A DISSERTATION ON

To study the effect of Angiotensin-Converting Enzyme 2 activator (ACE2A) on neurogenesis in Spontaneously Hypertensive Rats (SHR)

SUBMITTED TO THE

DEPARTMENT OF BIOENGINEERING

FACULTY OF ENGINEERING

INTEGRAL UNIVERSITY, LUCKNOW

IN PARTIAL FULLFILLMENT

FOR THE

DEGREE OF B.TECH.- M.TECH.

IN BIOTECHNOLOGY

By

SUMBUL MUEED

DEPARTMENT OF BIOENGINEERING

INTEGRAL UNIVERSITY

UNDER THE SUPERVISION OF

Dr. Kashif Hanif

Principal Scientist, Division of Pharmacology

CSIR-CENTRAL DRUG INSTITUTE, LUCKNOW- 226031 (UP)

DECLARATION FORM

I, **Sumbul Mueed**, a student of **B.Tech-M.Tech** (V year/X semester) Integral University have completed my six months dissertation work entitled **““**To study the effect of angiotensin-Converting Enzyme 2 activator (ACE2A) on neurogenesis in Spontaneously Hypertensive Rat (SHR) **”** successfully from CSIR-CDRI under the guidance of Dr. Kashif Hanif (Principle Scientist) Department of Pharmacology

I, hereby, affirm that the work has been done by me in all aspects. I have sincerely prepared this project report and the results reported in this study are genuine and authentic.

Name and Signature of Student with Date

Name and Signature of Course Coordinator with Date

सी.एस.आई.आर.-केन्द्रीय औषधि अनुसंधान संस्थान, लखनऊ
(वैज्ञानिक तथा औद्योगिक अनुसंधान परिषद्)
सेक्टर 10, जानकीपुरम विस्तार, सीतापुर रोड, लखनऊ – 226 031 (भारत)
CSIR - Central Drug Research Institute
(Council of Scientific & Industrial Research)
Sector 10, Janakipuram Extension, Sitapur Road, Lucknow - 226 031 (India)

CERTIFICATE

This is to certify that work embodied for the report on **"To study the effect of Angiotensin-Converting Enzyme 2 activator (ACE2A) on neurogenesis in Spontaneously Hypertensive Rats (SHR)"** *towards the fulfillment of the course requirement of 10th semester of B.Tech MTech (Biotechnology) has been carried out under my supervision.*

DATE:

Dr. Kashif Hanif
Principal Scientist
Department of Pharmacology
CSIR-Central Drug Research Institute

INTEGRAL UNIVERSITY

Established Under the Integral University Act 2004 (U.P. Act No.9 of 2004)
Approved by University Grant Commission
Phone No.: +91(0522) 2890812, 2890730, 3296117, 6451039, Fax No.: 0522-2890809
Kursi Road, Lucknow-226026 Uttar Pradesh (INDIA)

CERTIFICATE BY INTERNAL ADVISOR

This is to certify that **SUMBUL MUEED,** a student of **Dual Degree B. Tech – M. Tech Biotechnology, Integral University** has completed her six months dissertation work entitled **"To study the effect of Angiotensin-Converting Enzyme 2 activator (ACE2A) on neurogenesis in Spontaneously Hypertensive Rats (SHR)"** successfully. She has completed this work from **Department of Pharmacology division , CSIR-CDRI, Lucknow** under the guidance of Dr. Kashif Hanif**, Principal Scientist** Department of Pharmacology division. The dissertation was a compulsory part of her **Dual Degree B. Tech – M. Tech Biotechnology.**

I wish him good luck and bright future.

Dr. Iffat Zareen
Associate Professor
Department of Bioengineering
Faculty of Bioengineering

INTEGRAL UNIVERSITY

Established Under the Integral University Act 2004 (U.P. Act No.9 of 2004)
Approved by University Grant Commission
Phone No.: +91(0522) 2890812, 2890730, 3296117, 6451039, Fax No.: 0522-2890809
Kursi Road, Lucknow-226026 Uttar Pradesh (INDIA)

TO WHOM IT MAY CONCERN

This is to certify that **SUMBUL MUEED,** a student of **Dual Degree Biotechnology** (5th Year/ 10th Semester), Integral University has completed her six months dissertation work entitled **"To study the effect of Angiotensin-Converting Enzyme 2 activator (ACE2A) on neurogenesis in Spontaneously Hypertensive Rats (SHR)"** successfully. She has completed this work from **CSIR-CDRI** (Department of Pharmacology division) under the guidance of Dr. Kashif Hanif. The dissertation was a compulsory part of her **Dual Degree Biotechnology** Program. I wish him/her good luck and bright future.

Dr. Alvina Farooqui
Head
Department of Bioengineering
Faculty of Engineering

ACKNOWLEDGEMENT

After an intense period of six months, today is the day: writing this note of thanks is the finishing touch on my dissertation. It has been a period of intense learning for me, not only in scientific area but also on a personal level. Writing this dissertation had a big impact on me. I would like to reflect on the people who have supported and helped me so much throughout this period.

I am highly grateful to **S.W. Akhtar** Chancellor Integral University Lucknow and special thanks to **Dr. Syed Nadeem Akhtar** Pro Chancellor, **Prof Javed Musarrat** Vice Chansellor, **Prof Aqil Ahmad** Pro Voice Chansellor and **Prof Tahseen Usmani** Dean of Engineering Integral University Lucknow

I praise and thank the almighty God for the gift of life, a wonderful journey, and for providing me the opportunity and capability to proceed successfully in this study. It gives me immense pleasure to acknowledge my heartiest thanks to **S.W.Akhtar** Chancellor Integral university Lucknow and **Dr. Srinivasa Reddy** Director, CSIR-CDRI Lucknow, **Dr. (Er.) Alvina Farooki** (HOD, Department of Bioengineering), IntegralUniversity for providing me golden opportunity to work in such a prestigious research institute.

I would like to acknowledge and give my warmest thanks to my supervisor **Dr. Kashif Hanif,** Principal Scientist, Division of Pharmacology, CSIR-CDRI, Lucknow for his understanding, expert advice, knowledge, keen observation and valuable comments which made my work successful. I gratefully acknowledge him for his help and support during the course of dissertation.

I would like to thanks my internal advisor **Dr. Iffat Zareen and Dr. Snober Mir associate professor Integral University** for always guiding me and supporting me and giving me suggestions, which is so valuable for me. I would like to thank my course coordinator **Dr. Ashish Kalicharan and Dr. Roohi assistantant Professor Integral university Lucknow** for her support and guidance. Further, I would like tothank my professor **Dr. Soban Ahmad Faridi** for his encouragement, suggestion, and support which help me a lot in my thesis.

Now, I would like to thank my senior **Ms. Priya Tiwari** for her unconditional support and advice. Her invaluable guidance and her hardworking nature inspire me a lot without her help my thesis would never be completed.Thank you for motivating me. I really wanted to appreciate her for the support and time. Working with her was so interesting and memorable.

I would also thankall the lab members and Seniors **Ms. Pragya Sharma, Ms. Smita Prajapati, Mr. Zahid Rasool, and Mr. Adam Abdulkareem** for their helping nature and advices which means a lot to me.

My acknowledgment would be incomplete without thanking my parents and family without their support and encouragement I would not be able to do my training. They always motivate me. I feel blessed to have parentslike them.

Now, last but not least work.I would like to thank my friends. Their support and care helped me overcome setbacks and stay focused in my endeavours.

Finally, I would like to thank everyone who helped me either directly or indirectly for this study.

Sumbul Mueed

CONTENT

Abbreviations

HTN Hypertension

RAS Renin angiotensin System

ACE Angiotensin converting enzyme

ACE 2 Angiotensin converting enzyme 2

BSA Bovine serum albumin

DW Distilled water

ECLEnhanced chemiluminescence

GFAPGlial fibrillary acidic protein

PAGE Polyacrylamide gel electrophoresis

PBSPhosphate Buffer Saline

PVDF Polyvinylidene difluoride

SDSSodium dodecyl sulphate

TEMEDTetramethyl ethylenediamine

TNF-α Tumour necrosis factor-α

IL-10 Interleukin 10

TRISTris(hydroxymethyl)aminomethane

HRP Horse radish peroxidase

IAEC Institutional Animal Ethics Committee

i.p. Intraperitoneal

kDa Kilo Dalton

CHAPTER-1

INTRODUCTION

CHAPTER 1

Introduction

Hypertensionis characterized by persistently high blood pressure (BP) in the systemic arteries. BP is commonly expressed as the ratio of the systolic BP (that is, the pressure that the blood exerts on the arterial walls when the heart contracts) and the diastolic BP (the pressure when the heart relaxes). The BP thresholds that define hypertension depend on the measurement method(Oparil et. al 2018).Hypertension is the most important modifiable risk factor for all-cause of morbidity and mortality worldwide and is associated witan h increased risk of cardiovascular disease (CVD). Fewer than half of those with hypertension are aware of their condition, and many others are aware but not treated or inadequately treated, although successful treatment of hypertension reduces the global burden of disease and mortality. The aetiology of hypertension involves the complex interplay of environmental and pathophysiological factors that affect multiple systems, as well as genetic predisposition. Evaluation of patients with hypertension includes accurate standardized blood pressure (BP) measurement, assessing patients predicted risk of atherosclerotic CVD, evidence of target organ damage, detection of secondary causes of hypertension, and presence of comorbidities, including CVD and kidney disease. Lifestyle changes, including dietary modifications and increased physical activity, are effective in lowering BP and preventing hypertension and its CVD sequelae. (Mariaet.al, 2018). Hypertension is further divided into two types: Primary hypertension that has no causes and symptoms and Secondary hypertension which is caused by another medical condition (Lesley et.al, 2017). The increase in hypertensive subjects in India is expected to increase to 22.9% for men and 23.6% for women by 2025, respectively (Aanchala et al,2014).

Although often neglected, the brain is one of the main organs targeted and dysfunctional by hypertension(Goel et.al;2016). Hypertension is the leading risk factor for cerebrovascular events like decreased neurogenesis, and stroke(Goel et.al; 2016) and is increasingly associated with the development of dementia (Goel et.al; 2016). The relationship betweenhypertension and memory impairment is further strengthened when antihypertensive agentslike angiotensin-converting enzyme inhibitors and AT1 receptor blockers, improve memory functions in hypertensive subjects (Braszko et al. 2003; Fogari et al., 2004 and 2006) and in various rodent models of dementia (Tota et al., 2012). The dentate gyrus of the

hippocampus is one of the few places in the brain where neurogenesis occurs in adulthood. Nowadays, an increasing number of children and young adults are affected by hypertension, one of the factors in the development of cerebrovascular diseases and age-related cognitive deficits. (Pistikova et.al; 2017).

Hypertension is associated with neurodegenerative diseases and cognitive impairment. (Shih 2016 May). That's why we using spontaneous hypertensive rats to study the effect of hypertension on memory performance and adult hippocampal neurogenesis. Neuroinflammation, characterized by increased levels of cytotoxic cytokines, is an integral aspect of neurodegenerative disorders (Block et al., 2007). Since CNS is considered an immune-privileged tissue, therefore, in the brain the inflammatory response is mainly orchestrated by the activation of microglia and astrocytes (Muldoon et al., 2013; Cerbai et al., 2012; Jensen et al., 2013). Activation of microglia and astrocytes results in the sustained release of inflammatory cytokines like TNF-α, IL-1β, and IL-6, increased oxidative and nitrosative stress (Tansey et al; 2007), and culminating in the neuroinflammation and neuronal death.The renin-angiotensin system (RAS) plays a key role in the pathophysiology of hypertension (Schiffrin and Touyz 2004). Ang II, a most potent component of RAS, induces vascular remodeling and injury by causing vasoconstriction, cell growth, generation of reactive oxygen species (ROS), and inflammation (Schiffrin and Touyz 2004; Goel et al., 2015). Recently, neurodegenerative diseases like AD, have been associated with impaired adult hippocampal neurogenesis (generation of new neurons). Of the neurogenic zones in the adult brain, hippocampal neurogenesis draws the most attention, because of its involvement in cognitive function (Kempermann et al., 2015).

Previous studies have reported that hypertension leads to the development of neurodegenerative diseases like AD and Vascular dementia, the predominant form of cognitive impairmentin humans (Biebl et al., 2000; Kempermann et al., 2003). Carnevelle et al (2012) demonstrated that hypertension is induced by transverse aortic constriction induces neurodegeneration by increasing neuroinflammation, oxidative stress, BBB leakage and decreasing cerebral blood flow (CBF). Further, Neurodegenerative diseases like AD Display the typical progressive loss of neurons and gliosis (Glass et; al 2010) and the impairment in the neurogenesis (Saxe et al., 2006) i.e generation of new neurons. Chronic neurodegeneration hampers stem cell maintenance, proliferation, survival, and functional

integration. Neurogenesis adds particular functionality to the mammalian brain because of its involvement in cognitive functions. There is a need for the detailed study of adult neurogenesis to open up a totally new avenue of therapeutics. Few regions like the dentate gyrus of the hippocampus are very sensitive to age-related neurodegeneration (Saxe et al., 2006). Hence in this study, we are interested to see the effect of chronic hypertension on the markers of neurogenesis in the hippocampus region of the hypertension rat brain.

CHAPTER-2

REVIEW OF LITERATURE

CHAPTER 2

2. REVIEW OF LITERATURE

Definition

Hypertension (HTN) is defined as having a systolic blood pressure (SBP) of 130mmHg or higher and/or a diastolic blood pressure (DBP) of greater than 80mmHg (Iqbal et al., 2022) The most prevalent chronic medical illness defined by a sustained increase in arterial pressure is hypertension.

Systemic arterial hypertension (hereafter referred to as hypertension) is characterized by persistently high blood pressure (BP) in the systemic arteries. BP is commonly expressed as the ratio of the systolic BP (that is, the pressure that the blood exerts on the arterial walls when the heart contracts) and the diastolic BP (the pressure when the heart relaxes)(Suzanne, 2018) The BP thresholds that define hypertension depend on the measurement method Several aetiologies can underlie hypertension. The majority (90–95%) of patients have a highly heterogeneous ‘essential’ or primary hypertension with a multifactorial gene-environment etiology. A Positive family history is a frequent occurrence in patients with hypertension, with the heritability (a measure of how much of the variation in a trait is due to variation in genetic factors) estimated between 35% and 50% in the majority of studies(Suzanne, 2018). Genome-wide association studies (GWAS) have identified ~120 loci that are associated with BP regulation and together explain 3.5% of the trait variance These findings are becoming increasingly important as we search for new pathways and new biomarkers to develop more modern ‘omics’-driven diagnostic and therapeutic modalities for hypertension in the era of precision medicine. Hypertension is the leading single contributor to all-cause death and disability worldwide and is the most common preventable risk factor for cardiovascular disease (CVD; including coronary heart disease, heart failure, stroke, myocardial infarction, atrial fibrillation, and peripheral artery disease), chronic kidney disease (CKD), and cognitive impairment. The link between high blood pressure and an increased risk of cardiovascular disease is graded and continuous, beginning at 115/75 mmHg, well within the normotensive range. Successful hypertension prevention and treatment are critical for lowering illness load and enhancing longevity in the global population. It's more crucial to evaluate a person's projected atherosclerotic CVD (ASCVD) risk while treating

hypertension than just their blood pressure because people with high CVD risk have a higher risk of developing hypertension.

Epidemiology of Hypertension

Hypertension is the leading cause of cardiovascular disease and premature death worldwide. Owing to the widespread use of antihypertensive medications, global mean blood pressure (BP) has remained constant or has decreased slightly over the past four decades(T.Mills, 2020). By contrast, the prevalence of hypertension has increased, especially in low- and middle-income countries (LMICs). Estimates suggest that 31.1% of adults (1.39 billion) worldwide had hypertension in 2010. The prevalence of hypertension among adults was higher in LMICs (31.5%, 1.04 billion people) than in high-income countries (28.5%, 349 million people).

Variations in the levels of risk factors for hypertension, such as high sodium intake, low potassium intake, obesity, alcohol consumption, physical inactivity, and unhealthy diet, may explain some of the regional heterogeneity in hypertension prevalence. Despite the increasing prevalence, the proportions of hypertension awareness, treatment, and BP control are low, particularly in LMICs, and few comprehensive assessments of the economic impact of hypertension exist. Future studies are warranted to test implementation strategies for hypertension prevention and control, especially in low-income populations, and to accurately assess the prevalence and financial burden of hypertension worldwide.

Types of Hypertension

Hypertension can be classified broadly into two categories namely primary or essential hypertension and secondary hypertension.

Primary Hypertension

The patients with hypertension have no clear cause and symptoms mostly and are classified as having primary hypertension(Charles et.al, 2017).Essential or primary hypertension is defined as high BP in which secondary causes such as renovascular disease, renal failure,or other diseases are not present as like in secondaryhypertension. Essential hypertension is a heterogeneous disorder, with different patients having different causal factors that lead to high BP. (Oscar et.al, 2000).

Secondary Hypertension

Secondary hypertension is described as high blood pressure.The patients who have secondary hypertension have another medical condition thatsecondary hypertension causes (Lesley et.al 2017). Here, high blood pressure can be reduced by identifying and treating the main cause of the disease. It is further classified as-

- **Renal parenchymal hypertension:** Renal Parenchymal hypertension is caused by secondary hypertension. It accounts for up to 5% cases of systemic hypertension(Vachek et.al, 2021). Renal parenchymal hypertension can occur in acute and chronic kidney disease. It is a form of secondary hypertension.

- **Renovascular hypertension:**The primary cause of secondary hypertension, renovascular hypertension, occurs when blood pressure rises as a result of renal ischemia. As a result, patients can experience premature death. (Fenves et al., 2006).

- **Endocrine hypertension:** Endocrine hypertension is caused by high blood pressure. Primary aldosteronism, Cushing's syndrome, thyroid conditions, and iatrogenic hormone manipulation are a few of the disorders that can result in endocrine hypertension. Endocrine hypertension in patients is thought to be caused by primary aldosteronism. (Joseph et al, 2017)

- **Drug-induced hypertension:** There are a variety of medicines that cause hypertension, including glucocorticoids, which can cause blood pressure to rise (BP) Nonsteroidal anti-inflammatory medicines raise blood pressure. (Leeuw, 1997).

Pathophysiology of Hypertension

There are a number of BP-regulating elements that could lead to the development of hypertension. Among these include humoral (such as RAS or vasodepressor processes), peripheral auto-regulation deficiency, aberrant neuronal activity, and fluctuating levels of sodium and natriuretic hormone (Borzeck, 2010). Numerous other factors, including vascularremodelling, inflammation, and oxidative stress, have been suggested to affect the prevalence of hypertension (Dinh et al., 2014). RAS, a very important factor in its development and a target of organ damage, modulates the majority of these disorders.

Therefore, here we will discuss in detail the factors responsible inthe pathology of hypertension as well as brain RAS.

1. Increased Sympathetic Activity

Sympathetic activity is a key component of the pathophysiology of hypertension. It raises blood pressure by stimulating the adrenergic receptors found in the kidneys, heart, and peripheral vasculature. The main adrenergic receptors involved in controlling blood circulation are alpha and beta receptors. These receptors' function is to regulate blood pressure via determining vascular resistance, fluid retention, and cardiac output. In the most hypertensive patients, research has shown a large rise in the sympathetic drive and a decrease in the parasympathetic drive (Zubcevic et al, 2011).

2. Inflammation

Hypertension develops as a result of inflammation, which also contributes to its growth and maintenance. (David M. Patrick et al., February 2021). Renal abnormalities, endothelial cell dysfunction, and central nervous system dysregulation are the physiological mechanisms that play a part in raising blood pressure and leading to the development of hypertension. These are the systems that inflammation affects, which eventually causes the emergence of hypertension. Multiple cell types are involved in inflammation, which is seen as a complex process that secretes numerous substances that have contributed to the rise in hypertension. (David M. Patrick et al., February 2021). Clinical trials and research have shown a clear link between system.

3. Oxidative stress

Oxidative stresscontributes to the mechanism of hypertension. Additionally, oxidative stress is brought on by hypertension, which harms target organs. The importance of oxidative stress in the context of hypertension is related to the fundamental role that ROS and redox signalling play in molecular, cellular, and systems processes that result in endothelial damage, vascular dysfunction, cardiovascular remodelling, renal dysfunction, sympathetic nervous system excitation, immune cell activation, and systemic inflammation, all of which are

significant in the pathophysiology of hypertension. (Kathy K. Griendling and colleagues, 2021).

4. Endothelial dysfunction

The phrase "endothelial dysfunction" refers to circumstances that cause aberrant endothelium activation, anomalies between the endothelium and leukocytes, thrombocytes, and regulatory molecules, as well as worsening of endothelium-dependent vasodilatation. Control of the cardiovascular system depends on a healthy endothelium. As a result, it contributes significantly to the development of numerous diseases and cardiovascular issues, including atherosclerosis, pulmonary and systemic hypertension, cardiomyopathies, and vasculitides. (2016) Dildar Konukoglu et al).

5. Central RAS

Blood pressure is significantly regulated by the sympathetic nervous system (SNS) and the renin-angiotensin system (RAS). Endocrine, paracrine, and autocrine regulator roles are played by angiotensins. The activities of the RAS in peripheral physiology and pathophysiology are well known. As a key regulator of the cardiovascular (CV) system and an important pharmacological target for antihypertensive and other CV therapies, the presence and functional significance of the RAS in the brain are being recognized more and more. Through sympathetic activation and vasopressin production, all of the RAS's components are found in the brain and contribute to the control of blood pressure. Additionally, behaviour and neurological illnesses like Parkinson's and Alzheimer's are impacted by a relationship between neurotransmitters and the brain RAS. (Marc de Gasparo et al, 2013). Hypertension affects one-third of the adult population and is a growing problem due to the increasing incidence of obesity and diabetes. Brain RAS (renin-angiotensin system) hyperactivity has been implicated in the development and maintenance of hypertension in several types of experimental and genetic hypertension animal models. (Ji Gao et.al 2014).

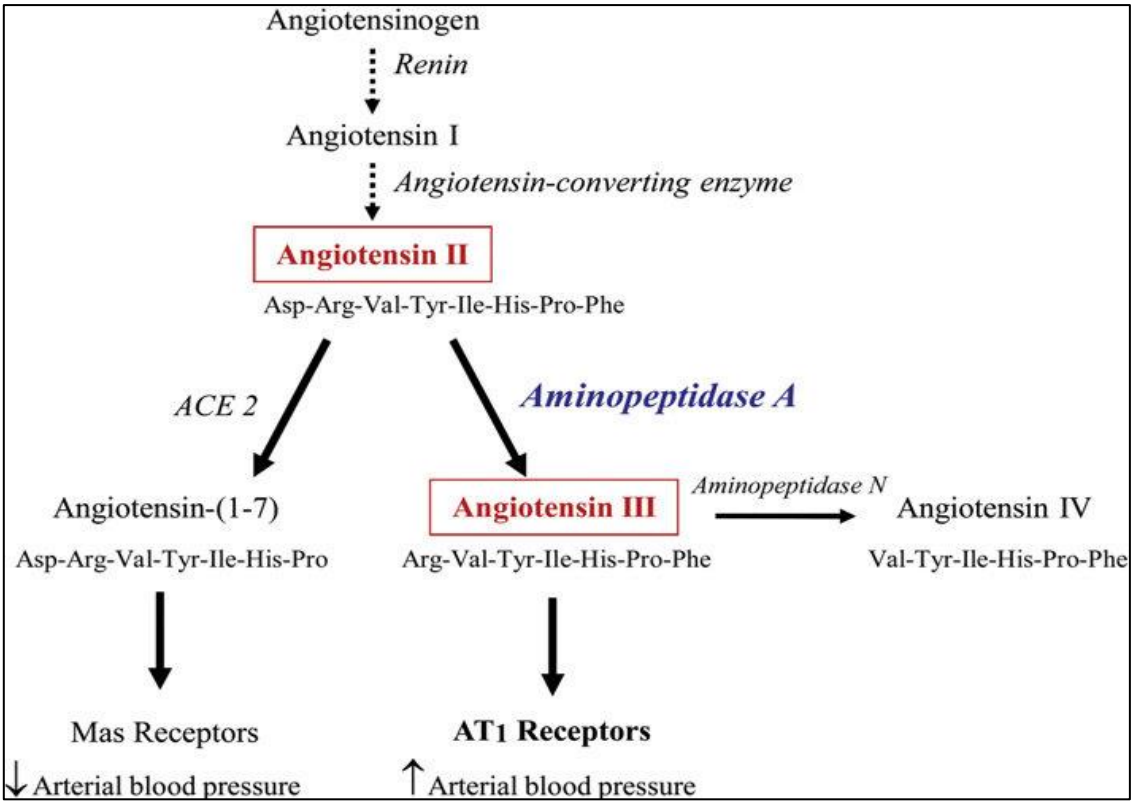


Fig 1. Showing Schematic diagram of the brain RAS (adopted from Ji Gao et.al 2014).

Components of the central RAS

A. Angiotensinogen

One of the basic effector molecules in RAS is ANG II. It influences different organ systems through some deleterious effects on the vasculature and its pressor properties and increasing blood pressure. ANG II-mediated vascular dysfunction and tissue injury response can be classified as the capacity of the peptide to induce a pro-inflammatory, pro-thrombogenic and pro-oxidative phenotype in both large and microscopic blood vessels.The vasomotor and inflammatory responses of ANG II are adjusted by the stimulation of AT-1R that are influenced on leukocytes, platelets, endothelial cells and vascular smooth muscle. (Chuanxin Su et.al 2021)

B. Renin

The renin–angiotensin system (RAS) has a critical role in cardiovascular physiology through its effects in regulating blood pressure and electrolyte balance. However, under

pathophysiological conditions, the effects of the RAS can intensify to trigger inflammation and structural remodelling, thus promoting cardiac and vascular damage. In the classical system, renin cleaves angiotensinogen to form angiotensin I, which is subsequently converted to angiotensin II by angiotensin-converting enzyme (ACE). (Maria Paz Ocaranza et.al 2020).

C. Angiotensin-converting enzyme (ACE)

ACE is found in most of the brain regions; however, it is highly expressed in circumventricular organs (CVOs) like the area postrema (AP), organum vasculosum laminae terminalis (OVLT), median eminence and subfornical organ (SFO) (Saavedra and Chevillard, 1982; Thunhorst et al., 1989; Chai et al., 1990). Angiotensin converting enzyme (ACE) inhibitors (ACEIs) are commonly used to treat high BP and heart failure (Saavedra, 2012). However (Amenta and colleagues et;al 2002) after reviewing the controlled clinical trials assessing the effect of anti-hypertensive treatment on cognitive functions in subjects with essential hypertension, inferred that treatment with angiotensin converting enzyme inhibitors (ACE) (captopril, perindopril and lisinopril) positively modulated memory functions independent of their BP lowering effects.Further, they observed that patients on ACEis showed superior results on memory functions than those on β-blockers and diuretics (Amenta et al., 2002).

D. Angiotensin converting enzyme 2(ACE2)

Brain angiotensin (Ang) converting enzyme-2 (ACE2) was discovered two decades ago as an RAS component, exhibiting a counter-regulatory role and opposing the adverse cardiovascular effects produced by Ang-II. Studies using synthetic compounds that can sustain the elevation of ACE2 activity or genetically overexpressed ACE2 in specific brain regions found various beneficial effects on cardiovascular function. More recently, ACE2 has been shown to play critical roles in neuro-inflammation, gut dysbiosis and the regulation of stress and anxiety-like behavior (Mazher Mohammed et.al 2020 Oct 16). In the present review, we aim to highlight the anatomical locations and functional implication of brain ACE2 related to its BP regulation via modulation of the sympathetic nervous system and discuss the recent developments and future directions in the ACE2-mediated central cardiovascular regulation. (Mazher Mohammed et.al 2020 Oct 16).

E. Angiotensin peptides

Presence of all the angiotensin peptides Ang I, Ang II, Ang III and Ang (1–7) has been described in CNS (Saavedra, 2005).

- **Ang II**

Ang II, the most studied angiotensins (Saavedra, 2005), is either synthesized directly by cathepsin G or tonin from angiotensinogen, or by hydrolysis of Ang I by ACE in the brain (Saavedra 2005). Central Ang II is known to regulate various physiological/homeostatic and behavioural processes like thirst, blood pressure and sexual behaviour (Tota et al., 2013; Saavedra, 2005). thirst, blood pressure and sexual behaviour (Tota et al., 2013; Saavedra, 2005). In addition, Ang II has been demonstrated to modulate various brain functions, like cerebroprotection, stress, depression, and memory (Saaveedra 2012). Further several reports have revealed that the level of Ang II is predominantly high in the hippocampus, brain region involved in the memory functions (Sirett et al. 1981; Saaveedra 2012; Tota et al., 2013). . Moreover, many evidences highlight the potential role of Ang II in the etiology of neurodegenerative diseases, like AD and PD (Saaveedra 2012).

- **Ang III**

Angiotensin (Ang) III, a biologically active peptide of the renin angiotensin system (RAS) is predominantly known for its central effects on blood pressure. Our understanding of the RAS has evolved from the simplified, classical RAS, a hormonal system regulating blood pressure to a complex system affecting numerous biological processes. (Annabelle Reaux-Le Goazigo et.al 2005).

- **Ang IV**

Angiotensin IV binds to a widely distributed binding site in the brain, but which is different from the known angiotensin II receptors AT1 and AT2. Angiotensin IV has been implicated in a number of physiological actions, including the regulation of blood flow, the modulation of exploratory behaviour, and processes attributed to learning and memory. Furthermore, angiotensin IV may also be involved in neuronal development. (Oliver von Bohlen und Halbach 2003). Ang IV, central administration, induces the exploratory locomotory

behaviour, improves recall in passive avoidance task and enhances memory retention in rodents (Wright and Harding 2012).

- **Ang (1-7)**

Ang-(1-7) [angiotensin-(1-7)] constitutes an important functional end-product of the RAS (renin-angiotensin system) endogenously formed from AngI (angiotensin I) or AngII (angiotensin II) through the catalytic activity of ACE2 (angiotensin-converting enzyme 2), prolyl carboxypeptidase, neutral endopeptidase or other endopeptidases. (Mariela M Gironacci et.al 2013 july).The Ang (1–7) has also been reported to show neuroprotective action in cerebral ischemia by suppression of inflammatory NFκB activation (Jiang et al., 2012).

F. Angiotensin receptors

Angiotensin receptors are present in various regions of brain and spinal cord (Saavedra, 2005). Presently, four major angiotensin receptor subtypes (AT1R, AT2R, Review of Literature 19 AT4R and Mas receptor) have been categorized in the brain.n. AT1R, AT2R and Mas receptor are GPCRs but AT4R is a tyrosine kinase (de Gasparo et al., 2000).

- **Angiotensin type 1 receptor (AT1R)**

AT1R is expressed predominantly in lamina terminalis, olfactory bulb, PVN, NTS, substantia nigra, piriform cortex and hippocampus (Saavedra, 2005, Lenkei et al., 1997). In brain, as in periphery, the most of the physiological or pathological events of RAS are the outcome of stimulation of AT1Rs by Ang II (Saaveedra 2005, 2012). In the brain, AT1R activation contributes in cerebral circulation, integrity of BBB, sympathetic activity, stress, inflammation, behaviour and cognition (Saaveedra 2005, 2012). In fact, treatment with AT1R blockers (ARBs) prevented CNS sympathetic activity, neuroinflammation, cognitive decline and even improve cognitive functions in hypertensive patients (Saaveedra 2005, 2012) and animals (Saaveedra 2005, 2012). The ARBs as compared to ACEis have been found more effective in enhancing cognitive functions, particularly episodic memory (Saaveedra 2012). Importantly, previous study from our lab demonstrated that AT1R blockade by candesartan prevented streptozotocin (i.c.v) induced cognitive decline in rats by modulation of CBF and

suppression of oxidative stress, free radical production and acetylcholine esterase activity (Tota et al., 2008).

- **Angiotensin type 2 receptor (AT2R)**

The angiotensin type 2 receptor, AT2R, has been described as having opposite effects to the angiotensin type 1 receptor, AT1R. Although the quantities of the AT2R found in the adult are low, its expression rises in pathological situations. (Gabriel Faria-Costa et.al 2014 july). AT2R expression in CNS is limited to certain specific areas like, medulla oblongata, septum, amygdale, thalamus, cerebellum, PVN, hippocampus and cortex (Lenkei et al., 1997; Von Bohlen und Halbach, 2003). In fact, AT2R functions are still unclear both in many physiological and pathophysiological situations. Until recently, physiological and pharmacological assessments of the AT2Rs were uncovered using either the antagonist PD123,319 or the more Review of Literature 20 common AT2R agonist CGP42112A (McCarthy et al.,2012, 2014; Guimond and Gallo-Payet, 2012). Therefore, many aspects regarding AT2R functions in physiological situations have emerged from indirect observations, by blockade of the AT1 receptor. Of late, studies have demonstrated that activation of AT2R, during AT1R blockade, becomes important for the anti-inflammatory and neuroprotective effects of ARBs (Saavedra et al., 2011, 2012).

- **Angiotensin type 4 receptor (AT4R)**

Angiotensin IV binds to a widely distributed binding site in the brain, but which is different from the known angiotensin II receptors AT1 and AT2. Angiotensin IV has been implicated in a number of physiological actions, including the regulation of blood flow, the modulation of exploratory behavior, and processes attributed to learning and memory. Furthermore, angiotensin IV may also be involved in neuronal development. Collectively, the available evidence suggests that angiotensin IV is a potent neuropeptide, involved in a broad range of brain functions. (Oliver von Bohlen und Halbach Jan 2003).AT4R is activated by Ang IV that mediates its activation by tyrosine phosphorylation (Handa, 2001). The expression of AT4R, in brain areas associated with memory functions; highlight its role in cognition (Von Bohlen und Halbach, 2003).

- **Mas Receptor**

A GPCR called Mas is where Ang-(1-7) performs its biological actions (Lazaroni et al., 2012). The hippocampus, piriform cortex, and amygdala all exhibit significant levels of expression (Lazaroni et al., 2012). Mas receptor antagonist A779 or Mas knockdown reduced Ang-(1-7) mediated increase in cognitive functions, demonstrating the role of Mas receptor in modulating cognition (Kostenis et al., 2005). Additionally, central Ang-(1-7) injection through Mas receptor activation demonstrated neuroprotection in a rodent model of stroke by reducing NFκB activation (Rojo et al., 2012).

Hypertension and the brain

More than one in four persons suffer from hypertension. As the first organ to suffer harm from hypertension, the brain, which can appear as dementia, subclinical cerebrovascular abnormalities, and stroke. Vascular dementia and the pathophysiology of Alzheimer's disease can both be brought on by hypertension-related small vessel dysfunction, which lowers the threshold at which signs and symptoms appear. Many hypertensive situations, such as hypertension encephalopathy, haemorrhagic stroke, or pre-eclampsia, may also show neurologically. (Dearbhla M. Kelly et al., April 2020). Strong links exist between arterial hypertension and the brain. The hypothalamus, which is connected to the brain, can swiftly adjust the blood pressure level to sustain cerebral blood flow. In order to prevent neurological issues like encephalopathy from occurring, an abrupt increase in blood pressure that exceeds the autoregulatory capacity requires an immediate intervention. (M Milicevic et.al 2008 May). A significant and modifiable risk factor for dementia and stroke is hypertension. The cerebral resistance vessels are altered by hypertension, which reduces their tolerance to extremely low blood pressure. (Hanne Christensen et al, June 2009). Beta-blockers perform less well than other kinds of antihypertensives in the primary prevention of stroke. ACE inhibitors and angiotensin blockers may be suggested as first-choice medications for secondary stroke prevention. However, choosing the right medication is less significant than lowering blood pressure. (Hanne Christensen et al, June 2009). The brain has a small amount of energy that it can store and a high baseline metabolic rate. Therefore, a constant flow of blood that is present at all times and in all conditions is necessary for the execution and maintenance of all brain activities.

The cerebral vasculature is intricately and extensively regulated by communication from neurons, glia, interneurons, and perivascular nerves as the infrastructure through which the brain obtains the supplies required to enable neuronal processing and preserve the environment required for neural homeostasis. (Kathryn M. Dunn et al. 2013, Oct.) Particularly susceptible to hypertensive damage is the brain. It has been established that hypertension causes dementia and Alzheimer's disease through causing neurodegeneration and cognitive impairment. It is becoming more and more clear that hypertensive neurodegeneration results from the harm that high blood pressure causes to the cerebral vasculature. (Kathryn M. Dunn et al. 2013, October).

Hypertension and neurodegeneration

Hypertension is the major risk factor for the development of neurodegenerative diseases like AD and vascular dementia, the predominant form of cognitive impairment in humans (Goel et al., 2015, 2016). In approximately 50% of dementia cases display a mixed pathology i.e.both neurodegenerative and vascular lesions (Schneider et al., 2009). In fact, the untreated elderly hypertensive patients perform poorly in cognitive tasks (Knecht et al., 2009). The relationship between hypertension and memory functions gets further accentuated when it is observed that antihypertensive therapy attenuated hypertension induced cognitive deficit, independent of various other risk factors like age, income level, education, gender, and history of stroke (Goel et al., 2015, 2016). The Kahrizak Elderly Study, carried out on 211 subjects, aged 65 years or older, reported that higher diastolic blood pressure increases the risk for cognitive impairment in elderly persons (Sharifi et al., 2011). Similarly, an inverse relationship between increased systolic blood pressure and poor cognitive performance was found in NHANES III clinical trial (Suhr et al., 2004). Further, a study on elderly hypertensive patients (1617) followed up for 5 years showed 38% fall in memory impairment in patients treated with antihypertensive agents (ACE and ARBs) in comparison to untreated patients (Murray et al., 2002). Moreover, various other clinical studies have cemented this inverse relationship between cognition and hypertension (Kivipelto et al., 2001; Knecht et al., 2009; Fogari et al., 2004, 2006). Experimental studies on cognitive functions have shown that hypertension associated angiotensin peptides like Ang II and its fragments Ang IV and Ang III (Wright et al., 1993, 1996; Yang et al., 2008; Tota et al. 2011, 2012) modulate the cognitive functions directly or by controlling the release of neurotransmitters like

acetylcholine (Wright et al., 1993, 1996; Yang et al., 2008; Tota et al. 2011, 2012). Importantly, previous study from our lab demonstrated that hypertension increases the susceptibility to neurodegeneration in the presence of an inflammatory stimulus (Goel et al., 2015). Further, (Carnevelle et al2012) demonstrated that hypertension induced by transverse aortic constriction, induces neurodegeneration by increasing neuroinflammation, oxidative stress, BBB leakage and decreasing cerebral blood flow (CBF). Further, as reported above, studies from our lab demonstrated that antihypertensive agents like ARB or ACEi improved memory functions in rodent models of dementia (Tota et al. 2011, 2012; Goel et al., 2015). Discussion so far has associated hypertension, particularly RAS, with cognitive functions, therefore to dissect the role of hypertension in neurodegeneration, we have to discuss/understand the impeccable aspect of neurodegeneration i.e. neuroinflammation and neurogenesis in greater detail.

Hypertension and Neurogenesis

Neurodegenerative diseases like AD Display the typical progressive loss of neurons and gliosis (Glass et; al 2010) and the impairment in the neurogenesis (Saxe et al., 2006) i.e generation of new neurons. Chronic neurodegeneration hampers the stem cell maintenance, proliferation, survival and functional integration. Neurogenesis adds particular functionality to the mammalian brain because of its involvement in cognitive functions. Neurogenesis adds particular functionality to the mammalian brain because of its involvement in cognitive functions. There is a need for the detailed study of adult neurogenesis to open up a totally new avenue of therapeutics.

Neurogenesis is the generation of new neurons in the sub-granular zone of the dentate gyrus in hippocampus (Altman and Das, 1965; Kaplan and Hinds 1977).

Adult neurogenesis can be divided into four stages

a. Quiescent adult neural stem cells (NSCs) maintenance and proliferation: Quiescent NSCs are slow-growing multipotent cells with limitless self-renewal in this phase.

b. Fate specification: When NSCs are triggered, they divide asymmetrically in the SGZ of the hippocampus, producing transient intermediate progenitors (TIP). TIPs are rapidly dividing cells that can develop into neurons but have limited self-renewal ability. TIPs give

rise to neuroblasts, which exit the cell cycle after a limited number of cell divisions, and a subset survives and develops into new-born neurons.

c. Immature neuron differentiation, maturation, and survival: The maturation process is gradual, and more than half of newborn neurons die during this time.Within the first several weeks following birth, apoptosis occurs (Cameron et al., 1993; Biebl et al., 2000; Kempermann et al., 2003). Those immature neurons that develop connections, on the other hand, are attracted into the network.

d. Integration into the existing brain circuit: As these newborn neurons mature, they are integrated into the existing brain circuit. Long-term potentiation is modulated by granular cells (Aimone et al.,2006).

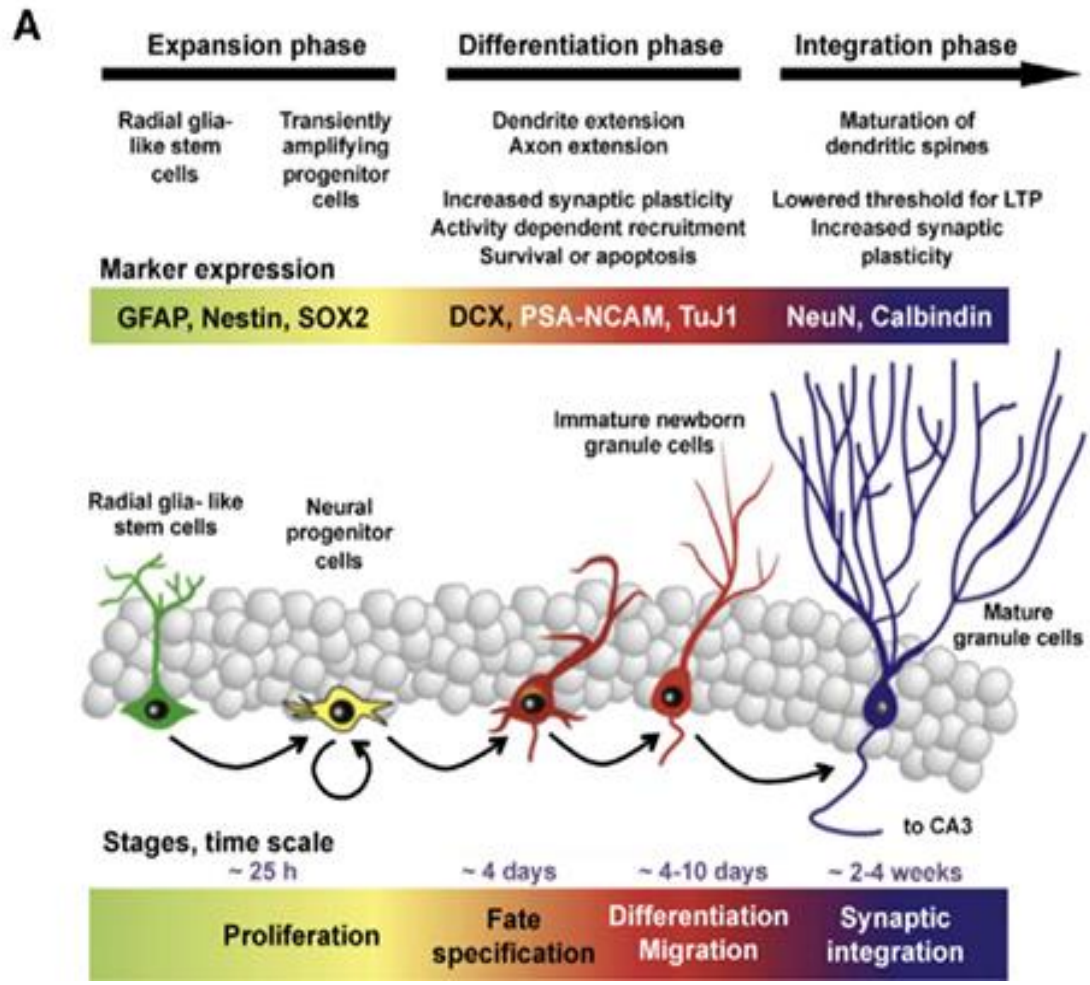


Fig2. Fig showing different stages of Neurogenesis adapted from (Marijn Schouten et;al 2012 Feb).

Effect of Chronic Hypertension on Neurogenesis

The brain is one of the main organs targeted and dysfunction by hypertension. (Goel et.al;2016). Hypertension is the leading risk factor for cerebrovascular events like decreased in Neurogenesis, stroke. (Goel et.al; 2016) and is increasingly associated with the development of dementia (Goel et.al; 2016). Relationship between hypertension and memory impairment is further strengthened when antihypertensive agent like angiotensin converting enzyme inhibitors and AT1 receptor blockers, improved memory functions in hypertensive subjects (Braszko et al. 2003; Fogari et al., 2004 and 2006) and in various rodent models of dementia (Tota et al., 2012). The dentate gyrus of the hippocampus is one of the few places in the brain where neurogenesis occurs in adulthood.

Hypertension is a chronic cardiovascular disease that is reaching epidemic proportions in western countries. Although hypertension is traditionally considered an ailment of old age, its prevalence is also increasing among children and youth (Falkner 2010). Nowadays it is well established that the hippocampus is specifically sensitive to the alterations in blood pressure (Sabbatini et al. 2002, Sabbatini et al. 2000). Importantly, hypertension can lead to the impairments in hippocampus-dependent processes such as learning and spatial orientation, even in the absence of clinical evidence of vascular damage (Harrington et al. 2000, Papademetriou 2005). Studies show that hypertension decreases (Pietranera et al. 2006, Pietranera et al. 2008, Pietranera et al. 2010, Shih et al. 2016) but also increases (Hwang et al. 2008, Kronenberg et al. 2007, Perfilieva et al. 2001) adult neurogenesis. Thus, question remains, however, if hippocampal sensitivity to blood pressure also includes alterations of adult neurogenesis in the dentate gyrus (DG). (A. PISTIKOVA et;al 2017 Nov).

Neurogenesis Pathways

- Wnt/β-Catenin Signalling Pathway
- The Notch signalling pathway
- The Rb pathway

Wnt/β-Catenin Signalling Pathway

The Wnt/-catenin signalling system is crucial for neural development. -catenin is a key component of the Wnt/-catenin signalling route, which not only transmits information in the cytoplasm but also translocate to the nucleus, where it activates target gene transcription. The target genes in neural tissues have not been fully identified, but current research has proven the impact of the Wnt/-catenin signalling pathway in adult neurogenesis, which is important for basic research and treatment of neuronal degenerative illnesses. (Lin Zhang, Xinyu Yang, and others, November 2010). Multiple elements of adult hippocampus neurogenesis are regulated by Wnt/-catenin signalling. Baicalin is a significant flavonoid molecule having a wide range of pharmacological properties, including anti-inflammatory, anti-apoptotic, and neuroprotective properties. The goal of this study was to look into the antidepressant properties of baicalin and its possible molecular mechanisms affecting hippocampal neurogenesis via the regulation of the Wnt/β-catenin signaling pathway. (Zhigang Xiao et;al 2021 August).

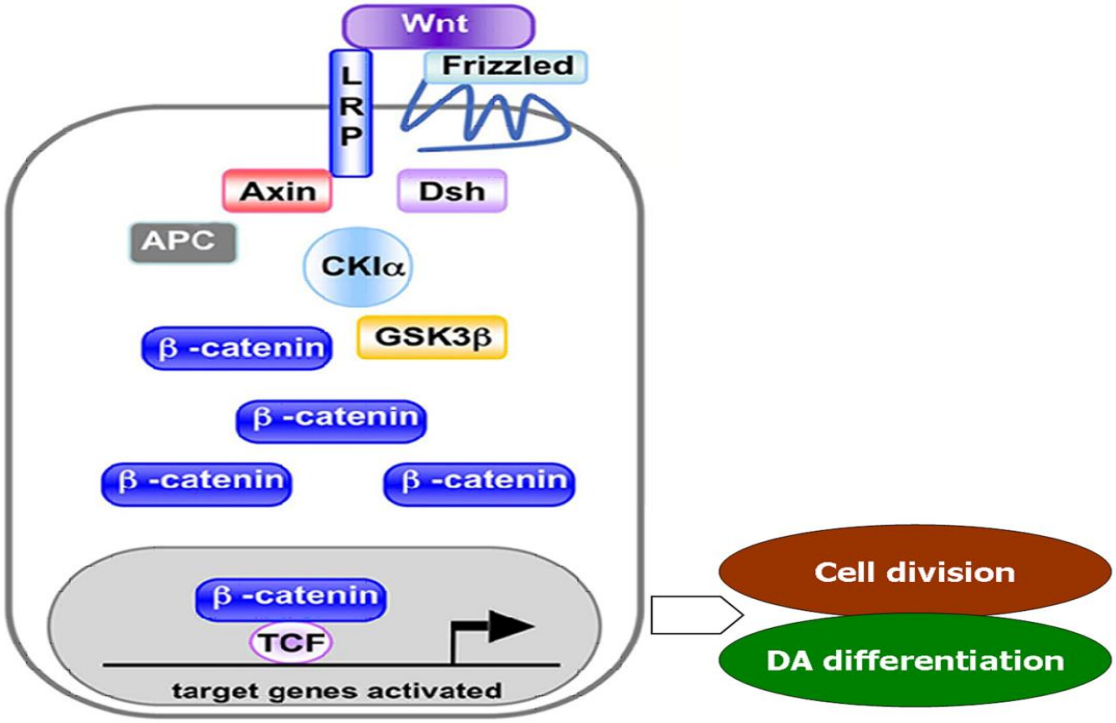


Fig3. The canonical Wnt/β-catenin signaling pathway in regulation of downstream target genes of DA neurogenesis (From Ref. 48, Ding, et al., 2011).

The Notch signalling pathway

In both the embryonic and adult brains, the Notch signalling pathway is important for neural stem cell maintenance and neurogenesis. Notch functions are highly reliant on proper control and cross-talk with other regulatory systems. Many neurodegenerative illnesses and brain disorders are linked to Notch signalling dysregulation. (Runrui Zhang et al., June 2017). Notch is a membrane-bound receptor with membrane-bound ligands that has evolved over time. Notch is a crucial integrator of environmental signals and can be thought of as amembrane-bound transcription factor' since ligand-mediated enzymatic cleavage results in nuclear signalling through canonical and non-canonical pathway.Notch is a membrane-bound transcription factor' that is thought to be a crucial integrator of environmental signals.

Notch controls the cell cycle in neural stem cells, ensuring that stem cell maintenance and daughter cell production are balanced. More neuronally devoted progeny are responsive to a variety of environmental signals, not only Notch ligands, indicating that Notch's ability to regulate proliferation is largely cell-type dependent. (Jessica L. Ables and colleagues, April 2011).

Notch signalling, in part through reelin–DAB (disabled homologous) signalling, also controls neuronal migration. According to this research, Notch-mediated migration modulation may also rely on indirect microtubule stability regulation. (Jessica L. Ables and colleagues, April 2011).

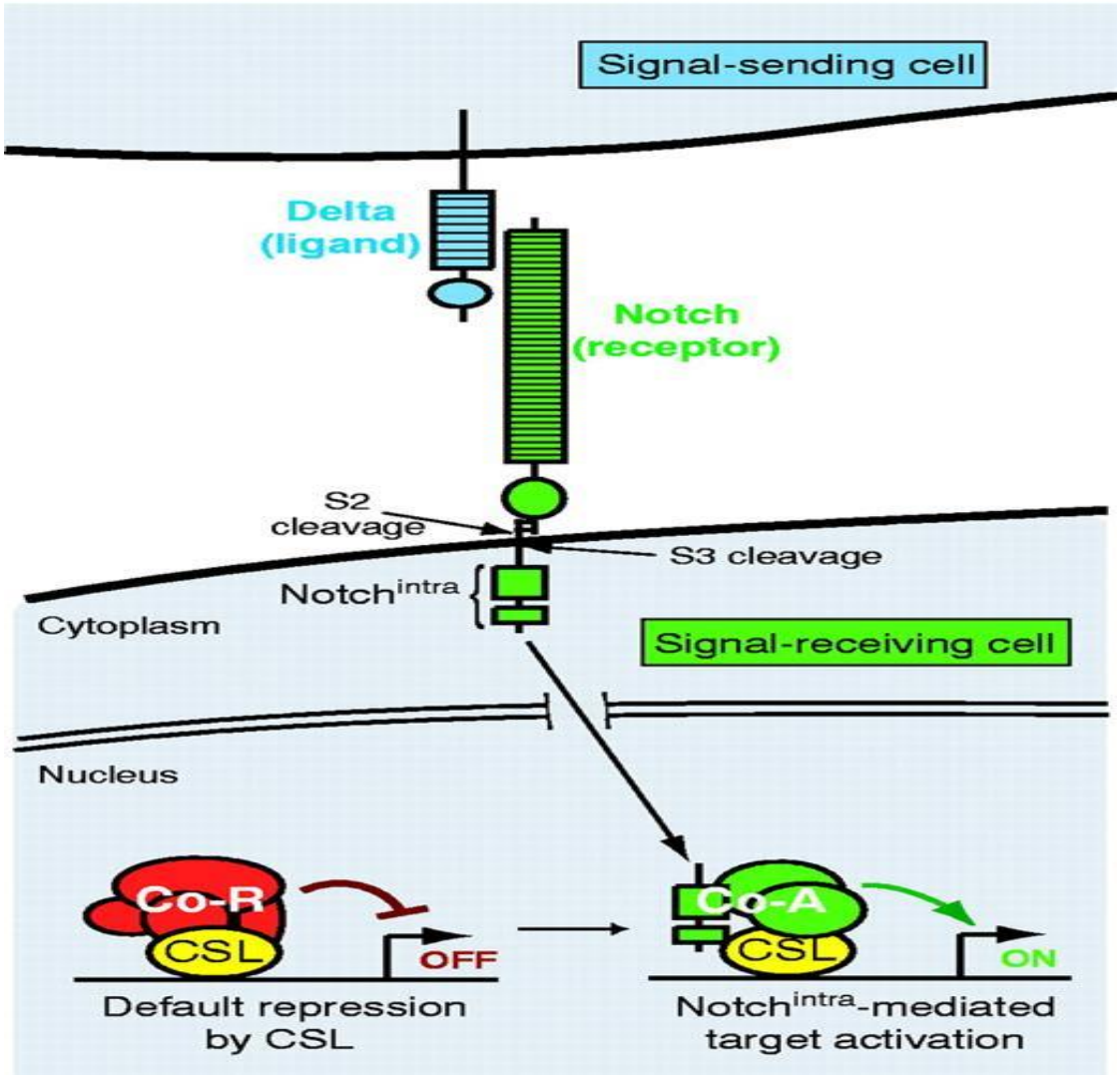


Fig4. Showing Basic operation of the Notch pathway adapted from (Eric C. Lai 2004 March)

The Rb pathway

Rb, the retinoblastoma protein, is known to have CNS-specific needs separate from its traditional role as a tumour suppressor. (Bensun C. Fong, R. Slack, and colleagues, January 2017). During embryogenesis, cell division is critical for the creation of the nervous system. Proliferating neural precursor cells commit to a neuronal fate and, as a result, undergo terminal mitosis and adopt a neuronal phenotype during this developmental process. (K L Ferguson et al., July 2001). A crucial regulator of the cell cycle

Retinoblastoma (Rb), a tumour suppressor protein, is involved in both terminal mitosis and neural development. Cell proliferation, cell fate determination and differentiation, as well as

programmed cell death, all play a role in neural development. (K L Ferguson et al., July 2001). The retinoblastoma protein, or pRb, is a tumour suppressor gene that regulates the G1-S cell cycle checkpoint (McClellan and Slack, 2006; Chen et al., 2009; Freedman et al., 2009). By binding and inhibiting E2F transcription factors, Rb regulates the transcription of genes needed for DNA replication and cell cycle advancement (Burkhart and Sage, 2008)

There are eight E2Fs, five of which can bind Rb (E2F1–5) and are regarded traditional Rb partners, and the other two are Rb-independent repressors (E2F6–8) (Dick and Dyson, 2006; Chen et al., 2009; Lammens et al., 2009). Through pocket protein binding, E2F1, 2, and 3 repress transcription and induce gene silencing, whereas E2F4 and 5 repress transcription and induce gene silencing (Dick and Dyson, 2006). Two unusual E2Fs, E2F7 and E2F8 (Lammens et al., 2009). can form homo and heterodimers which, in the absence of pocket proteins, bind and repress E2F target genes. The expression of E2F7 and 8 is induced by activating E2Fs and are believed to serve as a fine tuning mechanism to modulate E2F target gene regulation (Di Stefano et al., 2003; Christensen et al., 2005; Lammens et al., 2009), (Noël Ghanem et;al 2012 June).

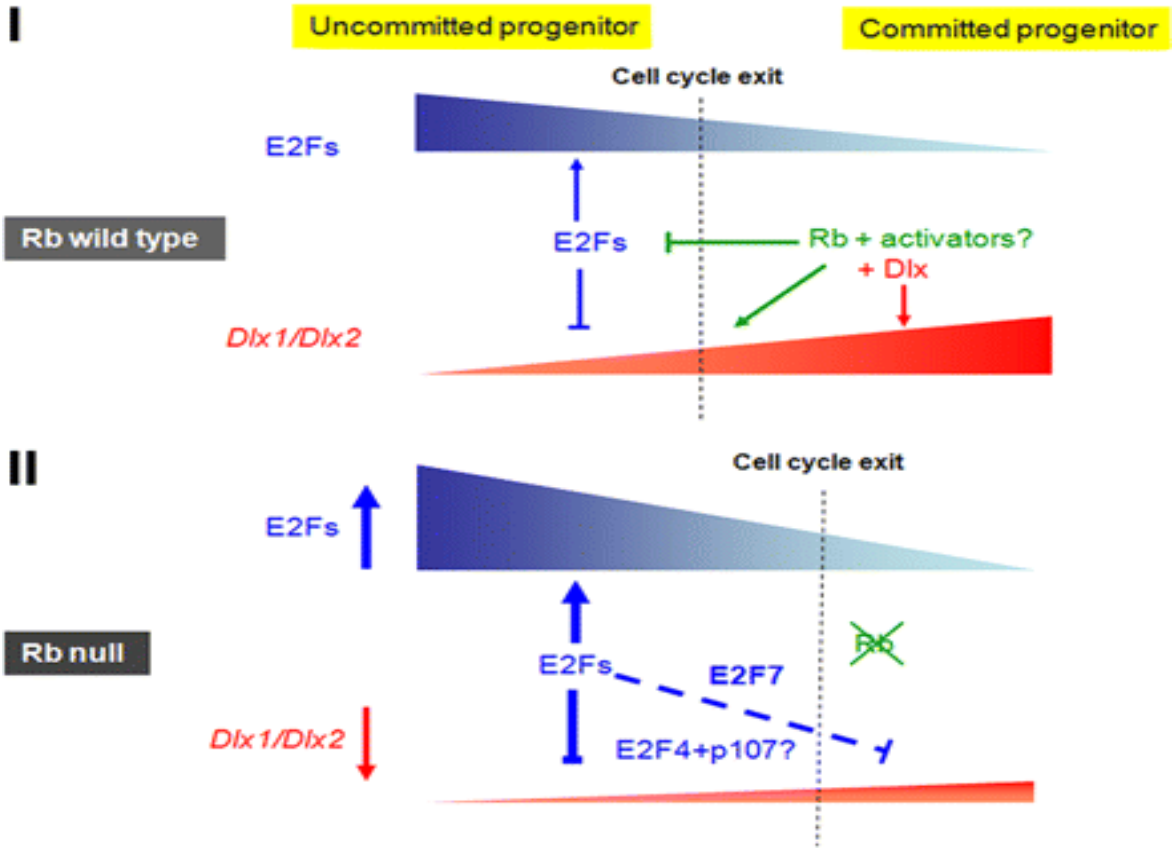


Fig5. showing the model of role of the Rb/E2F pathway in the coordination of proliferation and differentiation during neurogenesis. Adapted from (Noël Ghanem et;al 2012 June).

Regulation of Adult Neurogenesis in Mammalian Brain

A limited number of cells multiply, take on regional identities, and give rise to various cell types as the mammalian central nervous system (CNS) develops. This process is spatially and temporally controlled. (Maria Victoria Niklison-Chirou et al July 2020). These cells have been labelled as neural stem cells (NSCs) and have the capacity to differentiate into specialised brain cell types as neurons, astrocytes, and oligodendrocytes as well as to produce identical NSC progeny by symmetric cell division (self-renewal). The subventricular zone (SVZ) of the lateral ventricle, where new neurons are produced and eventually migrate to the olfactory bulb (OB), and the subgranular zone are two separate locations of the brain where adult neurogenesis is confined under physiological conditions(Maria Victoria Niklison-Chirou et;al 2020 July).

Despite being intricate and tightly controlled, neurogenesis may be broken down into six distinct stages. Stage 1 is known as the proliferation phase and occurs 1-3 days after birth. During this time, neural progenitor cells (NPCs) are capable of proliferating and

differentiating into a variety of cell types, but they are unable to self-renew. Stages 2-4, collectively referred to as the differentiation phase, take place about a week after birth. During this period, neural progenitors end the cell cycle and commit to the neuronal lineage. Immature neurons enter stage 5, also known as the migratory phase, following the commitment, to reach their destination. (Massimiliano Agostini et;al 2020 July). Between two and three weeks after delivery, this incident happens. Axonal projection lengthening and dendritic development begin in post-mitotic neurons. The final stage of adult neurogenesis is called synaptic integration, and it starts about four weeks after birth. During this stage, newly formed neurons create synaptic connections with those of the pre-existing circuits. In general, it takes 2-4 months for indistinguishable adult-born neurons to fully integrate with neighbouring cells and incorporate into the circuits of the hippocampus. (Massimiliano Agostini et;al 2020 July).

Epigenetic Mechanism in Neurogenesis

Neural stem cells multiply and give rise to neurons and glia in the embryonic and adult brain through highly controlled mechanisms. Different stages of neurogenesis are greatly influenced by epigenetic mechanisms, which include DNA and histone alterations as well as regulation by non-coding RNAs. Pathogenesis of numerous brain illnesses is also influenced by aberrant epigenetic control. (Bing Yao et al. 2016, September) Numerous traditional epigenetic mechanisms, such as DNA methylation, histone modifications, chromatin remodelling, and control carried out by non-coding RNAs like microRNAs (miRNAs) and long non-coding RNAs (lncRNAs), have been thoroughly studied (lncRNAs).

DNA Methylation in Neurogenesis

In order to produce 5-methylcytosine (5mC), which is a chemical alteration of the cytosine 5 carbon position, DNA methylation is required CpG islands26, areas with a high frequency of CG dinucleotides, have been the focus of DNA methylation research in the past. To preserve genomic stability, imprinted gene silence, and X-inactivation, the majority of CpG islands in mammals are hypomethylated. While DNMT3A and DNMT3B act as de novo methyltransferases to create new methylation patterns, DNMT1 predominantly copies the pre-existing methylation patterns for inheritance during DNA replication. (Kimberly M. Christian et;al 2016 June).

Histone Modification

In eukaryotes, DNA is wrapped around an octamer of histone proteins, which consists of two copies of each of the histone variations H2A, H2B, H3, and H4, to create a highly organised chromatin structure. By acting as docking stations to draw various epigenetic modifiers and transcription factors for transcriptional control, chemical covalent changes of the amino acids on the amino-terminal "histone tails" define the transcriptional environment. Furthermore, it has been proposed that interactions between histone and DNA modifications regulate the patterning and upkeep of the transcriptome landscape60. It is generally known that key functions in neurogenesis are played by histone methylation and acetylation on lysine residues(Bing Yao et;al 2016 Sep).

MicroRNAs and long non-coding RNAs in neurogenesis

In order to control the stability and translation of their target mRNA, microRNAs (miRNAs), a type of 20–25 nucleotide length non-coding RNAs, attach to either the 3′ untranslated region (UTR) or the coding sequence of the specific mRNA154. Numerous biological processes, including neurogenesis, have been revealed to be regulated by miRNAs154. For instance, miR-19 targets phosphatase and tensin homologue during embryonic neurogenesis to encourage the growth of radial glial cells (RGCs) and neural progenitor cells (NPCs). (Chuan He et;al 2016 June).

Chromatin Remodelling

For the regulation of gene expression, certain chromatin conformation is also necessary, along with chemical changes of histones and methylation of DNA. The right density and spacing of nucleosomes should be preserved because the presence of histones in the DNA creates a barrier to gene transcription. Nucleosome occupancy and composition are controlled by specialised ATP-dependent chromatin-remodelling complexes such as imitation switch (ISWI), chromodomain helicase DNA-binding (CHD), switch/sucrose non-fermentable (SWI/SNF also known as BRG1/BRM associated factor, BAF), and INO80. Chromatin remodelling complexes, which are made up of various protein combinations, have become significant regulators of neuronal growth. (Maria Victoria Niklison-Chirou et;al 2020).

CHAPTER- 3

AIM AND OBJECTIVES

AIM AND OBJECTIVES

AIM: To study the effect of Angiotensin-Converting Enzyme 2 activator (ACE2A) on neurogenesis in Spontaneously Hypertensive Rats (SHR)

Objectives:

1. To study the effect of Angiotensin-Converting Enzyme 2 activator (ACE2A) on hemodynamic parameters in spontaneously hypertensive rats (SHR).

2. To study the effect of Angiotensin-Converting Enzyme 2 activator (ACE2A) on the neuroinflammation in spontaneously hypertensive rats (SHR).

3. To study the effect of Angiotensin-Converting Enzyme 2 activator (ACE2A) on the parameters of oxidative stress and mitochondrial membrane potential in spontaneously hypertensive rats (SHR).

4. To study the effect of Angiotensin-Converting Enzyme 2 activator (ACE2A) on the markers of neurogenesis in spontaneously hypertensive rats (SHR).

CHAPTER-4

MATERIALS AND METHOD

MATERIALS AND METHODS

Animals

Male Spontaneously Hypertensive Rats (SHR) of 6 months old (250-350 gm) which is a well-accepted model of hypertension having major neurodegenerative changes in the brain has been used in this study. Wistar rats of similar age were used as control of SHRs. Animals were obtained from the Laboratory Animal Services Division of CSIR-Central Drug Research Institute, Lucknow. The animals were kept in polyacrylic cages with 7 Rats per cage and maintained under standard housing conditions (24-27 degrees Celsius) with a 12 hrs light and dark cycle.

Experimental setup

Group 1 – WISTAR

Group 2 – SHR

Group 3- SHR+ ACE2 activator (ACE2A) (10 mg/Kg)

Group 4- SHR+ ACE2 activator (ACE2A) (15 mg/Kg)

Drug administration

ACE2A was dissolved in a dose of 10mg/ml and 15mg/ml in normal saline. The dose of ACE2A was administrated by intraperitoneal injection daily for 4 weeks (28 days). About 28 rats were procured. Four groups were parted, each group comprising 7 rats.

Measurement of Body weight

Along with the dosing of the animals, their weight was also recorded weekly for a month to check the effect of the ACE2 activator on body weight.

Non-invasive blood pressure measurement (NIBP)

Non-invasive blood pressure method compared to other methods requires absolutely no surgery. The non-invasive rat blood pressure measurement consists of using a tail-cuff placed on the tail to occlude blood flow. These systems often come with a warming tray and snug restrainer to hold the animal. A comfortable and warm environment is necessary to produce peripheral vasodilatation and isolate the animal from external noise or other stimuli. This

makes conditioning any rodent a much easier task and lowers the risk of stressed animals influencing blood pressure results. In this study, we measured the blood pressure at the termination of the experiment. The hemodynamic parameters like systolic blood pressure (SBP), diastolic blood pressure (DBP), mean arterial blood pressure (MAP) and heart rate (HR) were recorded by using a tail-cuff placed on the tail to occlude blood flow.

Fig 6. Non-InvasiveBlood Pressure Measurement System (Columbus, USA)

Processing of brain tissue:

After the completion of the experiment, the rats were sacrificed with trans-cardiac perfusion. Rats were perfused through the heart with ice-cooled normal saline under ether anesthesia. The anesthetized rat was cut from its ventral side to expose the heart. The right auricle of the heart was punctured for efflux of blood following a slow injection of approximately 20 ml of ice-cooled normal saline through the left ventricle. The brain was immediately removed from each rat and cleaned with chilled normal saline and dissected hippocampus and cerebral cortex. In brief, the dissection of the brain was performed from the dorsal surface as follows: first the rhombencephalon (cerebellum, medulla and Pons) was separated by a transverse section from the rest of the brain. A transverse section was made at the level of the optic chiasm on the ventral surface. This section separated the cerebrum into two parts – the caudal portion and the rostral portion. Hippocampus and cerebral cortex were dissected from the caudal portion by opening the flaps. Collected brains were immediately stored at −80 °C for biochemical analysis and immunoblotting.

Western blot studies

Western blot is well-established technique to quantify protein expression. The brain was homogenized by using an Ultra-Turrax T25 homogenizer in five volumes of ice-cold lysate buffer (20 mM HEPES (pH- 7.5), 250 mM sucrose, 1mM dithiothreitol, 10Mm KCL, 1Mm EDTA, 1Mm EGTA, 1x protease inhibitor cocktail). The homogenates were then spun at 13000 rpm for 20 minutes and the resultant supernatant was used as whole cell lysate. The protein concentration of each sample was determined spectrophotometrically. An equal amount of proteins was separated on 10% or 12% SDS-PAGE and transferred to a polyvinylidene difluoride (PVDF) membrane. Protein ladder marker also loaded to confirm molecular weight of protein bands. Membranes were blocked with blocking buffer (5% BSA) for 2 h. Membranes were then incubated overnight at 4 Degree with primary antibody. After three washings for 5 minute each with TBST, the membrane was incubated with respective HRP-conjugated secondary antibodies for 2 h at room temperature. Blots were then developed by the ECL chemiluminescence detection system (Millipore, USA). The band intensity was measured using spot densitometry analysis.

Estimation of protein

Protein concentration was estimated at 560 nm wavelengths by the method of Bicinchoninic acid (BCA) kit (Thermo Fisher Scientific) in all the brain tissue samples using Bovine serum albumin (BSA) (1 mg/ml) as standard.

Estimation of Inflammatory cytokines by ELISA: Inflammatory cytokines in brain tissue were measured by ELISA following the protocol provided by the manufacturer (BD Bioscience, USA). For cytokines estimation in rat brain, tissues were homogenized in ice-cold tris buffer (pH 7.2) containing 50 mM Tris, 1 mM EDTA, 6 mM MgCl2, and 5% (w/v) protease inhibitor cocktail (Sigma-Aldrich, USA). After homogenization, samples were sonicated for 30 sec using an ultrasonic processor (Heat systems Ultrasonic inc.) at a setting of 20 duty cycles and then centrifuged at 20,000g for 20 min at 4^0C. Supernatants were collected and the level of the proinflammatory cytokine (TNF-α) was determined using a commercially available ELISA kit. The concentration of protein in the sample was determined by comparison with a standard curve of known protein concentrations. Cytokines level (TNF-α) were expressed as picogram per mg of protein.

Estimation of reduced glutathione (GSH): GSH level from different groups was estimated by using 0.2% DTNB reagent. In brief, the homogenized brain followed by ultra-sonication was centrifuged at 10,000 g for 5 min at 4^0C. An equal amount of brain homogenate (100 µl) from different groups was mixed with 50 µl of 0.2% DTNB reagent, followed by an incubation period of 10 min at RT. The absorbance was read at 412 nm.

Estimation of Mitochondrial membrane potential (ψm): The difference in the MMP was detected by JC-1 dye (2.5 µM) by using flow cytometry (Maurya et al., 2015).

RNA preparation and RT-PCR: Quantitative gene expression analysis was performed by using SYBR Green technology as described previously (Khanna et al., 2013). Briefly, the total RNA was extracted from different groups using TRIZOL (Sigma, USA) isolation procedure and cDNA was synthesized using RevertAid™ H Minus first-strand cDNA synthesis kit following the manufacturer's protocol (Invitrogen, USA). mRNA expression of key genes associated with apoptosis was quantified using specific primers. Real-time RT-PCR was carried out in QuantStudio 12K 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 GAPDH as an internal standard (Khanna et al., 2013) and the relative amount of mRNA was presented in the form of fold change over control.

Bax: Forward primer: AGTGTCTCAGGCGAATTGGC

Reverse primer: CACGGAAGAAGACCTCTCGG

β-actin: Forward primer:CCCGCGAGTACAACCTTCT

Reverse primer: CGTCATCCATGGCGAACT

Statistical analysis

Statistical analysis was done by using Prism software version 5.0 (Graph Pad Software, San Diego, CA, USA). All Results are expressed as mean ± SEM. Statistical significance was evaluated by *student's* t test and one-way analysis of variance (ANOVA) followed by Tukey's multiple comparison tests. A value of $p < 0.05$ was considered to be statistically significant.

CHAPTER-5

RESULTS

Results

1) Effect of ACE2A treatment on body weight in SHRs: Body weight was recorded every week till the 28th day of the experimental duration to see the effect of chronic hypertension or ACE2A on body weight. We did not find any change in the body weight of SHRs treated with ACE2A as comparison to SHR control rats.

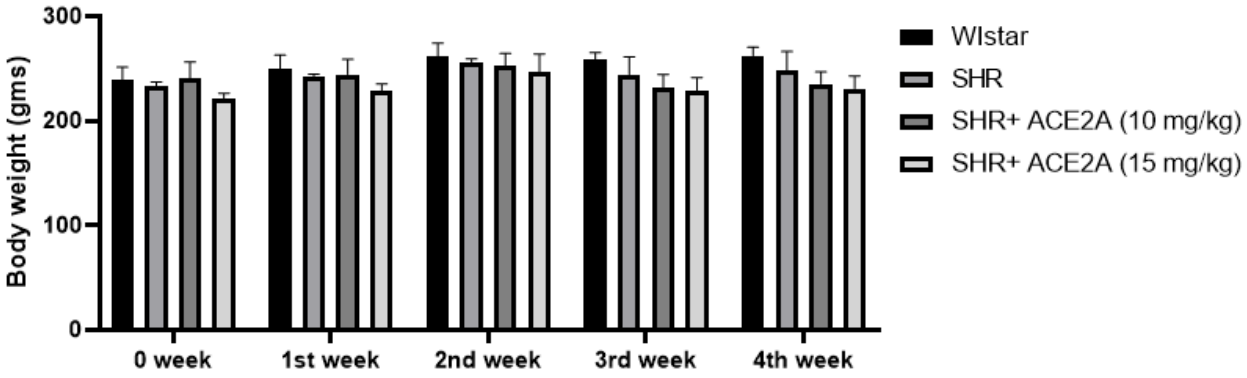
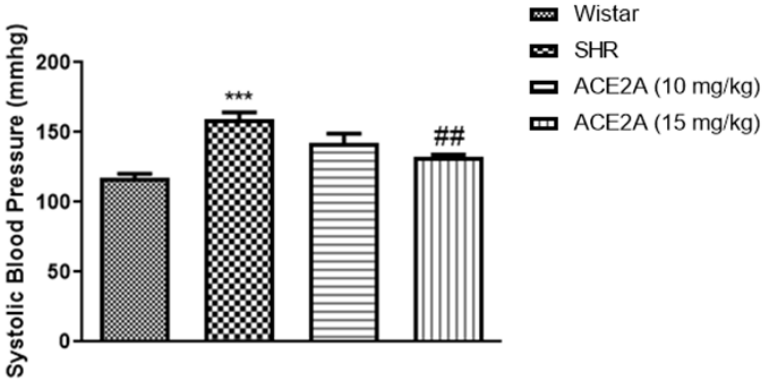
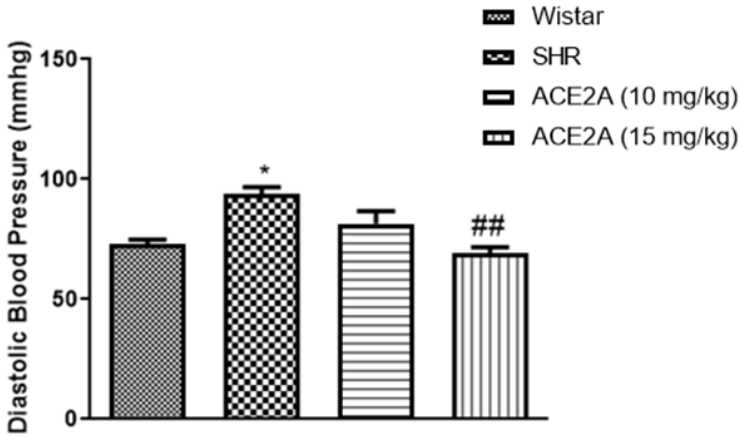


Figure 1: Effect of 2 different doses of ACE2A treatment on body weight of SHR measured weekly till 4th week (n=7 rats/group).

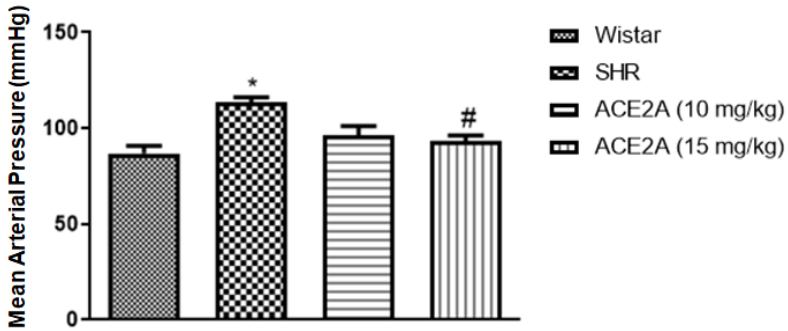
2) Effect of ACE2A treatment on hemodynamic parameters in SHRs: Effects of treatment with ACE2A in SHRs were recorded at the termination of the experiment by non-invasive blood pressure measurement.Systolic blood pressure (SBP), Diastolic blood pressure (DBP), Mean Arterial Pressure (MAP), and Heart Rate (HR) were significantly increased in SHRs which were significantly decreased by Angiotensin-converting enzyme 2 activator, treatment at 15 mg/kg for 4 weeks.



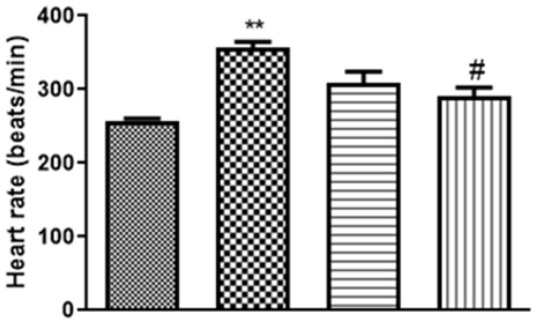
Systolic Blood Pressure (SBP)	
Wistar	117.3 ± 3
SHR	159.3 ± 4.8***
SHR + ACE2A (10 mg/kg)	142.6 ± 6.6
SHR + ACE2A (15 mg/kg)	132 ± 2##



Diastolic Blood Pressure (DBP)	
Wistar	73.0 ± 1.8
SHR	98.98 ± 6.9*
SHR + ACE2A (10 mg/kg)	81.55 ± 5.2
SHR + ACE2A (15 mg/kg)	69.39 ± 2.27##



Mean Arterial Pressure (MAP)	
Wistar	87 ± 3.8
SHR	113.3 ± 2.8*
SHR + ACE2A (10 mg/kg)	96.40 ± 4.8
SHR + ACE2A (15 mg/kg)	93.14 ± 3#



Heart Rate (HR)	
Wistar	257.4 ± 3.3
SHR	356.7 ± 7.77**
SHR + ACE2A (10 mg/kg)	308.9 ± 15.0
SHR + ACE2A (15 mg/kg)	291 ± 11.98#

Figure 2: Effect of 2 different doses of ACE2A treatment on haemodynamic parameter like **(A)**SBP, **(B)**DBP, **(C)**MAP, and **(D)**HR in SHR model of Hypertension(n=7 rats/group).

3) Effect of ACE2Atreatment on inflammatory cytokines in SHRs brain hippocampus: Effects of treatment with ACE2A in SHRs were estimated in the brain hippocampus. We found that ACE2A treatment both at 10 mg/kg and 15 mg/kg can significantly reduce the pro-inflammatory cytokines (TNF-α) in brain hippocampus of SHR rats.

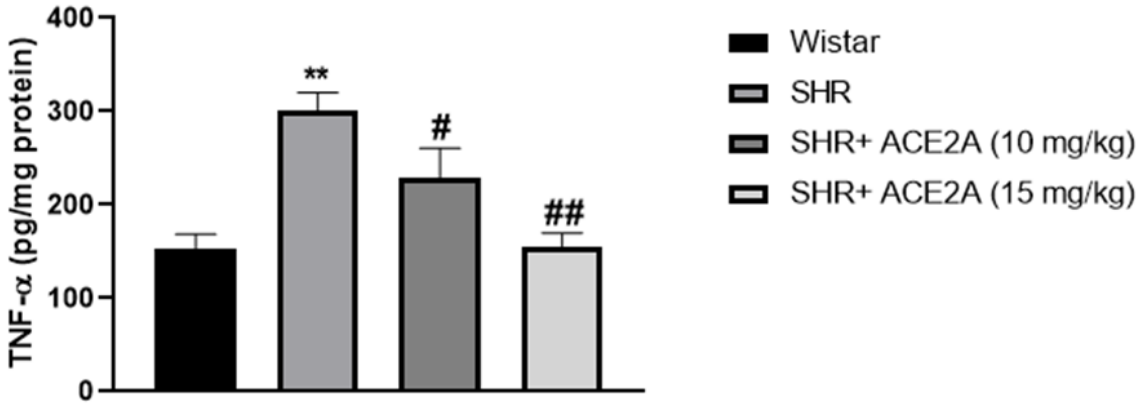


Figure 3: Effect of 2 different doses of ACE2A treatment onpro-inflammatory cytokine (TNF-α) in the hippocampus region of SHR model of Hypertension (n=3-4).

4) Effect of ACE2Atreatment on reduced glutathione level in SHR rat brain hippocampus: Effect of treatment with ACE2 activatorin chronic hypertension induced SHR rat brain on reduced glutathione level was estimated as per the protocol mentioned in the materials and methods sections and reading was done by spectrophotometer. We found that ACE2A increased the glutathione level in the ACE2A-treated SHR rat brain hippocampus as compared to SHR control.

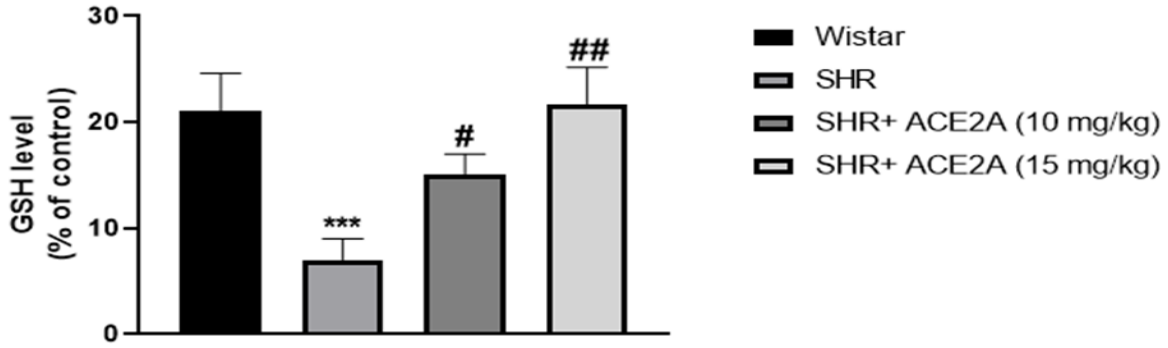


Figure 4: Effect of 2 different doses of ACE2A treatment onGSHlevel in hippocampus region of SHR model of Hypertension (n=3-4).

5) Effect of ACE2Atreatment on mitochondrial membrane potential (MMP) in SHR rat hippocampus: Effect of treatment with ACE2 activator treatment on MMP was determined by flow cytometry. We found the ACE2A reduced the mitochondrial depolarization (reduced MMP)significantly in ACE2A treated SHR rat brain hippocampus as compared to SHR control rat brain hippocampus.

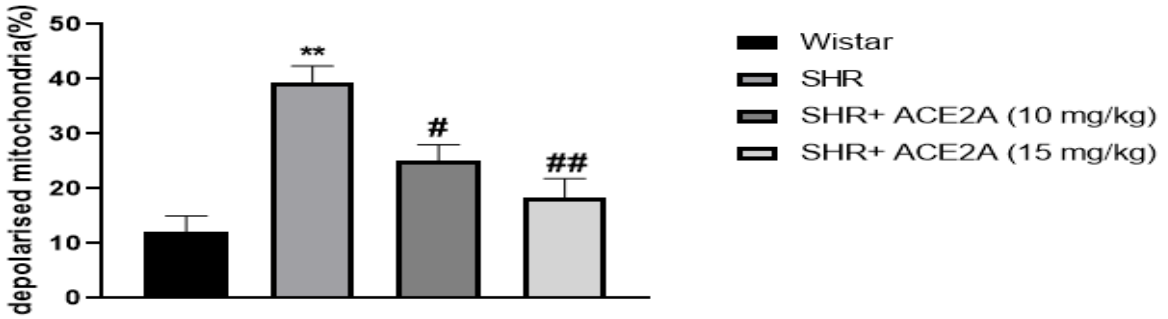


Figure 5: Effect of 2 different doses of ACE2A treatment on Mitochondrial membrane potential (MMP) determined by JC-1 staining by Flow cytometry (FACS) in hippocampus region of SHR model of Hypertension (n=3-4).

6) Effect of ACE2Atreatment on markers of apoptosis in SHR rat hippocampus: Effect of treatment with ACE2 activator treatment on apoptosis markers was determined by RT-PCR. We found the ACE2A can reduce the mRNA expression of pro-apoptotic marker, Bax significantly in ACE2A treated SHR rat brain as compared to SHR control.

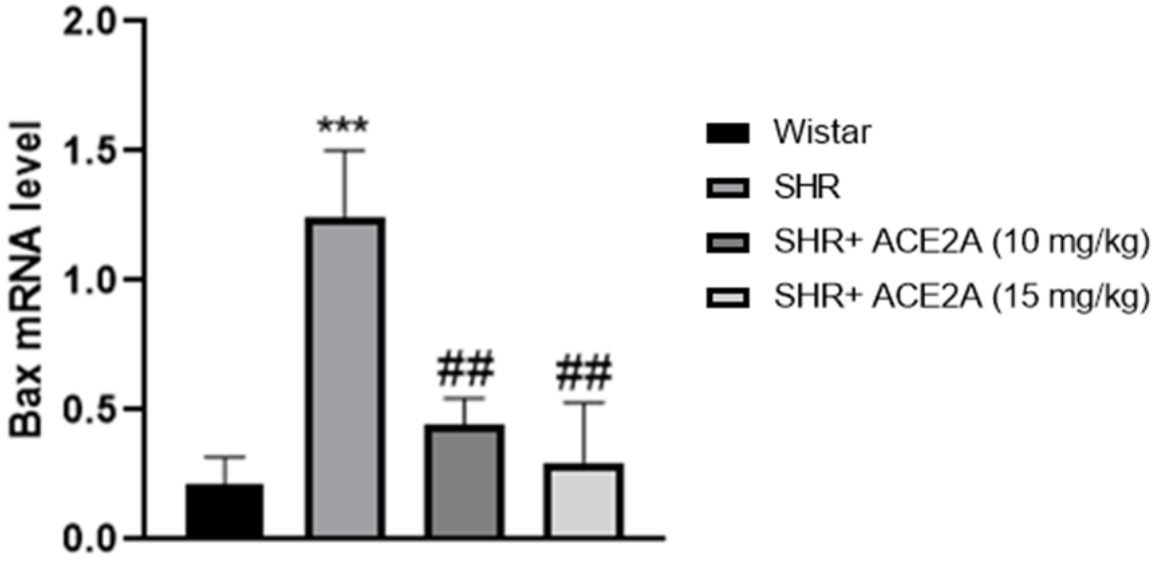


Figure 6: Effect of 2 different doses of ACE2A treatment on mRNA level of Bax in hippocampus region of SHR model of Hypertension (n=4).

7) Effect of ACE2Atreatment on markers of neurogenesis in SHR rat brain: Effect of treatment with ACE2 activator treatment on neurogenesis markerslike Wnt 3a, p-GSK3β was determined by western blotting. We found that ACE2A can increase the protein expression of Wnt 3a, p-GSK3β significantly in ACE2A-treated SHR rat hippocampus as compared to SHR control.

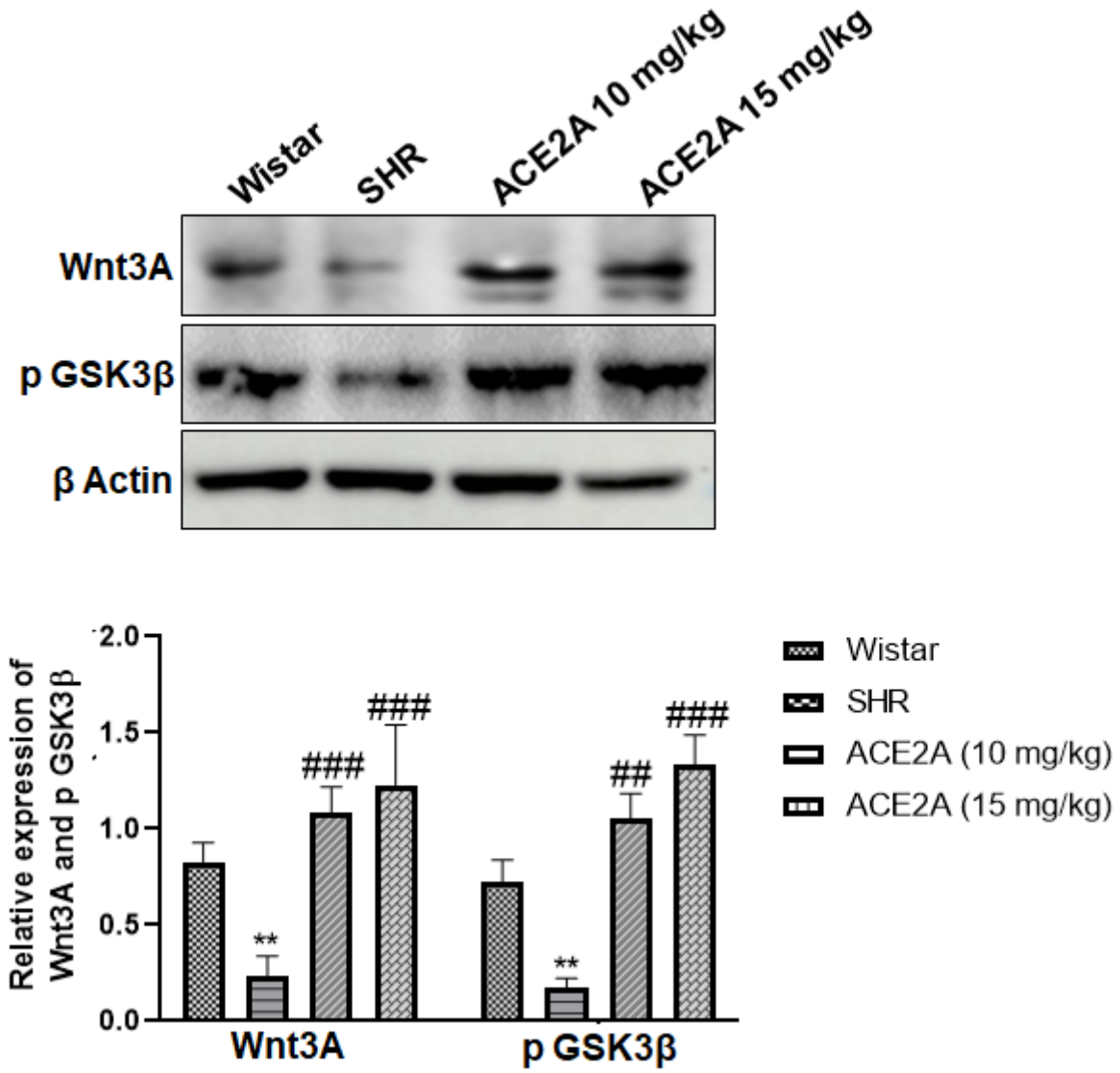


Figure 7: Effect of 2 different doses of ACE2A treatment on protein expression of neurogenesis markers Wnt3A and p-GSK3β in hippocampus region of SHR model of Hypertension (n=4).

CHAPTER-6

DISCUSSION

Discussion

Hypertension is an epidemic health challenge, a proven major risk factor of the development of cardiovascular disease and the leading cause of morbidity and mortality worldwide. Despite the availability of several classes of antihypertensive drugs, the treatment of hypertension often remains suboptimal. In addition, the prevalence of uncontrolled hypertension continues to rise globally. It is well established that neurodegeneration is an integral aspect of hypertension. Activation of the RAS is known to be a key mediator of hypertension, and interventions to block RAS activation are the most widely used of all blood pressure lowering agents.

In present study we have explored an ACE2 activator classical drug for trypanosomiasis and babesiosis in our in-vivo study. In this study, we used the SHR model which is a genetically hypertensive rat and well-established model to study hypertension induced changes. SHRs were treated with ACE2A for 28 days by intraperitoneal mode to check the effect of ACE2 activation in neurodegenerative and neurogenesis changes in model of hypertension.

We observed that ACE2 activator at the dose of 10 mg/kg and 15 mg/kg did not have any effect on body weight but ACE2A at the dose of 15 mg/kg was reducing all the haemodynamic parameters.However, we further found that ACE2A at both the doses of 10 mg/kg and 15 mg/kg is capable of reducing the inflammation by downregulating pro-inflammatory cytokine (TNF-α) in hippocampus of SHR rats treated with ACE2A. Wefurther checked the effect of ACE2A at both the doses of 10 mg/kg and 15 mg/kg on Glutathione level and found thatACE2A at both the doses of 10 mg/kg and 15 mg/kg is capable of upregulating the GSH level in hippocampus of SHR rats treated with ACE2A.It leads to conclude that ACE2 activation perform a crucial role in regulating the Neuroinflammation and oxidative stress.

Wefurther checked the mRNA level of neurodegenerative markers like Bax and found that ACE2A treatment at both the doses of 10 mg/kg and 15 mg/kg reduced the Bax expression. Further, the neurogenesis markers involved in Wnt-β catenin signalling like Wnt-3A and p-GSK3β were found to be upregulated by ACE2A treatment in SHR rat brain hippocampus. This implies that ACE2A treatment significantly upregulating the neurogenesis in SHR model of hypertension.

CHAPTER- 7

CONCLUSION

Conclusion

The study demonstrated that the ACE2 activation led to increase in the neurogenesis by suppressing the neurodegenerative markers, inflammation, oxidative stress burden, and by improving mitochondrial health in Spontaneously hypertensive rat model of hypertension. Therefore, potential drugs, may be screened for the activation of ACE2 and successful molecules can be taken up for future drug development program.

References

Oparil, S., Acelajado, M. C., Bakris, G. L., Berlowitz, D. R., Cífková, R., Dominiczak, A. F., Grassi, G., Jordan, J., Poulter, N. R., Rodgers, A., & Whelton, P. K. (2018). Hypertension. Nature reviews. Disease primers, 4, 18014. https://doi.org/10.1038/nrdp.2018.14

Anchala, R., Kannuri, N. K., Pant, H., Khan, H., Franco, O. H., Di Angelantonio, E., et al. (2014). Hypertension in India: a systematic review and meta-analysis of prevalence, awareness, and control of hypertension. J Hypertens, 32(6), 1170-1177.

Pistikova A, Brozka H, Bencze M, Radostova D, Vales K, Stuchlik A. The effect of hypertension on adult hippocampal neurogenesis in young adult spontaneously hypertensive rats and Dahl rats. Physiol Res. 2017 Nov 24;66(5):881-887. doi: 10.33549/physiolres.933562. Epub 2017 Jul 18. PMID: 28730828.

Shih YH, Tsai SF, Huang SH, Chiang YT, Hughes MW, Wu SY, Lee CW, Yang TT, Kuo YM. Hypertension impairs hippocampus-related adult neurogenesis, CA1 neuron dendritic arborization and long-term memory. Neuroscience. 2016 May 13;322:346-57. doi: 10.1016/j.neuroscience.2016.02.045. Epub 2016 Feb 26. PMID: 26921651.

Oparil, S., Acelajado, M. C., Bakris, G. L., Berlowitz, D. R., Cífková, R., Dominiczak, A. F., Grassi, G., Jordan, J., Poulter, N. R., Rodgers, A., & Whelton, P. K. (2018). Hypertension. Nature reviews. Disease primers, 4, 18014. https://doi.org/10.1038/nrdp.2018.14

Mills, K.T., Stefanescu, A. & He, J. The global epidemiology of hypertension. Nat Rev Nephrol 16, 223–237 (2020).

Iqbal AM, Jamal SF. Essential Hypertension. [Updated 2022 Feb 14]. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2022 Jan.

Charles L, Triscott J, Dobbs B. Secondary Hypertension: Discovering the Underlying Cause. Am Fam Physician. 2017 Oct 1;96(7):453-461. PMID: 29094913.

Carretero OA, Oparil S. Essential hypertension. Part I: definition and etiology. Circulation. 2000 Jan 25;101(3):329-35. doi: 10.1161/01.cir.101.3.329. PMID: 10645931.

Vachek J, Zakiyanov O, Oulehle K, Ciferská H, Tesař V. Renal parenchymal hypertension: relevant new aspects. Vnitr Lek. 2021 Winter;67(8):479-484. English. PMID: 35459368.

Fenves AZ, Ram CV. Renovascular hypertension: clinical concepts. Minerva Med. 2006 Aug;97(4):313-24. PMID: 17008836.

Pappachan JM, Buch HN. Endocrine Hypertension: A Practical Approach. Adv Exp Med Biol. 2017;956:215-237. doi: 10.1007/5584_2016_26. PMID: 27864805.

de Leeuw PW. Drug-induced hypertension. Recognition and management in older patients. Drugs Aging. 1997 Sep;11(3):178-85. doi: 10.2165/00002512-199711030-00003. PMID: 9303278.

Borzecki, A. M., Kader, B., & Berlowitz, D. R. (2010). The epidemiology and management of severe hypertension. Journal of human hypertension, 24(1), 9–18. https://doi.org/10.1038/jhh.2009.37

Dinh, Q. N., Drummond, G. R., Sobey, C. G., & Chrissobolis, S. (2014). Roles of inflammation, oxidative stress, and vascular dysfunction in hypertension. BioMed research international, 2014, 406960. https://doi.org/10.1155/2014/406960.

Vargas Vargas, R. A., Varela Millán, J. M., & Fajardo Bonilla, E. (2022). Renin-angiotensin system: Basic and clinical aspects-A general perspective. Endocrinologia, diabetes y nutricion, 69(1), 52–62. https://doi.org/10.1016/j.endinu.2021.05.012.

McCarthy CA, Facey LJ, Widdop RE. The protective arms of the renin-angiontensin system in stroke. Curr Hypertens Rep. 2014 Jul;16(7):440. doi: 10.1007/s11906-014-0440-1. PMID: 24816974.

Lu, H., Cassis, L. A., Kooi, C. W., & Daugherty, A. (2016). Structure and functions of angiotensinogen. Hypertension research : official journal of the Japanese Society of Hypertension, 39(7), 492–500. https://doi.org/10.1038/hr.2016.17.

Tewksbury DA. Angiotensinogen. Fed Proc. 1983;42:2724–8.

Okuyama A, Nonomura N, Nakamura M, Namiki M, Sonoda T. Renin-angiotensin system. Arch Androl. 1988;21(3):169-80. doi: 10.3109/01485018808986739. PMID: 3072932.

Le D, Brown L, Malik K, Murakami S. Two Opposing Functions of Angiotensin-Converting Enzyme (ACE) That Links Hypertension, Dementia, and Aging. Int J Mol Sci. 2021 Dec 7;22(24):13178. doi: 10.3390/ijms222413178. PMID: 34947975; PMCID: PMC8707689.

Atlas SA. The renin-angiotensin aldosterone system: pathophysiological role and pharmacologic inhibition. J Manag Care Pharm. 2007 Oct;13(8 Suppl B):9-20. doi: 10.18553/jmcp.2007.13.s8-b.9. PMID: 17970613.

Chappell M. C. (2016). Biochemical evaluation of the renin-angiotensin system: the good, bad, and absolute?. American journal of physiology. Heart and circulatory physiology, 310(2), H137–H152. https://doi.org/10.1152/ajpheart.00618.2015

Wright JW, Harding JW. The brain renin-angiotensin system: a diversity of functions and implications for CNS diseases. Pflugers Arch. 2013 Jan;465(1):133-51. doi: 10.1007/s00424-012-1102-2. Epub 2012 Apr 26. PMID: 22535332.

de Gasparo, M., Catt, K. J., Inagami, T., Wright, J. W., & Unger, T. (2000). International union of pharmacology. XXIII. The angiotensin II receptors. Pharmacol Rev, 52(3), 415-472.

Vauquelin, G., Michotte, Y., Smolders, I., Sarre, S., Ebinger, G., Dupont, A., et al. (2002). Cellular targets for angiotensin II fragments: pharmacological and molecular evidence. J Renin Angiotensin Aldosterone Syst, 3(4), 195-204.

Albrecht D. Physiological and pathophysiological functions of different angiotensins in the brain Br J Pharmacol. 2010 Apr; 159(7): 1392–1401.

Hallberg M, Nyberg F. Neuropeptide conversion to bioactive fragments – an important pathway in neuromodulation. Curr Protein Pept Sci. 2003;4:31–44.

Lavoie JL, Sigmund CD. Minireview: overview of the renin–angiotensin system – an endocrine and paracrine system. Endocrinology. 2003;144:2179–2183.

Hu J, Tiwari S, Riazi S, Hu X, Wang X, Ecelbarger CM. Regulation of angiotensin II type I receptor (AT1R) protein levels in the obese Zucker rat kidney and urine. Clin Exp Hypertens. 2009 Feb;31(1):49-63. doi: 10.1080/10641960802409788. PMID: 19172459.

Phillips MI, Speakman EA, Kimura B. Levels of angiotensin and molecular biology of the tissue renin angiotensin systems. Regul Pept. 1993 Jan 22;43(1-2):1-20. doi: 10.1016/0167-0115(93)90403-u. PMID: 8426906.

Chaudhary M. Anti-Hypertensive Potential and Epigenetics of Angiotensin II type 2 Receptor (AT2R). Curr Hypertens Rev. 2021;17(3):176-180. doi: 10.2174/1573402116999201209203015. PMID: 33302839.

Chai SY, Fernando R, Peck G, Ye SY, Mendelsohn FA, Jenkins TA, Albiston AL. The angiotensin IV/AT4 receptor. Cell Mol Life Sci. 2004 Nov;61(21):2728-37. doi: 10.1007/s00018-004-4246-1. PMID: 15549174.

Santos RA, Haibara AS, Campagnole-Santos MJ, Simões e Silva AC, Paula RD, Pinheiro SV, Leite MF, Lemos VS, Silva DM, Guerra MT, Khosla MC. Characterization of a new selective antagonist for angiotensin-(1-7), D-pro7-angiotensin-(1-7). Hypertension. 2003 Mar;41(3 Pt 2):737-43. doi: 10.1161/01.HYP.0000052947.60363.24. Epub 2003 Jan 20. PMID: 12623989.

Zubcevic, J., Waki, H., Raizada, M. K., & Paton, J. F. (2011). Autonomic-immune-vascular interaction: an emerging concept for neurogenic hypertension. Hypertension, 57(6), 1026-1033.

Patrick, D. M., Van Beusecum, J. P., & Kirabo, A. (2021). The role of inflammation in hypertension: novel concepts. *Current opinion in physiology*, *19*, 92–98. https://doi.org/10.1016/j.cophys.2020.09.016.

King, D. E., Egan, B. M., Mainous, A. G., 3rd, & Geesey, M. E. (2004). Elevation of Creactive protein in people with prehypertension. J Clin Hypertens (Greenwich), 6(10), 562-568.

Mattace-Raso, F. U., Verwoert, G. C., Hofman, A., & Witteman, J. C. (2010). Inflammation and incident-isolated systolic hypertension in older adults: the Rotterdam study. J Hypertens, 28(5), 892-895.

Harrison, D.G., Marvar, P.J., and Titze, J.M. 2012. Vascular inflammatory cells in hypertension. Front Physiol, 3: 128.

Rodriguez-Iturbe, B., Franco, M., & Johnson, R. J. (2013). Impaired pressure natriuresis is associated with interstitial inflammation in salt-sensitive hypertension. Curr Opin Nephrol Hypertens, 22(1), 37-44.

Solak, Y., Afsar, B., Vaziri, N. D., Aslan, G., Yalcin, C. E., Covic, A., et al. (2016). Hypertension as an autoimmune and inflammatory disease. Hypertens Res.

Chrysohoou, C., Pitsavos, C., Panagiotakos, D.B., Skoumas, J., and Stefanadis, C. 2004. Association between prehypertension status and inflammatory markers related to atherosclerotic disease: The ATTICA Study. Am J Hypertens, 17(7): 568-573.

Stumpf C, John S, Jukic J, Yilmaz A, Raaz D, Schmieder RE, Daniel WG, Garlichs CD. Enhanced levels of platelet P-selectin and circulating cytokines in young patients with mild arterial hypertension. J Hypertens. 2005; 23:995–1000.

Ward NC, Croft KD. Hypertension and oxidative stress. Clinical and Experimental Pharmacology & Physiology. 2006 Sep;33(9):872-876. DOI: 10.1111/j.1440-1681.2006.04457.x. PMID: 16922824.

Griendling, K. K., Camargo, L. L., Rios, F. J., Alves-Lopes, R., Montezano, A. C., & Touyz, R. M. (2021). Oxidative stress and hypertension. *Circulation Research*, *128*(7), 993-1020.

Konukoglu, D., & Uzun, H. (2016). Endothelial dysfunction and hypertension. *Hypertension: from basic research to clinical practice*, 511-540.

Gasparo, M. D., Speth, R. C., Baltatu, O. C., & Vanderheyden, P. (2013). Brain RAS: hypertension and beyond. *International Journal of Hypertension*, *2013*.

Su, C., Xue, J., Ye, C., & Chen, A. (2021). Role of the central renin-angiotensin system in hypertension (Review). *International journal of molecular medicine*, *47*(6), 95. https://doi.org/10.3892/ijmm.2021.4928.

Paz Ocaranza, M., Riquelme, J. A., García, L., Jalil, J. E., Chiong, M., Santos, R., & Lavandero, S. (2020). Counter-regulatory renin-angiotensin system in cardiovascular disease. *Nature reviews. Cardiology*, *17*(2), 116–129. https://doi.org/10.1038/s41569-019-0244-8.

Saavedra, J.M., and Chevillard, C. 1982. Angiotensin-converting enzyme is present in the subfornical organ and other circumventricular organs of the rat. Neurosci Lett, 29(2): 123-127.

Amenta, F., Mignini, F., Rabbia, F., Tomassoni, D., and Veglio, F. 2002. Protective effect of anti-hypertensive treatment on cognitive function in essential hypertension: analysis of published clinical data. J Neurol Sci, 203-204: 147-151.

Mohammed M, Berdasco C, Lazartigues E. Brain angiotensin converting enzyme-2 in central cardiovascular regulation. Clin Sci (Lond). 2020 Oct 16;134(19):2535-2547. doi: 10.1042/CS20200483. PMID: 33016313; PMCID: PMC7640374.

Saavedra JM (2012) Angiotensin II AT(1) receptor blockers as treatments for inflammatory brain disorders. Clin Sci (Lond) 123: 567-590.

Tota, S., Hanif, K., Kamat, P.K., Najmi, A.K., and Nath, C. 2012a. Role of central angiotensin receptors in scopolamine-induced impairment in memory, cerebral blood flow, and cholinergic function. Psychopharmacology (Berl), 222(2): 185-202.

Reaux-Le Goazigo A, Iturrioz X, Fassot C, Claperon C, Roques BP, Llorens-Cortes C. Role of angiotensin III in hypertension. Curr Hypertens Rep. 2005 Apr;7(2):128-34. doi: 10.1007/s11906-005-0087-z. PMID: 15748538.

von Bohlen und Halbach O. Angiotensin IV in the central nervous system. Cell Tissue Res. 2003 Jan;311(1):1-9. doi: 10.1007/s00441-002-0655-3. Epub 2002 Nov 22. PMID: 12483279.

Wright JW, Harding JW. The brain renin-angiotensin system: a diversity of functions and implications for CNS diseases.Pflugers Arch. 2013 Jan;465(1):133-51.

Gironacci MM, Longo Carbajosa NA, Goldstein J, Cerrato BD. Neuromodulatory role of angiotensin-(1-7) in the central nervous system. Clin Sci (Lond). 2013 Jul 1;125(2):57-65. doi: 10.1042/CS20120652. PMID: 23530669.

Jiang T, Gao L, Guo J, Lu J, Wang Y, Zhang Y. Suppressing inflammation by inhibiting the NF-κB pathway contributes to the neuroprotective effect of angiotensin-(1-7) in rats with permanent cerebral ischaemia. Br J Pharmacol (2012) 167:1520–32.

Faria-Costa G, Leite-Moreira A, Henriques-Coelho T. Cardiovascular effects of the angiotensin type 2 receptor. Rev Port Cardiol. 2014 Jul-Aug;33(7-8):439-49. English, Portuguese. doi: 10.1016/j.repc.2014.02.011. Epub 2014 Jul 31. PMID: 25087493.

von Bohlen und Halbach, O. (2003). Angiotensin IV in the central nervous system. *Cell and tissue research*, *311*(1), 1-9.

von Bohlen und Halbach, O., and Albrecht, D. 2006. The CNS renin-angiotensin system. Cell Tissue Res, 326(2): 599-616.

Handa, R.K. 2001. Characterization and signaling of the AT(4) receptor in human proximal tubule epithelial (HK-2) cells. J Am Soc Nephrol, 12(3): 440-449.

Lazaroni TL, Raslan AC, Fontes WR, de Oliveira ML, Bader M, Alenina N, Moraes MF, Dos Santos RA, Pereira GS.Angiotensin-(1-7)/Mas axis integrity is required for the expression of object recognition memory. Neurobiol Learn Mem. 2012 Jan;97(1):113-23. doi: 10.1016/j.nlm.2011.10.003.

Kostenis E., G. Milligan, A. Christopoulos, C.F. Sanchez-Ferrer, S. Heringer-Walther, P.M. Sexton, et al.G-protein-coupled receptor Mas is a physiological antagonist of the Angiotensin II type 1 receptor. Circulation, 111 (2005), pp. 1806–1813.

Kelly DM, Rothwell PM. Blood pressure and the brain: the neurology of hypertension. Pract Neurol. 2020 Apr;20(2):100-108. doi: 10.1136/practneurol-2019-002269. Epub 2019 Sep 26. PMID: 31558584.

Milicevic M, Krzesinski JM. Hypertension artérielle et cerveau [Hypertension and the brain]. Rev Med Liege. 2008 May-Jun;63(5-6):269-79. French. PMID: 18669192.

Christensen H, Strandgaard S. Hypertension og hjernen [Hypertension and the brain]. Ugeskr Laeger. 2009 Jun 8;171(24):2025-8. Danish. PMID: 19523368.

Dunn, K. M., & Nelson, M. T. (2014). Neurovascular signaling in the brain and the pathological consequences of hypertension. *American journal of physiology. Heart and circulatory physiology*, *306*(1), H1–H14. https://doi.org/10.1152/ajpheart.00364.2013.

Murray, M.D., Lane, K.A., Gao, S., Evans, R.M., Unverzagt, F.W., Hall, K.S., and Hendrie, H. 2002. Preservation of cognitive function with antihypertensive medications: a longitudinal analysis of a community-based sample of African Americans. Arch Intern Med, 162(18): 2090-2096.

Kivipelto, M., Helkala, E.L., Hanninen, T., Laakso, M.P., Hallikainen, M., Alhainen, K., Soininen, H., Tuomilehto, J., and Nissinen, A. 2001. Midlife vascular risk factors and late-life mild cognitive impairment: A population-based study. Neurology, 56(12): 1683-1689.

Knecht, S., Wersching, H., Lohmann, H., Berger, K., and Ringelstein, E.B. 2009. How much does hypertension affect cognition?: explained variance in cross-sectional analysis of non-demented community-dwelling individuals in the SEARCH study. J Neurol Sci, 283(1-2): 149-152.

Fogari R, Mugellini A, Zoppi A, Marasi G, Pasotti C, Poletti L, Rinaldi A, Preti P (2004) Effects of valsartan compared with enalapril on blood pressure and cognitive function in elderly patients with essential hypertension. Eur J Clin Pharmacol 59: 863-868.

Yang, R. I. Smolders, D. De Bundel et al., "Brain and peripheral angiotensin II type 1 receptors mediate renal vasoconstrictor and blood pressure responses to angiotensin IV in the rat," Journal of Hypertension, vol. 26, no. 5, pp. 998–1007, 2008.

Tota, S., Kamat, P.K., Saxena, G., Hanif, K., Najmi, A.K., and Nath, C. 2012b. Central angiotensin converting enzyme facilitates memory impairment in intracerebroventricular streptozotocin treated rats. Behav Brain Res, 226(1): 317-330.

Goel, R., Bhat, S.A., Rajasekar, N., Hanif, K., Nath, C., Shukla, R., 2015a. 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.

Tota, S., Kamat, P.K., Saxena, G., Hanif, K., Najmi, A.K., and Nath, C. 2012b. Central angiotensin converting enzyme facilitates memory impairment in intracerebroventricular streptozotocin treated rats. Behav Brain Res, 226(1): 317-330.

Frank-Cannon, T.C., Alto, L.T., McAlpine, F.E., and Tansey, M.G. 2009. Does neuroinflammation fan the flame in neurodegenerative diseases? Mol Neurodegener, 4: 47.

Pimplikar, S.W. 2014. Neuroinflammation in Alzheimer's disease: from pathogenesis to a therapeutic target. J Clin Immunol, 34 Suppl 1: S64-69.

Möller T. (2010). Neuroinflammation in Huntington's disease. J. Neural Transm. 117, 1001–1008 10.1007/s00702-010-0430-7.

Walker A., Kavelaars A., Heijnen C., Dantzer R. (2014). Neuroinflammation and comorbidity of pain and depression. Pharmacol. Rev. 66, 80–101 10.1124/pr.113.008144.

Cerbai F, Lana D, Nosi D, Petkova-Kirova P, Zecchi S, Brothers HM, et al. The neuronastrocyte- microglia triad in normal brain ageing and in a model of neuroinfl ammation in the rat hippocampus. PLoS One. 2012;7(9):e45250.

Sroga, J. M., Jones, T. B., Kigerl, K. A., McGaughy, V. M., & Popovich, P. G. (2003). Rats and mice exhibit distinct inflammatory reactions after spinal cord injury. J Comp Neurol, 462(2), 223-240.

Gogoleva VS, Drutskaya MS, Atretkhany KS. [The Role of Microglia in the Homeostasis of the Central Nervous System and Neuroinflammation]. Mol Biol (Mosk). 2019 Sep-Oct;53(5):790-798. Russian. doi: 10.1134/S0026898419050057. PMID: 31661478.

Subhramanyam CS, Wang C, Hu Q, Dheen ST. Microglia-mediated neuroinflammation in neurodegenerative diseases. Semin Cell Dev Biol. 2019 Oct;94:112-120. doi: 10.1016/j.semcdb.2019.05.004. Epub 2019 May 11. PMID: 31077796.

Li Y, Wei B, Liu X, Shen XZ, Shi P. Microglia, autonomic nervous system, immunity and hypertension: Is there a link? Pharmacol Res. 2020 May;155:104451. doi: 10.1016/j.phrs.2019.104451. Epub 2019 Sep 23. PMID: 31557524

Rodríguez V, deKloet AD, Sumners C (2016). Hypertension and Brain Inflammation: Role of RAS-Induced Glial Activation. Chapter: Hypertension and the Brain as an End-Organ Target pp 181-194.

Zarei-Kheirabadi M, Vaccaro AR, Rahimi-Movaghar V, Kiani S, Baharvand H. An Overview of Extrinsic and Intrinsic Mechanisms Involved in Astrocyte Development in the Central Nervous System. Stem Cells Dev. 2020 Mar 1;29(5):266-280. doi: 10.1089/scd.2019.0189. Epub 2020 Jan 28. PMID: 31847709.

Molofsky AV, Deneen B. Astrocyte development: A Guide for the Perplexed. Glia. 2015 Aug;63(8):1320-9. doi: 10.1002/glia.22836. Epub 2015 May 12. PMID: 25963996.

O'Connor AT, Clark MA. Astrocytes and the Renin Angiotensin System: Relevance in Disease Pathogenesis. Neurochem Res. 2018 Jul;43(7):1297-1307. doi: 10.1007/s11064-018-2557-0. Epub 2018 Jun 1. PMID: 29858771.

McGeer PL, McGeer EG. Inflammation and neurodegeneration in Parkinson's disease. Parkinsonism Relat. Disord. 2004; 10 (Suppl. 1), S3–S7.

Mélik-Parsadaniantz S, Rostène W. Chemokines and neuromodulation. J Neuroimmunol. 2008 Jul 31;198(1-2):62-8.

Pistikova A, Brozka H, Bencze M, Radostova D, Vales K, Stuchlik A. The effect of hypertension on adult hippocampal neurogenesis in young adult spontaneously hypertensive rats and Dahl rats. Physiol Res. 2017 Nov 24;66(5):881-887. doi: 10.33549/physiolres.933562. Epub 2017 Jul 18. PMID: 28730828.

Zhang, L., Yang, X., Yang, S., & Zhang, J. (2011). The Wnt/β-catenin signaling pathway in the adult neurogenesis. *European Journal of Neuroscience*, *33*(1), 1-8.

Xiao Z, Cao Z, Yang J, Jia Z, Du Y, Sun G, Lu Y, Pei L. Baicalin promotes hippocampal neurogenesis via the Wnt/β-catenin pathway in a chronic unpredictable mild stress-induced mouse model of depression. Biochem Pharmacol. 2021 Aug;190:114594. doi: 10.1016/j.bcp.2021.114594. Epub 2021 May 6. PMID: 33964281.

Zhang, R., Engler, A., & Taylor, V. (2018). Notch: an interactive player in neurogenesis and disease. Cell and Tissue Research, 371(1), 73-89.

Ables, J. L., Breunig, J. J., Eisch, A. J., & Rakic, P. (2011). Not (ch) just development: Notch signalling in the adult brain. Nature Reviews Neuroscience, 12(5), 269-283.

Ferguson KL, Slack RS. The Rb pathway in neurogenesis. Neuroreport. 2001 Jul 3;12(9):A55-62. doi: 10.1097/00001756-200107030-00001. PMID: 11435948.

Ferguson KL, Slack RS. The Rb pathway in neurogenesis. Neuroreport. 2001 Jul 3;12(9):A55-62. doi: 10.1097/00001756-200107030-00001. PMID: 11435948.

Niklison-Chirou, M. V., Agostini, M., Amelio, I., & Melino, G. (2020). Regulation of Adult Neurogenesis in Mammalian Brain. International journal of molecular sciences, 21(14), 4869. https://doi.org/10.3390/ijms21144869.

Yao, B., Christian, K. M., He, C., Jin, P., Ming, G. L., & Song, H. (2016). Epigenetic mechanisms in neurogenesis. Nature reviews. Neuroscience, 17(9), 537–549. https://doi.org/10.1038/nrn.2016.70