Molecular and Cellular Neuroscience 130 (2024) 103953



Contents lists available at ScienceDirect

Molecular and Cellular Neuroscience

journal homepage: www.elsevier.com/locate/ymcne





Activation of angiotensin converting enzyme 2 promotes hippocampal neurogenesis via activation of Wnt/β-catenin signaling in hypertension

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ARTICLE INFO

Keywords:
Hypertension
Neuroinflammation
Angiotensin converting enzyme 2
Diminazene Aceturate
Neurogenesis

ABSTRACT

Hypertension-induced brain renin-angiotensin system (RAS) activation and neuroinflammation are hallmark neuropathological features of neurodegenerative diseases. Previous studies from our lab have shown that inhibition of ACE/Ang II/AT1R axis (by AT1R blockers or ACE inhibitors) reduced neuroinflammation and accompanied neurodegeneration via up-regulating adult hippocampal neurogenesis. Apart from this conventional axis, another axis of RAS also exists i.e., ACE2/Ang (1–7)/MasR axis, reported as an anti-hypertensive and anti-inflammatory. However, the role of this axis has not been explored in hypertension-induced glial activation and hippocampal neurogenesis in rat models of hypertension. Hence, in the present study, we examined the effect of ACE2 activator, Diminazene aceturate (DIZE) at 2 different doses of 10 mg/kg (non-antihypertensive) and 15 mg/kg (antihypertensive dose) in renovascular hypertensive rats to explore whether their effect on glial activation, neuroinflammation, and neurogenesis is either influenced by blood-pressure. The results of our study revealed that hypertension induced significant glial activation (astrocyte and microglial), neuroinflammation, and impaired hippocampal neurogenesis. However, ACE2 activation by DIZE, even at the low dose prevented these hypertension-induced changes in the brain. Mechanistically, ACE2 activation inhibited Ang II levels, TRAF6-NFκB mediated inflammatory signaling, NOX4-mediated ROS generation, and mitochondrial dysfunction by upregulating ACE2/Ang (1–7)/MasR signaling. Moreover, DIZE-induced activation of the ACE2/Ang (1–7)/MasR axis upregulated Wnt/β-catenin signaling, promoting hippocampal neurogenesis during the hypertensive state. Therefore, our study demonstrates that ACE2 activation can effectively prevent glial activation and enhance hippocampal neurogenesis in hypertensive conditions, regardless of its blood pressure-lowering effects.

1. Introduction

Clinical trials and experimental investigations have demonstrated that hypertension is a significant risk factor for various CNS disorders, including stroke and AD, the most common form of dementia (Iadecola and Gottesman, 2019). Antihypertensive medications, such as angiotensin-converting enzyme inhibitors (ACEIs) and AT1 receptor blockers (ARBs), enhance memory functioning in hypertensive individuals, strengthening the link between hypertension and dementia (Ouk et al., 2021; Ho et al., 2021). Hypertension-induced glial cell-driven neuroinflammation is a hallmark neuropathological feature of neurodegenerative diseases (Guzman-Martinez et al., 2019). When activated, glial cells such as astrocytes and microglia trigger neuroinflammation by releasing inflammatory mediators like TNF-α, IL-1β, IL-6, and ROS, contributing to neuronal injury which ultimately leads to neurodegeneration (Kwon and Koh, 2020). Therefore, neuroinflammation, mediated by the activation of glia (microglia, astrocytes), is closely linked to the pathophysiology of neurodegenerative diseases like AD.

Further, neuroinflammation has been demonstrated to alter adult neurogenesis by regulating neural stem cell survival, proliferation, and maturation (Sung et al., 2020). Hence, neuroinflammation is one of the

Abbreviations: CNS, central nervous system; RAS, renin angiotensin system; HTN, hypertension; BP, blood pressure; ACE2, angiotensin converting enzyme 2; Ang II, angiotensin II; Ang (1–7), angiotensin (1–7); MasR, Mas receptor; AT1R, angiotensin type 1 receptor; DIZE, diminazene aceturate; AD, Alzheimer's disease; ROS, reactive oxygen species.

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https://doi.org/10.1016/j.mcn.2024.103953
Received 21 March 2024; Received in revised form 7 July 2024; Accepted 10 July 2024
Available online 14 July 2024
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possible mechanisms associated with hypertension and neurodegeneration. Hippocampal neurogenesis in the adult brain is a widely studied phenomenon due to its significant role in cognitive function. Nevertheless, there is much controversy regarding the effect of hypertension on adult hippocampal neurogenesis. Study conducted by Shih et al. and others has demonstrated a potential impairment of hippocampal neurogenesis during hypertension (Shih et al., 2016; Bhat et al., 2018; Pietranera et al., 2010). Apart from this, it has also been reported that hypertension promoted hippocampal neurogenesis (Perfilieva et al., 2001; Kronenberg et al., 2007). As a result, the impact of hypertension on neurogenesis remains uncertain and requires further investigation.

The key pathological mechanism that connects hypertension to neuroinflammation and subsequent neurodegeneration is the activation of the pro-inflammatory arm of Renin Angiotensin System (RAS) in the brain, specifically the Angiotensin Converting Enzyme/Angiotensin II/Angiotensin Type 1 receptor (ACE/Ang II/AT1R) pathway. Several studies have shown that inhibition of the ACE/Ang II/AT1R axis of RAS (by ARBs or ACEIs) is neuroprotective in the models of hypertension and neurodegeneration (Bhat et al., 2018; Goel et al., 2016; Tota et al., 2012). Further, neurodegeneration has been associated with impaired hippocampal neurogenesis. A previous study from our lab has reported that inhibition of ACE/Ang II/AT1R axis by AT1R blocker, Candesartan, independent of its BP-lowering property, reduced hypertension induced neurodegeneration and impaired adult hippocampal neurogenesis (Bhat et al., 2018). Apart from this conventional axis, another axis of RAS, i.e., Angiotensin Converting Enzyme 2/Angiotensin (1–7)/Mas receptor (ACE2/Ang (1–7)/MasR) axis, is reported as an anti-hypertensive and anti-inflammatory and hence imparted neuroprotection (Bennion et al., 2015). However, the neuroprotective role of this axis, either dependent or independent of their anti-hypertensive actions, in hypertension-induced glial activation and hippocampal neurogenesis in a rat model of chronic hypertension is still elusive and needs further exploration.

The discussion so far has demonstrated that hypertension-associated activation of the pro-inflammatory arm (ACE/Ang II/AT1R) of the brain RAS is the key pathogenetic mechanism linking hypertension to neuroinflammation and accompanying neurodegeneration, thereby impairing neurogenesis. However, the effect of another RAS axis, ACE2/Ang (1–7)/MasR, reported to be neuroprotective, has not been studied in detail on glial activation and neurogenesis in rat-model of chronic hypertension. Therefore, in this work, we studied the temporal activation of the glia in response to an increase in BP and their respective modulation by ACE2 activator, DIZE. Further, we dissected the involvement of ACE2 in hippocampal neurogenesis by using DIZE at both non-antihypertensive (10 mg/kg) and antihypertensive (15 mg/kg) doses.

2. Materials and methods

2.1. Reagents and antibodies

Diminazene Aceturate (DIZE) was purchased from Cayman Chemicals (USA). Primary antibodies, anti-β actin (Affinity biosciences), anti-GFAP (Merck), anti-Iba-1 (Abcam), anti-p65NFκB (Elabsciences), anti-IκB (Affinity biosciences), anti-TRAF6 (Abcam), anti-HO-1 (Santa-Cruz), anti-NRF2 (Santa-Cruz), anti-NOX4 (Santa-Cruz), anti-DCX (Invitrogen), anti-NeuN (Invitrogen), anti-BrdU (Merck), anti-Wnt-3A (Invitrogen), anti-p-Y-216-GSK-3β (Cell signaling technology), anti-p-S9-GSK-3β (Cell signaling technology), anti-β-catenin (Abcam), anti-p-β-catenin (Abcam), Alexa Fluor 594 and Alexa Fluor 488 IgG conjugate (Invitrogen) and secondary HRP-conjugated antibodies were purchased from Santa Cruz Biotechnology (USA).

2.2. Animals

Adult male Sprague-Dawley (SD) rats (180–200 g) procured from the Laboratory Animal Services Division of CSIR-Central Drug Research Institute (CDRI), Lucknow, India, were used in this study (approval no. IAEC/2019/17/REN). Experiments were performed according to internationally followed ethical standards and approved by the Institutional Animal Ethics Committee (IAEC) of CSIR- CDRI and Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), India. Rats were maintained under standard housing conditions (room temperature 24–27 °C and humidity 60–65 %) with a 12 h light and dark cycle. Food and water were available ad libitum. Twenty-eight rats were randomly divided into four groups (n = 7, for each): sham-operation, renovascular hypertension (HTN), HTN plus DIZE (10 mg/kg), and HTN plus DIZE (15 mg/kg).

2.3. Model of chronic hypertension

Chronic hypertension was induced in male SD rats (180–200 g), by partial occlusion of left renal artery, while in sham animals, renal artery was only exposed but not occluded as described previously (Bhat et al., 2018). Briefly, animals were anesthetized with pentobarbital (50 mg/kg i.p.). After a midline abdominal incision, the left renal artery was exposed and isolated over a short segment by a blunt dissection. After isolation, renal artery was constricted by making the loop of folded-in-two cotton thread tunneled through a plastic tube (0.5-mm internal diameter, 1.5–2.0-mm long). The artery was slowly inserted into the tube by pulling the ends of thread. The thread was tied in such a way that it did not constrict the artery but prevented its slippage from the plastic tube. Sham control rats underwent the same surgical procedure without the partial ligation of the renal artery. After surgery, Neosporin, an antifungal powder, and Betadine, a topical antiseptic, were applied at the site of the incision. The animals had access to food and water ad libitum.

2.4. Experimental plan and drug administration

After recovery of 1 week, animals were randomly distributed in following experimental groups: Group 1- Sham (normotensive); Group 2- Hypertensive (HTN); Group 3- HTN + DIZE (10 mg/kg, i.p.); Group 4- HTN + DIZE (15 mg/kg, i.p.). DIZE, an ACE2 activator, was freshly prepared daily in saline. DIZE at 10 and 15 mg/kg was administered intraperitoneally daily for 28 days starting from the first day after recovery (Fig. 1).

2.5. Measurement of hemodynamic parameters

BP of the rats was measured at 0, 14 and 28 days of DIZE treatment. Briefly, the rats were anesthetized with urethane (1.25 g/kg, i.p.) and placed on an isothermal pad to maintain normal body temperature during surgical procedures. The hemodynamic parameters like systolic blood pressure (SBP), diastolic blood pressure (DBP), mean arterial blood pressure (MAP), and heart rate (HR) were recorded by inserting fluid-filled catheters in the carotid artery. The catheter was attached to the pressure transducer coupled to the Data Acquisition System (Powerlab, AD Instruments, Australia). All animals were allowed to stabilize for 20 min before the final readings were taken. After taking hemodynamic parameters, the animals were sacrificed by transcardiac perfusion with ice-cold saline followed by 4 % paraformaldehyde in 0.1 M PBS. The brain was quickly removed for immunohistochemical studies as discussed below.

2.6. Cytokine estimation

The levels of cytokines TNF-α, and IL-10 were estimated using conventional ELISA kits (Elabscience, USA) by strictly following the manufacturer's guidelines. Cytokine level in brain tissues homogenate is expressed as pg of cytokines/mg protein (Bhat et al., 2017). In brief, hippocampus isolated from different groups were homogenized with Teflon homogenizer in phosphate buffered saline (PBS) having 5 % (w/v) protease inhibitor cocktail (Sigma). After homogenization, samples were sonicated for 10 s using an ultrasonic processor (Heat Systems-

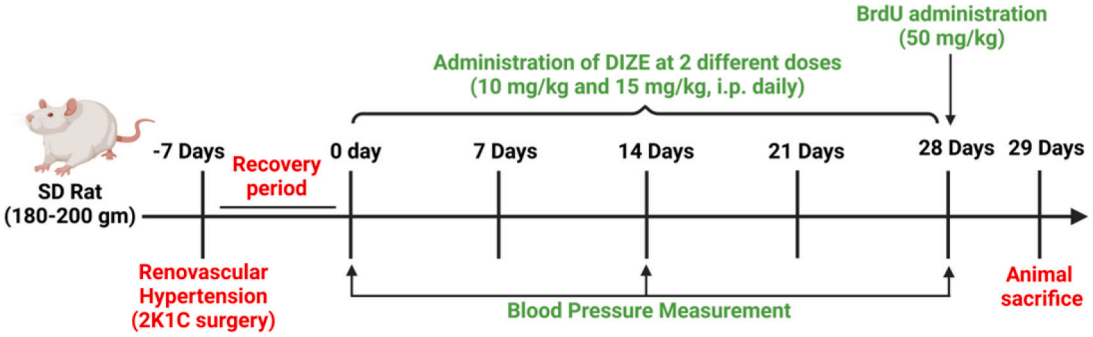


Fig. 1. Schematic representation of the experimental design.

Ultrasonic Inc.) at a setting of 20 duty cycles and then centrifuged at 20,000*g* for 15 min at 4 °C. The level of TNF-α and IL-10 were estimated in the supernatant according to the manufacturer's instructions.

2.7. Estimation of Ang II, Ang (1–7), and ACE2

The levels of Ang II, Ang (1–7) and ACE2 were estimated using conventional ELISA kits (Finetest, China) by strictly following the manufacturer's guidelines. Ang II, Ang (1–7) and ACE2 levels were estimated in hippocampal homogenate (Bhat et al., 2017). In brief, the hippocampus region from different treatment groups was homogenized in ice-cold PBS with 5 % (w/v) protease inhibitor cocktail (Sigma) followed by sonication (10 s, 20 cycles) using an ultrasonic processor (Heat Systems-Ultrasonic Inc.) and centrifugation at 20,000*g* (15 min) at 4 °C. The equal amount of supernatant was used to measure the level of Ang II, Ang (1–7) and ACE2. The data were expressed in the terms of pg/mg for brain tissue homogenate.

2.8. Estimation of ACE2 activity

ACE2 activity was estimated by a fluorometric assay kit in hippocampal homogenate as described by the manufacturer's instruction (AnaSpec, Sensolyte, USA). In brief, the hippocampus was homogenized in the assay buffer provided in the kit, followed by centrifugation at 20,000*g* (10 min) at 4 °C. The supernatant was used to estimate the ACE2 activity. Mca/Dnp fluorescence resonance energy transfer (FRET) test was performed under the fluorescent reader (Biotek, USA). Relative fluorescence unit (RFU), which was a ratio of fluorescence intensity at excitation/emission = 330 nm/390 nm were measured after 30 min of enzymatic activity. The results of ACE2 activity were calculated as RFU (Evans et al., 2020).

2.9. Estimation of reactive oxygen species (ROS)

2.9.1. By dichlorodihydrofluorescein diacetate (DCFDA)

DCFDA is a fluorogenic dye that measures hydroxyl, peroxyl and other reactive oxygen species (ROS) activity within the cell. Briefly, the hippocampus was digested with collagenase in HEPES solution at room temperature (RT). The obtained single-cell suspension was centrifuged at 500*g* for 5 min, and the resulting pellet was resuspended in HEPES and incubated with 10 μM DCFDA for 30 min at 37 °C for the estimation of cellular reactive oxygen species, using Fluorescence-activated cell sorting (FACS) Calibur (FACS Calibur, BD Bioscience). The cell population of interest was isolated with gating; data were analyzed and presented as the percentage of DCFDA-positive stained cell populations. Cells were treated with DMSO and 0.3 % H_2O_2 as negative and positive controls for oxidative stress, respectively.

2.9.2. By dihydroethidium (DHE)

DHE is a fluorescent dye for the detection of ROS generation and is specific for superoxide and hydrogen peroxide. The generation of reactive oxygen species (ROS) was determined by fluorescent-labeled dihydroethidium (DHE) in the cryosectioned brain hippocampus. Frozen slices (15 μm thick) were incubated in 5 μM DHE for 30 min at 37 °C in a dark humidified chamber. The sections were rinsed three times in PBS and were observed by using an inverted fluorescence microscope (Leica Microsystems, Germany) (Goel et al., 2016).

2.10. Estimation of mitochondrial membrane potential (MMP) and mitochondrial ROS (mtROS)

Briefly, the hippocampus was digested with collagenase in HEPES solution at RT. The obtained single-cell suspension was centrifuged at 500*g* for 5 min, and the resulting pellet was resuspended in HEPES and used for further studies. A viability test was done with trypan blue and 90 % of the viable population of cells was obtained. The difference in the mitochondrial membrane potential (Δψm) was determined by flow cytometry using JC-1 dye as described previously (Bhat et al., 2019). In brief, digested tissue suspension from different groups were incubated with JC-1 dye (2.5 μM) in a culture medium for 30 min at 37 °C. The cells were washed with PBS twice, and mitochondrial membrane potential (Δψm) was assessed using FACS Calibur (FACS Calibur, Becton Dickinson, NJ, USA) and analyzed by the Cell Quest program (Becton Dickinson, NJ, USA).

MitoSOX red (5 μM) was used to examine the mitochondrial ROS (mtROS) level by using flow cytometry (Bhat et al., 2019). The digested cells were incubated with MitoSOX red for 30 min. Finally, the cells were washed and data were acquired on a FACS Calibur (Becton Dickinson, NJ, USA) and quantified with the Cell Quest software.

2.11. Estimation of ATP

ATP was estimated in lysates from hippocampus of rat brain using ATP colorimetric/fluorometric assay kit (BioVision, Inc. California), according to manufacturer's instructions. Briefly, the hippocampus were lysed in 200 μl of ATP assay buffer and centrifuged at 15,000*g* for 2 min. To 50 μl of resulting supernatant, 50 μl of ATP reaction mixture (44 μl ATP assay buffer +2 μl ATP probe +2 μl ATP converter +2 μl developer mix) was added in a 96 well plate. After 30 min incubation, absorbance was measured at 570 nm in an ELISA reader (BIOTEK, USA). The concentration of ATP was calculated using ATP standard curve and expressed in nmols/mg protein.

2.12. Immunoblotting

Proteins extracted from brain hippocampus were processed for western blotting as described previously (Bhat et al., 2017). In brief, an equal amount of protein was separated on 10 % SDS-PAGE and transferred to the PVDF membrane. Membranes were blocked with blocking buffer (5 % BSA, 10 mM Tris pH 7.5, 100 mM NaCl, and 0.1 % tween-20) for 2 h. After blocking, the membranes were incubated with primary antibodies overnight at 4 °C. After washing, membranes were incubated

with respective HRP-conjugated secondary antibodies for 2 h at room temperature. The images were captured by Chemidoc (LAS 4010, GE Healthcare) and the band intensity was measured using spot densitometry analysis by My Image Analysis TM software (Thermo Scientific).

2.13. RNA preparation and RT-PCR

Quantitative gene expression analysis was performed by using SYBR Green technology as described previously. Briefly, the total RNA was extracted from hippocampus using TRIzol (Sigma, USA) isolation procedure and complementary DNA (cDNA) was synthesized using RevertAid™ H Minus first-strand cDNA synthesis kit following the manufacturer's protocol (Invitrogen, USA). Messenger RNA (mRNA) expression of MasR was quantified using the following primers: ACE2 (Forward- AATCGTAGGCTCTGGGCTTG and Reverse- GCTTCATAATCCCCTCGCCA), MasR (Forward- GATCGACAATCAACATCTAGGACTG and Reverse-AGACTGCATGTTTACCTGGCTG) and β-actin (Forward- CCCGCGAGTACAACCTTCT and Reverse- CGTCATCCATGGCGAACT). RT-PCR was carried out in QuantStudio 12 K Flex Real-Time PCR System (Applied Biosciences Indianapolis, USA). Relative mRNA expression was calculated by using comparative cycle threshold ($2^{-\Delta\Delta CT}$) method using β-actin as an internal standard and the relative amount of mRNA was presented in the form of fold change over control.

2.14. Bromodeoxyuridine (BrdU) injection

We used bromodeoxyuridine (BrdU) to label proliferating cells. On the 28th day of treatment, all groups of rats were treated with four intraperitoneal injections of BrdU (50 mg/kg in saline, Sigma-Aldrich, St. Louise, MO, USA) in a 4 h intervals because the half-life of BrdU is about 2 h and the number of BrdU+ cells peaks at about 15 h after BrdU injection and decreases afterwards (Bhat et al., 2018). Two hours after the last injection, the hemodynamic parameters were measured and rats were transcardially perfused with ice-cold 0.1 M PBS (pH 7.4) followed by 4 % paraformaldehyde in 0.1 M PBS. The brains were quickly removed for immunohistochemical studies as discussed below.

2.15. Immunohistochemistry

Brains were isolated, fixed overnight in 4 % PFA, and were serially incubated overnight in each 10 %, 20 % and 30 % sucrose solution at 4 °C. The serial coronal sections of hippocampal tissue of 15-μm thickness were collected by using cryostat (Micron International, GmbH, Germany) on the poly L-lysine coated slides. For BrdU staining, brains sections were washed with tris buffer saline (TBS, pH -7.5) followed by permeabilization with TBST (0.2 % Triton X-100) at room temperature for 60 min. For BrdU labeling, sections were incubated in 1 N − HCl for 10 min at 4 °C followed by incubation with 2 N − HCl at 37 °C for 20 min in order to DNA denaturation. These sections were incubated with borate buffer (0.1 M, pH 8.5) for 30 min in order to neutralize acidic reaction. Sections were blocked with 2.5 % BSA in TBST for 2 h at RT. The sections were incubated with primary antibodies, anti-mouse GFAP (1:100; Merck, USA), anti-goat Iba-1 (1:200; Abcam, USA), anti-mouse BrdU (1:200; Merck, USA), anti-rabbit DCX (1:300; Invitrogen, USA) and anti-rabbit NeuN (1:300; Abcam, USA) overnight. After rinsing with TBS, sections were incubated with Alexa Fluor 594 goat anti-mouse and Alexa Fluor 488 donkey anti-goat IgG conjugate (1:400) for 2 h. Finally, the sections were counterstained with DAPI and mounted with mounting media (Invitrogen). Brain sections were visualized using a fluorescence microscope (Leica Microsystems, Germany). The images were processed using the free image analysis software ImageJ (NIH, MD, USA) to measure the mean fluorescent intensity (Bhat et al., 2017).

2.16. Statistical analysis

Statistical analysis was performed with Prism software version 8.0 (Graph Pad Software, San Diego, CA, USA). All Results are expressed as mean ± SEM. Statistical significance was evaluated by one-way/two-way analysis of variance (ANOVA) followed by Tukey's multiple comparison test. A value of $p < 0.05$ was considered as statistically significant.

3. Results

3.1. ACE2 activator, DIZE, prevented the increase in the BP in the renovascular model of hypertension

Several studies have reported that renovascular hypertensive rats develop a significant increase in BP between 3 to 5 weeks after surgery (Campos et al., 2015; Bhat et al., 2018). Therefore, to study the effect of ACE2 activation on BP, preventive dosing with DIZE for 28 days was performed, after 1 week of recovery following surgery. Hemodynamic parameters such as systolic blood pressure (SBP) and heart rate (HR) were measured at 0, 14 and 28 days of treatment to monitor temporal changes in BP in a rat model of renovascular hypertension. A significant increase in SBP was observed on 14th day which remained raised until the end of 28th day in renovascular hypertensive rats (Fig. 2A). Furthermore, HR significantly increased at 28th days compared to that in sham (normotensive) rats (Fig. 2B). In contrast, administration of DIZE at an anti-hypertensive dose of 15 mg/kg prevented the rise of blood pressure in rats with renovascular hypertension. However, a dose of 10 mg/kg DIZE did not affect blood pressure, confirming that it was a non-antihypertensive dose (Fig. 2A and B). Therefore, both doses of DIZE were used for further studies to determine whether its beneficial effects are dependent or independent of its BP-lowering effect.

3.2. ACE2 activation ameliorated hypertension-induced astrocyte and microglial activation

To examine the time-dependent effect of ACE2 activator, DIZE on BP-induced glial activation markers, we performed immunohistochemical analysis of the hippocampus at 0, 14th and 28th days of treatment. The hippocampal region of the brain, which is associated with memory, shows increased activation of glial cells in response to hypertension, as evidenced by higher immunostaining levels of GFAP (an astroglia marker) and Iba-1 (a microglia marker) (Fig. 3A and B). Further, astrocytes had thin and fine processes with small cell body in normotensive sham rats; however, at 14th and 28th days after surgery, in the hippocampus of renovascular hypertensive rats, the astrocytes became hypertrophied (thick and dense processes and enlarged cell body) with intense GFAP immunostaining (Fig. 3A). In contrast to sham rats, where microglia have a non-activated/resting ramified shape with profusely branched processes, hypertensive rats showed activated microglia with an amoeboid shape and decreased branch length in the hippocampus region on 14th and 28th days of hypertension (Fig. 3B). The most noticeable changes in astrocyte and microglial morphology were observed after 28 days of hypertension. Treatment with DIZE at either a non-antihypertensive dose (10 mg/kg) or an anti-hypertensive dose (15 mg/kg) prevented astrocytic and microglial activation, as evidenced by decreased GFAP/Iba-1 immunostaining and reversed their respective morphological changes, such as increased hypertrophy in astroglia and decreased ramification in microglia in the hippocampus of hypertensive rats in a time-dependent manner (Fig. 3A and B).

3.3. ACE2 activation prevented hypertension-induced imbalance in inflammatory cytokines

In the hippocampus of hypertensive rats, levels of pro-inflammatory TNF-α increased over time while the level of the anti-inflammatory

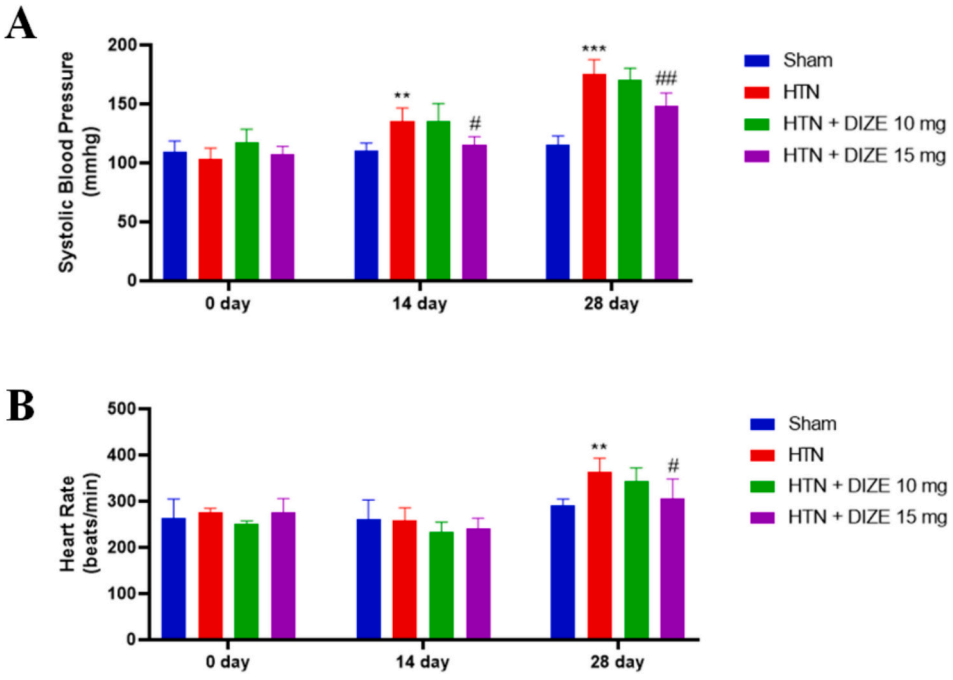


Fig. 2. ACE2 activator, DIZE, prevented the increase in BP in the established model of hypertension. The bar graphs show the quantification of relative hemodynamic parameters after treatment with DIZE in renovascular hypertensive rats. (A) Systolic blood pressure (B) Heart rate. Data are expressed as mean ± SEM of n = 8. Data were analyzed by two-way ANOVA, followed by Tukey's test (*P < 0.05, **P < 0.01, ***P < 0.001, $^{\#}$P < 0.05, $^{\#\#}$P < 0.01, $^{\#\#\#}$P < 0.001) *Sham vs HTN, $^{\#}$HTN vs HTN + DIZE.

cytokine IL-10 decreased in a time-dependent manner (Fig. 4A and B), similar to the markers of glial activation. Treatment with DIZE, either at non-antihypertensive (10 mg/kg) or an antihypertensive (15 mg/kg) dose, prevented the imbalance of inflammatory cytokines in the hippocampus region of the rat brain in a time-dependent manner, as evidenced by decreased levels of TNF-α and increased levels of IL-10 (Fig. 4A and B).

3.4. ACE2 activator, DIZE, up regulates ACE2/Ang (1–7)/MasR axis

Since we observed a significant and robust activation of glia and inflammatory imbalance at 28th day, therefore, the further estimations were done at this time point to elucidate the mechanism of glial activation by modulation of ACE2/Ang (1–7)/MasR axis in the rat model of renovascular hypertension. We found that ACE2 activator DIZE significantly blunted the effect of hypertension on ACE2 mRNA expression and activity and increased both of them (Fig. 5A and B). Further, treatment with DIZE also significantly up regulated MasR mRNA expression (Fig. 5C) and Ang (1–7) level (Fig. 5D) while reduced the Ang II level (Fig. 5E), hence increasing Ang (1–7)/Ang II ratio (Fig. 5F) in hippocampus region of the brain.

3.5. ACE2 activation prevented hypertension induced TRAF6-NFκB inflammatory signaling

Hypertension induced neuroinflammatory signaling is responsible for neuronal damage, synaptic dysfunction, and cognitive impairment (Ungvari et al., 2021). TRAF6-NFκB signaling plays a crucial role in neuroinflammation by inducing several pro-inflammatory responses in the brain (Dou et al., 2018). TRAF6 is a protein that functions as an E3 ubiquitin ligase and is involved in the activation of the NFκB signaling pathway. The activation of NFκB, which regulates the expression of various inflammatory mediators, is central to astrocyte and microglial activation (Bhat et al., 2017). This activation is typically prevented by IκB-α, which acts as a physiological inhibitor of NFκB and keeps it in an inactive state. In the present study, hypertension induced TRAF6 expression (Fig. 6A), resulting in the degradation of IκB-α (Fig. 6B) and the subsequent release of NFκB (increased expression of p65-NFkB expression) (Fig. 6C) in the hippocampus. Treatment with DIZE at both doses (10 mg/kg and 15 mg/kg) prevented the activation of the TRAF6-NFκB signaling cascade, as evidenced by reduced expression of TRAF6, stabilized IκB-α, and suppressed NFκB expression in the brain (Fig. 6A–C).

3.6. Activation of ACE2 prevented the increased oxidative stress burden in hypertension

Oxidative stress is one of the key fundamental mechanisms responsible for the development of hypertension (Dou et al., 2018). NADPH oxidase 4 (NOX4) is a major source of ROS generation, leading to increased production of hydroxyl and superoxide ROS in the brains of hypertensive rats. Hence, the effect of ACE2 on these markers were studied. Hypertension resulted in a significant up regulation of NOX4 (Fig. 7A) and DHE (Fig. 7D) expressions as well as increased number of DCFDA^{+} cells (Fig. 7E) in hippocampus region of the brain. However, DIZE at both doses attenuated the hypertension-induced ROS generation as evident by reduced DHE expression and DCFDA+ cell number in brain of hypertensive rats (Fig. 7D and E). Hypertension cause significant down regulation of anti-oxidative defense mechanism (Tarafdar and Pula, 2018) as evident from reduced expression of NRF2 (Fig. 7B) and HO-1 (Fig. 7C) in the brain, as compared to the sham group. On the contrary, DIZE at both non-antihypertensive (10 mg/kg) as well as antihypertensive (15 mg/kg) doses reversed the effect of hypertension on NOX4, NRF2, and HO-1 in the hippocampus (Fig. 7A–C).

3.7. ACE2 activation improved mitochondrial dysfunction

Mitochondrial dysfunction has been implicated in various neurogenerative diseases. Therefore, we examined the mitochondrial functions following DIZE treatment in hypertensive rats. Our findings indicate that hypertensive rats exhibited impaired mitochondrial functions, as evidenced by an increase in depolarized mitochondria (Fig. 8A

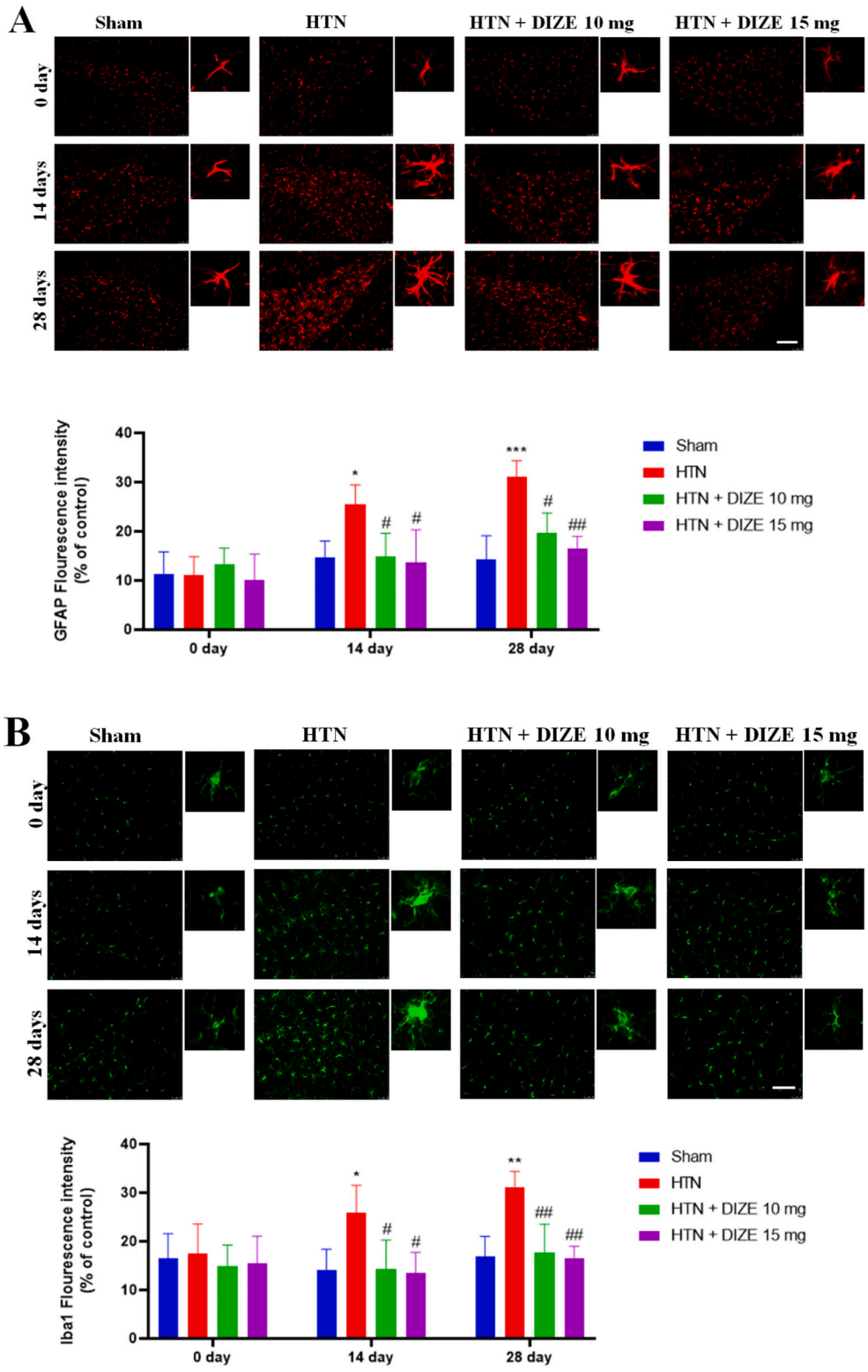


Fig. 3. ACE2 activation ameliorated hypertension-induced astrocyte and microglial activation. Representative photomicrographs show immunostaining of (A) GFAP and, (B) Iba1 in hippocampus region of rat brain at 0, 14 and 28 days of treatment. The bar graph shows quantification of immunofluorescence of GFAP and Iba1 in hippocampus. Scale bar, 50 μm. Data are expressed as mean ± SEM of n = 4–5. Data were analyzed by two-way ANOVA, followed by Tukey's test (*P < 0.05, **P < 0.01, ***P < 0.001, $^{\#}$P < 0.05, $^{\#\#}$P < 0.01, $^{\#\#\#}$P < 0.001). *Sham vs HTN, $^{\#}$HTN vs HTN + DIZE.

and C), an increase in mitochondrial ROS (as indicated by increased MitoSOX Red fluorescence) (Fig. 8B and D), and a decrease in cellular ATP levels (Fig. 8E). On the other hand, treatment with DIZE prevented hypertension-induced mitochondrial dysfunction in the hippocampus region of the brain, as shown by an increase in ATP levels, a high mitochondrial membrane potential, and suppression of mitochondrial ROS (Fig. 8A–E).

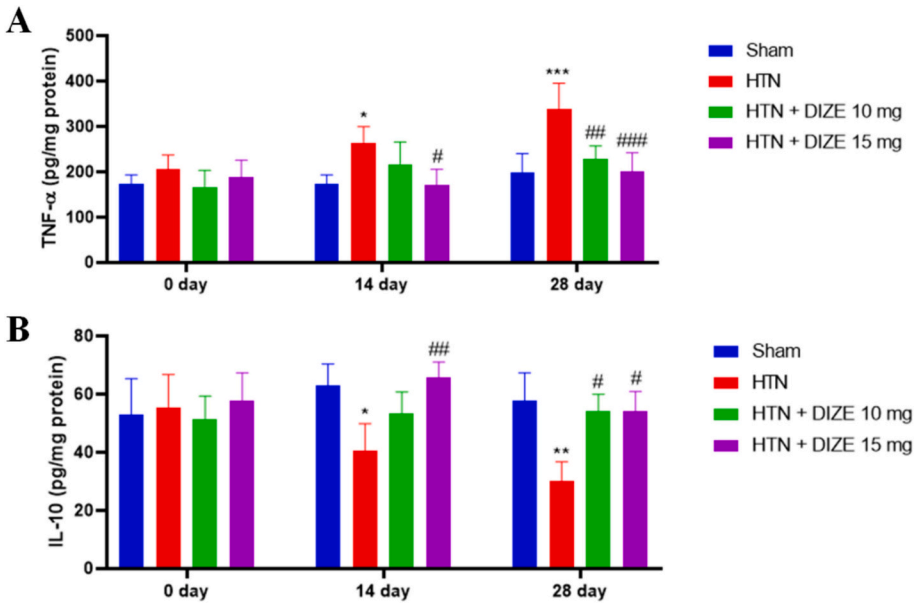


Fig. 4. ACE2 activation prevented hypertension-induced imbalance in inflammatory cytokines. The bar graphs show quantification of relative level of (A) TNF-α and, (B) IL-10 in hippocampus region of rat brain at 0, 14 and 28 days of treatment. Data are expressed as mean ± SEM of n = 4–5. Data were analyzed by two-way ANOVA, followed by Tukey's test (*P < 0.05, **P < 0.01, ***P < 0.001, $^{\#}$P < 0.05, $^{\#\#}$P < 0.01, $^{\#\#\#}$P < 0.001). *Sham vs HTN, $^{\#}$HTN vs HTN + DIZE.

3.8. ACE2 activation promoted hippocampal neurogenesis in the hypertensive rats

In the hippocampus of hypertensive rats, the double-immunostaining of BrdU (a marker of cell proliferation) and DCX (a marker of immature neurons) revealed a notable decrease in the number of BrdU+/DCX+ co-labeled cells compared to the sham group (Fig. 9A). Interestingly, DIZE was found to increase the expression of BrdU+/DCX+ co-labeled cells and the number of DCX+ cells in the hippocampus of hypertensive rats, independent of its blood pressure-regulating action (Fig. 9A). Further, the double-immunostaining of BrdU with NeuN, a marker of mature neurons showed that the number of $BrdU^{+}/NeuN^{+}$ co-labeled cells was significantly reduced in the hippocampus of hypertensive rats as compared to sham (Fig. 9B). However, DIZE treatment significantly up regulated $BrdU^{+}/NeuN^{+}$ co-labeled cells in the hippocampus of hypertensive rats (Fig. 9B). Taken together, these data clearly indicate that activation of ACE2 promotes hippocampal neurogenesis in hypertensive rats.

3.9. ACE2 activation promoted the Wnt/β-Catenin signaling in the hypertensive rats

The Wnt/β-Catenin pathway has been found to involved in the survival, proliferation, and differentiation of neuronal precursors (Maguschak and Ressler, 2012). This signaling pathway functions by inhibiting GSK-3β, thereby stabilizing cytosolic β-catenin and preventing its ubiquitination and subsequent proteasome degradation. Therefore, the potential link between hypertension and Wnt signaling has been studied. We observed that the expression of Wnt-3A decreased in response to hypertension, indicating impaired Wnt/β-catenin signaling (Fig. 10A). In the hippocampus of hypertensive rats, it was observed that there was an increase in the expression of GSK-3β, as indicated by an increase in the phospho-Y216/phospho-S9 GSK-3β ratio (Fig. 10B–D), which resulted in the degradation of β-catenin, indicated by an increase in its phosphorylation (Fig. 10E). Furthermore, there was a reduction in the nuclear translocation of β-catenin (Fig. 10F). In contrast, even at a low dosage (10 mg/kg), DIZE facilitated the activation of Wnt-3A, suppressed GSK-3β activation, and enhanced the stabilization and increased nuclear translocation of β-catenin in the hippocampus of hypertensive rats (Fig. 10A–F).

4. Discussion

Hypertension is reported to increase the susceptibility to neurodegeneration (Ben-Shabat et al., 2023). The ACE/Ang II/AT1R axis of RAS plays a critical role in hypertension induced neuroinflammatory and neurodegenerative changes (Abiodun and Ola, 2020). Previous studies from our lab and by others showed that inhibition of ACE/Ang II/AT1R axis imparted neuroprotection by attenuating glial activation and neuroinflammation and enhanced hippocampal neurogenesis in model of hypertension and neurodegeneration (Bhat et al., 2018; Goel et al., 2015; Kuber et al., 2023). However, the involvement of the counter regulatory axis of RAS, i.e. ACE2/Ang (1–7)/MasR has not been explored in hypertension-induced glial activation and impaired hippocampal neurogenesis. The findings of our study suggest that DIZE, an ACE2 activator, can activate the ACE2/Ang (1–7)/MasR pathway and mitigate the adverse effects of hypertension on the brain, including neuroinflammation, astrocytes and microglial activation, and impaired hippocampal neurogenesis, independent of its BP-lowering action. Mechanistically, ACE2 activation prevented the NOX4-induced oxidative stress, TRAF6-NFκB induced inflammation and mitochondrial dysfunction while promoting the Wnt/β-catenin signaling in hypertensive rats.

The ACE2/Ang (1–7)/MasR axis of RAS is known for its anti-hypertensive property (Liao and Wu, 2021). Hence, we studied the effect of ACE2 activator, DIZE on hemodynamic parameters in renovascular model of hypertension. Results showed that rats undergone renovascular surgery started showing a rise in BP from 3rd week of surgery as reported by Bhat et al. (Bhat et al., 2018, 2017). Also, treatment of hypertensive rats with lower dose of ACE2 activator, DIZE (10 mg/kg) did not affect any of the hemodynamic parameter following renovascular surgery but significantly decreased the same at 15 mg/kg dose of DIZE. Therefore, the dose of 10 mg/kg and 15 mg/kg DIZE were chosen to study if the beneficial effects of ACE2 activator, DIZE are either dependent or independent of its BP-lowering property. Earlier studies have also reported anti-hypertensive effect of DIZE in other models of hypertension (Sartorio et al., 2020; De Maria et al., 2016).

It is important to note that the brain is a significant target organ

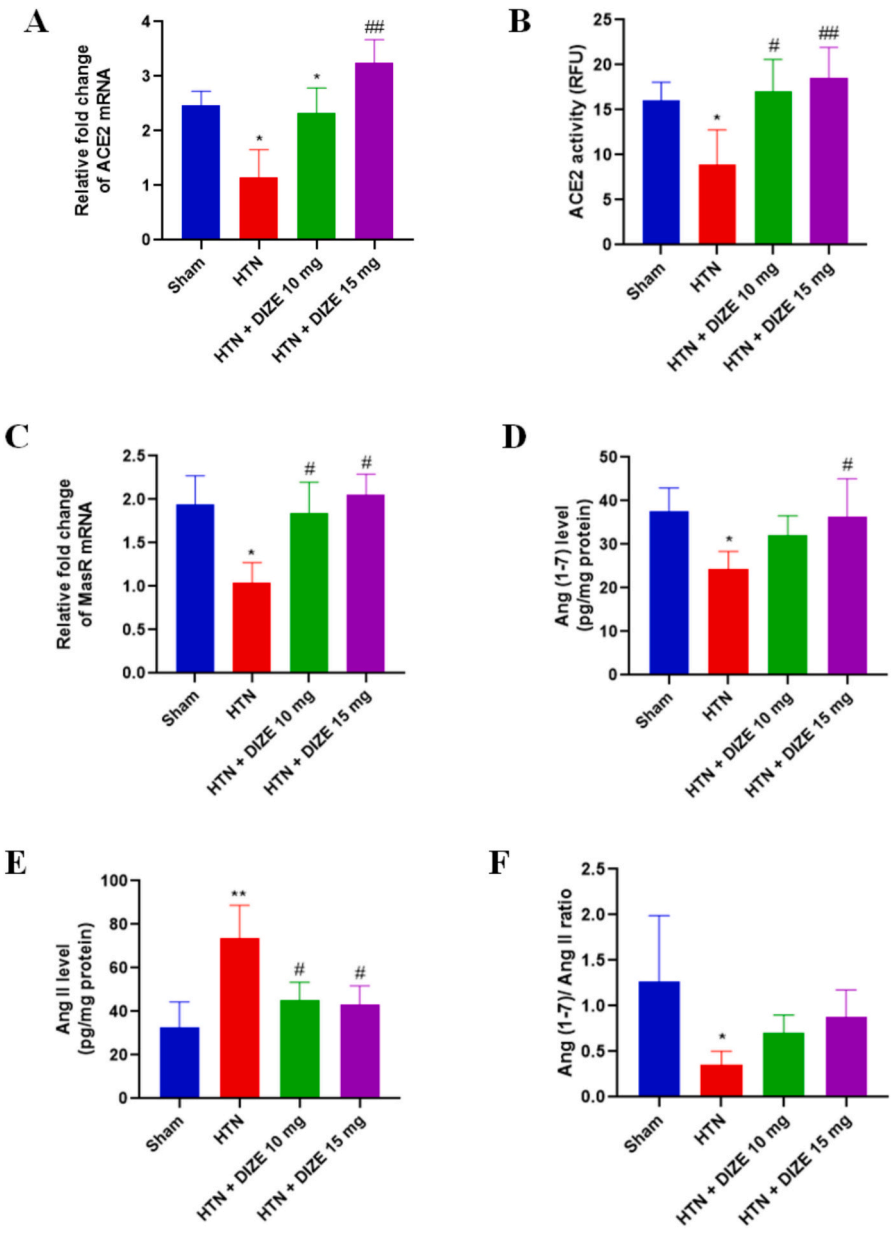


Fig. 5. ACE2 activator, DIZE upregulates ACE2/Ang (1–7)/MasR axis. The bar graphs show quantification of the relative level of (A) ACE2 mRNA expression, (B) ACE2 activity, (C) MasR mRNA expression, (D) Ang (1–7) level, (E) Ang II level, (F) Ang (1–7)/Ang II ratio in hippocampus region of rat brain. Data are expressed as mean ± SEM of n = 4–5. Data were analyzed by one-way ANOVA, followed by Tukey's test (*P < 0.05, **P < 0.01, ***P < 0.001, $^{\#}$P < 0.05, $^{\#\#}$P < 0.01, $^{\#\#\#}$P < 0.001). *Sham vs HTN, #HTN vs HTN + DIZE.

affected by high blood pressure, despite often being overlooked (Goel et al., 2015). Numerous studies have demonstrated that hypertension can contribute to the onset and progression of cognitive impairment by triggering neuroinflammatory processes in the brain (Ungvari et al., 2021; Bajwa and Klegeris, 2022; Santisteban et al., 2023). Hypertension induced inflammatory responses in brain are mainly orchestrated by the activation of astrocytes and microglia (Bhat et al., 2018). On similar lines, we found increased glial activation (astrocyte and microglial activation along with the morphological transformation) in hippocampus region of hypertensive rat brain which was significantly down regulated following DIZE treatment, independent of BP lowering effect. Similarly, the previous studies have demonstrated that activation of ACE2/Ang (1–7)/MasR axis is associated with reduced activation of astrocytes and microglia in model of LPS-induced neuroinflammation and 6-OHDA induced neurodegeneration (P. Tiwari et al., 2023; Gupta et al., 2022).

To mediate the inflammatory responses in brain, the activated glial cells can release various pro-inflammatory cytokines, including TNF-α, IL-1β, and IL-18 (P. Tiwari et al., 2023). Further, cytokines, particularly pro-inflammatory cytokines, also found to be involved in the development and progression of hypertension (Wen and Crowley, 2018). Therefore, in line with the temporal activation of astrocytes and microglia, we observed an increase in pro-inflammatory TNF-α and a decrease in anti-inflammatory IL-10 in the hippocampus of hypertensive rats. Further, activation of ACE2 by DIZE, even at the low dose (non-antihypertensive), reverted the effect of hypertension on these markers. Similarly, other studies have also reported that ACE2 activation by DIZE, prevented astrocyte and microglial activation and subsequent imbalance in inflammatory cytokines in models of hypertension and neurodegeneration (P. Tiwari et al., 2023; Sun et al., 2023). These results indicate that the anti-inflammatory action of ACE2 activation is independent of its blood pressure-lowering effect.

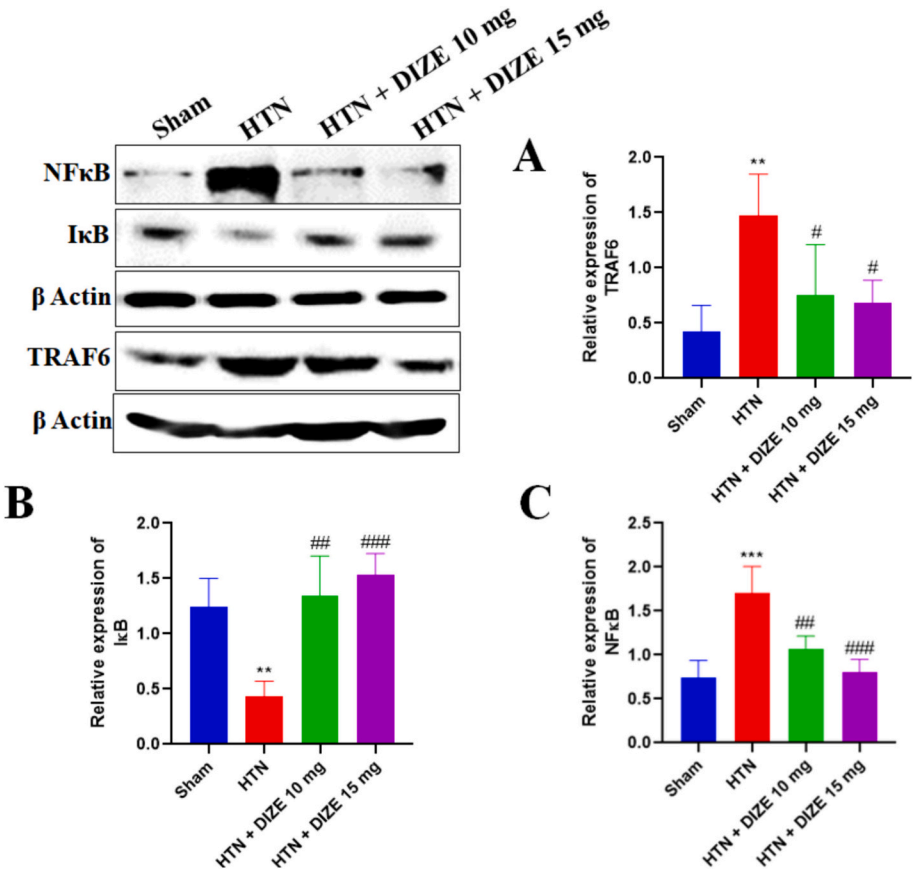


Fig. 6. ACE2 activation prevented hypertension induced TRAF6-NFκB inflammatory signaling. Representative immunoblot of (A) TRAF6, (B) IκB and, (C) NFκB in hippocampus region of rat brain. Data are expressed as mean ± SEM of n = 3–4. Data were analyzed by one-way ANOVA, followed by Tukey's test (*P < 0.05, **P < 0.01, ***P < 0.001, #P < 0.05, ##P < 0.01, ###P < 0.001). *Sham vs HTN, #HTN vs HTN + DIZE.

Renin angiotensin system (RAS) plays a vital role in maintaining physiological homeostasis in the brain (P. Tiwari et al., 2023). There is strong evidence to suggest that hypertension causes an over-activation of the ACE/Ang II/AT1R axis while simultaneously suppressing the ACE2/Ang (1–7)/MasR axis (Yim and Yoo, 2008; Ibrahim, 2006). In line with these findings, we also observed a reduction in the mRNA expression of ACE2 and MasR, as well as a decrease in Ang (1–7) level and ACE2 activity in the hippocampus of hypertensive rats. However, treatment with DIZE, the ACE2 activator, increased these parameters, even at non-antihypertensive doses, indicating its potential to modulate the RAS axis in the brain. Other studies are also in sync with our findings and reported that ACE2 activation can restore ACE2 and MasR expression, as well as Ang (1–7) levels thereby exhibiting a protective effect against neuroinflammatory changes in various models of neurological disorders, including hypertension, cerebral ischemia, stroke, and Alzheimer's disease (Bennion et al., 2015; P. Tiwari et al., 2023; V. Tiwari et al., 2023; Jiang et al., 2012).

Ang II, which belongs to the pro-inflammatory branch of the renin-angiotensin system (RAS) known as ACE/Ang II/AT1R axis, counteracts the effects of the ACE2/Ang (1–7)/MasR axis and contributes to neural injury, neuroinflammation, and cognitive impairment (P. Tiwari et al., 2023). Elevated levels of Ang II have been observed in hypertension and neurodegeneration models (Abiodun and Ola, 2020). Likewise, we found an increased level of Ang II in hippocampus of hypertensive rat brain which was significantly down regulated after DIZE treatment. Previous studies have also supported this observation by showing a decrease in Ang II levels after DIZE treatment in mouse models of AD, as well as in cases of LPS-induced neuroinflammation (Evans et al., 2020; P. Tiwari et al., 2023).

Glial activation is always associated with inflammatory signaling which plays a critical role in hypertension induced neurodegenerative changes (Bajwa and Klegeris, 2022). One of the primary pathways that contributes to inflammation is the activation of TRAF6, which regulates the expression of various cytokines, chemokines, and other factors that are involved in mediating and regulating the inflammatory process (Abdullah et al., 2018). Furthermore, there is also evidence showing that TRAF6 can mediate the inflammatory response through NF-κB signaling pathway (Dou et al., 2018). In concurrence with this, we found increased TRAF6 and NF-κB and reduced IκB expressions, inhibitor of NF-κB, in hippocampus of hypertensive rat brain which were reversed by ACE2 activation following DIZE treatment. In line with this, other studies have also reported that ACE2 activation reduced TRAF6 and NF-κB expressions in macrophages and model of AD, respectively (V. Tiwari et al., 2023; Shang and Wu, 2020).

Neuroinflammation leads to oxidative stress, resulting in reduced cellular antioxidant capacity and ultimately contributing to neurodegeneration (Uttara et al., 2009). Several reports have suggested that the activation of NADPH oxidase 4 (NOX4) mediated ROS generation and down-regulation of NRF2/HO-1 signaling are pathological hallmarks of glial activation during neuroinflammatory or neurodegenerative diseases such as AD and PD (Boonpraman et al., 2023). In the present study, we observed an association between hypertension-induced astrocyte and microglial activation and increased activation of NOX4, leading to elevated levels of ROS production (including superoxide and peroxynitrite). We also noted a reduction in NRF2 and HO-1 expression levels. However, even at a low dose of 10 mg/kg, ACE2 activation by DIZE prevented hypertension-induced NOX4 activation, ROS generation, and reductions in NRF2 and HO-1 levels in the brains of hypertensive rats. Similarly, previous studies have reported that ACE2 activation by DIZE resulted in reduced oxidative stress in rodent models

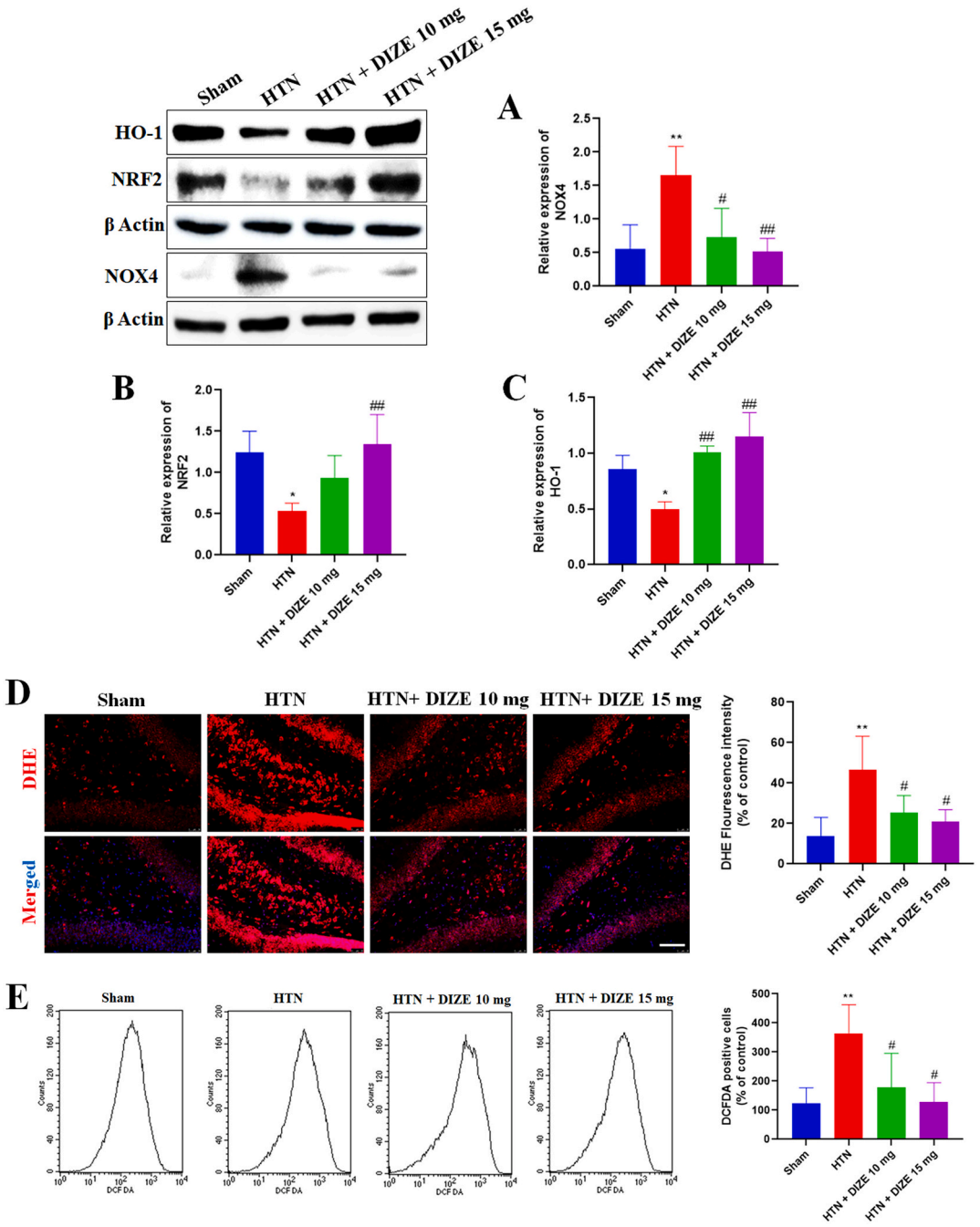


Fig. 7. Activation of ACE2 prevented the increased oxidative stress burden in hypertension. Representative immunoblot of (A) NOX4, (B) NRF2 and, (C) HO-1 in hippocampus region of rat brain. (D) Representative photomicrographs show DHE stained cells in the hippocampus region of the rat brain. The bar graph shows the fluorescence intensity of DHE stained cells. (E) Representative photomicrographs show ROS positive cells in the hippocampus region of the rat brain. The bar graph shows the fluorescence intensity of DCFDA stained cells. Scale bar- 50 μm. Data are expressed as mean ± SEM of n = 3–4. Data were analyzed by one-way ANOVA, followed by Tukey's test (*P < 0.05, **P < 0.01, ***P < 0.001, $^{\#}$P < 0.05, $^{\#\#}$P < 0.01, $^{\#\#\#}$P < 0.001). *Sham vs HTN, $^{\#}$HTN vs HTN + DIZE.

of dementia as well as in vitro cultures of astrocytes and microglia (P. Tiwari et al., 2023; V. Tiwari et al., 2023). Conclusively, these results suggest that ACE2 activation, independent of its BP lowering effect, possesses anti-oxidative property.

Oxidative stress has a detrimental effect on mitochondrial health, serving as a mechanism that links inflammation to cellular damage in both neuroinflammatory and neurodegenerative diseases (Picca et al., 2020). Studies indicate that hypertension is linked to increased mitochondrial membrane depolarization (i.e., a decrease in MMP), the generation of mitochondrial ROS (mtROS), and decreased ATP levels (Eirin et al., 2014). In line with this, we observed increased mitochondrial depolarization, mtROS as well as reduced ATP level during hypertension. Activation of ACE2 by DIZE restored the MMP, mtROS, and ATP level, indicating an improvement in mitochondrial function. These findings are consistent with previous studies that have reported improved mitochondrial function in endothelial cells and pancreatic β cells following exosomal-mediated ACE2 transfer (Wang et al., 2020; Shi et al., 2018).

In addition to neuroinflammatory changes, hypertension has also been shown to be responsible for impaired neurogenesis, which has been implicated in the pathogenesis of Alzheimer's disease (Bhat et al., 2018). In this study, we found evidence suggesting that hypertension has a

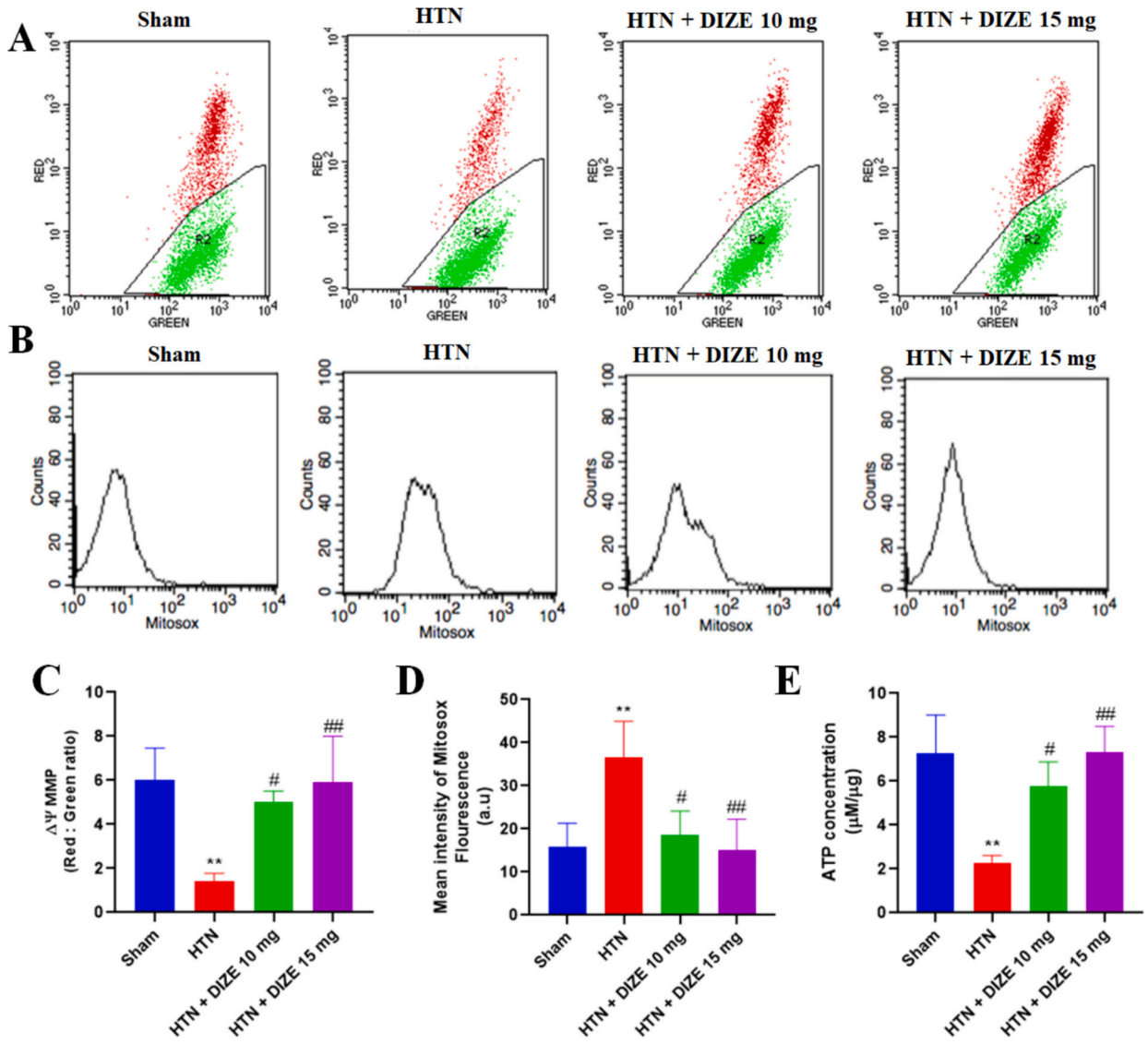
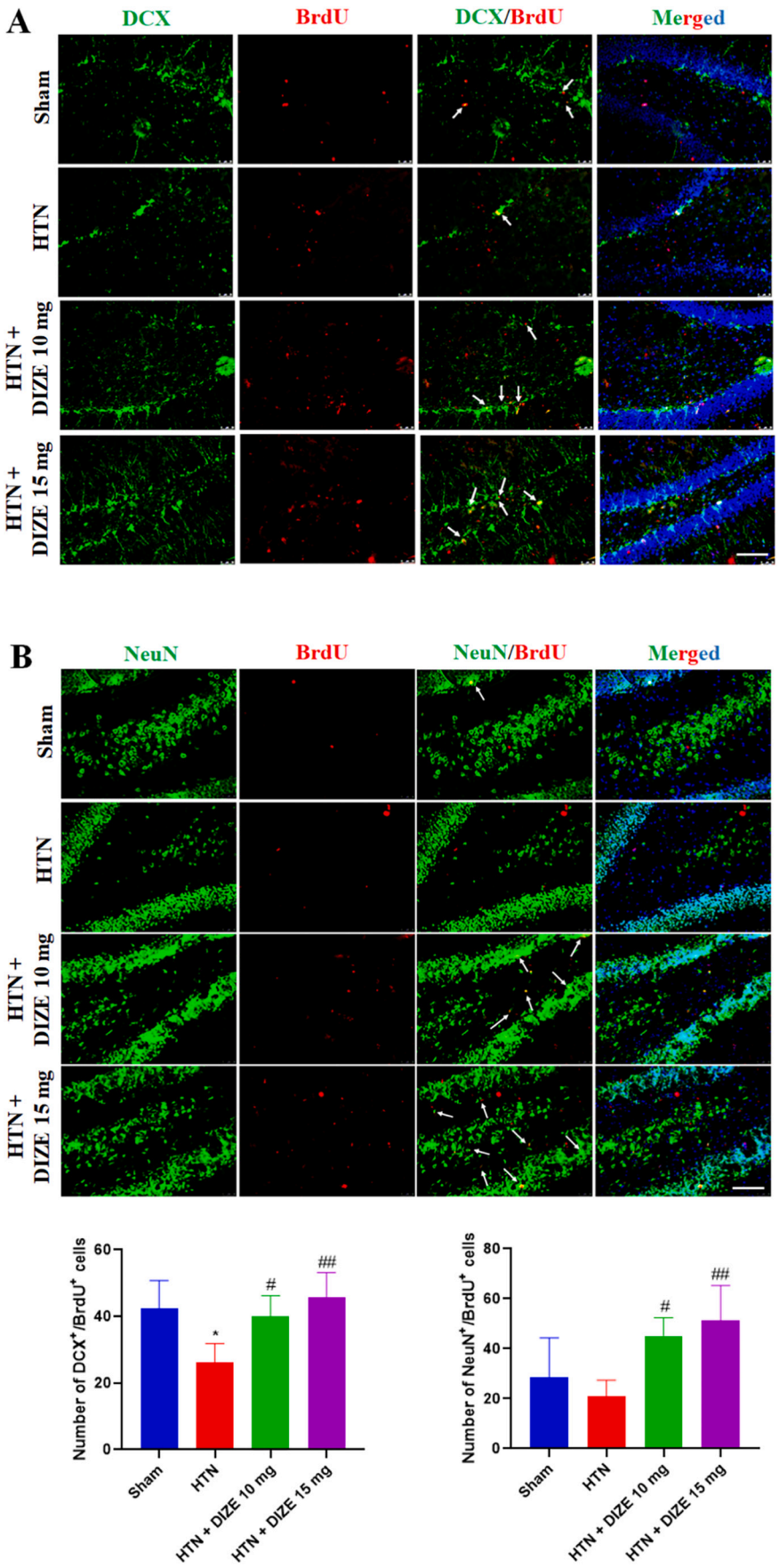


Fig. 8. ACE2 activation improved mitochondrial dysfunction. Representative photomicrographs show (A) MMP and, (B) mtROS in the hippocampus region of the rat brain as quantified using flow cytometry. The representative bar graph shows quantification of (C) MMP, (D) mtROS and, (E) ATP level in hippocampus. Data are expressed as mean ± SEM of n = 3–4. Data were analyzed by one-way ANOVA, followed by Tukey's test (*P < 0.05, **P < 0.01, ***P < 0.001, $^{\#}$P < 0.05, $^{\#\#}$P < 0.01, $^{\#\#\#}$P < 0.001). *Sham vs HTN, #HTN vs HTN + DIZE.

negative impact on adult hippocampal neurogenesis, as indicated by a reduction in the number of DCX+ and NeuN+ cells (markers of immature and mature neurons, respectively) that colocalized with BrdU (a marker of cell proliferation). ACE2 activation by DIZE reversed hypertension-induced impaired neurogenesis markers by increasing the number of cells positive for DCX and NeuN that were also positive for BrdU, indicating improved proliferation and differentiation of new neurons in the hippocampus. Similarly, previous report demonstrated decreased expression of neurogenesis markers in the brain of ACE2 deficient rat brain hippocampus (Klempin et al., 2018). Therefore, we conclude that ACE2 promotes neurogenesis in a hypertensive state.

In addition to neurogenic factors, the Wnt/β-catenin signaling pathway also plays a crucial role in neurogenesis (Zhang et al., 2011). Hypertension and neurodegeneration have been reported to impair hippocampal neurogenesis (Vallee et al., 2022). In our current investigation, we also observed a decline in the expression of Wnt-3A, a gene specific to the Wnt signaling pathway, within the hippocampus of hypertensive rats. Furthermore, we noted an elevation in the activation of GSK-3β (indicated by an increased p-Y216-GSK-3β/GSK-3β ratio and a decreased p-S9-GSK-3β/GSK-3β ratio), a pivotal component of the Wnt/β-catenin pathway responsible for promoting the degradation of β-catenin in the hippocampus of hypertensive rats. In contrast, ACE2 activation by DIZE even at a low dose of 10 mg/kg, promoted the expression of Wnt-3A, attenuated GSK-3β activation, and promoted the stabilization of cytosolic β-catenin in the hypertensive rats. Similar to us, a study by Zhang et al. (2020) has also reported an inverse relation between ACE2 expression and Wnt signaling in cancer progression (Li et al., 2020). Based on the observed results, it can be conferred that activation of the Wnt/β-catenin pathway by ACE2 activation improves hippocampal neurogenesis in hypertension.

To summarize, our study demonstrates that hypertension leads to a significant activation of astrocytes and microglia, resulting in neuroinflammation and impairment of neurogenesis in the brain. It is noteworthy that in hypertensive rats, ACE2 activation by DIZE prevented astrocyte and microglial activation, and the resultant neuroinflammation, independent of its effect on lowering blood pressure. ACE2 activation prevented the activation of NOX4, TRAF6 and NFκB, and promoted hippocampal neurogenesis in hypertension by activating Wnt/β-catenin signaling. This provides evidence that ACE2 activation has a dual effect of preventing neuroinflammation while promoting



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Fig. 9. ACE2 activation promoted hippocampal neurogenesis in the hypertensive rats. Representative photomicrographs show immunostaining of (A) DCX and BrdU, (B) NeuN and BrdU in the hippocampus region of rat brain. The bar graph shows the fluorescence intensity by colocalization of DCX/BrdU and NeuN/BrdU. Scale bar - 50 μm. Data are expressed as mean ± SEM of n = 3–4. Data were analyzed by one-way ANOVA, followed by Tukey's test (*P < 0.05, **P < 0.01, ***P < 0.001, $^{\#}$P < 0.05, $^{\#\#}$P < 0.01, $^{\#\#\#}$P < 0.001). *Sham vs HTN, $^{\#}$HTN vs HTN + DIZE.

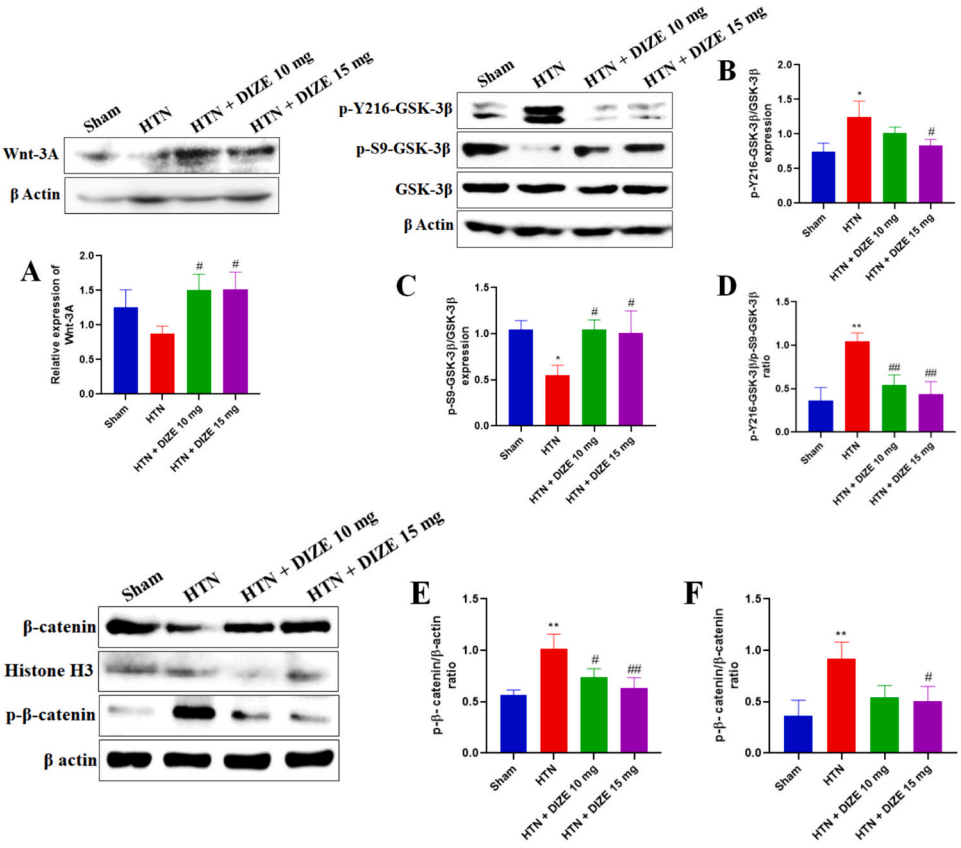


Fig. 10. ACE2 activation promoted the Wnt/β-Catenin signaling in the hypertensive rats. Representative immunoblot quantification of (A) Wnt3A, (B) p-Y216-GSK-3β/GSK-3β, (C) p-S9-GSK-3β/GSK-3β, (D) p-Y216-GSK-3β/p-S9-GSK-3β ratio, (E) p-β-catenin and, (F) p-β-catenin/β-catenin ratio in the hippocampus region of rat brain. Data are expressed as mean ± SEM of n = 3–4. Data were analyzed by one-way ANOVA, followed by Tukey's test (*P < 0.05, **P < 0.01, ***P < 0.001, $^{\#}$P < 0.05, $^{\#\#}$P < 0.01, $^{\#\#\#}$P < 0.001). *Sham vs HTN, $^{\#}$HTN vs HTN + DIZE.

neurogenesis in hypertensive rats and can be a target of therapeutic potential for neurodegeneration/neuroinflammation.

Funding

This work was supported by financial grants from the Council of Scientific and Industrial Research (CSIR) and the Department of Biotechnology (BT/PR27480/MED/122/138/2018), New Delhi, India.

Ethics statement

All studies were accomplished in accordance with the guidelines provided by Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Government of India.

CRediT authorship contribution statement

Priya Tiwari: Writing – original draft, Visualization, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Sumbul Mueed:** Methodology. **Adam Olaitan Abdulkareem:** Methodology. **Kashif Hanif:** Writing – review & editing, Supervision, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors have no conflict of interest to declare.

Data availability

Data will be made available on request.

Acknowledgement

The authors acknowledge Mr. A.L. Vishwakarma for assisting with Flow cytometry, Division of Sophisticated Analytical Instrument Facility, CSIR-CDRI. Awards of research fellowship to Ms. Priya Tiwari from University Grant Commission (UGC), New Delhi, India and Adam Olaitan Abdulkareem from Council of Scientific and Industrial Research (CSIR), New Delhi, India are greatly acknowledged. The CSIR-CDRI communication number of this article is 10828.

References

Abdullah, M., Berthiaume, J.M., Willis, M.S., 2018. Tumor necrosis factor receptor-associated factor 6 as a nuclear factor kappa B-modulating therapeutic target in cardiovascular diseases: at the heart of it all. Transl. Res. 195, 48–61.

Abiodun, O.A., Ola, M.S., 2020. Role of brain renin angiotensin system in neurodegeneration: an update. Saudi J Biol Sci 27 (3), 905–912.

Bajwa, E., Klegeris, A., 2022. Neuroinflammation as a mechanism linking hypertension with the increased risk of Alzheimer's disease. Neural Regen. Res. 17 (11), 2342–2346.
Bennion, D.M., et al., 2015. Neuroprotective mechanisms of the ACE2-angiotensin-(1-7)-Mas axis in stroke. Curr. Hypertens. Rep. 17 (2), 3.
Ben-Shabat, M., et al., 2023. Predisposition to cortical neurodegenerative changes in brains of hypertension prone rats. J. Transl. Med. 21 (1), 51.
Bhat, S.A., et al., 2017. Platelet CD40L induces activation of astrocytes and microglia in hypertension. Brain Behav. Immun. 59, 173–189.
Bhat, S.A., et al., 2018. Angiotensin receptor blockade by inhibiting glial activation promotes hippocampal neurogenesis via activation of Wnt/beta-catenin signaling in hypertension. Mol. Neurobiol. 55 (6), 5282–5298.
Bhat, S.A., et al., 2019. AT2R activation prevents microglia pro-inflammatory activation in a NOX-dependent manner: inhibition of PKC activation and p47(phox) phosphorylation by PP2A. Mol. Neurobiol. 56 (4), 3005–3023.
Boonpraman, N., et al., 2023. NOX4 as a critical effector mediating neuroinflammatory cytokines, myeloperoxidase and osteopontin, specifically in astrocytes in the hippocampus in Parkinson's disease. Redox Biol. 62, 102698.
Campos, R.R., Oliveira-Sales, E.B., Nishi, E.E., Paton, J.F., Bergamaschi, C.T., 2015. Mechanisms of renal sympathetic activation in renovascular hypertension. Exp. Physiol. 100 (5), 496–501. https://doi.org/10.1113/expphysiol.2014.079855.
De Maria, M.L., et al., 2016. Anti-hypertensive effects of diminazene aceturate: an angiotensin- converting enzyme 2 activator in rats. Protein Pept. Lett. 23 (1), 9–16.
Dou, Y., et al., 2018. Roles of TRAF6 in central nervous system. Curr. Neuropharmacol. 16 (9), 1306–1313.
Eirin, A., Lerman, A., Lerman, L.O., 2014. Mitochondrial injury and dysfunction in hypertension-induced cardiac damage. Eur. Heart J. 35 (46), 3258–3266.
Evans, C.E., et al., 2020. ACE2 activation protects against cognitive decline and reduces amyloid pathology in the Tg2576 mouse model of Alzheimer's disease. Acta Neuropathol. 139 (3), 485–502.
Goel, R., et al., 2015. Hypertension exacerbates predisposition to neurodegeneration and memory impairment in the presence of a neuroinflammatory stimulus: protection by angiotensin converting enzyme inhibition. Pharmacol. Biochem. Behav. 133, 132–145.
Goel, R., et al., 2016. Perindopril attenuates lipopolysaccharide-induced amyloidogenesis and memory impairment by suppression of oxidative stress and RAGE activation. ACS Chem. Neurosci. 7 (2), 206–217.
Gupta, S., et al., 2022. Angiotensin-converting enzyme 2 activation mitigates behavioral deficits and neuroinflammatory burden in 6-OHDA induced experimental models of Parkinson's disease. ACS Chem. Neurosci. 13 (10), 1491–1504.
Guzman-Martinez, L., et al., 2019. Neuroinflammation as a common feature of neurodegenerative disorders. Front. Pharmacol. 10, 1008.
Ho, J.K., et al., 2021. Blood-brain barrier crossing renin-angiotensin drugs and cognition in the elderly: a meta-analysis. Hypertension 78 (3), 629–643.
Iadecola, C., Gottesman, R.F., 2019. Neurovascular and cognitive dysfunction in hypertension. Circ. Res. 124 (7), 1025–1044.
Ibrahim, M.M., 2006. RAS inhibition in hypertension. J. Hum. Hypertens. 20 (2), 101–108.
Jiang, T., et al., 2012. Suppressing inflammation by inhibiting the NF-kappaB pathway contributes to the neuroprotective effect of angiotensin-(1-7) in rats with permanent cerebral ischaemia. Br. J. Pharmacol. 167 (7), 1520–1532.
Klempin, F., et al., 2018. Depletion of angiotensin-converting enzyme 2 reduces brain serotonin and impairs the running-induced neurogenic response. Cell. Mol. Life Sci. 75 (19), 3625–3634.
Kronenberg, G., Lippoldt, A., Kempermann, G., 2007. Two genetic rat models of arterial hypertension show different mechanisms by which adult hippocampal neurogenesis is increased. Dev. Neurosci. 29 (1–2), 124–133.
Kuber, B., Fadnavis, M., Chatterjee, B., 2023. Role of angiotensin receptor blockers in the context of Alzheimer's disease. Fundam. Clin. 37 (3), 429–445.
Kwon, H.S., Koh, S.H., 2020. Neuroinflammation in neurodegenerative disorders: the roles of microglia and astrocytes. Transl Neurodegener 9 (1), 42.
Li, S., et al., 2020. ACE2 attenuates epithelial-mesenchymal transition in MLE-12 cells induced by silica. Drug Des. Devel. Ther. 14, 1547–1559.
Liao, W., Wu, J., 2021. The ACE2/Ang (1-7)/MasR axis as an emerging target for antihypertensive peptides. Crit. Rev. Food Sci. Nutr. 61 (15), 2572–2586.
Maguschak, K.A., Ressler, K.J., 2012. A role for WNT/beta-catenin signaling in the neural mechanisms of behavior. J. NeuroImmune Pharmacol. 7 (4), 763–773.
Ouk, M., et al., 2021. The use of angiotensin-converting enzyme inhibitors vs. angiotensin receptor blockers and cognitive decline in Alzheimer's disease: the importance of blood-brain barrier penetration and APOE epsilon4 carrier status. Alzheimers Res. Ther. 13 (1), 43.
Perfilieva, E., et al., 2001. Gender and strain influence on neurogenesis in dentate gyrus of young rats. J. Cereb. Blood Flow Metab. 21 (3), 211–217.
Picca, A., et al., 2020. Mitochondrial dysfunction, oxidative stress, and neuroinflammation: intertwined roads to neurodegeneration. Antioxidants (Basel) 9 (8).
Pietranera, L., et al., 2010. Involvement of brain-derived neurotrophic factor and neurogenesis in oestradiol neuroprotection of the hippocampus of hypertensive rats. J. Neuroendocrinol. 22 (10), 1082–1092.
Santisteban, M.M., Iadecola, C., Carnevale, D., 2023. Hypertension, neurovascular dysfunction, and cognitive impairment. Hypertension 80 (1), 22–34.
Sartorio, C.L., et al., 2020. Acute hypotensive effect of diminazene aceturate in spontaneously hypertensive rats: role of NO and Mas receptor. Clin. Exp. Pharmacol. Physiol. 47 (10), 1723–1730.
Shang, N., Wu, J., 2020. Egg-derived tripeptide IRW attenuates LPS-induced osteoclastogenesis in RAW 264.7 macrophages via inhibition of inflammatory responses and NF-kappaB/MAPK activation. J. Agric. Food Chem. 68 (22), 6132–6141.
Shi, T.T., et al., 2018. Angiotensin-converting enzyme 2 regulates mitochondrial function in pancreatic beta-cells. Biochem. Biophys. Res. Commun. 495 (1), 860–866.
Shih, Y.H., et al., 2016. Hypertension impairs hippocampus-related adult neurogenesis, CA1 neuron dendritic arborization and long-term memory. Neuroscience 322, 346–357.
Sun, X., et al., 2023. Diminazene ameliorates neuroinflammation by suppression of astrocytic miRNA-224-5p/NLRP3 axis in Alzheimer's disease model. J. Inflamm. Res. 16, 1639–1652.
Sung, P.S., et al., 2020. Neuroinflammation and neurogenesis in Alzheimer's disease and potential therapeutic approaches. Int. J. Mol. Sci. 21 (3).
Tarafdar, A., Pula, G., 2018. The role of NADPH oxidases and oxidative stress in neurodegenerative disorders. Int. J. Mol. Sci. 19 (12).
Tiwari, P., et al., 2023a. Activation of angiotensin-converting enzyme 2 protects against lipopolysaccharide-induced glial activation by modulating angiotensin-converting enzyme 2/angiotensin (1-7)/Mas receptor axis. Mol. Neurobiol. 60 (1), 203–227.
Tiwari, V., et al., 2023b. ACE2/ANG-(1-7)/Mas receptor axis activation prevents inflammation and improves cognitive functions in streptozotocin induced rat model of Alzheimer's disease-like phenotypes. Eur. J. Pharmacol. 946, 175623.
Tota, S., et al., Role of central angiotensin receptors in scopolamine-induced impairment in memory, cerebral blood flow, and cholinergic function. Psychopharmacology, 2012. 222(2): p. 185–202.
Ungvari, Z., et al., 2021. Hypertension-induced cognitive impairment: from pathophysiology to public health. Nat. Rev. Nephrol. 17 (10), 639–654.
Uttara, B., et al., 2009. Oxidative stress and neurodegenerative diseases: a review of upstream and downstream antioxidant therapeutic options. Curr. Neuropharmacol. 7 (1), 65–74.
Vallee, A., Vallee, J.N., Lecarpentier, Y., 2022. WNT/beta-catenin pathway: a possible link between hypertension and Alzheimer's disease. Curr. Hypertens. Rep. 24 (10), 465–475.
Wang, J., Chen, S., Bihl, J., 2020. Exosome-mediated transfer of ACE2 (angiotensin-converting enzyme 2) from endothelial progenitor cells promotes survival and function of endothelial cell. Oxidative Med. Cell. Longev. 2020, 4213541.
Wen, Y., Crowley, S.D., 2018. Renal effects of cytokines in hypertension. Curr. Opin. Nephrol. Hypertens. 27 (2), 70–76.
Yim, H.E., Yoo, K.H., 2008. Renin-angiotensin system - considerations for hypertension and kidney. Electrolyte Blood Press 6 (1), 42–50.
Zhang, L., et al., 2011. The Wnt/beta-catenin signaling pathway in the adult neurogenesis. Eur. J. Neurosci. 33 (1), 1–8.