	25.2 ± 3.3	146 ± 2	196 ± 6	103 ± 9	109 ± 3
WKY-DBB	24.0 ± 2.3	23.8 ± 3.4	147 ± 3	170 ± 6	110 ± 6	107 ± 5
SHR-control	4.6 ± 0.2	4.8 ± 0.2	140 ± 4	272 ± 10	127 ± 9	167 ± 11
SHR-diabetes	22.7 ± 1.6	23.5 ± 4.0	142 ± 3	187 ± 9	129 ± 8	159 ± 9
SHR-DARB	19.8 ± 1.0	26.0 ± 4.3	138 ± 3	178 ± 6	125 ± 5	123 ± 8
SHR-DBB	20.0 ± 1.2	23.0 ± 2.9	138 ± 3	177 ± 7	130 ± 8	119 ± 10

observed in the SHRs, as SHR-control displayed low levels of GFAP ($P > 0.05$) and SHR-diabetes displayed increased GFAP in the retina ($P < 0.01$). The number of GFAP-positive fibers counted in the IPL was significantly higher in SHR-diabetes and WKY-diabetes ($P < 0.01$, Fig. 1a, b, c, d).

Treatment with valsartan or metoprolol decreased GFAP levels in SHR-diabetes ($P < 0.01$). Valsartan led to a greater decrease in the number of GFAP-positive fibers in the IPL compared to metoprolol ($P < 0.05$, Fig. 2A, B, C, D).

Valsartan prevented the retinal glial reaction in WKY-diabetes rats ($P < 0.05$), while treatment with metoprolol did not; these relationships were observed in the GCL and IPL-OPL. The number of GFAP-positive fibers in the IPL of WKY-diabetes rats was decreased by valsartan ($P < 0.05$), but not by metoprolol ($P > 0.05$).

Aquaporins

Aquaporins are the main water transport channel in the retina. In the retina of both control groups, AQP1 was present at minimal levels. After inducing diabetes, AQP1 increased ($P < 0.01$). This change was similar in SHR-diabetes and WKY-diabetes rats ($P > 0.05$, Fig. 1a, c). The effect of diabetes on AQP4 expression was more prominent in the retina of SHR ($P < 0.01$) than WKY ($P > 0.05$) rats. AQP4 expression was similar in SHR-control and WKY-control rats ($P > 0.05$, Fig. 1b, c).

Valsartan and metoprolol decreased retinal AQP1 and retinal glial AQP4 in SHR-diabetes ($P < 0.01$, Fig. 2A, C, and $P < 0.05$, respectively). The effect of valsartan was more pronounced. Metoprolol did not affect AQP4 expression in the GCL ($P > 0.05$), but did affect expression in the IPL-OPL ($P < 0.05$, Fig. 2B, C).

Increased AQP1 expression occurring in short-term DR has been reported in our previous studies.^{24,25} Treatment with valsartan in our study prevented the increase in AQP1 in WKY-diabetes rats ($P < 0.01$), but treatment with metoprolol did not have a significant effect ($P > 0.05$). AQP4 expression did not change after inducing diabetes, and was not affected significantly by treatment with valsartan or metoprolol.

Western Blot Analysis

Densitometric analysis revealed significantly higher expression of GFAP and AQP4 in SHR-diabetes compared to WKY-diabetes. No significant difference was found between the SHR-control and WKY-control groups. Expression of AQP1 was similar in the SHR-diabetes and WKY-diabetes groups. After treatment with valsartan, GFAP and AQP1 expression decreased significantly in the WKY-diabetes and SHR-diabetes groups, while AQP4 expression decreased only in SHR-diabetes rats. De-

creased expression of GFAP, AQP1, and AQP4 after treatment with metoprolol was observed only in SHR-diabetes rats.

DISCUSSION

WKY-control and SHR-control did not show any obvious differences in GFAP and AQP1,4 expression or distribution; however, it cannot be concluded that hypertension did not have an effect on macroglia activity. SHR experience three stages of hypertension with age. The 4-month-old SHR in the SHR-control group were in their early stage of hypertension. SHR start to show GFAP changes and neuron apoptosis in the retina after 6 months of age when they are in the middle stage of hypertension.²⁹ In our study, we evaluated the interaction of short-term hypertension and short-term diabetes on early DR.

Iandiev et al.³⁰ and we^{24,25} reported previously on AQP1 expression in astrocytes in DR. In SHR and genetically normotensive WKY, neuronal and glial changes have been demonstrated in the presence of hypertension.²⁹ The observation that these abnormalities were decreased partially with an antihypertensive drug^{31,32} suggests they may represent hypertension-related phenomena. Retinopathy was worsened by the combination of diabetes and hypertension. To our knowledge, no information exists in the literature on changes in retinal macroglia, especially changes in AQPs, in a model combining hypertension and diabetes.

Increased macroglia activity, as evidenced by enhanced GFAP expression and an increased number of GFAP-positive fibers, was observed in the retinas of the diabetic rats. In addition, inducing diabetes in hypertensive rats led to further macroglial activation in the GCL and IPL-OPL. Macroglial activation was decreased partially by anti-hypertensive agents with or without blockade of the RAS, but treatment with the ARB had a greater effect. These observations suggest that macroglial activation may be one of the mechanisms by which hypertension and diabetes interact to worsen retinal disease and that the RAS has a role.

We also found that AQP4 expression in Müller cells did not change significantly in the retinas of diabetic rats, but was enhanced greatly by combined diabetes and systemic hypertension in the GCL and IPL-OPL. Although AQP1 expression in the inner retina increased significantly in diabetes, expression was not different between WKY-diabetes and SHR-diabetes. This finding indicates that changes in AQP4 expression in Müller cells may have an important role in the mechanism by which hypertension and diabetes interact to affect retinal disease; AQP1 expression in astrocytes does not appear to contribute. In Müller cells, AQP4 located in the perivascular area and end foot processes is believed to form a multiprotein complex involving Kir4.1 that allows movement of water into and out of the cell. Most retinal pathologies are accompanied

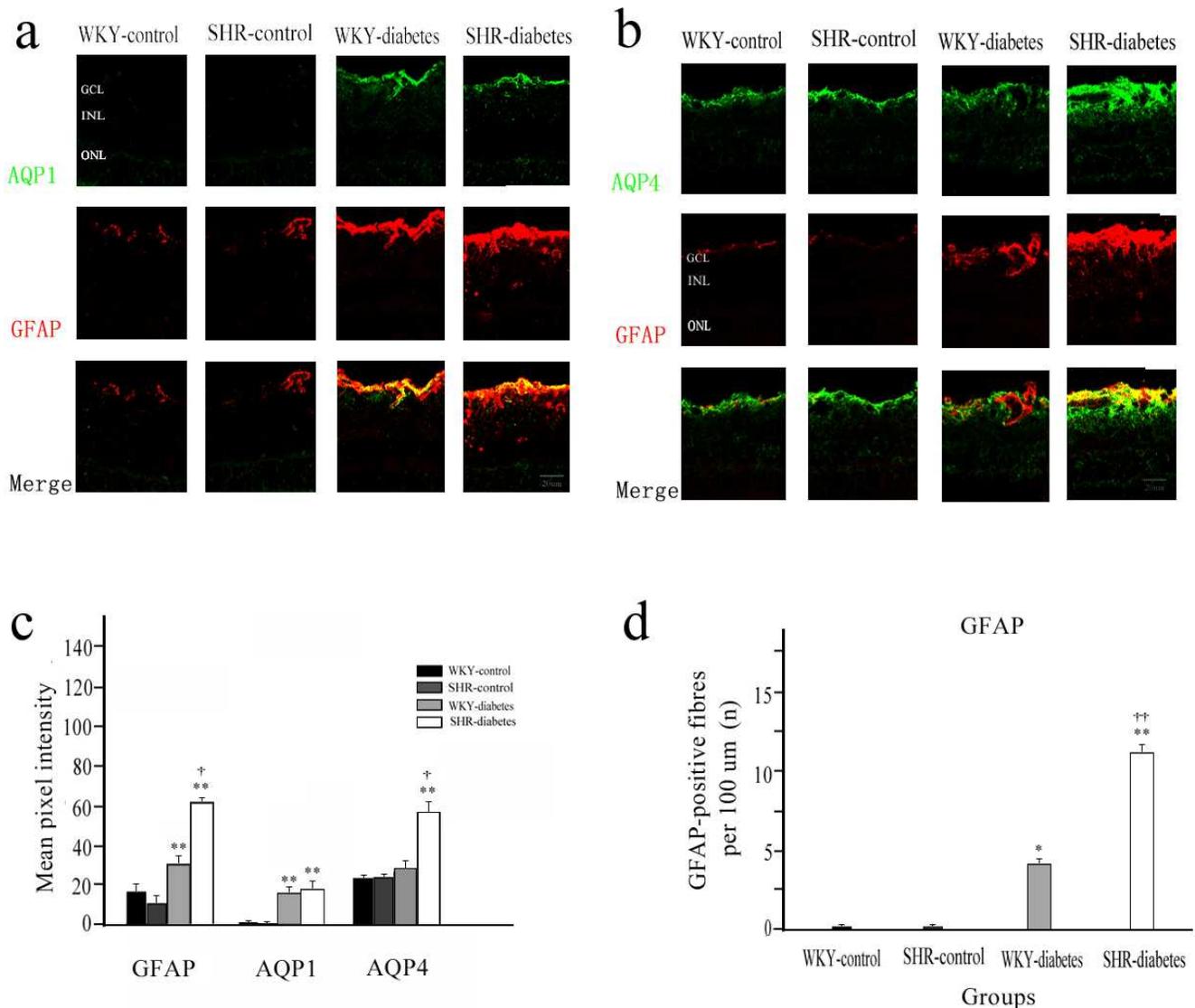


FIGURE 1. Results from immunohistochemistry demonstrating expression of AQP1, AQP4, and GFAP in the retinas of WKY-control, SHR-control, WKY-diabetes, and SHR-diabetes. (a) GFAP was expressed in the innermost retina in the control groups. Expression was increased in the diabetic groups. GFAP expression was higher in SHR-diabetes than WKY-diabetes. AQP1 expression was low in the control groups and increased in the diabetic groups. (b) AQP4 expression did not change in SHR-control or WKY-diabetes, but increased significantly in the retina of SHR-diabetes. (c) Quantification of GFAP, AQP1, and AQP4 intensity in the retina indicating that GFAP expression was higher in SHR-diabetes than WKY-diabetes. Quantification of AQP1 intensity in the GCL-OPL did not show significant differences in AQP1 expression between SHR-diabetes and WKY-diabetes. Quantification of AQP4 intensity in the GCL-OPL indicating that AQP4 was higher in SHR-diabetes compared to WKY-diabetes. (d) The number (*n*) of GFAP-positive fibers in the IPL was significantly increased in the diabetic groups. ***P* < 0.01 for WKY-diabetes versus WKY-control and SHR-diabetes versus SHR-control. †*P* < 0.05 and ††*P* < 0.01 for SHR-diabetes versus WKY-diabetes and SHR-control versus WKY-control. GCL-OPL from the top of the ganglion cell layer to the bottom of the outer plexiform layer. Scale bars = 20 μm.

by alterations in the amount and/or spatial distribution of AQP4 or Kir4.1. AQP4 in the end foot processes of Müller cells is thought to aid in the transport of water and ions between the vitreous body and retina, while AQP4 located in the perivascular area helps transport water and ions between the vasculature and retina.^{20,53} In our study, we observed that increases in AQP4 were decreased more by treatment with an ARB than a beta blocker. Expression of AQP4 located on the Müller end foot processes (in the GCL) was decreased only by ARB treatment. Therefore, this protein is sensitive to RAS regulation, but not to blood pressure. It is reasonable that the AQP4 responsible for water and ion transport between the vitreous body and retina was not affected by changes in

systemic pressure. The microvasculature in the retina is divided into three layers: a superior layer (GCL), inferior layer (INL), and a middle layer (ventral vasculature links the superior and inferior layers) in the IPL and INL,³⁴ which may be more sensitive to changes in blood pressure based on its structure. Also, the AQP4 located in the GCL was not sensitive to beta blocker treatment, and protein expression in the IPL-OPL layer was decreased partially by metoprolol. AQP4 located in the perivascular region of the IPL is more likely to be stimulated by changes in blood pressure.

Although no obvious changes in AQP4 in DR were detected in our current or previous studies, our experiments were done on short-term DR, which may be not long enough to induce

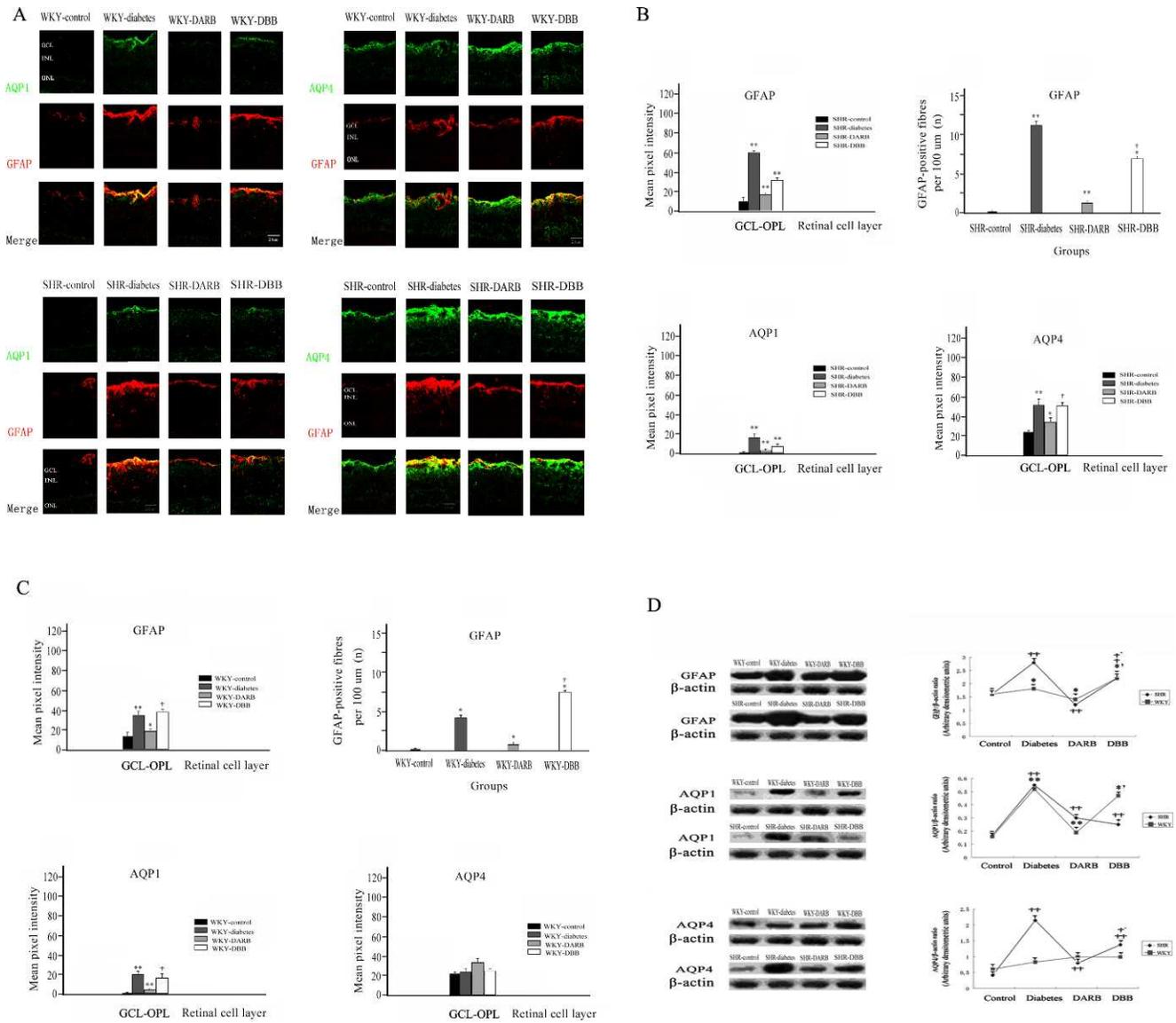


FIGURE 2. Immunohistochemistry results and Western blot analysis of effect of anti-hypertension medications on expression of AQP1, AQP4, and GFAP in the retinas of eight groups, including WKY-control, WKY-diabetes, WKY-DARB, WKY-DBB, SHR-control, SHR-diabetes, SHR-DARB and SHR-DBB. (A) Color images showing immunohistochemistry results: treatment with valsartan decreased GFAP and AQP1 expression in WKY-diabetes. AQP4 expression did not change significantly. Treatment with valsartan and metoprolol decreased GFAP and AQP1 expression in SHR-diabetes. AQP4 expression was reversed partially in SHR-diabetes by valsartan treatments but not changed after treatment with metoprolol. (B) Quantification of GFAP intensity in SHR rats indicating that GFAP expression was reversed partially by both treatments, and GFAP expression was decreased by valsartan more than by metoprolol. AQP1 intensity decreased significantly with both treatments but AQP4 intensity was decreased only by valsartan. The number (*n*) of GFAP-positive fibers in the IPL decreased more in SHR-DARB than in SHR-DBB. (C) Quantification of GFAP intensity in WKY rats indicating that GFAP expression was decreased by valsartan AQP1 intensity in GCL-OPL was significantly lower after treatment with valsartan. AQP4 intensity showed no differences. The number (*n*) of GFAP-positive fibers in the IPL significantly decreased in WKY-DARB. (D) Western blot analysis of GFAP, AQP1, and AQP4 based on the immunohistochemical examinations in the eight experimental groups. Bars represent the ratio of mean ± SEM band densities for GFAP/AQP1/AQP4 versus beta-actin expressed in arbitrary densitometric units from at least three independent experiments. **P* < 0.05, ***P* < 0.01 for WKY-diabetes versus WKY-control and WKY-DARB/WKY-DBB versus WKY-diabetes. ***P* < 0.05 for WKY-DBB versus WKY-DARB, †*P* < 0.05, ††*P* < 0.01 for SHR-diabetes versus SHR-control and SHR-DARB/SHR-DBB versus SHR-diabetes. ††*P* < 0.05 for SHR-DBB versus SHR-DARB. *n* = 5 in each group.

significant changes. Iandiev et al.³⁰ and Pannicke et al.³⁵ reported decreased AQP4 expression in the GCL within the first six months of DR. No work has been done to explain if decreased AQP4 is the reason for DR deterioration, or if it is the protective reaction of glia. Water flux through AQPs is bidirectional and driven by osmotic gradients. Hence, perivascular AQP4 might be involved in edema formation and

remission.²¹ It was reported that mice without this protein were protected from cellular brain edema.²² AQP4 was also up-regulated after brain ischemia or traumatic injury,²³ and its deletion led to vasogenic cerebral edema.³⁶ A few studies have focused on changes in AQP1,4 in the central nervous system during hypertension. It has been reported that AQP1 increases at six months of age and AQP4 increases at four months in the

SHR brain,^{36,37} indicating that AQP1,4 may have a major role in the pathogenesis of hypertensive cerebral injury. In our study, increased AQP4 was the result of a combination of diabetes and hypertension, as neither short-term diabetes nor hypertension alone induced significant changes in AQP4. Based on the fact that AQP4 was decreased by ARB, which is protective against DR in patients with hypertension, we predicted that increased AQP4 may be one of the reasons that DR is affected by the interaction between hyperglycemia and hypertension. In our study, AQP1 was not increased by early stage hypertension in DR. It is possible that the hypertension was not of sufficient duration, because increases in AQP1 were counteracted by anti-hypertensive agents, with or without blockade of the RAS. This indicates that AQP1 is sensitive to changes in blood pressure. More work should be done on animals with later stage hypertension to detect the effect of long-term hypertension on AQP1 in DR. In our study, we did not find the immunoreactivity for AQP1 in photoreceptors as described before for unknown reasons.

There is growing interest in the pathogenic actions of the RAS in DR.^{38,39} All components of the RAS have been localized in retinal vessels, and an impaired RAS and deficient vascular permeability and auto-regulation have been associated with DR.¹⁷ Increased Ang II present in diabetes acts on pericytes to cause changes in vessel diameter and blood flow, and also contributes to changes in vascular permeability.^{38,40} Multiple studies have shown that inhibiting the RAS prevents an increase in VEGF expression without reducing systemic blood pressure,¹⁵ and also prevents basement membrane thickening in retinal blood vessels.^{15,41} To our knowledge, no attention has been paid to the relationship between the RAS and macroglia in the retina, which has an important role in the progression of DR. In WKY-diabetes rats, AQP1 and GFAP increased, while AQP4 did not. Additionally, we demonstrated that these increases in AQP1 and GFAP could be decreased by treatment with an ARB, but not a beta blocker, indicating AQP1 and macroglia activity are linked to the RAS.

Controlling blood pressure in diabetic patients can slow the progression of DR. In the normotensive rats with DR (WKY-diabetes), AQP1 and GFAP were decreased only by treatment with an ARB. In the hypertensive rats with DR (SHR-diabetes), AQP1 and GFAP were decreased by the ARB and the beta blocker. This finding explains partially the clinic finding that treatment of normotensive diabetic patients with an ARB reduces the incidence of DR,⁴² and demonstrates the importance of decreasing blood pressure in diabetics with concomitant hypertension, as the beneficial effect occurs regardless of the treatment used, provided the blood pressure is controlled. Although prior studies confirmed that AQP1 is upregulated in DR,^{24,25,30,35} to our knowledge no work had been done to determine if that promoted or limited the progression of DR. Based on our results, we predict that increased AQP1 is damaging to the retina in DR, and that ARB treatment limits the progression of DR by inhibiting AQP1. Although no change in AQP4 was observed in WKY-diabetes after treatment with an ARB, it cannot be concluded that AQP4 was not linked to the RAS as AQP4 expression was decreased more by treatment with an ARB than a beta blocker in SHR-diabetes rats. Further work should be done on DR of longer duration which may exhibit changes in AQP4.

CONCLUSION

Müller cell and astrocyte activation are involved in the interaction of systemic hypertension with DR. Systemic hypertension increased GFAP and AQP4 expression. Lowering blood pressure in systemic hypertension, either with an ARB or

a beta blocker, decreases GFAP and AQP1,4 expression in DR. In DR without systemic hypertension, ARB treatment will limit the increase in GFAP and AQP1, while a beta blocker will not. This indicates that AQPs and macroglia are linked to the RAS in DR, and provides further support for the use of ARB treatment in early DR, especially in patients with retinal edema.

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