https://jksus.org

ScientificScholar®
Knowledge is power
Publisher of Scientific Journals

Journal of King Saud University – Science

2026 38 (3) 13822025

Research Article

Influence of type 1 diabetes mellitus on angiotensin-II converting enzymes and neurodegenerative factors in the hypertensive rat

Mohammad S Ola[a], Abeeb Abiodun Oyesiji[b], Dalia I AlDosari[a], Abdulaziz Alamri[a], Amani AlGhamdi[a], Abdullah S Alhomida[a], Sarfaraz Ahmad[c,*]

[a]Department of Biochemistry, College of Science, King Saud University, Riyadh, 11451, Saudi Arabia
[b]Department of Environmental Toxicology, Southern University A & M College, Health Research Centre, Baton Rouge, LA 70813, United States
[c]Department of General Surgery, Wake Forest University School of Medicine, 1 Medical Center Blvd., Winston Salem, NC 27157, United States

ARTICLE INFO

Keywords:
ACE
Brain
Diabetes
Hypertension
Neurodegeneration

ABSTRACT

Hypertension and diabetes are recognized as risks for cerebrovascular disease. The present study examined the expression and regulation of angiotensin-converting enzyme (ACE), ACE2, and chymase in the rat frontal brain cortex to assess their roles in neurodegeneration. Frontal cortex brain tissues from control, streptozotocin-induced diabetic, spontaneously hypertensive rats (SHR), and SHR-diabetic (SHR-D) rats were analyzed using immunoblot and biochemical techniques. The expressions of ACE, ACE2, chymase, neurotrophic factors (brain-derived neurotrophic factor (BDNF), tropomyosin receptor kinase B (TrkB), glutamine synthetase), apoptotic markers (caspase-3, cytochrome c), and oxidative stress markers were evaluated. ACE, ACE2, and chymase were expressed in all groups. Diabetic and hypertensive rats, alongside elevated chymase levels, exhibited significant ACE upregulation and ACE2 downregulation compared to controls. Neuroprotective factors BDNF, TrkB, and glutamine synthetase were markedly reduced in diabetic and hypertensive-diabetic rats, whereas apoptotic markers (caspase-3, cytochrome c) were significantly increased. Oxidative stress, measured by glutathione (GSH) and thiobarbituric acid reactive substances (TBARS), was higher in diabetic and hypertensive groups than in the controls. The combined effects of ACE upregulation, ACE2 downregulation, and increased chymase expression in the hypertensive-diabetic brain cortex may amplify pathological processes. These changes likely enhance oxidative damage and apoptotic pathways, which contribute to neurodegeneration. This study highlights how oxidative stress and the renin-angiotensin system interact critically in cerebrovascular and neurodegenerative disorders linked to diabetes and hypertension.

1. Introduction

The two most prevalent endocrine system disorders, hypertension and diabetes, frequently coexist and are known as comorbidities. Their interconnectedness poses a significant risk to the brain and other organs, potentially resulting in dementia, memory loss, and cognitive decline. Diabetes mellitus, characterized by high blood sugar levels, causes chronic inflammation and oxidative stress, resulting in endothelial damage and faster development of atherosclerosis. This impairs blood flow to the brain and raises the chance of having a stroke and cognitive decline (Beckman and Creager, 2016; UK Prospective Diabetes Study, 1998). Conversely, hypertension exerts excessive pressure on blood vessels, resulting in arterial stiffness and microvascular damage that exacerbates brain injury. Therefore, the concurrent presence of diabetes and hypertension synergistically poses a considerable threat to vascular and neuronal health, amplifying the risk of brain damage (Gasecki *et al.*, 2013; Hassing *et al.*, 2004; Yen *et al.*, 2022; Nagar *et al.*, 2022). Additionally, hypertension and diabetes share common genetic and environmental factors, suggesting there may be some overlaps in the prevention and treatment of these conditions (Long and Dagogo-Jack 2011). Thus, the combined impact of these comorbidities makes them a formidable challenge, emphasizing the necessity for diligent management of both conditions.

Hypertension, or high arterial blood pressure, is influenced by multiple regulatory systems, including the renin-angiotensin system (RAS). The RAS, a key hormonal pathway, consists of components such as renin, angiotensin-converting enzyme (ACE), chymase, and angiotensin II (Ang II) receptors, which collectively contribute to blood pressure regulation through vasoconstriction, fluid balance, and vascular remodeling (Ola *et al.*, 2017). The role of the systemic RAS and its components in managing hypertension is well-characterized; however, little is known about the local brain RAS and its pathophysiological function in the brain. Numerous studies have documented independent RAS components with distinct expression patterns and the de novo synthesis of RAS genes and components in the brain, which may significantly influence the progression of neurological diseases (Grobe *et al.*, 2010; Saavedra, 1992). Nonetheless, a few scientists continue to dispute the existence of the RAS in the brain (Van Thiel *et al.*, 2017). Activation of the brain RAS has been linked to neuronal apoptosis, increased oxidative stress, and vascular inflammation (Kishi *et al.*, 2010;

***Corresponding author:**
E-mail address: Sarfaraz.Ahmad@advocatehealth.org (S Ahmad)

Received: 31 August, 2025 **Accepted:** 10 December, 2025 **Epub Ahead of Print:** 12 March, 2026 **Published:** 17 March, 2026

DOI: 10.25259/JKSUS_1382_2025

Waki *et al.*, 2011). An elevated Ang II peptide hormone, a component of the RAS, is considered the primary source of pathophysiological actions through angiotensin receptor (AT1R) stimulation, leading to the production of reactive oxygen species, ultimately resulting in neurodegeneration (Waki *et al.*, 2011). ACE is essential to the RAS because it produces the angiotensin II peptide hormone. The expression of ACE has been detected in the frontal and parietal lobes, as well as several other brain regions, which may contribute to neurotransmitter signaling and cognitive processes such as learning and memory consolidation (Moran *et al.*, 2019; Zuliani *et al.*, 2005).

The counterregulatory enzyme ACE2 produces Ang-(1–7) by cleaving Ang II, a protective molecule that inhibits the harmful consequences of Ang II through its Mas receptor (MasR). Additionally, ACE2 is found in the neuronal cytoplasm, especially in areas connected with synaptic function, and its expression is significantly downregulated in the hippocampus and prefrontal cortex of Alzheimer's disease patients (Doobay *et al.*, 2007; Cui *et al.*, 2021). This downregulation correlates with impaired acetylcholine and glutamate signaling, suggesting that ACE2 may play a critical role in modulating neurotransmitter balance, which may influence memory, learning, and potentially cognitive decline (Doobay *et al.*, 2007; Cui *et al.*, 2021).

Another enzyme, chymase (a mast cell protease), primarily expressed in various tissues and cells, has been shown to generate Ang II directly from both Ang-(1–12) and Ang I at a significantly higher rate than ACE (Ola *et al.*, 2017; Ahmad *et al.*, 2013). Therefore, in this study, we aim to analyze the expression and regulation of these potential enzymes that may alter Ang II levels in the frontal brain cortex under hypertension and diabetic conditions in experimental animals, which could contribute to neuronal damage.

Diabetes mellitus has been independently linked to Ang II-mediated cerebral endothelial dysfunction, neuroinflammation, and neuronal damage, all of which contribute to the emergence of cognitive decline and disease of central nervous system disorders (Vargas *et al.*, 2012; Ola *et al.*, 2013). Ang II activates AT1R, leading to the generation of reactive oxygen species through NADPH oxidase (NOX), an enzyme found in various brain regions that may trigger apoptosis (Zhao *et al.*, 2015). Additionally, Ang II may promote apoptosis through mitochondrial ROS pathways, which upregulate both AMP-activated protein kinase and PARP-1, thereby activating Bax, a pro-apoptotic protein, leading to neuronal cell death (Kim *et al.*, 2017). Furthermore, AT1R-induced autophagy activation and cell death caused by mitochondrial pathways have been shown to result in the death of dopaminergic cells (Gao *et al.*, 2016). However, little is known about the effects of diabetes under hypertensive conditions, particularly concerning its influence on the mechanisms of neurodegeneration in the frontal cortex.

Recently, we reported that diabetes and hypertension appear to synergistically over-activate the Ang II/AT1R axis while suppressing the protective MAS receptor arm in the rat cerebral cortex (Oyesiji *et al.*, 2023). This study investigates how diabetes and hypertension might influence Ang II levels by ACE, ACE2, and chymase enzymes in the rat frontal cortex and their implications for neurodegeneration. These findings shed light on neuronal damage in the hypertensive-diabetic frontal cortex, potentially leading to new strategies for preventing strokes and other cerebrovascular diseases.

2. Materials and Methods

2.1 Induction of diabetes mellitus

Ten-week-old Wistar Kyoto (WKY) and transgenic hypertensive (SHR) male rats weighing 250-280 g were used in this investigation. The animal studies were conducted in part in collaboration with the Department of Surgery at Wake Forest University. The experimental animal protocol was approved by the Experimental Animal Care Committee (approval number KSU-SE-21 04), King Saud University, Riyadh, Saudi Arabia, and the Animal Care and Use Committee at Wake Forest University in accordance with NIH guidelines, and the study is also reported in accordance with ARRIVE guidelines (https://arriveguidelines.org). The WKY animals were sourced from King Saud University, College of Medicine, Animal Care Center, Riyadh, Saudi Arabia, and SHR rats from Harlan Laboratories in Indianapolis, USA. The rats were housed at an average temperature of 23°C in a controlled environment with free access to normal laboratory rat food and water. Both SHR and WKY rats were divided into two groups. A single intraperitoneal injection of streptozotocin (65 mg/kg; Sigma Aldrich, USA), freshly prepared in a 50 mM citrate-buffered solution (pH 4.5), was given to induce diabetes in one group of rats from each category. Consequently, four groups of rats were made: Group 1. Non-diabetic WKY rats control (C); Group 2. Diabetic-WKY (D); Group 3. Non-diabetic SHR (SHR-C); Group 4. SHR-Diabetic (SHR-D). Three days following the injection, body weight and blood glucose levels were assessed. Fasting blood glucose was checked using an ACCU-CHEK glucometer with tail vein blood samples to confirm diabetes. The study included diabetic animals with blood glucose levels of ≥ 250 mg/dl. Four to seven rats from each group were used in the experiments following four weeks of streptozotocin-induction.

2.2 Tissue harvesting and brain homogenate preparation

Rats in each group were fasted for 7 h, then deeply anesthetized with a high dose of sevoflurane (Tabuk Pharmaceutical, KSA) to induce euthanasia, followed by a blood sample obtained by cardiac puncture, and were intracardially perfused with 100 ml normal saline to wash out blood from tissues and confirm death before their brains were dissected. We used the prefrontal cortex and adjacent areas, 1 to 3 mm from the brain's surface. These consist of the prelimbic cortex located in the medial prefrontal cortex, the anterior cingulate cortex, a part of the medial prefrontal cortex, and the orbitofrontal cortex positioned more laterally. We used Paxinos and Watson Rat Brain Atlas for precise localization and coordinates. After isolating the frontal brain cortex, it was promptly rinsed with ice-cold saline, placed into labeled Eppendorf tubes, and stored at -70°C until analysis. Brain homogenates were prepared by applying a brief burst of ultrasonication in a 10 mM HEPES buffer (pH 7.4) containing 100 mM NaCl, 1 mM Na_3VO4, 10 mM Sodium purophosphate ($Na_4P_2O_7$), 10 mM NaF, 2 mM EDTA, 1 mM Phenylmethylsulfonyl fluoride (PMSF), 1% Triton X-100, and 0.2% sodium dodecyl sulphate (SDS) along with a protease inhibitor. The supernatants were collected after centrifuging the samples for 15 min at 15,000 g in a cooling centrifuge. The Lowry technique was then used to determine the protein concentrations in each brain sample.

2.3 Biochemical analysis

2.3.1 Glutathione assay

Glutathione (GSH) levels were measured in the frontal cortex samples from the four rat groups using the standard protocol established in our laboratory. The brain homogenate was deproteinized with metaphosphoric acid (2.5% w/v). This mixture was centrifuged at 15,000 rpm, and the supernatant was collected. To 50 μl of the frontal brain cortex supernatant, 50 μl of 4M triethanolamine was added per 100 μl. Then, 100 μl of 0.01M Ellman's reagent (DTNB) was included. Absorbance was recorded with a spectrophotometer at 412 nm within 5 min. A GSH standard curve was created, covering concentrations from 0 to 10 μM.

2.3.2 Thiobarbituric acid reactive substances (TBARS) assay

Malondialdehyde (MDA), an important marker of lipid peroxidation, is used as an indicator of oxidative stress. The TBARS assay kit was sourced from ZeptoMetrix, USA, and utilized for the MDA assay. The MDA-TBARS adduct, produced by the reaction at elevated temperatures, was measured colorimetrically at 532 nm. A total of 1.25 ml of reaction buffer was mixed with 50 μl of the frontal brain cortex homogenate. The mixture was subsequently heated at 95°C for 60 min. The absorbance of the supernatant was measured after the sample was cooled and centrifuged.

2.3.3 BDNF quantification by enzyme-linked immunosorbent assay (ELISA)

The BDNF levels were measured in the frontal cortex using ELISA kits (R&D Systems, MN, USA), following the manufacturer's instructions.

The brain homogenate was prepared as previously described. A two-fold diluted frontal brain cortex supernatant (150 μl/brain) was used for BDNF analysis. The concentration of BDNF was determined using a standard curve. The ELISA plate readings were performed by Autobio Labtech Instruments Co., Ltd., China.

2.4 Western blot analysis

The Western blotting technique was employed to measure protein expression in the frontal brain cortex of control, diabetic, hypertensive, and hypertensive-diabetic rats. We analyzed the expression using anti-ACE (Sc-23908, lot 2020, ACE2 (Sc-390851, lot H0420), mast cell chymase (Sc-59586), glutamine synthetase (GS; Sc-74430), and cytochrome c (Sc-13561) antibodies from Santa Cruz Biotechnology, USA), as well as anti-BDNF (MBS 2085794, MyBiosource) and anti-TrkB (Cat No: 4603S, Cell Signaling R&D Systems, Minneapolis, MN); anti-Bcl-2 (Sc7382), anti-Bcl-xL (Sc-56021), anti-caspase-3 (Sc-71481) and α-tubulin (Sc-58667), β-actin (Sc-47778; Cruz Biotechnology, Santa Cruz, CA). Frontal cortex protein samples (30 to 50 μg) were separated on 10-15% SDS-polyacrylamide gels and subsequently transferred onto PVDF membranes.

After blocking the membranes with 5% non-fat milk in tris-buffered saline with tween 20 (TBST), primary antibodies were incubated overnight at 4°C: anti-ACE/ACE2, anti-chymase, anti-BDNF, anti-TrkB, anti-caspase-3, anti-cytochrome c, anti-GS, anti-Bcl-2, and anti-Bcl-xL. Mouse monoclonal β-actin and α-tubulin antibodies served as internal controls, and all steps were performed as outlined above. After the overnight incubation, the membranes were washed at least three times with TBST and incubated with their respective secondary horseradish peroxidase-conjugated antibodies (Bio-Rad, California, USA; 1:2000). The membranes were rinsed four times with TBST, each for 5 min. Bands were then detected on a LI-COR C-DiGit Blot Scanner from Biosciences, Lincoln, Nebraska, USA, with enhanced chemiluminescence and Western blotting luminol reagents mixed in a 1:1 ratio. Membranes were washed and treated with a mouse monoclonal β-actin antibody (1:2000, Santa Cruz Biotechnology, Inc., Santa Cruz, CA) for internal control. All subsequent procedures were performed as previously mentioned. Densitometry analysis quantified protein bands using Image Studio Acquisition Software (Image-Lab 2.0.1 software). Band intensities were quantified by drawing a square or circle around the protein bands. The ratios of band intensities between the protein of interest and β-actin or tubulin were calculated. The band intensity ratio of the control protein sample to β-actin or tubulin is considered 100%. Band intensity ratios to β-actin were compared with the control ratios.

2.5 Statistical analysis

Data are presented as means ± SEM. Comparisons among multiple groups were conducted using one-way ANOVA and the Mann-Whitney test, while an unpaired t-test was employed for comparison analyses between two groups. Statistical analyses were carried out with SPSS version 29 and Microsoft Excel 365. A p-value under 0.05 was regarded as statistically significant.

3. Results

3.1 ACE and ACE2 protein expression levels in the control, diabetic, hypertensive, and hypertensive-diabetic rat frontal cortex

ACE is a vital enzyme that helps in producing angiotensin II (Ang II), a powerful vasoconstrictor that is essential for regulating blood pressure and cardiovascular as well as neurovascular functions. However, ACE2 serves as a counterregulatory enzyme that balances the effects of ACE by degrading Ang II to Ang (1-7), which has protective effects. We quantified the expression levels of these enzymes in the frontal brain cortex from control (non-diabetic WKY), WKY-diabetic, hypertensive (SHR-C), and hypertensive-diabetic (SHR-D) rats using Western blot analysis (Fig. 1). Densitometric analysis of the protein bands showed increases of 32% and 22% in ACE levels in the diabetic and hypertensive frontal cortex, respectively, compared to controls ($p<0.05$) (Fig. 1c). Moreover, ACE levels in hypertensive-diabetic rats more than doubled compared to controls ($p<0.01$). Additionally, ACE levels in hypertensive-diabetic rats were significantly higher than in hypertensive groups ($p<0.01$). No notable differences in ACE expression were seen between diabetic and hypertensive rats. Furthermore, the levels of ACE2 protein in the frontal brain cortex of Non-diabetic (C), diabetic, SHR-C, and SHR-D rats were assessed (Fig. 1c). Band density ratios indicated that ACE2 was reduced by 20% in diabetic rats ($P < 0.05$ vs. controls). Furthermore, ACE2 decreased by 44% and 52% in SHR-C and SHR-D rats, respectively, compared to controls ($P < 0.01$). Interestingly, ACE2 levels in hypertensive and hypertensive-diabetic rats were also considerably lower than diabetic groups ($p<0.05$).

3.2 Chymase protein expression levels in the non-diabetic, diabetic, hypertensive, and hypertensive-diabetic rat frontal cortex

Chymase is an alternative serine protease enzyme capable of producing Ang II independently of ACE, particularly in tissues where ACE activity is low or inhibited. Chymase plays a role in local Ang II production from Ang I, especially under pathological conditions. In this study, we measured the expression levels of chymase protein in the frontal cortex of control, diabetic, SHR-C, and SHR-D rats using Western blotting (Fig. 2). Densitometric analysis of protein bands indicated that chymase levels increased by 40%, 32%, and 70% in the diabetic, SHR-C, and SHR-D brain cortices, respectively, compared to controls. Additionally, the chymase level in SHR-D was significantly higher than in both diabetic and SHR-C rats ($p<0.05$).

3.3 BDNF, TrkB, and GS protein expression levels in the control, diabetic, hypertensive, and hypertensive-diabetic rat frontal cortex

BDNF and its receptor, TrkB, are essential for brain development, neuronal survival, synaptic plasticity, and cognitive functions. Dysregulation of BDNF-TrkB signaling is known to be implicated in various neurological disorders. In addition, glutamine synthetase (GS) is a critical enzyme in the brain that protects neurons from oxidative stress and excitotoxicity by maintaining low levels of extracellular glutamate and ammonia. However, an altered level of GS under pathological conditions may cause neurodegenerative diseases. The expression levels of BDNF, TrkB, and GS proteins in the cortex of control, diabetic, SHR-C, and SHR-D rats were quantified by Western blotting (Fig. 3). Densitometry analysis of the protein bands indicated a considerable decrease in BDNF levels (44, 35, and 48% in diabetic, SHR-C, and SHR-D rats, respectively; $p<0.01$) than to controls (Fig. 3b). In addition, no notable changes were observed in BDNF expression in the frontal brain cortex among the diabetic, SHR-C, and SHR-D groups. Additionally, the protein expression level of TrkB was also quantified in the brain cortex of these rats. The band intensity ratio indicated that the levels of TrkB were significantly lower in the diabetic and SHR-D groups compared to controls ($p<0.05$), while there was no significant difference in TrkB levels between the control and SHR-C groups (Fig. 3c). Furthermore, GS levels were also quantified in the cortex of all rat groups. The band intensity ratio data demonstrated a decrease in GS levels of approximately 65%, 40%, and 69% in the diabetic, SHR-C, and SHR-D groups, respectively, compared to controls ($p<0.01$) (Fig. 3d). Moreover, the GS level in SHR-C was significantly higher than in the diabetic and SHR-D groups ($p<0.05$).

3.4 Caspase 3 and cytochrome c protein expression levels in the control, diabetic, hypertensive, and diabetic-hypertensive-diabetic rat frontal cortex

Dysregulation of apoptotic markers, caspase-3, and cytochrome c plays critical roles in the execution of apoptosis and is thereby implicated in neurodegenerative diseases. Caspase-3 and cytochrome c protein expression levels were measured in the frontal cortex of control, diabetic, SHR-C, and SHR-D rats (Fig. 4). Analysis of the protein bands indicated a 3-fold increase in caspase-3 expression in the diabetic group and a 2.5-fold increase in the SHR-D group than to controls (Fig. 4c). However, only a 50% increase in caspase-3 levels was seen in the SHR-C group compared to the control. Additionally, caspase-3 levels were significantly higher in the diabetic and SHR-D groups compared to the SHR-C group ($p<0.01$). Moreover, cytochrome c levels doubled

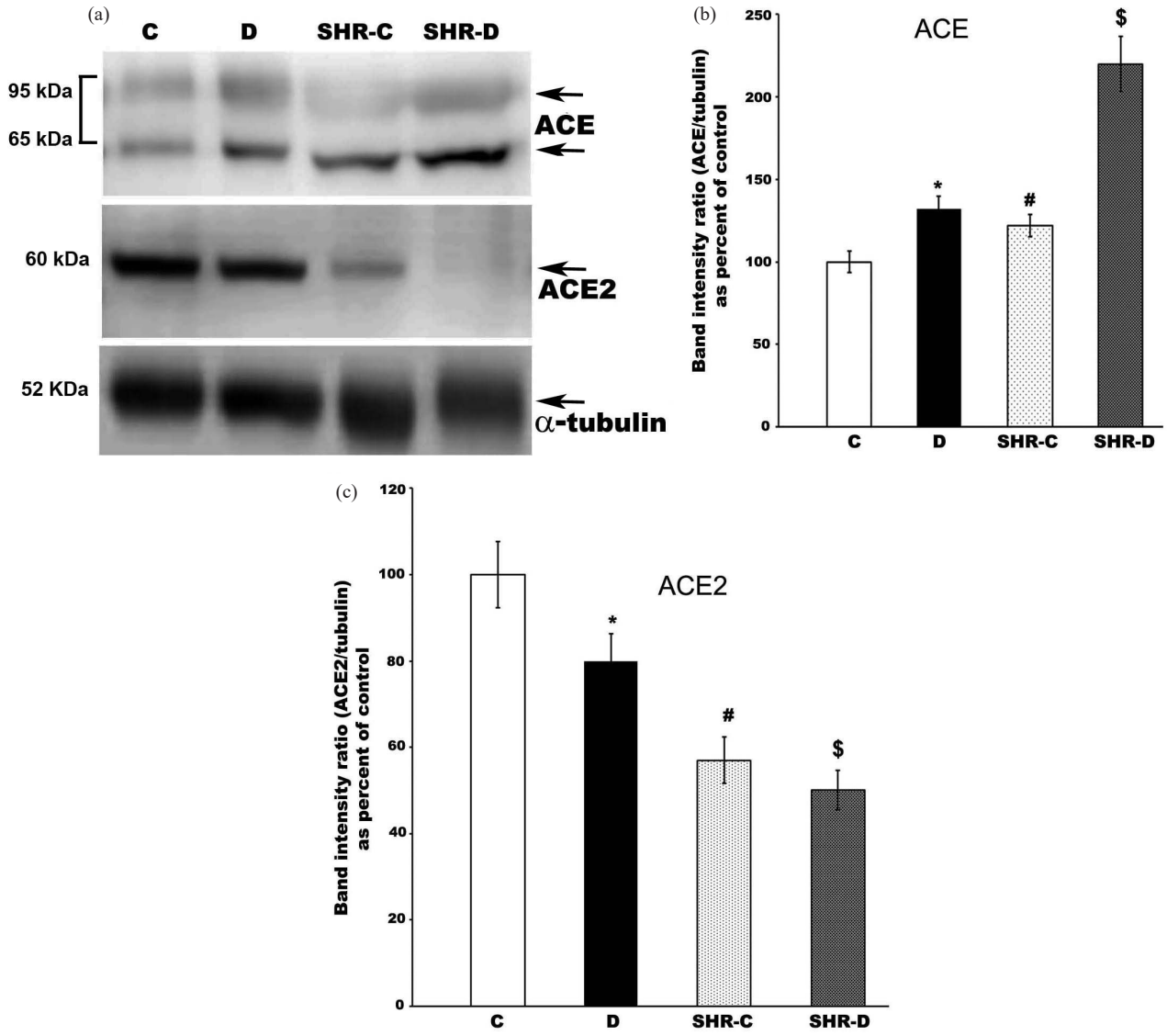

Fig. 1. Western blot analysis of ACE and ACE2 expression in the frontal cortices of control, diabetic, hypertensive, and hypertensive-diabetic rats. Band intensities were quantified using densitometry. Panel (a): represents ACE, ACE2, and α- tubulin band immunoblots. Panels (b), and (c) show the band intensity ratios of ACE and ACE2, respectively. C represents control; D represents diabetes; SHR-C represents spontaneously hypertensive rats-control; and SHR-D represents hypertensive-diabetic rats. ACE-data presented as means ± SEM; $^{*,\#}p < 0.05$ vs. control; $^{\$}p < 0.01$ vs. controls, diabetic and SHR-C groups. ACE2-data presented as means ± SEM; $^{*}p < 0.05$ vs. control; $^{\#,\$}p < 0.01$ vs. control; $^{\#,\$}p < 0.05$ vs diabetic group. (n = 5/group).

in diabetic rats compared to controls ($p<0.05$) (Fig. 4b). Notably, there was approximately a 5- and 6-fold increase in cytochrome c levels in SHR-C and SHR-D rats, respectively, compared to controls. In both SHR-C and SHR-D groups, cytochrome c levels were considerably higher than in diabetic groups ($p<0.01$). Interestingly, the cytochrome c level was also considerably higher in the SHR-D group than in the SHR-C group ($p<0.05$).

3.5 Bcl-2 and Bcl-xL expression levels in the frontal cortex of control, diabetic, hypertensive and hypertensive-diabetic rats

Bcl-2 and Bcl-xL are anti-apoptotic proteins that support neuronal survival and protect the brain from injury and disease. Dysregulation of Bcl-2 and Bcl-xL levels is implicated in the pathogenesis of neurodegenerative diseases. The expression levels of Bcl-2 and Bcl-xL were examined in the frontal cortex of control, diabetic, SHR-C, and SHR-D rats using Western blot analysis (Fig. 5). The density of the protein bands indicate that levels of Bcl-xL were considerably reduced by 30%, 60%, and 35% in the brain cortex of the diabetic, SHR-C, and SHR-D groups, respectively, as compared to controls ($p< 0.01$) (Fig. 5b). Furthermore, the Bcl-xL expression in the SHR-C group was significantly reduced than to those in the diabetic and SHR-D groups ($p<0.05$). Similarly, the expression of the anti-apoptotic protein Bcl-2 was reduced by nearly 50%, 36%, and 72% in the brain cortex of the diabetic, SHR-C, and SHR-D groups, respectively, compared to the control group ($p < 0.01$) (Fig. 5c). Additionally, Bcl-2 levels in the SHR-D group were significantly reduced when compared to the diabetic and SHR-C groups ($p<0.05$).

3.6 Quantification of BDNF in the control and diabetic, and hypertensive-diabetic rats frontal brain cortex by ELISA

The levels of BDNF in the homogenate of the brain cortex of control, diabetic, SHR-C, and SHR-D rats were assessed using the ELISA technique. The expression of BDNF in the brains of diabetic, SHR-C, and SHR-D rats was significantly decreased than the corresponding non-diabetic control group ($p < 0.01$) (Fig. 6a). However, no significant variation in BDNF levels were exhibited among diabetic, hypertensive, and hypertensive-diabetic rats.

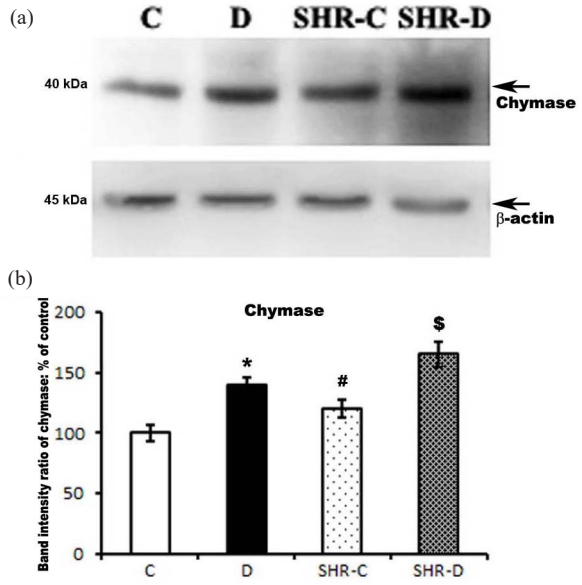

Fig. 2. Western blot analysis of chymase expression in the frontal cortices of control, diabetic, and hypertensive-diabetic rats. Band intensities were quantified using densitometry. The upper panel (a) represents Western blots of chymase and β-actin bands. The lower panel (b) represents the bar graph of the band intensity ratios of chymase in C, D, SHR-C, and SHR-D groups. C represents control; D (diabetes); SHR-C (spontaneously hypertensive rats-control); and SHR-D (spontaneously hypertensive-diabetic rats). Data presented as means ± SEM; $^{*,\#}p < 0.05$ vs. control, $^{\$}p < 0.01$ vs. control, $^{\$}p < 0.05$ vs. diabetic and SHR-C, (N = 4/group).

3.7 Quantification of GSH and TBARS in the control, diabetic, and hypertensive-diabetic rats frontal brain cortex

GSH is a critical antioxidant that shields the brain from oxidative stress, detoxifies harmful substances, and maintains redox balance. Meanwhile, TBARS serve as a biomarker for oxidative damage. Altered levels of GSH and TBARS are associated with neurodegenerative diseases. The levels of glutathione (GSH) and TBARS were quantified in the frontal brain cortex of control rats, as well as diabetic, SHR-C, and SHR-D rats, to assess the severity of oxidative stress (Figs. 6b and c). The antioxidant GSH level significantly decreased by 28% and 39% in the diabetic and SHR-D brain cortex, respectively, than to controls ($p < 0.01$). The GSH level in the SHR-D group decreased slightly but not significantly compared to the diabetic and SHR-C groups. Additionally, lipid peroxidation, indicated by TBARS levels, increased significantly in the diabetic, SHR-C, and SHR-D brain cortex than to non-diabetic controls ($p < 0.01$). Nonetheless, TBARS levels were insignificantly different among the diabetic, SHR-C, and SHR-D groups.

4. Discussion

Recently, we reported dysregulated levels of critical constituents of the RAS (AT1R and MAS receptors) in the rat brain cortex in the context of diabetes and hypertension (Oyesiji *et al.*, 2023). In this study, we investigated the expression and regulation of the angiotensin-converting enzyme (ACE), ACE2, and chymase enzymes in the rat frontal brain cortex under diabetic and hypertensive conditions that may implicate the etiology of neurodegenerative factors. We selected the frontal cortex to study the RAS system and its regulation in hypertensive and diabetic rats, as this region is commonly examined for its roles in cognition, emotion, and neurological diseases. Interestingly, we found an altered

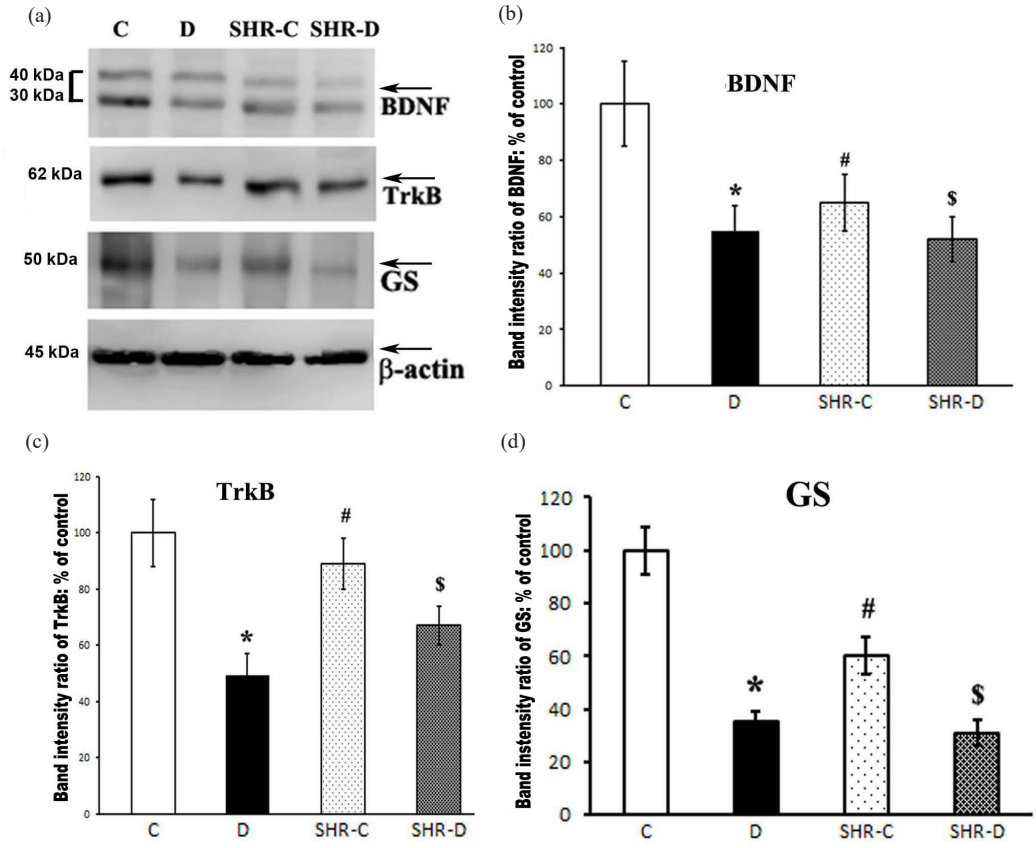

Fig. 3. Western blot analysis of BDNF, GS, and TrKB expression in frontal cortices from control, diabetic, and diabetic-hypertensive rats. The band intensities were quantified by densitometry. Panel (a) shows immunoblots of BDNF, GS, TrkB, and β-actin bands. Panel (b) shows a bar graph of band intensity ratios of BDNF in the C, D, SHR-C, and SHR-D groups. Panel (c) shows the band intensity of the level of TrkB in all the groups. Panel (d) shows the level of GS in all the groups. GS represents glutamine synthetase; BDNF represents brain-derived neurotrophic factor; C represents control; D represents diabetes; SHR-C represents spontaneously hypertensive rats-control; and SHR-D represents hypertensive-diabetic rats. Data presented as means ± SEM; BDNF ($^{*,\$,\#}p < 0.01$; vs. control); TrkB ($^{*,\$}p < 0.05$ vs. control, $^{\#}p < 0.05$ vs. diabetic and SHR-D); GS ($^{*,\#,\$}p < 0.01$ vs. control, $^{\#}p < 0.05$ vs. diabetic and SHR-D), (n = 5/group).

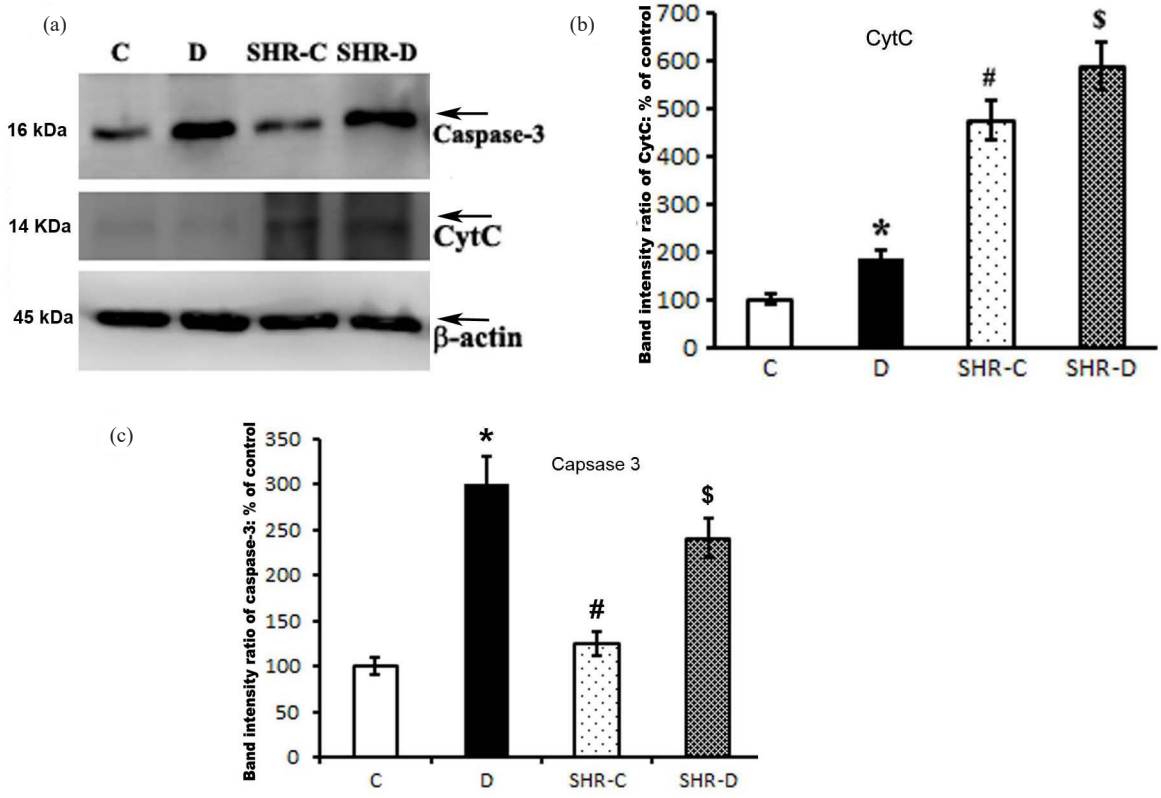

Fig. 4. Western blot analysis of caspase-3 and cytochrome c expression in the frontal cortices of control, diabetic, and hypertensive-diabetic rats. Panel (a) represents immunoblots of cytochrome c, caspase 3, and β-actin bands. The band intensities were measured using densitometry. Panel (b) represents the band intensity ratio of cytochrome c. Panel (c) represents the band intensity ratio of caspase-3. C represents control; D represents diabetes; SHR-C represents spontaneously hypertensive rats-control; and SHR-D represents spontaneously hypertensive-diabetic rats. Data presented as means ± SEM; $^{*,\#,\$}p < 0.01$ (caspase-3 vs. controls; $^{*,\$}p < 0.01$ vs. SHR-C); $^{*,\#,\$}p < 0.01$ (cytochrome c vs. controls, $^{\#,\$}p < 0.01$ vs. diabetic), (n = 4/group).

expression of the Ang II-forming enzymes in the brain cortex of diabetic and hypertensive-diabetic rats. This imbalance in the RAS system could potentially lead to increased oxidative stress and apoptosis, ultimately contributing to cognitive decline. Numerous previous researches have indicated that both hypertension and diabetes disrupt the two arms of the RAS system, which comprise ACE/angiotensin-II (Ang-II)/(AT1R) and ACE2/Ang 1-7/AT2R, linked to various neurological disorders of the retina and brain (Ola *et al.*, 2017; Oyesiji *et al.*, 2023; Saavedra, 2012; Abiodun and Ola, 2020). Indeed, our study indicates that components of both arms of the RAS system are dysregulated in brain cortex of hypertensive-diabetic rats.

The ACE enzyme was found to be highly expressed in the frontal cortex of the rat brain. ACE expression increased in the diabetic brain cortex, like those observed under hypertensive conditions (Estato *et al.*, 2013). However, ACE expression was heightened in the hypertensive brain influenced by diabetes. The additive effect of heightened ACE expression in the hypertensive brain due to diabetes underscores the complex interplay between hypertension and diabetes. These two conditions frequently coexist and amplify each other's pathological effects. Their combined impact can create a synergistic effect through ACE upregulation, potentially leading to severe cognitive consequences in the brain cortex. Reports indicate that diabetes-induced factors, including hyperglycemia, insulin resistance, and dyslipidemia, can affect ACE activity in the brain (Henriksen, 2007). In individuals with diabetes, increased ACE activity may raise levels of Ang II, contributing to inflammation, oxidative stress, and endothelial damage in cerebral blood vessels, ultimately leading to diabetic encephalopathy and cognitive decline (Bernstein *et al.*, 2014). Hypertension is linked to increased ACE activity in the brain, resulting in elevated levels of angiotensin that can cause cerebrovascular remodeling, endothelial dysfunction, and neuroinflammation, thereby contributing to cognitive impairment and a heightened risk of stroke and dementia (Bernstein *et al.*, 2007; Wang *et al.*, 2007). Animal research has shown that inhibition of ACE activity or blockade of angiotensin II receptors in the brain can mitigate hypertension-related cerebrovascular dysfunction and cognitive decline (Yamada *et al.*, 2011). Thus, targeting ACE in the brain may provide therapeutic opportunities to alleviate these adverse effects and enhance outcomes in individuals with diabetes and hypertension.

On the other hand, ACE2 counteracts the effects of ACE by converting angiotensin II into angiotensin-(1-7), which has vasodilatory, anti-inflammatory, and antioxidative properties (Zuliani *et al.*, 2005; Zheng *et al.*, 2014; Chen *et al.*, 2017). We observed a significant reduction in ACE2 levels in the brain cortex of rats with diabetes and hypertension. The decrease in ACE2 expression in the hypertensive brain, which is further diminished under the influence of diabetes, represents a notable additive effect that may worsen cerebrovascular and neuronal dysfunction. The decline in ACE2 expression in the brain may disrupt the angiotensin II/angiotensin-(1-7) ratio, leading to increased vasoconstriction, inflammation, and neurodegeneration. This reduction in ACE2 levels in the diabetic brain is supported by another study showing lower ACE2 expression across various brain regions, including the hypothalamus and cerebral vasculature, under diabetic conditions (Chen *et al.*, 2017). Decreased ACE2 activity may contribute to neuroinflammation, oxidative stress, and endothelial dysfunction, which are hallmark features of diabetic encephalopathy and cognitive decline (Tran *et al.*, 2022). The additive effect of diabetes on an already ACE2-deficient hypertensive brain may elevate Ang II and diminish the protective effects of angiotensin (1-7), thereby exacerbating neuroinflammation and increasing the risk of cognitive decline, stroke, and neurodegenerative diseases (Jiang *et al.*, 2013). Restoring ACE2 activity in the brain could be a potential therapeutic strategy for preventing or treating diabetic neurovascular complications (Wang *et al.*, 2007). ACE2 may also play a role in neuronal survival and function by promoting BDNF production, a vital factor for neuronal growth and repair (Wang *et al.*, 2016). However, the exact role of ACE2 in

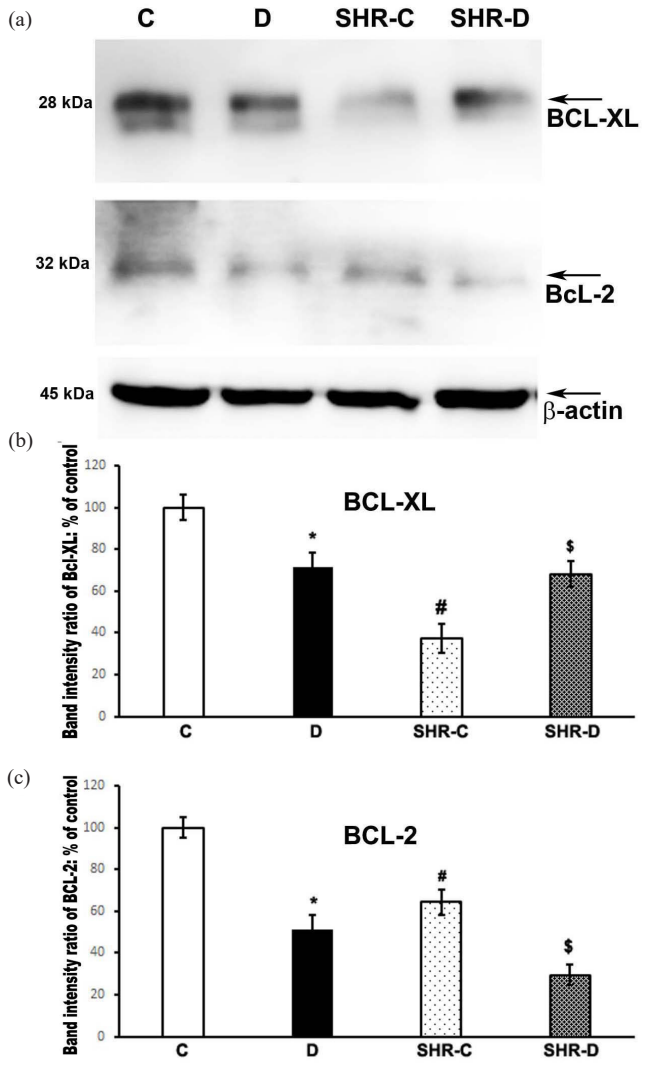

Fig. 5. Western blot analysis of Bcl-2 and Bcl-xL expression in the frontal cortices of control, diabetic, and hypertensive-diabetic rats. Panel (a) represents a blot image of Bcl-2, Bcl-xL, and β-actin. The band intensities were quantified by densitometry. Panels (b) and (c) display data as band intensity ratios of the proteins to β-actin. Data are expressed as means ± SEM; Bcl-xL (*, #, $, $p < 0.01$, significantly different from controls; *,$ $p < 0.05$ vs. SHR-C); Bcl-2 (*, #, $, $p < 0.01$ vs. control; $ $p < 0.05$ vs. diabetic and SHR-C). Bcl-2 refers to B-cell lymphoma 2. BCL-xL means Bcl-2-associated XL protein. C represents control, D is diabetic, SHR-C indicates hypertensive rats-control, SHR-D indicates hypertensive-diabetic rats; n = 5 per group.

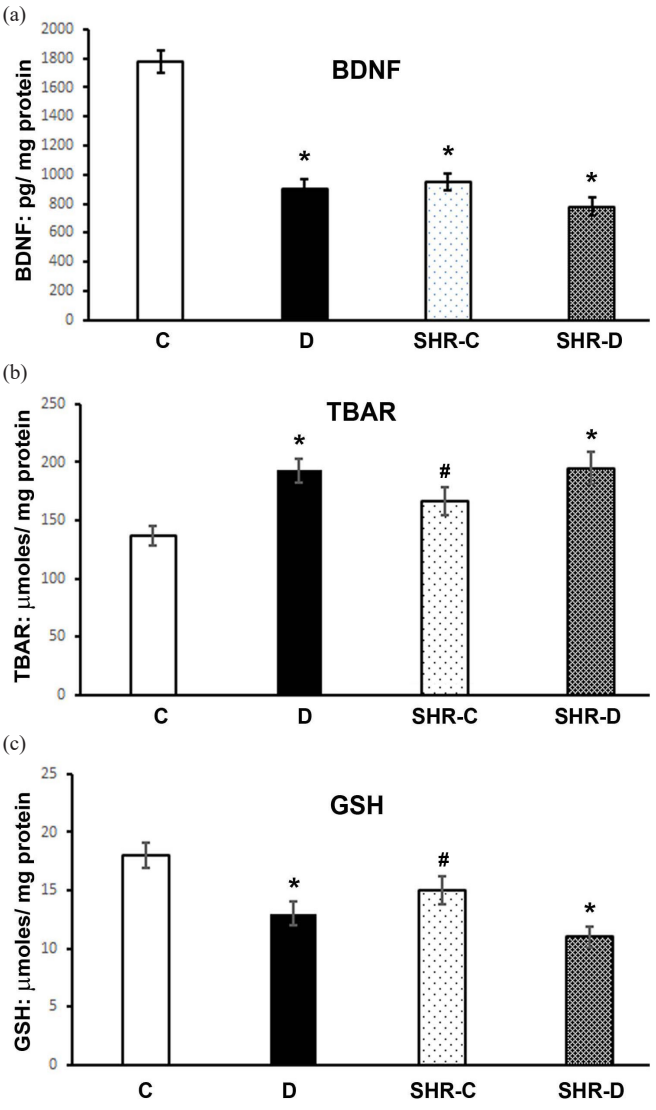

Fig. 6. (a-c) Quantification of brain-derived neurotrophic factor (BDNF), glutathione (GSH), and thiobarbituric acid reactive substances (TBARS) in the brain cortices of control (C), diabetic (D), hypertensive (SHR), and hypertensive-diabetic (SHR-D) rats. Values are expressed as means ± SEM; BDNF: (*$p < 0.01$, compared to control); TBARS: (*,#$p < 0.05$ vs. control); GSH: (*,#$p < 0.05$ vs. control), n = 7 per group.

hypertension-related brain dysfunction and cognitive impairment has yet to be fully addressed.

Chymase-mediated angiotensin II formation bypasses the ACE pathway, establishing it as a crucial alternative mechanism for angiotensin II generation in hypertensive states (Ola *et al.*, 2017). We analyzed the regulation of chymase, which has become a key factor in the pathophysiology of various cardiovascular and neurological conditions, including diabetes and hypertension (Ferrario *et al.*, 2021; Hendrix *et al.*, 2013; Ahmad *et al.*, 2013). Our results indicate that the chymase protein level was elevated in the rat frontal cortex under diabetic and hypertensive conditions. Notably, a synergistic effect on chymase expression in the frontal cortex was observed when hypertensive rats were made diabetic. This additive upregulation of chymase underscores its role as a key contributor to the compounded neurovascular risks in individuals with both conditions. Several studies have reported that chymase is implicated in increasing oxidative stress, disrupting the blood-brain barrier, and activating the pro-inflammatory cytokine interleukin-1β (IL-1β) in the brain, potentially exacerbating neuroinflammation and neuronal dysfunction in diabetes (Hendrix *et al.*, 2013; Caughey, 2023). Animal studies have demonstrated that chymase inhibition or blockade reduces hypertension-induced cerebrovascular dysfunction and cognitive decline, emphasizing the potential therapeutic utility of targeting chymase in hypertensive brain diseases (Hendrix *et al.*, 2013; Ahmad *et al.*, 2013; Caughey, 2023). Therefore, targeting chymase may present therapeutic opportunities for alleviating these adverse effects and improving outcomes in diabetic and hypertensive conditions.

Diabetes is known to increase the production of reactive oxygen species (ROS) and decrease antioxidant levels, resulting in cell damage and neurodegeneration. This study demonstrated a significant increase in lipid peroxidation in the frontal cortex of diabetic, hypertensive, and hypertensive-diabetic rats. The level of the endogenous antioxidant glutathione also declined in the frontal brain cortex of diabetic, hypertensive, and hypertensive-diabetic rats compared to the controls, echoing findings from various organs in diabetes (Girouard, 2007). Oxidative stress caused by diabetes and hypertensive conditions is known to promote apoptosis. In fact, we observed higher levels of cytochrome c and caspase-3 expression, along with lower levels of the anti-apoptotic proteins Bcl-2 and Bcl-xL in the brain cortex of diabetic and spontaneously hypertensive rats. Both Bcl-2 and Bcl-xL are anti-apoptotic proteins, and their expression is tightly regulated

under various pathological conditions. In SHR-D, elevated levels of angiotensin II (Ang II) have been reported compared to normotensive controls (Singh *et al.*, 2008). Additionally, persistent hyperglycemia in diabetic rats contributes to metabolic stress. These factors are known to differentially influence the regulation of apoptotic pathways in fibrotic and inflammatory tissue environments. The observed downregulation of Bcl-2 and upregulation of Bcl-xL in SHR diabetic rats may reflect a compensatory or adaptive response to sustained hemodynamic stress and chronic hyperglycemia. These conditions act as primary factors driving the altered expression patterns, contributing to the complex apoptotic signaling seen in SHR-D rats compared to non-diabetic and non-hypertensive controls. The increased levels of pro-apoptotic factors, along with decreased anti-apoptotic proteins in the diabetic brain cortex, may be linked to heightened neurodegeneration and diminished axonal regeneration, which are consistent indicators of apoptosis (Mohr *et al.*, 2002).

BDNF is essential for neuronal survival and maintenance (Ola *et al.*, 2013). It interacts with the TrkB receptor, triggering signaling pathways that promote cell proliferation and survival (Yoshii and Constantine-Paton, 2010). In contrast, proBDNF, the precursor to BDNF, induces apoptosis and reduces neurotransmission by activating the p75 receptor (Teng *et al.*, 2005). Our lab previously identified a reduction in neurotrophic factors in the retinas of diabetic rats, alongside a decrease in TrkB expression levels (Ola *et al.*, 2013). Consistent with earlier studies, we also noted a decline in BDNF expression under diabetic and hypertensive conditions, which correlated with increased ACE and chymase levels and a reduced level of ACE2 (Wang *et al.*, 2016). Moreover, the levels of TrkB and GS were diminished in the brains of both the diabetic and hypertensive-diabetic groups, potentially resulting in a lack of neurotrophic support that may contribute to neurodegeneration in the brain.

The analyses of protein expression for critical enzymes that control Ang II peptide levels and regulate the RAS system indicate that these enzymes are highly expressed in the rat frontal cortex. A comparison of ACE and chymase expression showed a notable increase in their levels in the frontal cortex under diabetic and hypertensive-diabetic conditions, while the ACE2 level declined. The compounded effect of heightened ACE and chymase expression, combined with reduced ACE2 expression in the hypertensive-diabetic brain cortex due to diabetes, may worsen pathological consequences, leading to neuronal dysfunction. Consequently, diabetes and/or hypertension may disrupt early neurotrophic support in the brain cortex. This disruption could impair signaling through TrkB and GS in later stages, causing damage to the neurons. The dysregulated RAS components in hypertensive-diabetic conditions might diminish neurotrophic effects by enhancing apoptotic and oxidative stress pathways, resulting in neurodegeneration in the brain.

5. Conclusions

The combined effects of ACE upregulation, ACE2 downregulation, and increased chymase expression in the hypertensive-diabetic brain cortex may intensify the pathophysiology of neuronal damage and encephalopathy. These changes likely elevate oxidative stress and apoptosis pathways, leading to neurodegeneration. However, further research is necessary to better understand how diabetes impacts hypertensive signaling pathways, which may influence inflammation and contribute to neurodegeneration in the diabetic brain cortex.

CRediT authorship contribution statement

Abeeb Oyesiji Abiodun, Mohammad Shamsul Ola and Sarfaraz Ahmad: Conceived and designed the experiments. **Dalia I AlDosari and Abeeb Oyesiji Abiodun:** Performed the experiments. **Mohammad Shamsul Ola, Abdul Aziz AlAmri, Abdul Aziz AlAmri and Abdullah S. Alhomida:** Contributed reagents/materials tools. **Mohammad Shamsul Ola and Sarfaraz Ahmad:** Wrote the paper, and **Mohammad Shamsul Ola, Abdul Aziz AlAmri, Abdullah S. Alhomida, and Amani AlGhamdi:** Revised and edited the manuscript. All authors contributed to the article and approved the submitted version.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

All data generated or analyzed during this study are included in this manuscript. However, raw data in the study can be available from the corresponding author upon reasonable request.

Declaration of generative AI and AI-assisted technologies in the writing process

The authors confirm that there was no use of artificial intelligence (AI)-assisted technology for assisting in the writing or editing of the manuscript and no images were manipulated using AI.

Acknowledgement

The authors thank the Ongoing Research Funding support (ORF-2025-710) from King Saud University, Riyadh, Saudi Arabia.

Funding

Ongoing Research Funding (No. ORF-2025-710); King Saud University, Riyadh, Kingdom of Saudi Arabia.

References

Abiodun, O.A., Ola, M.S., 2020. Role of brain renin angiotensin system in neurodegeneration: An update. Saudi J Biol Sci 27, 905-912. https://doi.org/10.1016/j.sjbs.2020.01.026

Ahmad, A., Crupi, R., Campolo, M., Genovese, T., Esposito, E., Cuzzocrea, S., 2013. Absence of TLR4 reduces neurovascular unit and secondary inflammatory process after traumatic brain injury in mice. PLoS One 8, e57208. https://doi.org/10.1371/journal.pone.0057208

Ahmad, S., Wei, C.C., Tallaj, J., D'Italia, L.J., Moniwa, N., Varagic, J., Ferrario, C.M., 2013. Chymase mediates angiotensin-(1-12) metabolism in normal human hearts. J Am Soc Hypertens 7, 128-136. https://doi.org/10.1016/j.jash.2012.12.003

Beckman, J.A., Creager, M.A., 2016. Vascular complications of diabetes. Circ Res 118, 1771-1785. https://doi.org/10.1161/CIRCRESAHA.115.306884

Bernstein, K.E., Koronyo, Y., Salumbides, B.C., Sheyn, J., Pelissier, L., Lopes, D.H.J., Shah, K.H., Bernstein, E.A., Fuchs, D.T., Yu, J.J.Y., Pham, M., Black, K.L., Shen, X.Z., Fuchs, S., Koronyo-Hamaoui, M., 2014. Angiotensin-converting enzyme overexpression in myelomonocytes prevents Alzheimer's-like cognitive decline. J Clin Invest 124, 1000-1012. https://doi.org/10.1172/jci66541

Caughey, G.H., 2023. Update on mast cell proteases as drug targets. Immunol Allergy Clin North Am 43, 777-787. https://doi.org/10.1016/j.iac.2023.04.006

Chen, J.L., Zhang, D.L., Sun, Y., Zhao, Y.X., Zhao, K.X., Pu, D., Xiao, Q., 2017. Angiotensin-(1–7) administration attenuates Alzheimer's disease-like neuropathology in rats with streptozotocin-induced diabetes via Mas receptor activation. Neuroscience 346, 267-277. https://doi.org/10.1016/j.neuroscience.2017.01.027

Cui, H., Su, S., Cao, Y., Ma, C., Qiu, W., 2021. The altered anatomical distribution of ACE2 in the brain with Alzheimer's disease pathology. Front Cell Dev Biol 9, 684874. https://doi.org/10.3389/fcell.2021.684874

Doobay, M.F., Talman, L.S., Obr, T.D., Tian, X., Davisson, R.L., Lazartigues, E., 2007. Differential expression of neuronal ACE2 in transgenic mice with overexpression of the brain renin-angiotensin system. Am J Physiol Regul Integr Comp Physiol 292, R373-R381. https://doi.org/10.1152/ajpregu.00292.2006

Estato, V., Obadia, N., Carvalho-Tavares, J., Freitas, F.S., Reis, P., Castro-Faria Neto, H., Lessa, M.A., Tibiriçá, E., 2013. Blockade of the renin-angiotensin system improves cerebral microcirculatory perfusion in diabetic hypertensive rats. Microvasc Res 87, 41-49. https://doi.org/10.1016/j.mvr.2013.02.007

Ferrario, C.M., Groban, L., Wang, H., Cheng, C.P., VonCannon, J.L., Wright, K.N., Sun, X., Ahmad, S., 2021. The Angiotensin-(1-12)/Chymase axis as an alternate component of the tissue renin angiotensin system. Mol Cell Endocrinol 529, 111119. https://doi.org/10.1016/j.mce.2020.111119

Gao, Q., Jiang, T., Zhao, H.R., Wu, L., Tian, Y.Y., Ou, Z., Zhang, L., Pan, Y., Lu, J., Zhang, Y.D., 2016. Activation of autophagy contributes to the angiotensin ii-triggered apoptosis in a dopaminergic neuronal cell line. Mol Neurobiol 53, 2911-2919. https://doi.org/10.1007/s12035-015-9177-3

Gąsecki, D., Kwarciany, M., Nyka, W., Narkiewicz, K., 2013. Hypertension, brain damage and cognitive decline. Curr Hypertens Rep 15, 547-558. https://doi.org/10.1007/s11906-013-0398-4

Girouard, H., Park, L., Anrather, J., Zhou, P., Iadecola, C., 2007. Cerebrovascular nitrosative stress mediates neurovascular and endothelial dysfunction induced by angiotensin II. Arterioscler Thromb Vasc Biol 27, 303-309. https://doi.org/10.1161/01.ATV.0000253885.41509.25

Grobe, J.L., Grobe, C.L., Beltz, T.G., Westphal, S.G., Morgan, D.A., Xu, D., de Lange, W.J., Li, H., Sakai, K., Thedens, D.R., Cassis, L.A., Rahmouni, K., Mark, A.L., Johnson, A.K., Sigmund, C.D., 2010. The brain Renin-angiotensin system controls divergent efferent mechanisms to regulate fluid and energy balance. Cell Metab 12, 431-442. https://doi.org/10.1016/j.cmet.2010.09.011

Hassing, L.B., Hofer, S.M., Nilsson, S.E., Berg, S., Pedersen, N.L., McClearn, G., Johansson, B., 2004. Comorbid type 2 diabetes mellitus and hypertension exacerbates cognitive decline: Evidence from a longitudinal study. Age Ageing 33, 355-361. https://doi.org/10.1093/ageing/afh100

Hendrix, S., Kramer, P., Pehl, D., Warnke, K., Boato, F., Nelissen, S., Lemmens, E., Pejler, G., Metz, M., Siebenhaar, F., Maurer, M., 2013. Mast cells protect from post-traumatic brain inflammation by the mast cell-specific chymase mouse mast cell protease-4. FASEB J 27, 920-929. https://doi.org/10.1096/fj.12-204800

Henriksen, E.J., 2007. Improvement of insulin sensitivity by antagonism of the renin-angiotensin system. Am J Physiol Regul Integr Comp Physiol 293, R974-R980. https://doi.org/10.1152/ajpregu.00147.2007

Intensive blood-glucose control with sulphonylureas or insulin compared with conventional treatment and risk of complications in patients with type 2 diabetes (UKPDS 33) UK prospective diabetes study (UKPDS) group. Lancet 1998; 352, 837-853. https://doi.org/10.1016/S0140-6736(05)60424-2

Jiang, T., Gao, L., Lu, J., Zhang, Y.D., 2013. ACE2-Ang-(1-7)-Mas axis in brain: A potential target for prevention and treatment of ischemic stroke. Curr Neuropharmacol 11, 209-217. https://doi.org/10.2174/1570159X11311020007

Kim, J.H., Ko, P.W., Lee, H.W., Jeong, J.Y., Lee, M.G., Kim, J.H., Lee, W.H., Yu, R., Oh, W.J., Suk, K., 2017. Astrocyte-derived lipocalin-2 mediates hippocampal damage and cognitive deficits in experimental models of vascular dementia. Glia 65, 1471-1490. https://doi.org/10.1002/glia.23174

Kishi, T., Hirooka, Y., Konno, S., Ogawa, K., Sunagawa, K., 2010. Angiotensin II type 1 receptor-activated caspase-3 through ras/mitogen-activated protein kinase/extracellular signal-regulated kinase in the rostral ventrolateral medulla is involved in sympathoexcitation in stroke-prone spontaneously hypertensive rats. Hypertension 55, 291-297. https://doi.org/10.1161/HYPERTENSIONAHA.109.138636

Long, A.N., Dagogo-Jack, S., 2011. Comorbidities of diabetes and hypertension: Mechanisms and approach to target organ protection. J Clin Hypertens (Greenwich) 13, 244-251. https://doi.org/10.1111/j.1751-7176.2011.00434.x

Mohr, S., Xi, X., Tang, J., Kern, T.S., 2002. Caspase activation in retinas of diabetic and galactosemic mice and diabetic patients. Diabetes 51, 1172-1179. https://doi.org/10.2337/diabetes.51.4.1172

Moran, C., Xie, K., Poh, S., Chew, S., Beare, R., Wang, W., Callisaya, M., Srikanth, V., 2019. Observational study of brain atrophy and cognitive decline comparing a sample of community-dwelling people taking angiotensin converting enzyme inhibitors and angiotensin receptor blockers over time. J Alzheimers Dis 68, 1479-1488. https://doi.org/10.3233/JAD-180943

Nagar, S.D., Pemu, P., Qian, J., Boerwinkle, E., Cicek, M., Clark, C.R., Cohn, E., Gebo, K., Loperena, R., Mayo, K., Mockrin, S., Ohno-Machado, L., Ramirez, A.H., Schully, S., Able, A., Green, A., Zuchner, S., Jordan, I.K., Meller, R., 2022. Investigation of hypertension and type 2 diabetes as risk factors for dementia in the All of Us cohort. Sci Rep 12, 19797. https://doi.org/10.1038/s41598-022-23353-z

Ola, M.S., Nawaz, M.I., El-Asrar, A.A., Abouammoh, M., Alhomida, A.S., 2013. Reduced levels of brain derived neurotrophic factor (BDNF) in the serum of diabetic retinopathy patients and in the retina of diabetic rats. Cell Mol Neurobiol 33, 359-367. https://doi.org/10.1007/s10571-012-9901-8

Ola, M.S., Alhomida, A.S., Ferrario, C.M., Ahmad, S., 2017. Role of tissue renin-angiotensin system and the chymase/angiotensin-(1-12) axis in the pathogenesis of diabetic retinopathy. Curr Med Chem 24, 3104-3114. https://doi.org/10.2174/0929867324666170407141955

Ola, M.S., Ahmed, M.M., Abuohashish, H.M., Al-Rejaie, S.S., Alhomida, A.S., 2013. Telmisartan ameliorates neurotrophic support and oxidative stress in the retina of streptozotocin-induced diabetic rats. Neurochem Res 38, 1572-1579. https://doi.org/10.1007/s11064-013-1058-4

Oyesiji Abiodun, A., AlDosari, D.I., Alghamdi, A., Aziz Al-Amri, A., Ahmad, S., Ola, M.S., 2023. Diabetes-induced stimulation of the renin-angiotensin system in the rat brain cortex. Saudi J Biol Sci 30, 103779. https://doi.org/10.1016/j.sjbs.2023.103779

Saavedra, J.M., 2012. Angiotensin II AT(1) receptor blockers ameliorate inflammatory stress: A beneficial effect for the treatment of brain disorders. Cell Mol Neurobiol 32, 667-681. https://doi.org/10.1007/s10571-011-9754-6

Saavedra, J.M., 1992. Brain and pituitary angiotensin. Endocr Rev 13, 329-380. https://doi.org/10.1210/edrv-13-2-329

Singh, V.P., Le, B., Khode, R., Baker, K.M., Kumar, R., 2008. Intracellular angiotensin II production in diabetic rats is correlated with cardiomyocyte apoptosis, oxidative stress, and cardiac fibrosis. Diabetes 57, 3297-3306. https://doi.org/10.2337/db08-0805

Teng, H.K., Teng, K.K., Lee, R., Wright, S., Tevar, S., Almeida, R.D., Kermani, P., Torkin, R., Chen, Z.Y., Lee, F.S., Kraemer, R.T., Nykjaer, A., Hempstead, B.L., 2005. ProBDNF induces neuronal apoptosis via activation of a receptor complex of p75NTR and sortilin. J Neurosci 25, 5455-5463. https://doi.org/10.1523/JNEUROSCI.5123-04.2005

Tran, S., Kuruppu, S., Rajapakse, N.W., 2022. Chronic renin-angiotensin system activation induced neuroinflammation: Common mechanisms underlying hypertension and dementia? J. Alzheimers. Dis. 85, 943-955. https://doi.org/10.3233/JAD-215231

van Thiel, B.S., Góes Martini, A., Te Riet, L., Severs, D., Uijl, E., Garrelds, I.M., Leijten, F.P.J., van der Pluijm, I., Essers, J., Qadri, F., Alenina, N., Bader, M., Paulis, L., Rajkovicova, R., Domenig, O., Poglitsch, M., Danser, A.H.J., 2017. Brain renin-angiotensin system: Does it exist?. Hypertension 69, 1136-1144. https://doi.org/10.1161/HYPERTENSIONAHA.116.08922

Vargas, R., Rincón, J., Pedreañez, A., Viera, N., Hernández-Fonseca, J.P., Peña, C., Mosquera, J., 2012. Role of angiotensin II in the brain inflammatory events during experimental diabetes in rats. Brain Res 1453, 64-76. https://doi.org/10.1016/j.brainres.2012.03.021

Waki, H., Gouraud, S.S., Maeda, M., Raizada, M.K., Paton, J.F., 2011. Contributions of vascular inflammation in the brainstem for neurogenic hypertension. Respir Physiol Neurobiol 178, 422-428. https://doi.org/10.1016/j.resp.2011.05.004

Wang, X.L., Iwanami, J., Min, L.J., Tsukuda, K., Nakaoka, H., Bai, H.Y., Shan, B.S., Kan-No, H., Kukida, M., Chisaka, T., Yamauchi, T., Higaki, A., Mogi, M., Horiuchi, M., 2016. Deficiency of angiotensin-converting enzyme 2 causes deterioration of cognitive function. NPJ Aging Mech Dis 2, 16024. https://doi.org/10.1038/npjamd.2016.24

Wang, J., Ho, L., Chen, L., Zhao, Z., Zhao, W., Qian, X., Humala, N., Seror, I., Bartholomew, S., Rosendorff, C., Pasinetti, G.M., 2007. Valsartan lowers brain beta-amyloid protein levels and improves spatial learning in a mouse model of Alzheimer disease. J Clin Invest 117, 3393-3402. https://doi.org/10.1172/JCI31547

Yamada, K., Horita, T., Takayama, M., Takahashi, S., Takaba, K., Nagata, Y., Suzuki, N., Kanda, T., 2011. Effect of a centrally active angiotensin converting enzyme inhibitor, perindopril, on cognitive performance in chronic cerebral hypo-perfusion rats. Brain Res 1421, 110-120. https://doi.org/10.1016/j.brainres.2011.09.016

Yen, F.S., Wei, J.C., Yip, H.T., Hwu, C.M., Hsu, C.C., 2022. Diabetes, hypertension, and the risk of dementia. J Alzheimers Dis 89, 323-333. https://doi.org/10.3233/JAD-220207

Yoshii, A., Constantine-Paton, M., 2010. Postsynaptic BDNF-TrkB signaling in synapse maturation, plasticity, and disease. Dev Neurobiol 70, 304-322. https://doi.org/10.1002/dneu.20765

Zhao, H.R., Jiang, T., Tian, Y.Y., Gao, Q., Li, Z., Pan, Y., Wu, L., Lu, J., Zhang, Y.D., 2015. Angiotensin II triggers apoptosis via enhancement of NADPH oxidase-dependent oxidative stress in a dopaminergic neuronal cell line. Neurochem Res 40, 854-863. https://doi.org/10.1007/s11064-015-1536-y

Zheng, J.L., Li, G.Z., Chen, S.Z., Wang, J.J., Olson, J.E., Xia, H.J., Lazartigues, E., Zhu, Y.L., Chen, Y.F., 2014. Angiotensin converting enzyme 2/Ang-(1-7)/mas axis protects brain from ischemic injury with a tendency of age-dependence. CNS Neurosci Ther 20, 452-459. https://doi.org/10.1111/cns.12233

Zuliani, G., Cherubini, A., Volpato, S., Atti, A.R., Blè, A., Vavalle, C., Di Todaro, F., Benedetti, C., Ruggiero, C., Senin, U., Fellin, R., 2005. Treatment with angiotensin-converting enzyme inhibitors is associated with a reduction in short-term mortality in older patients with acute ischemic stroke. J Gerontol A Biol Sci Med Sci 60, 463-465. https://doi.org/10.1093/gerona/60.4.463.