PERIPHERAL ANTIHYPERTENSIVE MECHANISMS OF *ZINGIBER OFFICINALE* VAR. *RUBRUM* IN SPONTANEOUSLY HYPERTENSIVE RATS

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by

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LIST OF SYMBOLS & ABBREVIATIONS

%	percentage
$[Ca^{2+}]_e$	extracellular calcium ion
$[Ca^{2+}]_i$	intracellular calcium ion
µg/kg	microgram per kilogram
µg/kg/min	microgram per kilogram per minute
µL	microlitre
µM	micromolar
ACE	angiotensin converting enzyme
ACh	acetylcholine
Ang II	angiotensin II
ANOVA	analysis of variance
ARASC	Animal Research and Service Centre
ANS	autonomic nervous system
ARB	angiotensin II receptor blocker
ATP	adenosine triphosphate
AT_1	angiotensin II type 1
b.w	body per weight
Ca^{2+}	calcium ion
$CaCl_2$	calcium chloride anhydrous
cAMP	cyclic adenosine monophosphate
cAMP-PK	cAMP-dependent protein kinases
cGMP-PK	cGMP-dependent protein kinases
CCB	calcium channel blocker
cGMP	cyclic guanosine monophosphate

C_{max}	maximal contraction response
CNS	central nervous system
CO_2	carbon dioxide
COX	cyclooxygenase
DAG	diacylglycerol
DBP	diastolic blood pressure
EC_{50}	median effective concentrations
EDCF	endothelium-derived contracting factor
EDHF	endothelium-derived hyperpolarising factor
EDNO	endothelium-derived nitric oxide
EDRF	endothelium-derived relaxing factor
EGTA	ethylene glycol-bis (β-aminoethyl ether)-N,N,N′,N′ tetraacetic acid)
E_{max}	maximal contractile response
ER	endoplasmic reticulum
et al.,	from the latin *et cetera*, which means “and others”
g	gram
g/L	gram per litre
GTP	guanosine triphosphate
HPLC	High Performance Layer Chromatography
i.p.	intraperitoneal injection
i.u/mL	international units per millilitre
i.v.	intravenous injection
IP_3	inositol triphosphate
K_{ATP}	ATP-sensitive potassium

K^+	potassium ion
KCl	potassium chloride
KH_2PO_4	potassium dihydrogen orthophosphate
LDL-receptor	low-density lipoprotein receptor
L-NAME	n^{ω}-nitro-l-arginine methyl ester
M	molar
MAP	mean arterial pressure
mg	milligram
mg/kg	milligram per kilogram
mg/mL	milligram per millilitre
$MgCl_2$	magnesium chloride
$MgSO_4$	magnesium sulphate
min	minute
ml	millilitre
MLC	myosin light chain
MLCK	myosin light chain kinase
mm	millimetre
mmHg	millimetre of mercury
mmol/L	millimoles per litre
mol/L	moles per litre
n	the number of rats
NaCl	sodium chloride
$NaHCO_3$	sodium bicarbonate
NO	nitric oxide
NOS	nitric oxide synthase

O_2	oxygen
°C	degree Celsius
$P < 0.05$	statistically significant
pD_2	sensitivity
PE	phenylephrine
PGI_2	prostacyclin
PKC	protein kinase C
PNS	peripheral nervous system
RhoK	rho-kinase
R_{max}	maximal relaxation response
rpm	revolutions per minute
SEM	standard error of the mean
SBP	systolic blood pressure
sGC	soluble guanylyl cyclase
SHRs	spontaneously hypertensive rats
SNP	sodium nitroprusside
SR	sarcoplasmic reticulum
TPR	total peripheral resistance
T_a	baseline tension obtained from precontraction by PE
T_b	absolute tension obtained after adding extracts
U/L	units per litre
USM	Universiti Sains Malaysia
WHO	World Health Organisation
WKY	Wistar-Kyoto rats
ZOVR	*Zingiber officinale* var. *rubrum*

α	alpha
β	beta
μmol/L	micromoles/litre

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MEKANISME ANTIHIPERTENSI PERIFERAL *ZINGIBER OFFICINALE* VAR. *RUBRUM* DALAM TIKUS HIPERTENSI SPONTAN

ABSTRAK

Hipertensi dikaitkan dengan kerosakan organ akhir yang akhirnya boleh menyebabkan strok, kegagalan jantung dan buah pinggang. Kebolehubahan tekanan darah mungkin merupakan faktor penentu yang paling penting untuk kerosakan organ hujung. *Zingiber officinale* var. *rubrum* (*ZOVR*) (*Halia bara*) ialah suatu ubat tradisional Melayu yang didakwa mempunyai kesan antihipertensi. Kajian ini menyelidik tentang kesan *ZOVR* (250 mg/kg b.w) terhadap tindak balas dan kereaktifan vaskular dalam tikus hipertensi spontan (SHR) dan mekanisme yang terlibat. SHR diberi ekstrak *ZOVR* secara oral setiap hari selama 28 hari dan tekanan darah sistolik tak invasif, tekanan arteri purata, kadar denyut jantung dan berat badan diukur. Kereaktifan dan daya tindak balas vaskular dinilai menggunakan cincin aorta terasing, tikus terbius dan tikus terbius yang di"pithed". Agonis dan antagonis yang bersesuaian (L-NAME, indometasin, metalina biru, atropina, glibenclamida, hexamesonium, prazosin dan propranolol) untuk kajian mekanisme digunakan. Ketiga-tiga ekstrak mentah *ZOVR* mengurangkan tekanan arteri purata (MAP) secara signifikan dibandingkan dengan verapamil semasa rawatan oral selama 28 hari, dengan ekstrak petroleum eter (ZOP) menghasilkan kesan antihipertensi yang paling tinggi dan dipilih untuk proses fraksinasi. Tiada perubahan fizikal atau tingkah laku, atau kematian diperhatikan dalam tempoh rawatan 28 hari ini melalui pemberian ekstrak secara oral. Dalam kajian gelang aorta SHR, fraksi *n*-heksana (HFZOP) memberi kesan vasorelaksasi melalui penglibatan faktor relaksasi endotelium (EDRFs) seperti nitrik

oksida terbitan endotelium (EDNO) dan prostasiklin, laluan siklase guanilil terlarut cGMP, reseptor muskarinik, saluran K^+, reseptor adrenergik α_1, dan pengurangan mobiliti Ca^{2+} luar sel dan dalam sel. Manakala, dalam keseluruhan kajian haiwan menggunakan SHR, sebahagian besar mekanisme relaksasi aruhan fraksi *n*-heksana telah dikenalpasti dalam SHR yang tidak di"pithed", telah diperantarakan secara periferal dengan kawalan refleks pusat yang tiada atau minimum. Fraksi *n*-heksana memberi kesan vasorelaksasi melalui penglibatan laluan NO dan prostasiklin, reseptor muskarinik, reseptor adrenergik α_1, dan desensitisasi atau penghalangan reseptor kolinergik nikotinik pada ganglia autonomi juga diperlihatkan. Mekanisme relaksasi oleh aruhan fraksi *n*-heksana disetempatkan pada tahap vaskular dan bebas dari kawalan refleks pusat. Sebatian aktif yang bertanggungjawab untuk aktiviti antihipertensi *ZOVR* dan HFZOP dikenalpasti sebagai 6-gingerol, 8-gingerol, dan 6-shogaol. Rumusannya, *ZOVR* menunjukkan potensi sifat antihipertensi.

PERIPHERAL ANTIHYPERTENSIVE MECHANISMS OF *ZINGIBER OFFICINALE* VAR. *RUBRUM* IN SPONTANEOUSLY HYPERTENSIVE RATS

ABSTRACT

Hypertension has been associated with end-organ damage that may eventually lead to stroke, heart, and renal failure. Blood pressure variability is possibly the most important determining factor for the end-organ damage. *Zingiber officinale* var. *rubrum (ZOVR) (Halia bara)* is a Malay traditional medicine claimed to have antihypertensive effects. This study investigated the effects of *ZOVR* (250 mg/kg b.w) on vascular responsiveness and reactivity in spontaneously hypertensive rats (SHRs) and the mechanisms involved. SHRs were orally fed daily for 28 days with *ZOVR* extracts and non-invasive systolic blood pressure, mean arterial pressure, heart rate and body weight were measured. Vascular reactivity and responsiveness were assessed using isolated aortic rings, anaesthetised non- and pithed rats, respectively. Appropriate agonists and antagonists (L-NAME, indomethacin, methylene blue, atropine, glibenclamide, hexamethonium, prazosin and propranolol) for the mechanism study were employed. All three crude extracts of *ZOVR* significantly reduced the mean arterial pressure (MAP) of SHRs in comparison or comparable with verapamil during the 28-day daily oral feeding of *ZOVR* extracts, with petroleum ether extract (ZOP) produced the most antihypertensive effect and selected for fractionation. No physical or behavioural changes or deaths were observed during the 28-day treatment duration of oral feeding by extracts. In the SHR's aortic rings study, *n*-hexane fraction (HFZOP) exerted its vasorelaxation through involvements of endothelium-derived relaxing factors (EDRFs) such as endothelium-derived nitric oxide (EDNO) and prostacyclin, soluble guanylyl

cyclase of cGMP pathway, muscarinic receptors, K^+ channels, α_1-adrenergic receptors, and reduction of extracellular and intracellular Ca^{2+} mobility. In the whole animal study using SHRs, the most of *ZOVR n*-hexane fraction-induced relaxation mechanisms identified in the completely non-pithed SHRs, and it were peripherally and not centrally mediated. *ZOVR n*-hexane fraction exerted its vasorelaxation through involvements of NO and prostacyclin pathways, muscarinic receptors, α_1-adrenergic receptors, and desensitisation or blockage of nicotinic cholinergic receptors in the autonomic ganglia. *ZOVR n*-hexane fraction-induced relaxation mechanisms were localised at the vascular level and independent of central reflex regulation. Active compounds responsible for the antihypertensive activity of *ZOVR* and HFZOP were identified as 6-gingerol, 8-gingerol, and 6-shogaol. In conclusion, *ZOVR* exhibited potential antihypertensive properties.

CHAPTER 1

INTRODUCTION

1.1 *Overview of usage of natural products in the management of hypertension*

Hypertension is a cardiovascular disease (CVD) in which the blood pressure in the arteries is elevated. It is a significant risk factor for the development of cardiovascular disease in many developing countries (WHO, 2018). Several medications are used to treat hypertension, collectively referred to as synthetic drugs, such as a thiazide-type diuretic, beta-blocker, calcium channel blocker, and angiotensin II receptor antagonists (Chobanian *et al.,* 2003). Most of these drugs are usually associated with specific side effects (Terra, 2003; Wykretowicz *et al.,* 2008). Several traditional medicines support the use of natural products in preventing cardiovascular diseases. The action of natural products depends upon the balance between multiple compounds, which often have different mechanisms, to give a final therapeutic effect (Ghayur and Janssen, 2010). As a result, there is much interest using natural products as alternative medicine in treating chronic diseases, especially hypertension (Tabassum and Ahmad, 2011), in the search for plants with antihypertensive activity, less adverse side effects (Tabassum and Ahmad, 2011) and have developed a good reputation (White & Carrier, 1990). More high-quality primary and clinical research is needed to incorporate natural products as complementary therapies into mainstream medicine.

1.2 *Research problem statement*

About 1.56 billion people (29% of the world's adult population) are predicted to have hypertension by 2025 (Kearney *et al*., 2005). Hypertension, one of the main risk factors for CVD (WHO, 2018), contributed to 1.9% of the mortality rate in Malaysia in 2020 (Malaysia, 2021) and about 17.9 million deaths per year globally in 2016 (WHO, 2016). Two national scale studies in Malaysia found that high prevalence of hypertension was due to low levels of awareness, treatment, and hypertension management (Lim *et al.,* 2004; Rampal *et al.,* 2008). This information showed that hypertension remained at risk, both globally and locally. A comprehensive program is urgently needed to solve the growing problem of hypertension.

There are claims by traditional medicine practitioners about the blood pressure-lowering abilities of herbal plants that contribute to their scientific clarification. One of the herbal plants traditionally claimed by the Malaysian as an alternative treatment for hypertension is *Zingiber officinale* Rosc. (true ginger). *Zingiber officinale* Rosc. is a popular herbal plant and reported to possess anti-inflammatory, analgesic, antipyretic, antimicrobial, hypoglycemic, antioxidant, hepatoprotective, and hypercholesterolemic activities (Langner *et al.,* 1998; Ali *et al.,* 2008). It also traditionally claimed and scientifically supported to have blood pressure-lowering effect (Ghayur *et al.,* 2005; Manosroi *et al.,* 2013). Another member of the Zingiberaceae family, *Zingiber officinale* var. *rubrum* (*ZOVR*), which is commonly known as red ginger or *Halia bara* (Malaysia), also has been traditionally used to treat rheumatism, osteoporosis, asthma, cough, stomach discomfort, tumors, and as a postpartum medicine (Hassan and Mahmood, 2006; Ibrahim *et al.,* 2008). Previous studies have reported that *ZOVR* has antioxidant and anticancer (Ghasemzadeh *et al.,* 2015), anti-tumor (Vimala *et al.,* 1999), antimicrobial (Norazian *et al.,* 2001), antibacterial (Sivasothy *et al.,* 2011), cytotoxic

activity (Fitriana and Susantiningsih, 2014) and antihypertensive effects (Theilade, 1996). However, not all mechanisms of these effects of *ZOVR* have been thoroughly studied, including its antihypertensive effects at the peripheral cardiovascular level. Therefore, this study will further contribute to the fundamental knowledge of the mechanism of antihypertensive properties of *ZOVR*, particularly at the peripheral vascular level.

1.3 *Significance of studies*

Zingiber officinale var. *rubrum* is a herb traditionally used for its blood pressure-lowering effects. Hence, the finding of this study contributes to the scientific knowledge of *Zingiber officinale* var. *rubrum*, specifically on its antihypertensive properties. Mechanisms involved in these properties are established, and active compounds are identified. The potential of *Zingiber officinale* var. *rubrum* as an alternative treatment for hypertension may contribute towards the enhancement of health and vitality.

1.4 *Research objectives*

This study aimed to investigate the antihypertensive effect and the mechanisms of *Zingiber officinale* var. *rubrum* at the peripheral vascular level using spontaneously hypertensive rats.

The specific objectives of the study were as follows:

1. To evaluate the vascular reactivity of extracts/ fractions of *Zingiber officinale* var. *rubrum* and mechanisms involved using aortic rings of SHRs.

2. To assess the antihypertensive activity and mechanisms of extracts/ fractions of *Zingiber officinale* var. *rubrum* in SHRs using anaesthetised SHRs.

3. To assess the antihypertensive activity and mechanisms of extracts/ fractions of *Zingiber officinale* var. *rubrum* in SHRs using anaesthetised pithed SHRs.

4. To analyse the bioactivity constituents of fractions of *Zingiber officinale* var. *rubrum* responsible for its antihypertensive activity using selected marker compounds.

1.5 *Hypotheses*

1. *Zingiber officinale* var. *rubrum* exhibited potential antihypertensive properties in SHRs by reducing the MAP in SHRs.
2. Antihypertensive mechanisms of *Zingiber officinale* var. *rubrum* involved NO and cyclooxygenase pathways, muscarinic and α- and β-adrenergic, receptors and intracellular and extracellular calcium.
3. Central baroreflex control had no influence on the *Zingiber officinale* var. *rubrum's* antihypertensive mechanisms at the peripheral vascular level.
4. 6-gingerol, 8-gingerol, and 6-shogaol were possible bioactive compounds responsible for the antihypertensive activity of *Zingiber officinale* var. *rubrum.*

1.6 *Overview of the study*

Figure 1.1 summarises the overall experimental procedures of this study.

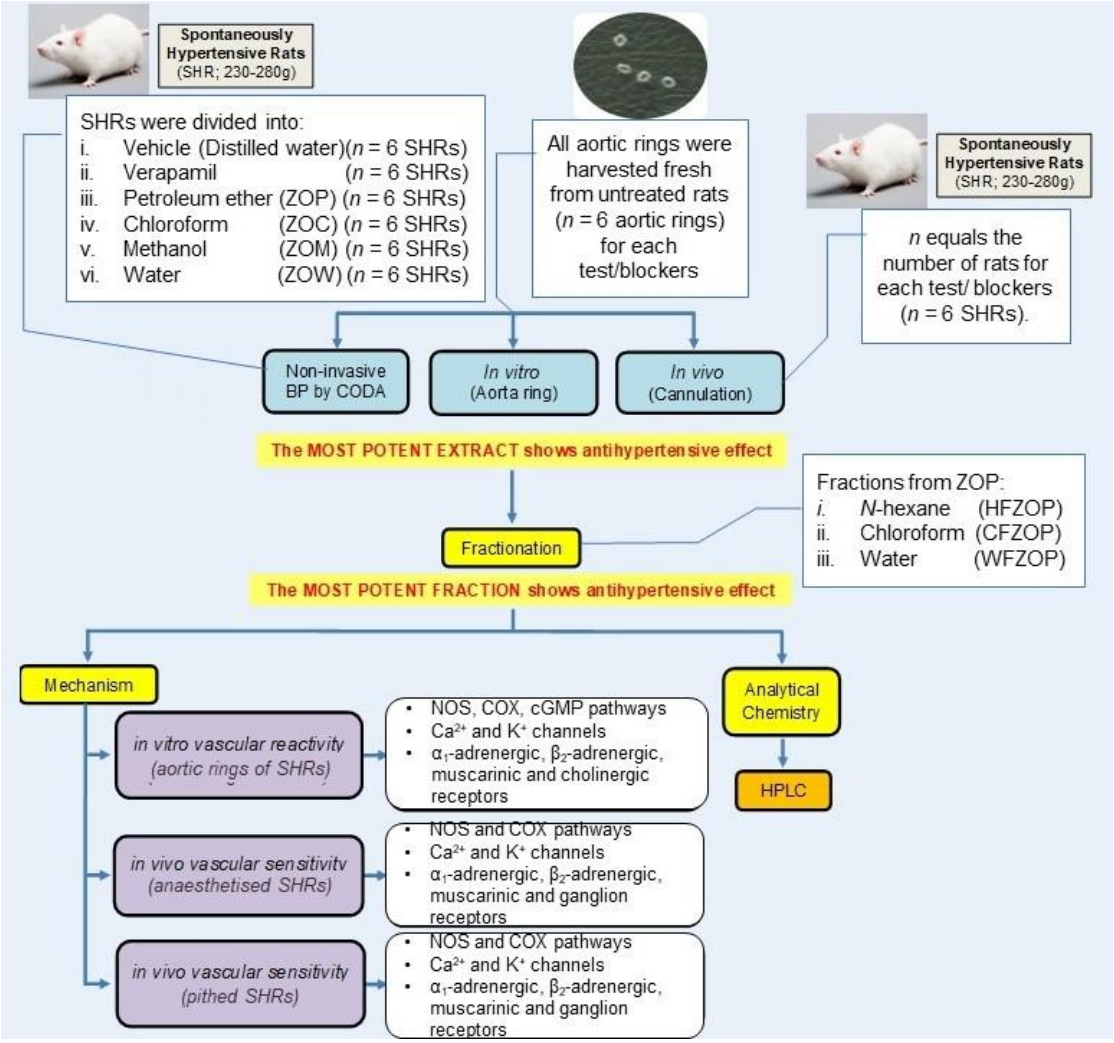

Figure 1.1 Overview of the study methodology. ZOP = *ZOVR* petroleum ether extract, ZOC = *ZOVR* chloroform extract, ZOM = *ZOVR* methanol extract, ZOW = *ZOVR* water extract, HFZOP = *ZOVR n*-hexane fraction, CFZOP = *ZOVR* chloroform fraction, WFZOP = *ZOVR* water fraction, COX = cyclooxygenase, NOS = nitric oxide synthase, cGMP = cyclic adenosine monophosphate, SHRs = spontaneously hypertensive rats, HPLC = High Performance Layer Chromatography, Ca^{2+}= calcium ion, K^{+}= potassium ion.

CHAPTER 2

LITERATURE REVIEW

2.1 *Hypertension*

Hypertension is a cardiovascular disease described as systolic blood pressure (SBP) above 140 mmHg or diastolic blood pressure (DBP) above 90 mmHg. In most cases, the increase in blood pressure is attributed to an increase in total peripheral resistance (TPR) when cardiac output and heart rate are not high. Epidemiological reports have shown that the higher the pressure (systolic or diastolic or both), the higher the incidence of CVD (Park *et al.,* 2018). Su and Miao (2005) indicated that variability in blood pressure might be associated with end-organ damage, including cardiac hypertrophy, atherosclerosis, and early-stage renal lesion. Hypertension can be caused by many factors, including an increase in fluid body volume, blood vessel resistance, and other factors that trigger an increase in blood pressure (Takahashi and Smithies, 2004). Attempts have been made to control elevated blood pressure as strategies to control arterial hypertension (Lang *et al.,* 2001).

2.1.1 Prevalence and risk factors

About 7.1 million deaths annually can be attributed to hypertension (Singh *et al.,* 2000). The World Health Organisation (WHO, 2002) stated that hypertension is now a widespread risk factor for cardiovascular disease in developing and developed countries. After five years, the WHO (2007) estimated that 30.0% of the 58 million deaths worldwide in 2005 were due to various types of cardiovascular disease (CVD), with hypertension being a significant risk factor.

Today's lifestyle has become a significant threat to public health. Modifiable risk factors for hypertension include excessive salt intake, smoking, alcohol consumption, renal impairment, and the contraceptive pill (Pilakkadavath and Shaffi, 2016). Risk factors that cannot be modified include a family history of hypertension; age over 65 years, and co-existing diseases such as diabetes or kidney disease (Ranasinghe *et al.*, 2015). Data from the Malaysian Community Salt Study (MyCoSS) found that people with older age, higher body mass index (BMI), and diabetes were more likely to have hypertension (Zaki *et al*., 2021).

2.1.2 Pathogenesis

Hypertension can be genetic or caused by environmental factors such as poor diet, obesity, tobacco use, excessive alcohol consumption, and a sedentary lifestyle (Weber, 2014; WHO, 2019). Nevertheless, the relative role of each of these factors in the development of hypertension may vary from person to person. Several population-based studies have demonstrated that genetics is one of the most critical factors in the development of hypertension (Boehme *et al*., 2002; Oparil *et al.*, 2003). It is now generally accepted that hypertension is more common in individuals with a positive family history of hypertension (Boehme *et al*., 2002), and it has been reported that paternal and maternal history of hypertension determined systolic and diastolic blood pressure in offspring (Mitsumata *et al.*, 2012).

2.1.3 Pathophysiology

Several physiological mechanisms are involved in maintaining normal blood pressure, and their disruption may play a role in developing essential hypertension. Various interrelated factors likely contribute to elevating blood pressure in hypertensive patients, and their relative roles may vary from person to person. Factors that have been extensively studied include salt intake (Feldstein, 2009), obesity and insulin resistance

(Rao *et al.,* 2015), the renin-angiotensin system (Santos *et al.,* 2012) and the sympathetic nervous system, genetics, endothelial dysfunction, and neurovascular abnormalities (Beevers *et al.,* 2001; Touyz, 2011).

2.1.3.a Cardiac output and peripheral vascular resistance

Maintenance of normal blood pressure depends on the balance between cardiac output and peripheral vascular resistance. Most patients with essential hypertension have normal cardiac output but increased peripheral resistance (McEniery *et al., 2005)*. Peripheral resistance is not determined by the large arteries or the capillaries but by the small arterioles whose walls contain smooth muscle cells. The contraction of smooth muscle cells is thought to be related to increase in intracellular calcium concentration, which may explain the vasodilator effect of drugs that block calcium channels (Lacolley *et al.,* 2017). Prolonged smooth muscle constriction is thought to cause structural changes with the thickening of arteriolar vessel walls, possibly mediated by angiotensin, leading to an irreversible increase in peripheral resistance (Touyz *et al.,* 2018).

2.1.3.b Renin-angiotensin-aldosterone system

The renin-angiotensin system is the most important of the endocrine systems that influence blood pressure control (Chopra *et al.,* 2011). The juxtaglomerular apparatus of the kidney secretes renin in response to glomerular under perfusion or reduced salt intake. It is also released in response to stimulation by the sympathetic nervous system. Renin converts the renin substrate (angiotensinogen) into angiotensin I, a physiologically inactive substance that is rapidly converted into angiotensin II in the lungs by the angiotensin-converting enzyme (ACE). Angiotensin II is a potent vasoconstrictor and leads to increased blood pressure. It also stimulates the release of aldosterone from the zona glomerulosa of the adrenal gland, leading to a further increase in blood pressure associated with sodium and water retention (Atlas, 2007). The

circulating renin-angiotensin system is not thought to be directly responsible for the increase in blood pressure in essential hypertension. In particular, many hypertensive patients have low renin and angiotensin levels II (especially elderly and black people), and drugs that block the renin-angiotensin system are not particularly effective (Turgut *et al.,* 2010).

2.1.3.c Autonomic nervous system

Stimulation of the sympathetic nervous system can cause both constriction and dilation of the arterioles. Therefore, the autonomic nervous system plays an important role in maintaining normal blood pressure (Joyner *et al.,* 2008). It also plays an important role in mediating short-term changes in blood pressure in response to stress and physical exertion (McEwen and Gianaros, 2010). Hypertension is likely related to an interaction between the autonomic nervous system and the renin-angiotensin system, along with other factors, including sodium, circulating volume, and some of the more recently described hormones (Takahashi *et al.,* 2011).

2.1.3.d Endothelial dysfunction

Vascular endothelial cells play a crucial role in cardiovascular regulation by producing several potent local vasoactive substances, including nitric oxide (vasodilator molecule) and endothelin (vasoconstrictor peptide). Endothelial dysfunction is associated with essential hypertension in humans (Grover-Páez and Zavalza-Gómez, 2009).

Modulating endothelial function is an attractive therapeutic option to minimise some of the important complications of hypertension. Clinically effective antihypertensive therapy appears to restore impaired nitric oxide production but not impaired endothelium-dependent vascular relaxation or response to endothelial agonists (Virdis *et al.,* 2011).

2.1.3.e Vasoactive substances

Many other vasoactive systems and mechanisms that influence sodium transport and vascular tone are involved in maintaining normal blood pressure (Wadei and Textor, 2012). However, what role they play in developing essential hypertension needs to be clarified. Bradykinin is a potent vasodilator that is inactivated by angiotensin-converting enzymes. Consequently, the ACE inhibitors might exert part of their effect by blocking bradykinin inactivation (Ancion *et al.,* (2019).

Endothelin is a recently discovered potent vascular endothelial vasoconstrictor that can cause a salt-dependent increase in blood pressure. It also activates the local renin-angiotensin system (Kisanuki *et al.,* 2010). Endothelium-derived relaxing factor, nitric oxide diffuses through the vessel wall into smooth muscle, causing vasodilatation.

The atrial natriuretic peptide is a hormone secreted by the heart's atria in response to increased blood volume. It increases sodium and water excretion from the kidney as a kind of natural diuretic. A defect in this system can lead to water retention and high blood pressure (Pandey, 2005).

Sodium transport through the cell walls of vascular smooth muscle is also thought to influence blood pressure through its interaction with calcium transport (Hübner *et al.,* 2015). Ouabain may be a naturally occurring steroid-like substance that is thought to interfere with sodium and calcium transport in cells, causing vasoconstriction (Lingrel, 2010).

2.2 *Nervous system*

The nervous system is a system of organs comprising a network of specialized cells known as neurons. The neurons organize the actions and transfer signals to the different parts of their bodies. The nervous system is composed of the central and peripheral nervous systems.

2.2.1 Central nervous system

The central nervous system (CNS) consists of the brain and spinal cord. Anatomically, at the base of the brain is the brainstem, which expands from the spinal cord to the diencephalon of the cerebrum. The brainstem connects the brain with the spinal cord and includes the midbrain, pons, and medulla oblongata. Posterior to the brainstem lies the cerebellum, which plays a significant role in motor function. The cerebrum is the largest part of the brain and is divided into two cerebral hemispheres and several subcortical structures. The cerebrum controls all voluntary actions in the human body (Widmaier *et al.*, 2006; Tortora and Derrickson, 2006).

The spinal cord is a brain extension from the foramen magnum at the base of the skull and is surrounded by the vertebral bodies that make up the spinal column. The central butterfly-shaped structures of the spinal cord are made up of gray matter. The external or surrounding tissue of gray matter is made up of white matter, which consists of a group of myelinated motor and sensory axons. It contains a pathway that connects the brain with the rest of the body. Groups of afferent neurons transmit sensory impulses from the peripheral nervous system to the central part. Meanwhile, the efferent neurons transmit motor impulses from the central part to the peripheral nervous system. On the other hand, all other neurons, termed interneurons, will be responsible for integrating the information by afferent neurons and formulating the response by efferent neurons

to comprise higher cognitive functions (Widmaier *et al.,* 2006; Tortora and Derrickson, 2006).

2.2.2 Peripheral nervous system

The peripheral nervous system (PNS) comprises nerves branching from the spinal cord and expanding to all body areas. The PNS is divided into the somatic nervous system and the autonomic nervous system (ANS). The somatic system carries sensory and motor information and voluntary movement to and from the CNS. The autonomic system controls aspects of the body that are usually not under voluntary control. The ANS is further divided into three divisions, the sympathetic nervous system, parasympathetic nervous system, and enteric nervous system, which are antagonistic to one another. The sympathetic system activates the "fight or flight" response, while the parasympathetic activates the "rest and digest" response. The motor outflow of both systems is formed by two serial connected sets of neurons, preganglionic neurons and postganglionic neurons (ganglion cells). Preganglionic neurons originate in the brainstem or the spinal cord, and postganglionic neurons lie outside the CNS in collections of nerve cells called autonomic ganglia (Loewy and Spyer, 1990; Guyenet, 2006).

This research will elucidate both sympathetic and parasympathetic nervous systems. The enteric nervous system, a branch of the peripheral nervous system, works independently via its primary connection to the vagus nerve to regulate the gastrointestinal system (Loewy and Spyer, 1990).

2.2.3 Central baroreflex and hypertension

The baroreflex or baroreceptor reflex is one of the body's homeostatic mechanisms that help maintain blood pressure at a nearly constant level (Fernandez *et al.,* 2015). The baroreflex provides a rapid negative feedback loop in which increased blood pressure causes a decrease in heart rate (Wehrwein and Joyner, 2013).

The sympathetic nervous system (SNS) partly controls blood pressure. Experimental data in animals and humans suggest that overactivity of the SNS can lead to the development and maintenance of hypertension (Fisher *et al.,* 2009).

The sympathetic nervous system modulates cardiovascular homeostasis by affecting blood pressure, vascular resistance, heart rate, and cardiac contractility (Urroz Lopez *et al.,* 2022). In addition, it also regulates renal function by controlling sodium and fluid reabsorption. Overactivation of the sympathetic nervous system can contribute to chronic elevation of blood pressure. Left untreated can lead to end-organ damage such as myocardial infarction, stroke, cardiomyopathy, and renal failure (Kumar *et al.,* 2014).

The baroreflex influences the release of vasopressin and renin, and thus, the sodium and water balance of the kidneys and the extracellular fluid volume (Harrison-Bernard, 2009).

In the arterial baroreflex system, when the baroreflex sensors indicate elevating blood pressure, it activates the parasympathetic nervous system while inhibiting the sympathetic nervous system by increasing vagal tone, which lowers heart rate and vascular resistance (Lovic *et al.,* 2014). In the setting of hypotension, the opposite reaction occurs, resulting in vasoconstriction, tachycardia, and increased contractility of the heart.

In the renal sympathetic nervous system, the activation of high-pressure baroreceptors produces afferent signals that stimulate cardioregulatory centers in the brain and lead to the activation of efferent pathways in the sympathetic nervous system. Activation of the renal sympathetic nervous system stimulates the release of renin and angiotensin II, activating the renin-angiotensin-aldosterone system. Activating the sympathetic nervous system, like angiotensin II, causes peripheral and renal vasoconstriction. Angiotensin II constricts blood vessels and stimulates the release of aldosterone from the adrenal gland. It also increases tubular sodium reabsorption and causes myocardial cell remodeling. Aldosterone, in addition to increasing sodium reabsorption and the secretion of potassium and hydrogen ions in the collecting ducts, also can have direct effects on the heart (Izzo, 2008).

2.3 *Cardiovascular system*

The cardiovascular system is comprised of the pulmonary circulation and systemic circulation. From the right ventricle, pulmonary circulation moves to the lungs and returns to the left atrium. The systemic circulation begins from the left ventricle, travels to all peripheral organs and tissues, and then returns to the right atrium. In systemic circulation, the large artery exits the left side of the heart via the aorta. The large vein reaches the right side through the superior vena cava and inferior vena cava. Microcirculation involves blood flow in the smallest blood vessels like arterioles, capillaries, and venules (Brownley *et al*., 2000).

Established as a silent killer as it showed no symptoms, hypertension affects the structures and functions of small muscular arteries, arterioles, and other blood vessels (Escobales *et al*., 2005; Lee and Oh, 2010).

The heart receives a generous supply of sympathetic and parasympathetic nerve fibers. The sympathetic postganglionic fibers release noradrenaline (NA), while the

parasympathetic nervous system releases acetylcholine (ACh). Stimulating the sympathetic nervous system causes increases in heart rate, myocardial contractility, pupil dilatation, bronchiole dilatation, and blood vessel constriction. Conversely, parasympathetic stimulation decreases heart rate, constricts the pupils, increases secretion of the eye glands, increases peristalsis, increases secretion of salivary and pancreatic glands, and constricts bronchioles. The receptors for noradrenaline (NA) on cardiac muscle are mainly beta-adrenergic, whereas the receptors for acetylcholine are muscarinic cholinergic (Brodde *et al.*, 2001; Olshansky *et al*., 2008). The parasympathetic postganglionic fibers are cholinergic. ACh can bind to the nicotinic receptors and muscarinic receptors. Muscarinic receptors are found in the membranes of effector cells at the end of postganglionic parasympathetic nerves and the ends of cholinergic sympathetic fibers (Guyenet, 2006; Gordan *et al.*, 2015). Meanwhile, the nicotinic receptors are found at synapses between pre- and post-ganglionic neurons of the sympathetic and parasympathetic pathways. Compared to the muscarinic receptors, nicotinic receptors produce fast and excitatory responses (Loewy and Spyer, 1990; Gordan *et al*., 2015).

Blood pressure directly influences cardiac output and total peripheral resistance (TPR) (Mayet and Hughes, 2003; Wehrwein and Joyner, 2013). Blood pressure will increase if the cardiac output and total peripheral resistance increase one or both, and vice versa. Mean arterial pressure (MAP) defines the average pressure in the arterial system. It is the sum of the values of diastolic blood pressure and one-third of the way between diastolic and systolic blood pressure (Tortora and Derrickson, 2006). Cardiac output is the volume of blood pumped by each ventricle of the heart in litres per minute and can be influenced by both the flow rate and the blood volume. The cardiac output is calculated by multiplying the heart rate and the stroke volume. The heart rate is the

number of beats per minute, whereas the stroke volume is the blood volume expelled by each ventricle with each beat (Charkoudian et al., 2005). Cardiac output will increase if the heart rate increases. The greater the circulating blood volume, the higher the blood pressure. Blood pressure measures the force the heart uses to pump blood through the heart muscle and into the body. Total peripheral resistance against blood flow and is regulated by the arteries. Regulation of blood pressure is necessary to maintain an adequate blood supply to the heart and the brain by the vascular system. Blood pressure is generated by the contraction of the ventricles. Therefore, blood pressure can be defined as the force exerted on the blood vessels by the blood that is being pumped by the heart during ventricular contraction and relaxation. Blood pressure measurement is one of the essential parameters in animal cardiovascular research. Both direct invasive and indirect non-invasive methods are used in blood pressure measurement in experimental animals, such as rats.

2.3.1 The vascular system

The connection between the heart and the tissue is the blood vessels. The vascular wall consists of three layers: the intima (inner layer), the tunica media (middle layer), and the outer tunica layer (Levick, 2013). Blood vessels are divided into arteries, capillaries, and veins according to function, location, and size.

Once considered a simple barrier between the wall of the blood and the vessel, the endothelium is now considered a dynamic organ that connects the entire vascular system. Endothelial cells lie on the intima, which is inside the vasculature. They control vascular function by responding to hormones, neurotransmitters, and vasoactive factors that affect vasomotion, thrombosis, platelet aggregation, and inflammation (Galley and Webster, 2004). The term endothelium was coined in 1865 by embryologist Wilhelm

Hirsch. It means the cell lining of the lumen in the vascular system and the body's inner cavities, such as the pericardium, pleura, peritoneum, or joints (Mas, 2009). Since the 1970s, the vascular endothelium has played a significant role in regulating smooth muscle function. Nowadays, endothelium also applies to the blood lining and lymph vessels (Laubichler *et al*., 2007).

Different substances come in contact with vascular endothelial cells causing the production and release of endothelial factors, which eventually cause the vascular smooth muscle to contract or relax. In 1983, Furchgott reported that blood vessels released a vasoactive substance called endothelium-derived relaxing factor (EDRF) in the presence of endothelium. Later, Ignarro *et al.,* (1987) identified the EDRF as nitric oxide (NO), a compound reported by Ferid Murad in 1977 (Katsuki *et al.*, 1977), which promotes the relaxation of smooth muscle cells. More important observations were made shortly afterward of new EDRFs, including prostacyclin (PGI_2) (Vane and Corin, 2003), endothelium-derived hyperpolarising factors (EDHFs) (Campbell and Gauthier, 2002), and endothelium-derived contracting factors (EDCFs), such as endothelin (Virdis *et al.,* 2010).

2.3.2 Endothelium and the cardiovascular system

The most common disease among the populations of developing countries is cardiovascular disease. The endothelium-impaired function may play a major role in cardiovascular diseases such as hypercholesterolemia and atherosclerosis. As a result, the vessel wall in these circumstances may promote inflammation, oxidation of lipoproteins, smooth muscle proliferation, extracellular matrix deposition or lysis, accumulation of lipid-rich material, platelet activation, and thrombus formation. All these effects of endothelial dysfunction may lead to cardiovascular disease development and clinical expression (Park & Park, 2015).

2.3.3 The physiology of endothelium

The structure and functional integrity of the endothelial cell is important for maintaining the vessel wall and circulatory function. The endothelium is semipermeable and regulates the transfer of small and large molecules. Endothelial cells are dynamic, and both have synthetic and metabolic functions (Figure 2.1).

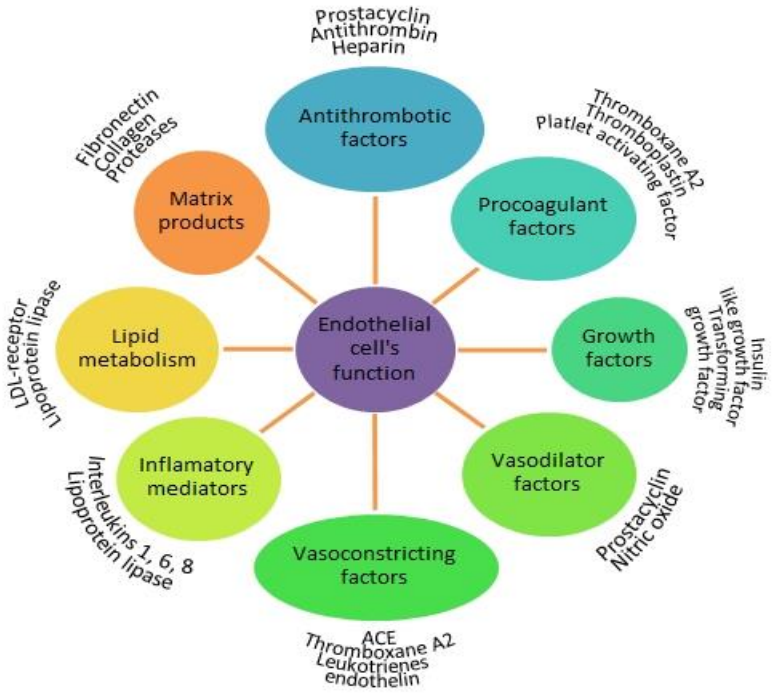

Figure 2.1 The functions of endothelial cells (modified from Galley and Webster, 2004). ACE; angiotensin converting enzyme, LDL-receptor; low-density lipoprotein receptor.

The endothelium is an active metabolic system that maintains vascular homeostasis by (a) tone modulator, (b) regulating the transportation of solute to cell components of vessel walls, local cellular growth, and extracellular matrix deposition, (c) protecting the vessel against potentially harmful effects of substances and cells circulating in the blood, and (d) regulates the homeostatic, inflammatory, and repair the reactions to local injury (Chiu and Chien, 2009).

The typical cell layer of the endothelium has a different biological function, such as maintaining a balanced coagulation and fibrinolysis, expression of adhesion molecules for cells in the immune system, metabolism of enzymes (noradrenaline and 5-hydroxytryptamine), and conversion of enzymes (angiotensin I and bradykinin). Besides, endothelium cells regulate the underlying smooth muscle layer and vascular tones by releasing EDRFs such as NO, PGI_2, and EDHF, as well as vasoconstrictive factors like endothelin, superoxide, and thromboxane (Duffy *et al.,* 2004; Cahill & Redmond, 2016). These properties are due to the endothelial cells' ability to sense humoral and hemodynamic stimuli.

2.3.3.a Endothelium-derived relaxing factors (EDRFs)

In hypertension, morphological changes occur in endothelial cells. The endothelium regulates the tone of the underlying vascular smooth muscle by releasing the production of vasodilator mediators (De Vriese *et al.,* 2000).

2.3.3.a.i Nitric oxide and cGMP pathways (NO/cGMP pathways)

NO is produced by many cells in the body and has an important role as a signaling molecule. NO production contributes to endothelium-dependent relaxation in large, isolated arteries, including coronary, systemic mesenteric, pulmonary, and cerebral arteries. Nevertheless, its production by vascular endothelium is important in blood flow regulation. As indifferent cardiovascular diseases, abnormal NO production can adversely affect blood flow and other vascular functions. NO is essential for maintaining normal blood pressure (Huang *et al.,* 1995). Therefore, its relationship to essential hypertension has been the subject of many studies.

Based on Figure 2.2, ACh binds to G protein receptors initiating IP3 production and releasing Ca^{2+} from the endoplasmic reticulum in the nitric oxide and cGMP pathways (NO/cGMP pathways). Ca^{2+} and calmodulin form a complex that stimulates

NO synthase to produce NO. NO diffuses into smooth muscle cells and activates soluble guanylyl cyclase (sGC) to convert guanosine triphosphate (GTP) to cGMP. Activated cGMP then activates protein kinase G that induces phosphorylation of several muscle proteins to induce muscle relaxation. cGMP also decreases the release of intracellular Ca^{2+} from the SR, and this causes smooth muscle relaxation (Vanhoutte, 1998; Sanders *et al.*, 2000).

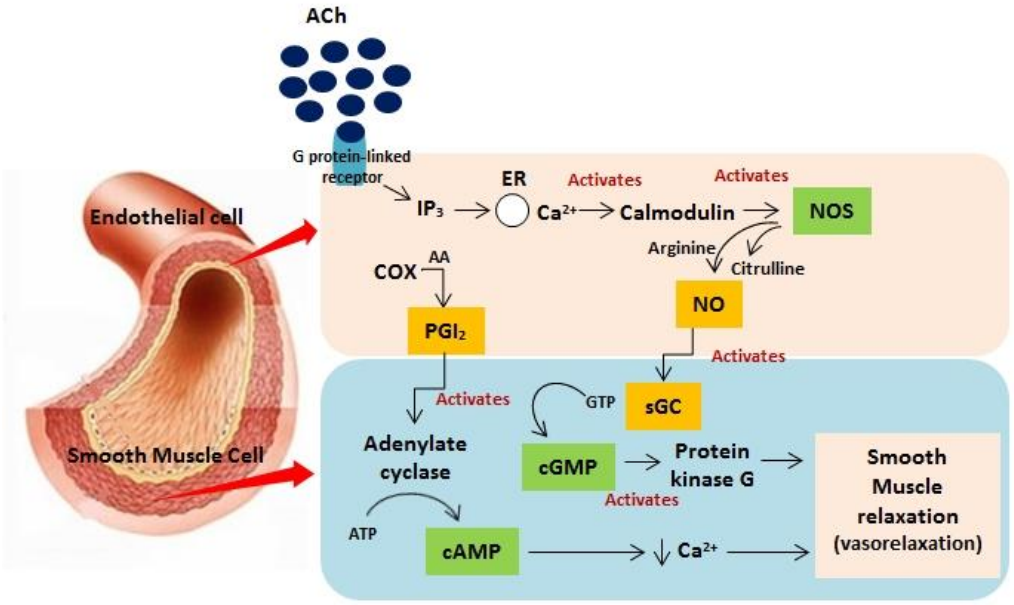

Figure 2.2 Mechanism of NO and cGMP pathways (modified from Vanhoutte, 1998; Sanders *et al.*, 2000). IP_3; inositol triphosphate, ER; endoplasmic reticulum, Ca^{2+}; calcium ion, NOS; niric oxide synthase, NO; nitric oxide, sGC; soluble guanylyl cyclase, cGMP; cyclic guanosine monophosphate, GTP; guanosine triphosphate, ATP; adenosine triphosphate, cAMP; cyclic adenosine monophosphate, AA; arachidonic acid.

2.3.3.a.ii Cyclooxygenase (COX) pathway

Prostacyclin is formed in endothelial cells following the activation of phospholipase A2, cyclooxygenase (COX), and prostacyclin synthase. Prostacyclin induces vascular smooth muscle relaxation by activating adenylate cyclase and increasing cyclic adenosine monophosphate (cAMP) production. Prostacyclin's

contribution to endothelium-dependent relaxation in blood vessels is not as significant as NO, but its effect is additive to NO. As shown in Figure 2.2, COX converts arachidonic acid to prostacyclin (PGI_2) during vasorelaxation. PGI_2 diffuses into smooth muscle cells and stimulates adenylate cyclase, which leads to increased cAMP production. cAMP decreases the amount of intracellular Ca^{2+} release and induces smooth muscle relaxation (Vanhoutte and Boulanger, 1995).

2.3.3.b Endothelium-derived hyperpolarising factor (EDHF)

The hyperpolarisation of smooth muscle cells induced by EDHF derives from increased the movement of potassium ions. As for NO, the release of EDHF requires an increase in intracellular Ca^{2+} in endothelial cells. The contribution of hyperpolarisation in endothelium-dependent vascular relaxation depends on the size of arteries and is prominent in resistance vessels (Nakashima *et al.*, 1993). In large arteries, both mediators may lead to endothelium-dependent relaxations, but under normal circumstances, the position of NO is dominant. EDHF can mediate close to normal endothelium-dependent relaxation if NO synthesis is inhibited (Cohen and Vanhoutte, 1995).

2.3.3.c Endothelium-derived contracting factors (EDCFs)

The endothelium is not the primary site of synthesis and release of EDRFs only. However, the endothelium is also a source of contracting factors, namely endothelium-derived contracting factors (EDCFs). EDCFs released by the endothelial cells are responsible for responding to a specific mechanical and chemical stimulus to the smooth muscle. These include endothelin-1 (ET-1), angiotensin II (Ang II), thromboxane A2, noradrenaline, serotonin, and reactive oxygen species (ROS) (Schiffrin, 2001; Verma and Anderson, 2002).

2.3.4 Vascular smooth muscle

2.3.4.a Vascular smooth muscle contraction

Vascular smooth muscle contraction plays a significant role in regulating vascular resistance and blood pressure, and its dysregulation may lead to vascular disorders, such as hypertension and coronary heart disease. The increased peripheral vascular resistance in essential human hypertension could be caused by structural changes in the resistance vessels (Mayet and Hughes, 2003; Hamilton, 2007). The increased blood pressure may cause an enhanced wall or lumen ratio and is responsible for the increased vascular reactivity in vasoconstrictor stimuli in hypertensive, compared to normotensive states (Weiss and Lundgren, 1978). Endothelium-dependent contractions can be interpreted either by the withdrawal of the release of EDRFs or by the production of diffusible vasoconstriction substances (EDCFs).

Salamanca and Khalil (2005) have clarified the mechanism of vascular smooth muscle contraction through the binding of an agonist to its receptor. Receptors' stimulation at the plasma membrane enhances the production of inositol triphosphate (IP_3) and diacylglycerol (DAG). IP_3 stimulates calcium ions (Ca^{2+}) release from the sarcoplasmic reticulum (SR). At the same time, the agonist activates extracellular Ca^{2+} influx through Ca^{2+} ion channels. Then, Ca^{2+} binds to calmodulin, which activates the myosin light chain kinase (MLCK), induces MLC phosphorylation, and initiates vascular smooth contraction. Other important pathways of vascular smooth muscle contraction include the RhoA/Rho-kinase pathway, which inhibits MLC phosphatase and further enhances the Ca^{2+} sensitivity. α_1-adrenergic receptors are linked to G_q-proteins. Activating the α_1-adrenergic receptor by phenylephrine (PE) activates phospholipase C (PLC), causing the production of IP_3 and DAG from inositol phospholipids. IP_3 triggers the release of intracellular Ca^{2+}, which results in smooth

muscle contraction (Figure 2.3) (Amberg and Navedo, 2013). PE-dependent stimulation of adrenergic receptors on the vascular smooth muscle cell plasma membrane (VSMC PM) leads to activation of PLC and the production of IP_3. IP_3 then promotes the IP_3 receptor's opening and resulting Ca^{2+} release events. Ca^{2+} release increase contraction by increasing intracellular calcium concentration ($[Ca^{2+}]_i$) and altering plasma membrane potential (Figure 2.3).

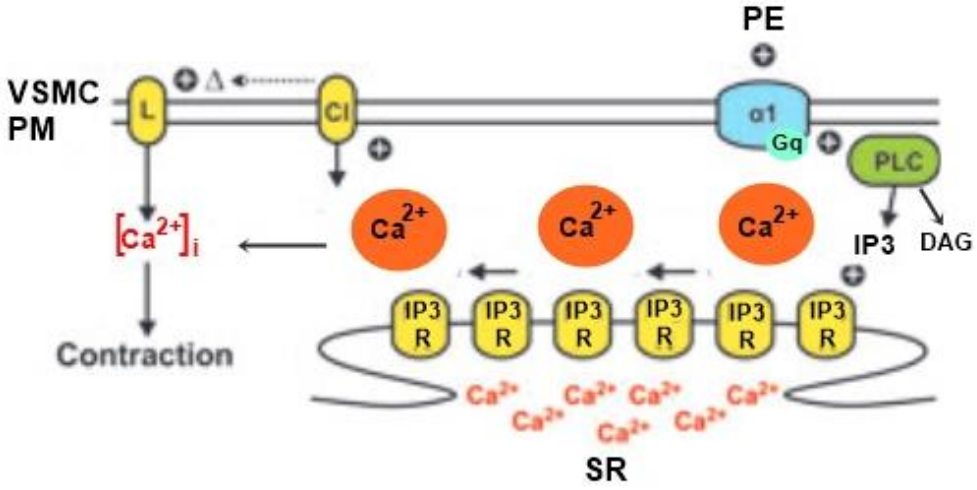

Figure 2.3 Vascular smooth muscle contraction (modified from Amberg and Navedo, 2013). VSMC PM = vascular smooth muscle cell plasma membrane, PLC = phospholipase C, IP3 R = IP_3 receptors, $[Ca^{2+}]_I$ = intracellular calcium concentration, DAG = diacylglycerol , α_1 = α_1-adrenergic receptor, G_q = G_q-proteins.

2.3.4.b Vascular smooth muscle relaxation

There are two ways that lead to smooth muscle relaxations; by removing the contractile stimulus or by using a substance that inhibits the contractile mechanism. As a result, $[Ca^{2+}]_I$ drops and MLC phosphatase activity increases. Relaxation of smooth muscle cells is induced by a decrease in the $[Ca^{2+}]_i$. The SR and the plasma membrane contain Ca, Mg-ATPase; the sarcoplasmic reticular, which involved in the removal of Ca^{2+} from cytosol. The uptake of Ca^{2+} is dependent on ATP hydrolysis. After being

phosphorylated, Ca, Mg-ATPase will bind two Ca^{2+} ions, which are then translocated to the luminal side of SR and released. Mg^{2+} then will bind to the ATPase's catalytic site for the enzyme's activity. Receptor-operated and voltage-operated Ca^{2+} channels (ROCC) which induced smooth muscle contraction by influx the Ca^{2+} will be inhibited and increased the relaxation of smooth muscle (Webb, 2003).

2.3.5 Antihypertensive agents – vasodilator drugs

Antihypertensive agents or drugs are a class of medications used to treat high blood pressure. Many antihypertensive drugs currently in use dilate blood vessel and make it easier for blood to flow. As the name implies, vasodilator drugs relax the smooth muscle of the blood vessels and stimulate the vasomotor core in the brain, causing smooth muscle relaxation in the blood vessels walls or acting on smooth muscle cells locally (Toda and Okumura, 2011).

Vasodilator drugs are categorised by site or mode of action. The right antihypertensive agent is important for drug treatment with minimal side effects. Nitrates, thiazide diuretics, angiotensin-converting enzyme (ACE) inhibitors, Ca^{2+} channel blockers, beta (β)-blockers, alpha (α)-blockers, and angiotensin II receptor blockers (ARBs) are some of the most effective and commonly used vasodilators in the treatment of hypertension are, as outlined in Table 2.1.