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INSIGHT INTO AUTONOMIC DYSFUNCTIONS WITH NOVEL INTERVENTIONS: FOCUSING ON VASCULAR TONE AND BREATHING REGULATIONS

by

SHUANG ZHANG

Under the Direction of Chun Jiang, PhD

ABSTRACT

The autonomic nervous system (ANS) controls most involuntary functions of the body. Dysfunctions of the ANS can be life-threatening. However, several critical questions related to cardiovascular and breathing regulations remain unclear.

One of the open questions is how the system lose control of the vascular tones under certain circumstances. Using the septic shock model induced by lipopolysaccharide (LPS) in isolated and perfused mesenteric arterial rings, we found the vascular hyporeactivity is attributed to the decreased vasoconstriction to α-adrenoceptor agonists. The endotoxin-induced vasodilation can be intervened with endothelin-1 (ET-1), serotonin (5-HT) or vasopressin, which have never been used in clinical treatment.

It is unclear how the excitability of endothelium affects vascular tones. Using optogenetics and transgenic mice with channelrhodopsin expression in endothelial cells (ECs), we found selective activation of the ECs induces a fast, robust, reproducible and long-lasting vasoconstriction in isolated and perfused hearts and kidneys.

Breathing control by the ANS within the brain becomes abnormal in certain genetic diseases, such as Rett syndrome with defected norepinephrine (NE) system in locus coeruleus (LC). The LC neurons are hyperexcitable while NE release is deficient. Using optogenetics and double transgenic mice with *Mecp2* null and channelrhodopsin expression in LC neurons, we found the NE-ergic modulation of hypoglossal neurons was impaired in transgenic mice, which cannot be improved with optostimulation, suggesting that LC neuronal hyperexcitability may not benefit the NE modulation in Rett syndrome.

Collectively, our results provide insight into the autonomic dysfunctions using experimental interventions that have barely been used before.

INDEX WORDS: Autonomic nervous system, Dysfunction, Vascular tones, Breathing, Septic shock, Lipopolysaccharide, Endothelium, Optogenetics, Transgenic mice, Rett syndrome

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SHUANG ZHANG

A Dissertation Submitted in Partial Fulfillment of the Requirements for the Degree of

Doctor of Philosophy

in the College of Arts and Sciences

Georgia State University

2016

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May 2016

DEDICATION

To my parents Shengguo Zhang and Hui Zhao, who has always been there for me and showed me how to persevere through all of the life's trails.

All I have and will accomplish are only possible due to their love and sacrifices.

To my aunt Xingfang Zhao

A lovely woman has been very supportive in my educational pursuits.

ACKNOWLEDGEMENTS

First and foremost, I would like to thank my advisor, Dr. Chun Jiang. Dr. Jiang is my research advisor and my mentor, who has been motivating, supporting and pushing me to become a better scientist. Without his expert guidance and help, I would not be where I am today.

Secondly, I would like to thank all of my committee members, Dr. Bingzhong Xue, Dr. Didier Merlin, Dr. Andrew Clancy, and Dr. Deborah Baro for their valuable suggestions and advice has helped me to conduct better research and improved my dissertation.

I would also like to thank other faculty members at GSU. Thanks to Dr. Ritu Aneja, and Dr. Julia Hilliard for their help when I took the qualifying examination. Dr. Blaustein and Nancy Russell, Dr. Matthew Brewer and Stephanie Gutzler for providing wonderful student teaching experience. Thank you, Dr. Phang Tai and Dr. Yi Pan for their suggestion on my academic development.

I also thank the staff at GSU. LaTesha Warren provided professional assistance and guidance. Barry Grant for his help with maintenance and repairs. Debby Walthall, Sonja Young, Ping Jiang, Gemia Cameron have been helpful to access research facilities. Tameka Hudson, Stephanie Lewis, Tamara Gross, and Karon Mackey-Conyers for their help with program issues. Department of animal resources staff for their assistance and advice for training and maintaining of experimental animals.

Thank you to the many friends and colleagues I have made in GSU, including but not limited to Dr. Yan Li, Johnny Garretson, Dr. Lei Zhong, Dr. Stephen Estes, Dr. Liana Artinian, Maksim Kvetny. Lab members, Dr. Weiwei Shi, Dr. Yang Yang, Casey Trower, Dr. Ningren

Cui, Dr. Max Oginsky, Dr. Xin Jin, Dr. Xiaotao Jin, Dr. Shanshan Li, Christopher M. Johnson, Dawn Wu, Vivian Zhong, Hao Xing.

I acknowledge Molecular Basis of Disease (MBD) Fellowship program for their financial support for my study and research.

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20-HETE	20-hydroxyeicosatetraenoic acid
5-HT	serotonin
AA	arachidonic acid
AAV	adeno-associated virus
AC	adenylate cyclase
ACh	acetylcholine
ADMA	dimethylarginine
Ang II	angiotensin II
ANS	autonomic nervous system
AP	area postrema
Arch	archaerhodopsin
ATP	adenosine triphosphate
AVP	arginine vasopressin
BH_4	tetrahydrobiopterin
CA	coronary artery
Ca^{2+}	calcium
CAG	cytomegalovirus-immediate-early
CAM	calmodulin
cAMP	cyclic adenosine monophosphate
CAN	Ca^{2+}-activated non-selective cation channels
CCHS	congenital central hypoventilation syndrome
cdh5	cadherin-5

cGMP	cyclic guanosine monophosphate
ChR	channelrhodopsin
CMV-IE	enhancer/chicken β-actin/rabbit β -globin hybrid promoter
CNP	C-type natriuretic peptide
CNS	central nervous system
CO	carbon monoxide
COX	cyclooxygenase
DA	Dopamine
DAG	diacylglycerol
DMEM	Dulbecco's Modified Eagle Medium
DPSS	diode-pumped solid state
DRG	dorsal respiratory groups
ECs	endothelial cells
ED	endothelium-denuded
EDHF	endothelium-derived hyperpolarizing factor
EETs	epoxyeicosatrienoic acids
EI	endothelium-intact
eNOS	endothelial NOS
ER	endoplasmic reticulum
ET-1	endothelin-1
ET_A	endothelin A receptors
ET_B	endothelin B receptors
FBS	fetal bovine serum

FR	firing rate
GABA	γ-aminobutyric acid
GAPDH	glyceraldehyde 3-phosphate dehydrogenase.
GC	guanylyl cyclase
GDP	guanosine diphosphate
GI	gastrointestinal
GIRK	G-protein-regulated inwardly rectifying K^+ channel
GPCR	G protein-coupled receptors
GTP	guanosine triphosphate
H_2CO_3	carbonic acid
H_2O_2	hydrogen peroxide
Hb	deoxygenated
HbO_2	oxygenated hemoglobin
HCO_3^-	bicarbonate
Hist	histamine
HNs	hypoglossal neurons
IKCa	intermediate conductance calcium-activated potassium channels
$O2^-$•	superoxide
iNOS	inducible NOS
IP_3	inositol (1,4,5) trisphosphate
Isop	isoproterenol
I-V	current-voltage
J receptors	Juxtacapillary receptor

K^+	potassium
Kir	inward rectifier K^+ channels
L2-3	Lumbar area 2-3
LC	locus coeruleus
LDL	low-density lipoprotein
LPS	lipopolysaccharide
M receptor	muscarinic receptor
MAPK	mitogen-activated protein kinase
MBD	methyl- binding domain
MECP2	Methyl CpG binding protein 2
MEK	MAPK kinase
MHPG	3-methoxy-4-hydroxyphenylethylene
MLCK	myosin light chain kinase
NA	nucleus ambiguus
NE	norepinephrine
NE-ergic	Norepinepherinergic neurons
nNOS	neuronal NOS
NO	nitric oxide
NOS	NO synthases
NpHR	halorhodopsin
NPY	neuropeptide Y
NTS	neucleus tractus solitaries
O_2	oxygen

OH•	hydroxyl radical
$ONOO^{-}$•	peroxynitrite
PA	pulmonary artery
PBS	phosphate buffer solution
PC-PLC	phosphatidylcholine-specific phospholipase C
PE	phenylephrine
PGH_2	prostaglandin H2
PGI_2	prostacyclin
PIP_2	phosphatidylinositol 4,5-biphosphate
PI-PLC	inositol triphosphate specific phospholipase C
PKC	protein kinase C
PLC	phospholipase C
PNS	peripheral nervous system
PolyA	bovine growth hormone polyadenylation signal and flippase recognition target flanked phosphoglycerate kinase-Neo-polyA cassette;
pre-BötC	pre-Bötzinger complex
PRG	pontine respiratory group
RAAS	renin-angiotensin-aldosterone system
ROK	Rho-kinase
ROS	reactive oxygen species
RTN	retrogtrapezoid nucleus
RTT	Rett syndrome
SIDS	sudden infant death syndrome

SKCa	small conductance calcium-activated potassium channels
SoNS	somatic nervous system
SR	sarcoplasmic reticulum
T1	thoracolumbar region 1
TASK	TWIK-related acid-sensitive K^+ channels
TH	tyrosine hydroxylase
TRD	transcriptional repression domain
TRP	transient receptor potential channels
TXA_2	thromboxane A2 VEGF
VEGFs	Vascular endothelial growth factors
VSM	vascular smmoth muscle
VT	tidal volume
WT	wild type

1 SPECIFIC AIMS AND HYPOTHESES

The autonomic nervous system (ANS) plays a critical role in maintaining physiological homeostasis. The ANS acts through sympathetic and parasympathetic innervation to target organs and tissues, including cardiovascular, respiratory, nervous, digestive, and reproductive systems. Therefore, dysfunctions of ANS can affect many parts of the body. Some are temporary while many worsen over time and can be life-threatening, especially when they affect blood pressure, heart beat, and breathing activity.

Although the understanding of the ANS function and dysfunction of has been significantly improved over the last century, there are still critical problems related to cardiovascular function and breathing activity to be resolved. To my understanding, the key resolving these problems is to intervene the ANS effectively under physiological and pathological conditions so that these problems can be well accessed, dissected, and understood. Therefore, it is necessary to explore innovative research approaches to the ANS. I hypothesize that certain dysfunctions in vascular tones and breathing can be better understood with alternative interventions. Thus, we proposed studies to address three specific aims.

Specific Aim 1: Interception of septic shock with pharmacological tools in LPS induced vasodilation model.

Blood vessels lose response to vascular regulators including epinephrine (EPI)/norepinephrine (NE) released from sympathetic nerve terminals and the adrenal gland under certain pathological conditions, such as septic shock. Approximately, 200,000 people die of severe sepsis or septic shock in the US each year (Martin et al., 2003). Septic shock is characterized by excessive vasodilation and vascular hyporeactivity to vasoconstrictors,

especially α-adrenergic agonists. Without effective therapeutic intervention, this leads to uncontrolled hypotension, hypoperfusion, tissue hypoxia, multiple organ failures and death (Dellinger et al., 2013; Vincent et al., 2006). The irreversible hyporeactivity could result from extensive injuries to the vascular smooth muscle (VSM), enhanced endothelium-dependent vasodilation, or disrupted contraction response to certain types of vasoconstrictor. Therefore, in this study, we investigated all of the potential mechanisms using *in vitro* mesenteric septic model by application of lipopolysaccharide (LPS). This study provides insight into the pathological development of septic shock and has an impact on potential clinical treatment for the disease.

Specific Aim 2: Intervention to vascular tones via optogenetic approach to vascular endothelium.

Although accumulating evidence suggests that the primary function of the endothelium in the vasculature is vasodilation, how vascular endothelial depolarization affects vascular tones is still unclear. The major obstacle is that these cells cannot be selectively activated without affecting VSM cells. The information of endothelium activation is necessary for the understanding vascular physiology as well as the development of several vascular diseases. Using optogenetics as an innovative intervention to vascular endothelium of transgenic mice. Therefore, we studied the function of endothelium with direct stimulation and inhibition. Our results demonstrate a novel intervention to endothelium and reveal the effects of endothelial depolarization on vascular tones and organ perfusion.

Specific Aim 3: Intervention to NE-ergic defects in Mecp2 knockout mice by optostimulation of locus coeruleus neurons.

Another important function of the ANS is respiratory regulation where several groups of neurons in brainstem play a role. Dysfunction in the brainstem neuronal networks underscores certain diseases. Rett syndrome (RTT) is a neurodevelopmental disease that is caused by mutation of a transcriptional regulator, Methyl CpG binding protein 2 (*MECP2*) and characterized by life-threatening breathing abnormalities. The breathing disorders in RTT mouse model with *Mecp2* disruption are found to be associated with the defects in locus coeruleus (LC) neurons located in the pons (Roux et al., 2010; Zhang et al., 2010). This group of neurons is the principle site of norepinephrine NE-ergic projection in the CNS. The NE synthesis enzymes are found deficient in both RTT patients and animal model (Viemari et al., 2005). However, the causal relationship between NE deficiency and LC neuron disruption in the etiology of RTT breathing disorder is unclear. Based on one of most widely used *Mecp2* gene knock-out RTT mice model, we developed a new double transgenic RTT mouse model with light sensitive opsin knock-in in the NE-ergic neurons. Our results showed that the double transgenic mice had significant RTT phenotypes, including breathing disorder, lower body weight, and hypomotility. The NE-ergic projection to hypoglossal neurons (HN) was disrupted in these mice with significant breathing disorders comparing to control TH-ChR mice. This study opens a new avenue for the study of causality in etiology of neurodevelopmental diseases.

2 INTRODUCTION

2.1 Autonomic nervous system

2.1.1 Overview and anatomy of the autonomic nervous system

The function of the nervous system is coordinating the voluntary and involuntary actions of the body. In vertebrate species, the nervous system mainly consists of two components, the

central nervous system (CNS) containing the brain and spinal cord, and the peripheral nervous system (PNS) consisting of nerves that connect the CNS to the different parts of the peripheral system. Nerves that carry sensory impulses from different parts of the body to the brain are sensory/afferent nerves while those nerves transmitting information from the CNS to muscles and organs are motor/efferent nerves. The PNS includes two parts, the somatic nervous system (SoNS) and the autonomic nervous system (ANS). The SoNS associates with skeletal muscles to control voluntary function of the body while the ANS controls involuntary/autonomic function, such as the activity of the cardiomyocytes, smooth muscle, and the internal organs (Schmidt and Thews, 1989). Thus, dysfunction of the ANS can lead to disorder in multiple organs and systems.

The ANS controls most involuntary function, including blood pressure, heart beat, respiration, gastrointestinal (GI) tract motility, pupillary diameter, and accommodation, urinary and bowel excretion, salivation, perspiration, metabolic and endocrine physiology, and sexual arousal. This system has two components: sympathetic and parasympathetic systems that are the primary control of the fight-or-flight response and the freeze-and-dissociate response (Schmidt and Thews, 1989). Both sympathetic and parasympathetic divisions require a sequential two-neuron efferent pathway, which the preganglionic neurons must synapse onto the ganglionic neurons first. Then the ganglionic neurons innervate the target organs. The sympathetic system has short preganglionic fibers originating from the spinal cord from T1 to L2-3. Long ganglionic fibers run from the sympathetic ganglia adjacent to the spinal cord to effector organs. The parasympathetic division has a craniosacral outflow, where the long preganglionic fibers exit cranial nerves of the brainstem, including oculomotor, facial, glossopharyngeal, and vagus nerves, and sacral spinal cord (S2-S4). The vagus nerve contains about 75% of all

parasympathetic fibers. Parasympathetic ganglia are located within the effector organs, so the ganglionic fibers are short. This anatomy structure allows the sympathetic division functions quickly with short preganglionic nerves, whereas the parasympathetic division functions without an immediate reaction but a more specific and localized response with long preganglionic nerves projected into the effector organs (Drake et al., 2005).

2.1.2 Function and regulation of the ANS

The sympathetic and parasympathetic divisions have a complementary function to each other. In both systems, preganglionic nerves are cholinergic that release acetylcholine (ACh) as neurotransmitters to stimulate the ganglionic cells. In response to the stimulation, their ganglionic nerves release different neurotransmitters into the target organs and induce opposite effects. In the sympathetic nervous system, norepinephrine (NE) is secreted by most adrenergic ganglionic fibers and acts on different receptors in target organs to promote a fight-or-flight response, such as diverting blood away from the GI tract through vasoconstriction, relaxing the coronary vessels of the heart, increasing heart rate and contractility of the cardiomyocytes, and dilating bronchioles of the lung to increase oxygen (O_2) exchange (Pocock and Richards, 2006). In contrast, ACh is secreted from cholinergic ganglionic fibers of the parasympathetic nervous system to promote a "rest and digest" response, which calms the nerves down to regular function. When activated, the parasympathetic division functions to increase blood flow to the GI tract through vasodilation, reduce heart rate, and constrict respiratory airways (Pocock and Richards, 2006).

There are exceptions in sympathetic neurotransmission. The sweat glands are stimulated by ACh released from ganglionic fibers, and the renal blood vessels are innervated by dopamine (DA) released from dopaminergic nerves (Noback et al., 2005). Also, there is no ganglionic

neuron innervating adrenal medulla. Instead, the preganglionic neuron releases ACh to activate nicotinic receptors located in the adrenal medulla, which in turn stimulate the adrenal medulla releasing hormones EPI and NE into the bloodstream. These hormones then act on adrenoceptors to trigger a widespread sympathetic activity (Wakade and Wakade, 1983). A third sub-division neurons using nitric oxide (NO) as neurotransmitters have been found to be essential in autonomic function, especially in the gut and the lungs, and named as 'non-adrenergic and non-cholinergic' neurons (Belvisi et al., 1992).

At the effector organs, most sympathetic ganglionic neurons release NE acting on adrenoceptors in different tissues and organs but the sweat glands. The adrenoceptors are G protein-coupled receptors (GPCR), which are the largest and most diverse group of membrane receptors distributed throughout the whole body in eukaryotes (Eisen et al., 2013). GPCR receive messages from external signaling molecules, such as neurotransmitters, to be activated and induce a variety of cellular signaling pathways. When GPCR is stimulated by ligand molecule, a conformational change of the GPCR occurs so that it interacts with a nearby G protein. G protein is a specialized heterotrimeric protein consisting of three different subunits: an alpha subunit (G_α), a beta subunit (G_β), and a gamma subunit (G_γ). Two of them, G_β and G_γ, are attached to the plasma membrane. G_α subunit binds to inactive guanosine diphosphate (GDP) forming G_α-GDP complex in the absence of a signal stimulus. This complex binds to a nearby GPCR until the external signal acts on the GPCR. At this point, guanosine triphosphate (GTP) replaces the GDP bound to the G protein so that the G protein is activated. Consequently, the G_α-GTP complex dissociates from the $G_{\beta\gamma}$ dimer. Both of these two subunits can interact with other membrane proteins involved in different signaling transductions. (Wettschureck and Offermanns, 2005).

G proteins can be classified into four major subfamilies depending on their different G_{α} subunits and associated downstream signaling pathways: $G_{\alpha s}$, $G_{\alpha i}$, $G_{\alpha q/11,}$ and $G_{\alpha 12/13}$. The effector of $G_{\alpha s}$ pathway is a direct stimulation of the membrane-associated enzyme adenylate cyclase (AC), which catalyzes the formation of the second messenger cyclic adenosine monophosphate (cAMP) from cytosolic adenosine triphosphate (ATP). The downstream signal of this pathway is protein kinase A (PKA), which in turn, phosphorylates many different downstream signals (Tamaki, 2007; Taylor et al., 2008). In contrast, activation of $G_{\alpha i}$ inhibits AC from generating cAMP. Thus, activation $G_{\alpha s}$ and $G_{\alpha i}$ signaling pathways have a compensatory effect on each other. $G_{\alpha q/11}$ activates membrane-associated phospholipase C-β (PLC_{β}), which in turn, catalyzes the formation of inositol (1,4,5) trisphosphate (IP_3) and diacylglycerol (DAG), which both can increase intracellular Ca^{2+} concentration. IP_3 binds to its receptors located in the membrane of the endoplasmic reticulum (ER) so that Ca^{2+} inflexed from the ER into the cytosol, while DAG activates protein kinase C (PKC), which can also be activated by increased intracellular Ca^{2+} (Kadamur and Ross, 2013). The $G_{\alpha 12/13}$ pathway is through RhoGEF superfamily, which binds to and allosterically activates the cytosolic small GTPase, Rho (Kozasa et al., 1998). Once bound to GTP, Rho then activates various proteins, such as Rho-kinase (ROK), which is involved in myosin phosphorylation and contraction of VSM (Noda et al., 1995). Most GPCRs that couple to $G_{\alpha 12/13}$ also couple to other sub-classes, often $G_{\alpha q/11}$ (Takashima et al., 2008). In addition to involvement in the $G_{\alpha i}$ cascade, $G_{\beta\gamma}$ targets on various ion channels, such as G protein-regulated inwardly rectifying K^+ channel (GIRK), and P/Q- or N- type voltage-gated Ca^{2+} channels (Takahashi et al., 1996).

There are mainly two types of adrenoceptors, α and β with several subtypes. The α receptors have the subtypes α_1, a $G_{\alpha q}$ coupled receptor, and α_2, a $G_{\alpha i}$ coupled receptor, while the

β receptors have the subtypes β_1, β_2, and β_3, which are all linked to Gs proteins. The β_2 also couples to $G_{\alpha i}$ proteins (Johnson and Liggett, 2011). These receptors play different roles in different tissues. The general function of α receptors is vasoconstriction. Specifically, the α_1 receptors are located in almost all of tissues throughout the body with a particularly important function in smooth muscles (Bulbring and Tomita, 1987). Activation of α_1-adrenergic receptors also mediates cardiac stimulation, cellular proliferation, activation of glycogenolysis and gluconeogenesis in adipose tissue and liver, secretion of sweat gland, and Na^+ reabsorption from the kidney (Graham et al., 1996). Noticeably, the α_1 receptors located in the CNS are involved in regulation of neuronal activity (Carette, 1999). The α_2 receptors are mostly located in some VSM, platelets, and fat cells. In addition to the general effect of the α receptors, the activation of the α_2 receptors have individual functions, including mediating synaptic transmission in pre- and postsynaptic nerve terminals to decrease release of ACh and NE, inhibiting lipolysis in adipose tissue, inhibiting insulin-releasing in pancreas inducing glucagon release from the pancreas, contracting sphincters of the GI tract, and increasing thrombocyte aggregation (Molinoff, 1984).

The β_1 receptors are mainly located in the heart, the β_2 receptors distribute in respiratory, uterine, and VSM, and the β_3 receptors are mainly exist in fat cells. The β_1 and β_2 receptors mediate cardiovascular response to NE released from sympathetic nerve terminals and to circulating adrenaline. When β_1 receptors are activated, the cardiac output increases through the augmentation of the heart rate, conduction velocity, stroke volume, and the renin secretion from the kidney and ghrelin secretion from the stomach increase as well. The activation of the β_2 receptor leads to vascular and nonvascular smooth muscle relaxation, lipolysis in adipose tissue, dilation of arteries to skeletal muscle, glycogenolysis and gluconeogenesis, insulin secretion, thickened secretions from salivary glands, increases in renin secretion from kidney, and

relaxation of bronchioles. The β_3 receptors are responsible for the enhancement of the lipolysis in adipose tissue. Taken together, the α receptors are mainly distributed throughout the vascular and nonvascular smooth muscle (SM) while the β receptors are mainly expressed in the heart and some SM (Minneman et al., 1981; Molinoff, 1984).

In the parasympathetic division, ACh released from ganglionic fibers acts on the muscarinic (M) cholinergic receptors of effector organs. There are five types of M receptors, M1-M5, which also function through binding to G proteins (Hulme et al., 1990). The M1, M4, M5 receptors are mainly expressed in the neural system and contribute to the CNS effects. The M2 receptors locate in the heart regulating cardiac function. The M3 receptors distribute in many parts of the body, such as endothelial cells (ECs), lungs, and GI tract, inducing vasodilation, bronchoconstriction, and intestinal motility and dilating sphincters. Besides, the M3 receptors also located in many glands helping glandular secretions (Caulfield and Birdsall, 1998).

Depending on the distribution of adrenoceptors and muscarinic receptors, the ANS maintains physiological homeostasis through the sympathetic and parasympathetic innervating target organs and tissues. Dysfunction of ANS can occur alone or result from other diseases, such as Parkinson's disease, alcoholism, and diabetes. These problems affect many parts or the whole system. Some are temporary, but many worsen over time and can be life-threatening, especially when they are associated with blood pressure, breathing activity, and heart beating. This dissertation is mainly focused on the cardiovascular and respiratory system of the ANS.

2.1.3 Neural control of the cardiovascular and respiratory systems

Cardiovascular control is indispensable for the timely needs of the body to survive. The control of the cardiovascular system can be grouped as neural, humoral, and local control (Sparks and Rooke, 1987). The neural control is fundamentally important because it regulates

multiple cardiovascular functions quickly that involves the blood flow, the contractility of the heart, and the arterial pressure (Thomas, 2011). Structurally, the neural control system consists of the medulla oblongata, afferent and efferent autonomic nerves, and peripheral sensory receptors. The medulla is a major control center of autonomic functions that contains vasomotor, cardiac and respiratory centers. These three control centers together belong to the sympathetic division. The vasomotor centers regulate vasoconstriction and vasodilation while the cardiac centers consisted of cardioacceleratory and cardioinhibitory centers regulate cardiac output. Respiratory centers located in medulla regulate rate and depth of breathing (Clark, 2005).

The activity of medullary neurons is modulated by afferent signals from the peripheral system and other brain regions, such as the hypothalamus and higher centers. The medullary parasympathetic center including nucleus ambiguus (NA) and the medullary relay center neucleus tractus solitaries (NTS) responds to cardiorespiratory afferents (Dampney, 1994). The medullary parasympathetic center receives afferents via NTS and, in turn, sends inhibitory regulatory information through the vagal fiber to the heart to decrease heart rate and the contractile force (Taylor et al., 1999). Medullary relay center NTS receives signals from sensory receptors, such as baroreceptors and chemoreceptors in peripheral system. Then the NTS relays the information to vasomotor center and cardiac vagal center, those control sympathetic and parasympathetic outputs, respectively (Paton et al., 2001).

2.2 Regulation of vascular tone

2.2.1 Anatomy and physiology of the vasculature

The vasculature is part of the circulatory system that transports blood throughout the whole body. There are three types of blood vessels, the arteries, which deliver the oxygenated blood from the heart to peripheral tissues; the capillaries, which enable the exchange of water

and molecules between the blood and peripheral tissues; and the veins, which carry deoxygenated blood from the capillaries back to the heart.

Vascular tone refers to the degree of vasoconstriction of a blood vessel relative to its maximally dilated state. All blood vessels exhibit basal vascular tone to maintain adequate perfusion to peripheral organs. The basal vascular tone varies in different organs and tissues. Those organs with a large vasodilatory capacity, such as myocardium, skeletal muscle, skin, splanchnic circulation system, have high vascular tone whereas organs with relatively low vasodilatory capacity, such as cerebral and renal circulation systems, have low vascular tone (Brayden and Nelson, 1992). The vascular tone determines the resistance of the circulation so that it plays a critical role in the regulation of blood pressure and organs perfusion. The vascular tone is determined by a balance between vasoconstrictor and vasodilator factors, including neurotransmitters, paracrine molecules, and hormones.

The arteries and veins regulate blood flow to downstream organs by contracting or dilating the muscular layer, which is mainly determined by the ANS innervation. Sympathetic vasoconstrictor fibers have tonic discharge to maintain basal vascular tone (Joyner et al., 1992). When sympathetic innervation is increased beyond the tonic level, the vasoconstrictor fibers produce vasoconstriction in most arteries, arterioles, and veins (Thomas, 2011). NE as the primary neurotransmitter released from vascular sympathetic fibers induces direct rapid vasoconstriction in VSMC through increasing intracellular Ca^{2+} concentration (Guimaraes and Moura, 2001). Some other neurotransmitters, such as neuropeptide Y (NPY) or ATP can also be released as cotransmitter potentiating the effect of NE (Pablo Huidobro-Toro and Veronica Donoso, 2004). Besides, the sympathetic nervous system also exerts indirect prolonged effects on cardiovascular system through humoral systems, the adrenal medulla and the renin-

angiotensin-aldosterone system (RAAS). After receiving sympathetic preganglionic innervation, the adrenal medulla releases circulating catecholamines, epinephrine, and NE, into the blood stream, which in turn, activate vascular adrenergic receptors and cause vasoconstriction. The RAAS releases renin into the blood after stimulated by sympathetic fibers. Renin helps to convert angiotensinogen into angiotensin II (Ang II), which is a potent vasoconstrictor. Ang II also increases blood volume through stimulation of aldosterone release (Ebert and Stowe, 1996). Collectively, the vasoconstriction increases peripheral resistance and diastolic blood pressure, which leads to a decrease in venous capacity, an increase in venous return, end-diastolic volume, stroke volume, cardiac output, and systolic blood pressure.

The vasodilation, an opposite process of vasoconstriction, is attributed to inhibited sympathetic effect and activated parasympathetic effect (Thomas, 2011), which lead to a decrease in discharges of sympathetic vasoconstrictor nerves, activation of sympathetic cholinergic vasodilator nerves, and activation of parasympathetic vasodilator nerves. Indeed, the vasodilation is produced by drawbacks in the tonic discharge of vasoconstrictor nerves in most of the vascular beds (Boron and Boulpaep, 2009). Otherwise, blood vessels located in some organs, such as skeletal muscles, receive innervation from both sympathetic vasoconstrictor adrenergic nerves and sympathetic vasodilator cholinergic nerves, which are not tonic active but only activated by stress, such as exercise and giving birth. Blood vessels do not receive parasympathetic innervation except those located in sexual organs and salivary gland (Pocock and Richards, 2006).

2.2.2 Function and dysfunction of the vascular smooth muscle

The VSMC is the essential component of the vasculature. The major function of VSMC is to maintain the local blood pressure by vasocontraction and vasodilation. The most

characteristic feature of VSMC is the capability of contraction that is slow, sustained, and tonic (Tanaka et al., 2008). The VSMC is primarily innervated by the sympathetic nervous system through adrenoceptors, α_1, α_2, and β_2 (as described above). The sympathetic constrictor fiber release NE that bind to α_1 receptors causing vasoconstriction. The α_1 receptors are activated by low blood pressure and defensively restore the blood pressure through vasoconstriction. Activation of α_2 receptors leads to vasoconstriction as well. In contrast, stimulation of β_2 receptors causes vasodilation and decreasing blood pressure. Under certain physiological conditions, such as pregnancy, exercise, and vascular injury, the VSMC is also involved in vascular remodeling, including synthesizing extracellular matrix components and increasing proliferation and migration (Louis and Zahradka, 2010). Taken together, the VSMC is involved in the short-term regulation of the vasomotor and long-term vascular remodeling.

The contraction in the excitable VSMC can be triggered by mechanical, electrical, and chemical stimuli. The VSMC contraction is induced by an increase in intracellular Ca^{2+} concentration due to Ca^{2+} release from intracellular sarcoplasmic reticulum (SR) (Laporte et al., 2004) and Ca^{2+} influx from the extracellular space through the membrane potential-dependent Ca^{2+} channels (Hill-Eubanks et al., 2011). Increased intracellular Ca^{2+} binds to calmodulin (CAM) to form a Ca^{2+}-CAM complex, which activates myosin light chain kinase (MLCK) and MLC phosphorylation. Phosphorylated MLC catalyzes interaction between two contractile proteins, myosin, and actin, forming cross-bridge, which leads to vasoconstriction (Kamm and Stull, 1989). On the other hand, vasodilation in VMSCs resultes from a decrease in intracellular Ca^{2+} concentration due to Ca^{2+} uptake by SR through Ca^{2+} pump and Ca^{2+} extrusion via Na^{+}/Ca^{2+} exchanger and plasmalemmal Ca^{2+} pump. The decreased intracellular Ca^{2+} leads to dissociation

of Ca^{2+}-CAM complex and dephosphorylation of MLC, which causes vasodilation (Laragh and Brenner, 1995).

In addition to intracellular Ca^{2+}, another major determinant of the maintained agonist-induced contraction of VSMC is activation of PKC, which increase the myofilament force sensitivity to intracellular Ca^{2+} (Nishimura et al., 1990) and MLC phosphorylation so that the VSMC contraction can be maintained with a smaller increase in intracellular Ca^{2+} concentration (Khalil, 2010). PKC with different isoforms and subcellular distribution undergo differential translocation during cell activation. The interaction of a PKC isoform with its substrate can trigger a cascade of protein kinases that ultimately stimulate VSMC contraction (Andrea and Walsh, 1992). PKC translocation to cell surface may trigger activation of cytosolic mitogen-activated protein kinase (MAPK) and MAPK kinase (MEK). After that, both MEK and MAPK translocate to the surface membrane and form a surface kinase complex, where MEK phosphorylates MAPK at both Thr and Tyr residues so that MAPK return to the cytoskeleton and phosphorylate the actin-myosin interaction and VSM contraction (Khalil et al., 1995). PKC may also phosphorylate CPI-17, an inhibitor of MLC phosphatase, to increases MLC phosphorylation and enhance VSMCs contraction (Woodsome et al., 2001). PKC also phosphorylate an actin-binding protein calponin to reverses its inhibition of actin-activated myosin ATPase so that the VSM contraction is enhanced (Kim et al., 2008).

The ROK signaling pathway enhances the myofilament force sensitivity to intracellular Ca^{2+} concentration as well (Kizub et al., 2010). GPCR agonist, particularly those coupling to $G_{\alpha 12/13}$, can activate the small G protein RhoA, which in turn stimulates the activity of ROK. ROK then phosphorylates and inactivates MLC phosphatase so that the VSMC contraction is enhanced (Somlyo and Somlyo, 2003). ROK signaling pathway is independent of intracellular

Ca^{2+} concentration change, which is therefore considered as Ca^{2+}-sensitization mechanism (Gong et al., 1997).

All of these Ca^{2+}-dependent and Ca^{2+}-sensitization mechanisms work synergistically for VSMC contraction. The collaboration alters under pathological conditions. Increased membrane permeability to Ca^{2+} has been reported in VSMC isolated from animal models of hypertension (Crews et al., 1999; Sugiyama et al., 1986). PKC has been suggested to play a role in the pathogenesis of several cardiovascular diseases, including coronary and cerebral vasospasm, hypertension, atherogenesis, and diabetic vascular complications (Ito et al., 1994; Salamanca and Khalil, 2005). These vascular disorders are characterized by increased contractile or hypertrophic response of the VSMC, which is resulted from increased production of and/or sensitivity to vasoactive stimuli and vascular growth factors, such as AngII, vasopressin, NE, and platelet-derived growth factor. These molecules bind to receptors and activate the G protein mediated PLC – DAG – PKC signaling pathway (Griendling et al., 1986; Takagi et al., 1988). Dysfunction of ROK signaling pathway is important in vascular hyperactivity associated with hypertension as well. The ROK inhibitors, Y-27632, and fasudil, normalized arterial pressure in animal models of hypertension (Uehata et al., 1997). An increased RhoA/ROK activity has been found in arteries from animal models of hypertension (Seko et al., 2003). In addition, mechanical stress on the vessel wall is increased that stimulates the VSMC proliferation in hypertension (Hishikawa et al., 1994). The stretch-induced activation of MAPK and the VSMC growth were inhibited by ROK inhibition (Numaguchi et al., 1999; Zeidan et al., 2003). Indeed, excessive RhoA/ROK activity also participated in the endothelial dysfunction and the decreased NO production associated with arterial diseases (Zhou and Liao, 2009). In pulmonary hypertension, studies on human pulmonary endothelial cells demonstrated that hypoxia-induced decrease in endothelial

NOS (eNOS) expression is mediated by ROK (Takemoto et al., 2002). Studies have also shown that activation of the RhoA/ROK pathway contributes to both vasoconstriction and vascular remodeling associated with pulmonary hypertension (Guilluy et al., 2005). Taken together, these findings indicate a prominent role of ROK in hypertension.

2.2.3 Function and dysfunction of the vascular endothelium

The endothelium is a specialized active organ lining the luminal side of vasculature throughout whole body (Villar et al., 2006). It is acknowledged that the primary function of the endothelium is maintaining vascular homeostasis and preventing initiation and/or development of cardiovascular diseases by releasing a variety of vasoactive substances. These substances not only alter the vascular tone and growth acting on the VSMCs but also regulate the activities of circulating blood cells and govern vascular permeability.

Cytoplasmic Ca^{2+} concentration is a key regulator of endothelial function, including the synthesis and release of vasoactive substances, vascular remodeling and permeability, cell proliferation and angiogenesis (Busse et al., 2002; Tran and Watanabe, 2006). Increased intracellular Ca^{2+} concentration responding to receptor activation activate downstream second messengers and a variety signaling pathways. Theoretically, the change of intracellular Ca^{2+} concentration is determined by membrane potential, the activity of various ionic pumps and channels for excitable cells, such as the VSMC and immune cells. But how endothelium release vasoactive substances remain unknown as the ECs are considered as “non-excitable”.

The most significant endothelium-derived vasodilators include NO (Palmer et al., 1987), prostacyclin (PGI_2) (Moncada et al., 1976), and the endothelium-derived hyperpolarizing factor (EDHF) (Chen et al., 1988). NO is converted from L-arginine by NO synthases (NOS) In the presence of cofactors tetrahydrobiopterin (BH_4) (Forstermann and Munzel, 2006). NO then

diffuses into the VSMC and causes vasodilation via activation of soluble guanylyl cyclase (GC) and cyclic guanosine monophosphate (cGMP) (Moncada et al., 1991). There are three forms of NOS, predominant eNOS, lesser extent neuronal NOS (nNOS), and inducible NOS (iNOS) expressed in inflammatory cells. The eNOS is the predominant NOS isoform responsible for most of NO production in the vasculature, where the NO dilates all types of blood vessels through GC – cGMP pathway (Forstermann et al., 1994). The nNOS is mainly expressed in nervous tissue and skeletal muscle type, facilitating intercellular communication (Forstermann and Sessa, 2012). The iNOS is located in immune and cardiovascular systems defending the immune system against pathogens (Hauser et al., 2004). Shear stress is the major activator of eNOS in normal physiology for adequate organ perfusion (Corson et al., 1996). The enzyme is also activated by several signaling molecules, such as bradykinin, adenosine, vascular endothelial growth factors, and serotonin (5-HT) (Govers and Rabelink, 2001). NO is also involved in the regulation of vascular permeability, synthesis of monocyte and lymphocyte adhesion molecules, platelets aggregation, tissue oxidation and inflammation, activation of thrombogenic factors, cell growth, proliferation and migration, and atherogenesis (Esper et al., 2006).

PGI_2 is formed primarily in ECs. Indeed, it is a metabolic product of arachidonic acid (AA) catalyzed by cyclooxygenase (COX) (Gryglewski, 1983). Two isozymes of COX have been identified, COX-1 and COX-2. COX-1 is a constitutive enzyme expressed in the endothelium that contributes to the maintenance of vascular homeostasis. COX-2 is an inducible isozyme expressed in the cardiovascular system during pathogenesis (Parente and Perretti, 2003). PGI_2 induce vasodilation of the VSMC through AC – cAMP – PKA singling pathway, which results in a decrease in MLCK (Mitchell and Warner, 2006).

EDHFs induce vasodilation by hyperpolarizing the VSMC independently from NO and PGI_2. Although how EDHFs induce vasodilation is unclear, several candidates of EDHF have been proposed, including epoxyeicosatrienoic acids (EETs), K^+, reactive oxygen species (ROS), H_2O_2, the spread of electronic current, and C-type natriuretic peptide (CNP) (Khazaei et al., 2008). It is widely acceptable that EDHFs are released from the ECs through the opening of small conductance calcium-activated potassium channels (SKCa) and intermediate conductance calcium-activated potassium channels (IKCa) (Edwards et al., 1998). Indeed, these vasodilators compensate for a loss of NO-mediated vasodilator tone, particularly in the microcirculation (Edwards et al., 2010; Feletou, 2011). EETs diffuse from ECs to VSMC and open Ca^{2+}-activated K^+ channel causing the VSMC hyperpolarization and vasodilation (Pratt et al., 2001). Bradykinin or ACh stimulate EETs synthesis in the ECs (Fisslthaler et al., 1999).

ROS are oxygen derived molecules, including superoxide ($O2^{-}$•), hydroxyl radical (OH•), hydrogen peroxide (H_2O_2), and peroxynitrite ($ONOO^{-}$•), which some are vasodilators and some are vasoconstrictors. Thus, the ROS have a critical impact on both vascular physiology and pathophysiology. The generation of ROS is in a balance between oxidant and antioxidant reactions in ECs, and the imbalance between leads to endothelial dysfunction. Under stress or pathological conditions, the production of ROS increases. ROS mediated vasodilation is through the activation of K^+ channels (Barlow and White, 1998; Ellis and Triggle, 2003). K^+ induced vasodilation is found related to Na^+/K^+-ATPase activation, which increases extracellular K^+ concentration and hyperpolarization of the VSMC (De Mey and Vanhoutte, 1980; Fleming, 1980). Another involved factor is gap junctions, which enables electrical coupling between ECs and VSMC through a bi-directional delivery of vasoactive signals (Emerson and Segal, 2000).

These factors work in a complex but integrated manner to maintain the vascular homeostasis (Emerson and Segal, 2000). Indeed, each factor can interact with and manipulate any components of the synthesis process of other mediators. Basal NO tonically inhibits the effect of EDHFs until the NO diminishes, whereas the response of EDHFs then increases to maintain the absolute vasodilation of the arterial system (Bauersachs et al., 1996; Nishikawa et al., 2000). Similarly, the endothelium-dependent vasodilation induced by PGI_2 is significant only after the NOS is inhibited. This mechanism may also contribute to the basal NO tonic inhibitory effect (Wu et al., 2001).

The endothelium regulates vasomotion not only by releasing vasodilators, but also by secreting vasoconstrictors, such as endothelin-1 (ET-1), constricting prostaglandins, prostaglandin H2 (PGH_2), thromboxane A2 (TXA_2) and 20-hydroxyeicosatetraenoic acid (20-HETE), constricting ROS, and Ang II (Khazaei et al., 2008). ET-1 is a strong vasoconstrictor and produced in the ECs only (Inoue et al., 1989). Many factors including hypoxia, adrenaline, shear stress and ischemia, can stimulate the release of ET-1, which binds to GPCR coupled ET-1 receptors, ET_A and ET_B receptors. The vasoconstrictor ET_A receptors are located on VSMC and vasodilator ET_B receptors are located on ECs (Boulanger and Luscher, 1990; Herrmann and Lerman, 2001). ET-1 binds to G-protein coupled ET_A receptor and induces vasoconstriction through PLC – IP3 – DAG signaling pathway (Kiely et al., 1997; Pernow et al., 1996). Both PGH_2 and TXA_2 are derived from AA that trigger vasoconstriction through inhibited cAMP and/or increase cytosolic Ca^{2+} concentration in the VSMC (Halushka et al., 1989; Smith et al., 1994). PGH_2 is catalyzed by COX-1 and -2 from AA and then is converted into TXA_2 by TX synthase (Stankevicius et al., 2003). 20-HETE is another major metabolite of AA that has a central role in the regulation of pulmonary, renal and cerebral vascular function (Makita et al.,

1996). An increase in cytoplasmic Ca^{2+} activates PLA_2, which catalyzes AA into 20-HETE. Then 20-HETE diffuse from the endothelium to the VSMC and promote vasoconstriction through inhibiting large conductance KCa channels and Ca^{2+} influx (Gebremedhin et al., 2000). Some ROS are vasodilators and/or vasoconstrictors depending on the species, the location, and the concentration, such as H_2O_2 produced by both the VSMC and ECs (Ellis and Triggle, 2003). These vasoconstrictors act on local tissue predominantly, but may also have a systemic impact on blood pressure.

The endothelium is also important for controlling thrombosis and thrombolysis, coagulation, platelet-leukocyte interaction. Under physiological conditions, the ECs provide a non-thrombogenic inner layer to maintain hemostasis of blood fluidity by promoting different anticoagulant and antiplatelet mechanisms. (Stern et al., 1991). Once the endothelium is activated, clot formation, bleeding or thrombosis may occur through the induction of procoagulant and the suppression of anticoagulant mechanisms (Atherton and Born, 1972). After that, the damaged endothelium areas induce platelet adhesion to and leukocyte accumulation, which in turn, leads to extravasation of white blood cells to the sites of inflammation/infection. Then the platelet-leukocyte interaction and aggregation, which lead to vascular occlusion occur in the endothelium finally (Smyth et al., 2009). The endothelium also plays an important role in the regulation of vascular growth, cell proliferation, and angiogenesis. Vascular endothelial growth factors (VEGFs), angiopoietins, and ephrin family have been identified as regulators of endothelial growth. VEGF is required to initiate the formation of the blood vessels by vasculogenesis/angiogenic sprouting. Then the angiopoietins and ephrin are recruited for further remodeling and maturation (Michiels, 2003).

Endothelial dysfunction was originally identified as impaired vasodilation to specific stimuli, such as ACh or bradykinin. Indeed, the dysfunction of the endothelium also involves a proinflammatory and prothrombic state (Endemann and Schiffrin, 2004). In vascular diseases, endothelial dysfunction is defined as an imbalance between vasodilators and vasoconstrictor produced by the endothelium (Deanfield et al., 2005). Endothelial dysfunction has been implicated in resulting from and/or contributing to the pathology of several diseases, including hypertension, hypercholesterolemia, diabetes, septic shock, atherosclerosis, angiogenesis, and stroke. It also results from environmental factors, such as from smoking, air pollution, and aging (Endemann and Schiffrin, 2004).

The dysfunction of the endothelium involves a number of mechanisms. Reduced NO has been reported associated with the impaired endothelial function. NO, one of the most important vasodilators released from the endothelium, inhibits growth, inflammation, and platelets aggregation (Taddei et al., 2003). Decrease in NO under pathological conditions is attributed to several reasons: 1) Reduced activity of eNOS decreases the bioavailability of NO (Koppenol et al., 1992). 2) ROS quenches NO to form peroxynitrite, which is a cytotoxic oxidant affecting protein function in endothelium (Griendling and FitzGerald, 2003). Peroxynitrite also mediates oxidation of low-density lipoprotein (LDL) and degradation of the eNOS cofactor BH_4 (Milstien and Katusic, 1999). Under this situation, the oxygenase function to produce NO is inhibited, which is known as eNOS uncoupling, whereas the reductase function of eNOS is activated so that a lot of more ROS are produced (Landmesser et al., 2003). The consequence of this process is the deleterious effect on endothelial and vascular function. Exaggerated ROS also link to the proinflammatory state of the vessel wall by upregulating adhesion and chemotactic molecules that further decreases NO bioavailability (Landmesser et al., 2003). Moreover, ROS is involved

in mediating endothelial injury through apoptosis and anoikis (detachment of ECs) (Taniyama and Griendling, 2003). 3) A relative new mechanism related to NO reduction and endothelial dysfunction is asymmetric dimethylarginine (ADMA), an endogenous competitive inhibitor of eNOS.

Moreover, it is reported that $ONOO^{-}\cdot$ inactivates the prostacyclin synthase, which inhibits the effect of vasodilator PGI_2 and increases the release of vasoconstrictors, PGH_2, and TXA_2 (Zou et al., 2004). The chronic action of ROS inhibits the activity of KCa channel, which is involved in EDHF-mediated vasodilation (Xiao et al., 2001). Ang II has been noticed to increase ROS level by stimulating NADPH oxidase and promotes vascular inflammation and endothelial dysfunction (Diep et al., 2002; Rajagopalan et al., 1996). In addition to disabled vasodilation, ROS also promotes the contraction of the VSMC through promoting mobilization of Ca^{2+}, increasing the sensibility of the contractile proteins to Ca^{2+} (Suzuki and Ford, 1992), activating endothelial enzymes that can synthesize endothelium-derived contracting factors (Vanhoutte et al., 2005). A significant increase in ET-1 is also found in many cardiovascular diseases, such as hypertension, atherosclerosis, coronary artery disease, and renal failure (Bohm and Pernow, 2007).

Taken together, a reduced bioavailability of NO, an alteration in the generation of prostanoids, and the impairment of endothelium-dependent hyperpolarization, as well as an increased release of ET-1, can individually or in association contribute to endothelial dysfunction.

2.2.4 Disorder of vascular tones in multiple cardiovascular diseases

It is critical to maintaining basal vascular tone and adequate blood pressure under the physiological condition for proper organ function. Low blood pressure (hypotension) leads to

organ failures whereas elevated blood pressure (hypertension) causes cardiovascular diseases, such as hypertension, stroke, and chronic renal failure, which affect about 1 billion population worldwide (Porth et al., 2009). The arterial blood pressure is determined by cardiac output (blood volume pumped by the heart) and the peripheral resistance of the vasculature (vascular tone).

Up to date, there still many unanswered questions related to regulation of vascular tone. One of the most important unsolved problems associated with vascular tone dysfunction is the acute circulatory failure and vascular hyporeactivity in septic shock. Septic shock is persistent hypoperfusion and organ dysfunction in severe sepsis, a whole body infection (Dellinger et al., 2013). Sepsis affects approximately 750,000 people in the US each year (Goyette et al., 2004). Even with international guidelines for the management, septic shock is still the primary cause of death in ICU (Angus et al., 2001). Usually, application of vasoactive drugs, such as catecholamines and vasopressin, is the major approach to intervening low blood pressure and restore hemodynamic stability (Dunser et al., 2003). However, these vasoactive drugs cannot effectively restore the blood pressure in septic shock patients (Hollenberg, 2007). The irreversible hypotension in septic shock, therefore, leads to hypoperfusion to major organs, multiple organ failures, and death. Therefore, it is urgently needed to find out the underlying mechanisms and effective management for the hyporeactive vascular tone in septic shock.

Another mysterious mechanism related to vascular function is the function of the endothelium. Although a plenty of studies have done related to the mechanisms of endothelium under health and disease in multiple species and arteries, whether the change of membrane potential regulates the release of the vasoactive substances from the ECs remains elusive. Most cells are excitable, such as the VSMCs, neurons, and endocrine cells, which secrete hormones

and transmitters via depolarization and firing activity (Hill-Eubanks et al., 2011; Lowe et al., 1988; Ozawa and Sand, 1986). It is known that the membrane potential can affect a number of ion channels, transporters, intracellular Ca^{2+}, intracellular pH, etc. The activity of these molecules, in turn, change cellular functions. However, the ECs are non-excitable. They release the endothelium-derived vasoactive substances while cytosolic Ca^{2+} concentration increases. But the primary function of the ECs in response to direct depolarization remains unknown.

To solve these unknown questions, innovative approaches are needed so that we can reveal the underlying mechanisms without interference from existing barriers, including irreversible hyporeactivity of blood vessels and hypotension in septic shock and non-excitability and inaccessibility of the ECs that located on the lumen side of blood vessels.

2.2.5 Intervention to vasculature: from tradition to optogenetics

The typical research approaches to study the vascular function includes vascular tension measurement of different arteries and regular molecular biology analysis, such as western blot, PCR, and real-time PCR. To dissect the potential signaling pathways, agonists and antagonists of receptors, inhibitors of enzymes and second messengers are commonly employed and combined with traditional approaches. The most classic and widely used approach is isolated tissue bath system, which has over 150-year history in academic research. The therapies formed based on this approach have treated millions of people with cardiovascular diseases, such as hypertension, heart failure, diabetes, GI diseases, and so on (Jespersen et al., 2015). The advantage of the method includes follows: 1) The experiments can be performed in almost all kinds of acutely dissociated and cultured tissues and organs. Under these situations, the tissues are still living and functioning, either contraction or relaxation, that is relevant to the body response. 2) Underlying cellular and molecular mechanisms, including drug-receptor interaction, signaling pathways,

second messenger activation, the VSMC excitability, and the change in tissue function, can be evaluated and dissected with different agonists and antagonists in dose-dependent manner. The agonists or antagonists can be combined and applied to the tissue for revealing the signaling pathway and/or potential treatment in vitro. The advantage is the integration of all these steps, whereas other techniques may only allow each of these steps (Kenakin, 1984). Indeed, this is a pharmaceutical research approach that allows researchers to look up the effective drugs within an active dose range. Besides, the retaining tissue function permits calculation of important pharmacological variability that is more meaningful in a tissue versus a cellular setting (Jespersen et al., 2015). 3) Technically, this method is real time so that the researcher can observe the experiments and the results at the same time. Then the researcher can make the decision for next steps and/or troubleshoot during experiments rapidly. 4) A multiple-baths system allows measurement on target tissues from both experimental and control animal at the same time. Likewise, one drug can be tested on multiple tissues in one day. Besides, the experimental design can be extensively modified by introducing of external stimuli in addition to drugs, such as electrode stimulation on innervating nerves of the tissue (Heppner et al., 2009), thermal (Fitch and Yasunobu, 1975) or pH regulation (Hyvelin et al., 2004) in the water bath, and deprivation on certain extracellular ions (K^+ or Ca^{2+}). Moreover, the tissues after the test can be collected and rapidly freeze during a response for further signal transduction studies using western blot.

However, as every coin has two sides, the traditional approach has certain weaknesses that may contribute to the unsolved questions about the regulation of vascular tones. One of the most significant bottleneck problems is the accessibility to endothelium bypassing the second signaling pathway. The evaluation of the endothelial function with isolated tissue bath system is

the application of ACh, which erects a concentration-related relaxation through the M receptor in ring segments pre-contracted with NE. This vasodilation is abolished by endothelium denudation. Besides, the application of exogenous blockers and/or activators in different concentrations is very likely to interfere the primary vascular tone. Moreover, the dissection of blood vessels out of different organs in different species, especially with small size, could be a challenge. The tissues including the VSMC and ECs may experience certain levels of damage during surgical dissection, or placement of rings on setup hooks. Furthermore, some drugs, such as Ang II, cause receptor desensitization, which could mask the real effect from tissues (Thomas et al., 1996).

Therefore, an innovative research approach that can overcome these problems is needed. One of the most promising research tools is optogenetics, which has been applied in neuroscience widely in recent years (Boyden et al., 2005; Fenno et al., 2011). Generally, optogenetics is a combination of genetics, electrophysiology, and optics to control well-defined events within specific cells of living tissues by expressing an opsin protein in the target cells/tissues. By using the corresponding wavelength light stimulation on the target cells/tissues, this approach allows researchers to control membrane potentials and excitability of the opsin-expressing cells, which has been achieved in various types of neurons (Williams and Deisseroth, 2013), glia (Figueiredo et al., 2011), myocardium (Arrenberg et al., 2010), skeletal muscles (Sakar et al., 2012). This innovative research tool with high temporal and spatial resolution to access the target cells/tissues while ruling out the internal and external interferences, including activation of tissue nearby and associated second signaling pathways.

The control on spatial precision is achieved by optogenetic actuators, light-sensitive proteins, which contain three different types, channelrhodopsin (ChR), halorhodopsin (NpHR),

and archaerhodopsin (Arch) (Warden et al., 2014). The millisecond-scale temporal precision is determined by optogenetic sensors of Ca^{2+}, chloride, or member voltage (Boyden et al., 2005).

ChRs are endogenous sensory photoreceptors in unicellular green algae controlling movement in response to light. They function as light-gated ion channels. Expression of ChRs in cells of other organisms enables light to control electrical excitability, intracellular acidity, Ca^{2+} influx, and other cellular processes (Boyden et al., 2005). Most types of ChRs are nonspecific cation channels that conduct H^+, Na^+, K^+, Ca^2 and lead to cell depolarization extremely fast and robust. Structurally, ChRs contain a seven-transmembrane proteins, which is GPCR, with the light-isomerizable chromophore all-*trans*-retinal covalently lined to the rest of the protein (Nagel et al., 2002; Nagel et al., 2003). After absorbing blue light with a wavelength at ~470 nm, the intracellular all-*trans*-retinal complex absorbs a photon, which induces a conformational change from all-trans to 13-cis-retinal. This process induces pore opening of the transmembrane protein so that cation influx (Nagel et al., 2003). Each type of ChRs has unique intrinsic membrane properties, which have a significant impact on light stimulation, including channel conductance, ion selectivity, kinetics (opening and closing rates of ChR), desensitization and recovery, light sensitivity, spectral response and membrane trafficking and expression level (Lin, 2011). Channelrhodopsin-2 (ChR2), a member of ChRs, was the first opsin used to control neural activities and demonstrated by several groups around the same time (Boyden, 2011; Boyden et al., 2005). ChR2 works in a comparatively narrow wavelength range that is maximally excited by 470 nm blue light. It induces a rapid on-rate and moderate channel closing rate. The main deficiency of ChR2 is the high level of desensitization, which reduces the current by about 80% at physiological pH. This desensitization recovers in 25 s in the dark completely. (Ishizuka et al., 2006; Lin et al., 2009; Nagel et al., 2003). So far, the ChR2 is still the most widely used in

optogentic studies. Further development of opsin protein is concentrating on the magnitude and kinetics of photocurrent for more rapid, accurate, and efficient control.

Similarly, NpHRs are light-gated chloride channels, found in archaea originally (Lanyi et al., 1990). NpHRs share similar sequence to ChRs that also has a seven transmembrane protein of retinylidene protein (Zhang et al., 2011a). In contrast to ChRs, NpHRs silence excitable cells with brief pulses of yellow light. After stimulation with yellow light at ~580 nm wavelength, NpHRs move chloride ions into the cytoplasm causing cell hyperpolarization. Therefore, ChRs and NpHRs together create a powerful toolbox, which enables multiple color optical activation, silencing, and desynchronization of cellular activity (Han and Boyden, 2007). Another optogenetic tool is Archs, which respond to ~525 nm yellow light stimulation and are commonly used for neuronal inhibition by pumping H^+ out of target cells (Chow et al., 2010).

The action spectrum for each type of opsin is broad. The blue light activating ChRs has a peak activation wavelength of 470 nm, but the wavelength of 430-520 nm works with over 50% efficacy. The yellow light activating NpHRs and green light activating Archs work at the wavelength of 500-600 nm with over 50% efficacy (Chow et al., 2010; Han and Boyden, 2007; Nagel et al., 2003). This characteristic enables optogenetic control of larger target tissue volume. The magnitude of photocurrent depends on the speed of ion flux through a single opsin proteins and the expression level of the opsin proteins in the membrane. The ChRs mediate larger photocurrent using photonic energy to gate the channel opening, whereas the NpHRs and Archs transport ions through photon absorption.

There are two choices of the light source, laser and LED light source, with their advantages and drawbacks according to the requirements of the experimental design (Warden et al., 2014). Diode lasers and diode-pumped solid state (DPSS) lasers are more commonly used in

neuroscience research field for the last decade. The main advantage of lasers is the intrinsically highly collimated beam, which can focus to a very small point and provide with high efficient coupling to very thin optical fibers (< 50 μm in diameter). Lasers also have a narrow spectral width, which can activate opsin protein specifically (Britt et al., 2012). However, some disadvantages of laser light source may handicap their application in optogenetic studies including 1) Lasers are costly, fragile and need high maintenance (Warden et al., 2014). 2) They introduces noise because of simultaneous emission at close wavelengths. 3) Most DPSS lasers do not have the well-defined temporal controller, which is critical for optogenetic studies. 4) Lasers have random spatial intensity variation at illumination site, which is called speckle pattern that introduce noise in response to even minor changes in temperature and/or current in the lasers or at fiber banding. 5) The collimation beam can be dangerous and cause eye and skin injury. 6) The focused collimated beam can be a disadvantage if they are applied to wide field microscopy (Hertsens, 2005).

The LED illumination system provide with many advantages without most of above drawbacks of lasers, including 1) LEDs illumination is homogeneous and stable. 2) Most LEDs current drivers come with a microseconds delay when switch on/off, which is much faster than millisecond pulses required for the optogenetic application. 3) LEDs illumination intensity can be controlled manually. 4) LEDs are comparatively cheaper, smaller, and reliable. The main drawback of this light source is that the coupling of the light source with fiber optic cable always loses efficiency. The coupling efficiency of the LED light sources is less than 10% comparing to the 50% of the laser light source (Warden et al., 2014). Taken together, increasing coupling efficiency could lead to that the LED light sources become the most optimal light source for

optogenetic studies. Thus, it is necessary to evaluate the above characteristics based on the experimental design before making the final decision on which type of light source is needed.

In addition to the light source itself, the light intensity, stimulation duration, optic fiber type and diameter, and target tissue depth and texture are also important to perform the optogenetic studies. Light intensity (mW/mm^2) is the light power from an optic fiber at the surface of the tissue. It decreases with tissue depth (Aravanis et al., 2007). A safe range for *in vivo* experiments on neurons is up to ~75 mW/mm^2 for short pulses at ~0.5-50 ms (significant tissue damage in the cortex was observed with sustained stimulation > 500 ms duration at intensity > 100 mW/mm^2) (Cardin et al., 2010). Besides, it is necessary to consider the duration and strength of the light pulse in the CNS as a continuous light application can cause abnormal levels of neural activity and excitotoxicity, which may lead to local tissue damage during an experiment (Cardin et al., 2010). But in peripheral system, especially in the vasculature, it is possible to apply light stimulation continuously because of the different characteristic of the VSMC and ECs (Wu et al., 2015; Zhang et al., 2015). In addition, the power transmitted by the optic fiber varies with the different type of fibers, such as multimode or single-mode. The spread of light from the optic fiber is partially determined by the numerical aperture. The low numerical aperture corresponds to a narrow angle light spread and vice versa. The radius (r) of the optic fiber also has an impact on the power delivered to the tissue as the area of the initial cross-section of the light at the tip of the fiber increases as the distance between the light fiber and the tissue becomes further (Cardin et al., 2010). The potential impact of light stimulation may come from blood and device. Current opsins operate at the visible light wavelength range from 450 nm to 600 nm, where the hemoglobin, oxygenated (HbO_2) and deoxygenated (Hb) in blood, are the major light absorbers as well (Han, 2012). Furthermore, various tissue damages can be attributed

to optic fiber insertion procedure and heat produced from the device. Minimize devices size can reduce mechanical tissue damage. Heat generated by the device can introduce tissue damage although it remains difficult to evaluate the exact damage from heat. Most optogenetic studies used light intensity up to ~300 mW/mm^2, or a few mW of total light intensity *in vivo* without noticing heat damage (Han, 2012).

2.2.6 Animal models and transgenic mice

Transgenic mice with high-level expression of cellular markers in specific tissues is a versatile and powerful platform for the application of optogenetic approaches. Several techniques have been widely employed to generate transgenic animal models, including breeding, viral based gene delivery, and non-viral based gene delivery approaches.

Cre-loxP recombination system has been demonstrated effective for conditional expression of optogenetic probes from a defined genomic locus (Madisen et al., 2012; Zeng and Madisen, 2012). Two 34 base pair loxP recognition sequences flanking genes of interest are catalyzed by Cre enzyme, which is used to restrict the expression of genes in target cells. This technology can be applied to carry out deletions, insertion, translocation, and inversions at specific sites in the DNA of cells depending on the location and orientation of loxP sequences (Zheng et al., 2000).

To generate desired transgenic mice, crossing cell-specific Cre expressed mice with opsin expressed downstream of loxP-flanked (floxed) stop cassette mice is widely utilized to date (Madisen et al., 2012). The cell specificity is achieved by the availability of cell-type specific Cre drive lines either commercially and/or academically. The Cre-responsive allele driven by a cell-specific strong and ubiquitous promoter is inserted into a modified *Gt(ROSA)26Sor* locus, which is a ubiquitously expressed locus that can be targeted easily (Irion et al., 2007). We used

this system to generate transgenic mice with optic accessibility to either the VSMC or ECs specifically. The advantage of this method to get transgenic mice is 1) Most of the breeding pairs are available commercially from companies, such as Jackson Laboratory, Allen Brain Institute, and so on. 2) The expression of ChR2 lasts lifelong in transgenic mice. 3) The expression level can be controlled maintained by manipulating parental zygosity. 4) The transgene can be visualized with fluorescence reporter, such as eYFP.

Another way to express optogenetic probe is virus injection. In genetically intractable species, the most commonly used technology is virus transduction. The viral injection introduced the expression of ChR2 usually lasts for couple month maximally. But the time to express light sensitive opsin in mice can be controlled by researchers. Among all of the virus vectors, Lentivirus and adeno-associated virus (AAV) are widely engineered with excellent transduction efficiency and little/no toxicity (Atasoy et al., 2008). However, there are drawbacks associated with intrinsic stability of the viral particles that cannot be overcome easily: 1) Packing of viral particles is increasing difficult with larger DNAs. 2) The injected virus could be dangerous to the host DNA without persistent expression. 3) The injection may not cover all of the required neurons within the target region (Madisen et al., 2012).

Nowadays, more and more nonviral methods have been developed for gene delivery, such as using cationic lipid, cationic polymer, nanoparticles, carbon nanotubes, gene guns, and calcium phosphate nanoparticles (Bhakta et al., 2005; Boussif et al., 1995; Felgner et al., 1987; Kam et al., 2005; Kneuer et al., 2000; Nishikawa and Huang, 2001; Roy et al., 2003; Roy et al., 2005; Salem et al., 2003). Although these methods have low transduction efficiency *in vivo* in general, a potential advantage is that this method can introduce larger DNAs into cells and enable targeting specific cells.

2.3 Regulation of cardiac function in ANS

2.3.1 Anatomy and physiology of the Cardiac Muscle

The heart pumps blood to the whole body through the circulatory system. Blood provides the body with O_2 and nutrients and assists in the removal of metabolic waste. The coronary circulation is the driving force of the heart.

The heart wall contains three layers, epicardium, myocardium, and endocardium. The innermost layer of the heart wall endocardium is made of a lining of ECs and connective tissues that cover the heart chambers and valves. The endocardium joins to the middle layer myocardium through a layer of connective tissue. The major component of the myocardium is cardiac muscles, which are involuntary striated muscles that produce autorhythmicity of the heart beating. This unique capability is the driving force initiating cardiac action potentials at a certain frequency. The cardiac action potentials spread rapidly to trigger the contraction of the entire heart. The myocardium is supplied with blood vessels and nerve fibers those are involved in the modulation of the autorhythmicity by the ANS and endocrine hormones. The outermost layer of the heart wall is pericardium that is made of an inner serous membrane (epicardium) and an outer fibrous membrane. These two layers of membranes enclose the pericardial cavity with pericardial fluid that lubricates the surface of the heart (Boron and Boulpaep, 2009; Clark, 2005).

As one of three major types of muscle, the others being skeletal and smooth muscle, cardiac muscle is composed of cardiomyocytes (also known as myocardiocytes or cardiac myocytes), which have striations similar to those in skeletal muscle cells, whereas they contain only one nucleus (Olivetti et al., 1996), which is different from multinucleated skeletal muscle cells (Okazaki and Holtzer, 1966). Cardiomyocytes contain high mitochondria content allowing them to produce ATP quickly and highly resistant to fatigue (McBride et al., 2006), and

specialized organelle myofibrils that consist of long chains of sarcomeres, the fundamental contractile units of muscle cells (Severs, 2000). There are two types of cardiac muscle cells: cardiomyocytes and modified cardiomyocytes, the pacemaker cells of the conducting system. The major component of atrium and ventricles are cardiomyocytes that are contractile while the pacemaker cells make up about 1% of the cardiac muscle. The pacemaker cells are less contractive and smaller than the cardiomyocytes because they contain fewer myofibrils. They distribute throughout the heart and are responsible for heart beating, including spontaneously generating and sending out electrical impulses, transferring electrical impulses from cell to cell, and receiving and responding to electrical impulses from the brain. (Arrigo and Avanzino, 1979). Indeed, cardiac pacemaker cells function the way more similar to neurons. All of the cardiomyocytes are connected by intercalated discs, a porous junction between cells allowing quick delivery of action potentials from the pacemakers. The intercalated discs enable Na^+, K^+, and Ca^{2+} to diffuse from cell to cell easily so that the depolarization and repolarization are easy in the myocardium (Pocock and Richards, 2006).

2.3.2 Cardiac function and the neural control

Cardiac Output is the volume of blood pumped by the heart per minute (ml/min). The cardiac output can be evaluated by the heart rate (beats/min) and stroke volume (ml/beat). Heart rate is mainly determined by the pacemaker SA node, and the automaticity of the SA node is determined by spontaneous changes in cationic conductance, including Ca^{2+}, Na^+, and K^+ (Irisawa et al., 1993). This intrinsic automaticity is regulated by neural and humoral factors. Neural mechanism primarily involves the sympathetic adrenergic and parasympathetic cholinergic branches of the ANS. Activation of the parasympathetic system decreases heart rate. The increase in heart rate can be attributed to withdrawal of vagal tone and/or activation of

sympathetic nerves innervating SA node. The humoral factors include circulating catecholamines that act on β1 adrenoceptors located on SA nodes, renin-angiotensin system, circulating thyroxin, vasopressin, atrial natriuretic peptide, ET-1, and changes in core body temperature. The stroke volume is the volume of blood pumped out of the heart with each beat (Mangoni and Nargeot, 2008).

The stroke volume is regulated by ventricular contractility, end-diastolic volume (preload), and afterload (Bishop et al., 1976). Ventricular contractility determines the force of blood ejection and the stroke volume. Sympathetic activation increase ventricular contractility, which in turn, enhance the stroke volume, whereas the parasympathetic stimulation has no significant impact on stroke volume (Pocock and Richards, 2006). Preload/end-diastolic volume is the volume of a ventricle at the very end of filling and right before systole begins. Preload is variable because the ventricles are flexible and the blood flowing into changes when ventricles relax. Less blood flowing into the ventricles leads to reduced end-diastolic volume and vice versa. Preload is the most concerned affecting stroke volume through the Frank-Starling mechanism. The Frank-Starling mechanism refers to that the cardiomyocytes respond to stretch by contracting more forcefully because of increased expenditure of ATP energy. Therefore, an increase in preload enhances cardiac muscle stretching force and stroke volume (Pocock and Richards, 2006). Afterload is also known as aortic pressure during ejection that directly influences the blood volume flowing into the aorta during systole. If the aortic pressure increases, then the blood volume that flows into the aorta during systole reduces (Pocock and Richards, 2006).

The pacemakers located in the SA node of the conduction system initiates action potentials on its own, forming the basal rhythm of the heart. Then the action potentials are

conducted from the SA node to AV node. The conduction of impulses slows at the AV node allowing sufficient time for atrial depolarization and contraction. The impulses then enter the base of ventricle through the bundle of His, which contain the left and right branches along the interventricular septum. The branches divide into an extensive Purkinje fibers that continually conduct the impulses throughout the ventricles resulting in rapid depolarization of both ventricles. This conducting system within the heart is important for rapid and organized depolarization of ventricles, which indeed, to generate systolic pressure efficiently. The conducing system is regulated by ANS through AV node mostly (Pocock and Richards, 2006). Sympathetic activation increase conduction velocity of the AV node by increasing the rate of depolarization of action potentials. Adjacent cells are affected by this faster depolarization, leading to a faster conduction of impulses. Then the conducting time between atria and ventricles is reduced. In contrast, parasympathetic activation decreases the slope of AV nodal action potentials, which reduces the velocity of conduction throughout the heart (Gordan et al., 2015).

The ANS regulate cardiac function spontaneously in response to the signals from sensory receptors located in the peripheral system. These sensory receptors monitor physiological function and generate actions potentials in response to stimuli, including mechanical, chemical and thermal changes. The sensory receptors respond to stretching of the blood vessel wall, or cardiac chambers are called baroreceptors, which are located in the heart and major blood vessels. The other type of sensory receptors in the peripheral system is chemoreceptors, which are small, highly specialized and are primarily located at the bifurcation of the common carotid arteries and aorta. The chemoreceptors directly respond to the change of partial pressure of O_2 and CO_2 in the blood (Pocock and Richards, 2006). Both sensory receptors convey their status via afferent systems to the NTS, which relays the information to other brain regions that

coordinate the activity of efferent motor pathways targeting on cardiac muscles, smooth muscles, and other tissues (Critchley and Harrison, 2013).

The sympathetic fibers release NE, which binds to β adrenergic receptors located in the heart, and activates GPCR and downstream AC – cAMP – PKA signaling pathway. There are two types of β adrenergic receptors involved in sympathetic innervation in the heart. β_1 receptors couple to G_s-protein and activate cAMP primarily, whereas β_2 receptors couple to both G_s and G_i-proteins. Collectively, the activation of β receptors in the heart increases the slope of diastolic depolarization in the SA node and the conduction in the AV node, which leads to increased heart rate. Activation of β receptors also increases intracellular Ca^{2+} concentration through membrane Ca^{2+} channels and Ca^{2+} releasing from the SR, resulting in increased force of contraction. In addition, associated Ca^{2+} reuptake into the SR accelerates relaxation. Taken together, sympathetic effects on cardiac contractibility, heart rate, and impulse conduction result in larger stroke volume (Triposkiadis et al., 2009). The parasympathetic stimulation causes ACh release, which activates M_2 muscarinic receptors, then K^+ conductance increase of nodal cells. Then the intrinsic heart rate decreases. Given the ability to regulate both cardiac rate and stroke volume, ANS remotely modulate cardiac function to meet a short-term change in the body's needs (Harvey and Belevych, 2003).

For example, when blood pressure rises, baroreceptors are activated and sending corresponding signals to the brain control center. The sympathetic stimulation is inhibited. In parallel, the activity of the parasympathetic system, including vagus and the NA that influence heart rate, is stimulated. As a result, the stimulator noradrenergic effects of sympathetic innervation to the cardiac pacemaker and cardiac muscle is reduced, whereas the cholinergic parasympathetic innervation to the heart is activated. This process inhibits the pacemakers

discharge rate and slows down the ventricular conduction system. Finally, the heart rate and effectiveness of the myocardial contraction reduce, the peripheral arterioles dilate, and the blood pressure decreases. When hypoxia or hypercapnia occurs, a lower level O_2 or a higher level CO_2 will be detected by chemoreceptors. In response to the increased firing frequency from peripheral chemoreceptors, the central control enhances sympathetic stimulation to arterioles and veins so that the blood vessel contract and the blood pressure increases. Indeed, the chemoreceptors simultaneously stimulate central respiratory control centers to increase O_2 supply and reduce the CO_2 level.

Taken together, the neural control of the heart has a profound influence to maintain the homeostasis. The regulation on the heart rate and conduction velocity are performed with the innervation to the SA and AV nodes by both parasympathetic and sympathetic fibers while the contractility and relaxation are mainly mediated by sympathetic fibers innervating atrial and ventricular myocytes.

2.3.3 Coronary circulation and regulation

While providing the blood to the whole system, the cardiomyocytes need to be supplied with O_2 and nutrients, and a way of removing metabolic waste. This is achieved by the coronary circulation. The major vessels of the coronary circulation are the right main coronary artery and the left main coronary, which divide into left circumflex and anterior descending branches. Both left and right coronary arteries originate from aortic root right above the aortic valve (called the coronary ostia). The two coronary arteries and their branches lie on the surface of the heart (also known as epicardial coronary vessels). As in all vascular beds, the arteries and arterioles branch into numerous capillaries that lie adjacent to the cardiac myocytes. The large coronary arteries conduct blood with little resistance, whereas the small coronary arteries are the principle

resistance vessels of the heart that change their diameter to regulate the coronary blood flow (Pocock and Richards, 2006).

Factors that have an impact on coronary circulation include mechanical, metabolic reflex, and neural effects (Rubio and Berne, 1975). Mechanical influence is passive that is mostly determined by the myocardial extravascular compression. The metabolic factors affect coronary flow include lactic acid, adenosine, O_2, CO_2, K^+, and a variety of endothelium-derived vasoactive molecules in cardiac muscles (Feigl, 1983). Hormonal factors mainly involve 5-HT, vasopressin, and bradykinin. 5-HT has a different effect on the coronary circulation. It increases coronary blood flow through vasoconstriction effect in coronary arteries and vasodilation in coronary arterioles. Similar, vasopressin promote a different response in the coronary system, but collectively it induces coronary vasoconstriction (Tune, 2014). Bradykinin induces significant vasodilation of coronary arterioles through multiple signaling pathways (Groves et al., 1995). The reflex control contains carotid sinus reflex that promotes coronary vasodilation through inhibiting parasympathetic discharge and activating sympathetic nerves in response to the decrease of blood pressure. Carotid body chemoreceptor reflex induces coronary vasodilation through activating vagal parasympathetic nerves in response to hypoxia and hypercapnia (Feigl, 1983).

The neural factors affect coronary flow through direct and/or indirect ways. The direct effect is moderate involving both sympathetic and parasympathetic divisions. The sympathetic stimulation acts on both α and β receptors expressed in the coronary vessels causing slight vasoconstriction. The distribution of parasympathetic vagus nerves is slight in coronary vessels so that the innervation triggered vasodilation is mild as well. In contrast, indirect effect plays a far more important role in the regulation of coronary flow through an effect on extravascular

compression. The sympathetic innervation increases both heart rate and myocardial contractility, which in turn, positively increase coronary flow. Increased heart rate and myocardial contractility also result in high metabolism and more metabolites that can dilate coronary vessels and increase coronary flow (Tune, 2014).

2.3.4 Disorder and intervention of the cardiac function

According to WHO and the CDC reports, heart disease is the leading cause of death in the western societies. Over 11% of the US adults are diagnosed with heart disease and about 25% of all death in the US nowadays are caused by heart disease (CDC, 2013). Since the heart is one of the most important and complex organs, dysfunction of the heart lead to multiple and lethal diseases, such as heart attack, heart failure, arrhythmias, atherosclerosis, atrial fibrillation, cardiomyopathy, congenital heart defects, etc.

The research approaches to study the cardiac function are quite similar to that of vasomotor, including molecular biology, electrophysiology, and heart perfusion. Otherwise, the innovative application of optogenetics is way earlier in the heart since 2010. Bruegmann et al. generated ChR2 expressed cardiomyocytes *in vitro* with optical pacing and transgenic mice with ChR2 expression in cardiac muscles (Bruegmann et al., 2010). At the same year, another group led by Dr. Emilia Entcheva at Stony Brook University in New York developed a non-viral cell delivery system, tandem cell unit, for expression of rhodopsin channels *in vitro* (Jia et al., 2011). These innovative development in the study of heart diseases has many advantages over the traditional approaches, including 1) application in both *in vitro* and *in vivo* systems; 2) stimulating and silencing cardiomyocytes with different rhodopsin protein expression but the interference from exogenous drugs; 3) feasibility of micro-manipulation in coronary circulation

system with optogenetics; 4) highly precision on spatial and temporal control over cardiomyocytes.

2.4 Regulation of respiration

2.4.1 Anatomy and Physiology of Respiratory System

In addition to regulation of vascular tone and cardiac function, ANS also plays a critical role in the respiratory system to maintain levels of PO_2 and PCO_2 in the arterial blood through both peripheral and central chemoreceptors. As important as heart beat and vasomotor, the respiration is crucial to every human being. The major function of the respiratory system is to provide adequate O_2 into the blood stream and take CO_2 out of the body. The structure of respiratory system contains upper respiratory tract and lower respiratory tract. The upper respiratory tract involves nostrils, nasal cavities, pharynx, epiglottis, and larynx while the lower respiratory tract includes trachea, bronchi, bronchioles, and lungs (Pocock and Richards, 2006).

Homeostasis is maintained by the respiratory system in two ways, gas exchange and regulation of blood pH. Gas exchange includes external and internal respiration along partial pressure gradients. External respiration is the exchange of O_2 and CO_2 occurring between external environment/atmosphere and capillaries in the lungs. This is efficient because of bulk exchange between alveoli and capillaries. Internal respiration is the intracellular use of O_2 to make ATP. It provides the body with O_2 for further cellular respiration while eliminates CO_2. Maintaining the acid/base balance of the blood is critical for the system to survive as well. Normal blood pH ranges from 7.38 to 7.42. For human beings, if the blood pH drops below 7.2 or increase above 7.4, then the brain would cease functioning normally. Blood pH below 6.9 or above 7.9 for a short period would be fatal.

The body copes with the change of blood pH through the buffer, respiratory, and renal systems. The primary defense for blood pH is the buffers that are molecules that take in or release ions, in turn, to maintain the H^+ concentration at a certain level (Adrogue and Adrogue, 2001). There are two types of buffers inside and outside of cells. The extracellular buffers contain bicarbonate (HCO_3^-) and ammonia while the intracellular buffers are proteins and phosphate. Among all of these buffers, the most important buffer is the bicarbonate system (a mixture of bicarbonate and CO_2). The CO_2 forms carbonic acid (H_2CO_3), which can be dissolved in water and acts as acid giving away H^+ when needed (Garrett et al., 2013), such as in alkalosis, which is an excess of base. HCO_3^- is a base that can take in H^+ when blood pH is too low (Garrett et al., 2013), such as acidosis, which is an excess of acid or the CO_2 concentration is high in the blood. Protein molecules contain basic and acidic groups and can act as H^+ acceptors or donors, respectively. The phosphate ($H_2PO_4^-$ and HPO_4^{2-}) buffer is an important intracellular buffer that moderately efficient at physiological pH level (Garrett et al., 2013).

The acid-base imbalance that overcomes the buffer system can be compensated rapidly and effectively by the ventilation rate, which is the second defense. The change of the ventilation rate indeed alters the CO_2 concentration in the blood, which in turn, contribute to the blood pH. The renal system as the third defense to compensate the change of blood pH has a slower effect through excretion of excess acid and reabsorb of filtered bicarbonate (Adrogue and Adrogue, 2001).

2.4.2 Regulation of Respiration

The respiratory control system consists of three basic elements: a central controller, sensors, and effectors. The central controller includes brainstem and other parts of the brain, such as cortex. The efferent nerves from respiratory centers descend and terminate in the motor

neurons located in the cervical and thoracic segment of spinal cord. Two sets of nerve fiber arise from the motor neurons and innervate effectors. The effectors are respiratory muscles including the diaphragm, intercostal muscles, and accessory muscles, which receive efferent signals from central control centers. The sensors gather information and send afferent input to the respiratory centers. The sensors include peripheral and central chemoreceptors and pulmonary receptors. Afferent signals are transmitted through vagus and glossopharyngeal nerve to respiratory center in the brainstem (West and West, 2012).

The respiratory center is composed of several groups of neurons located in the medulla oblongata and pons of the brainstem. The dorsal respiratory groups (DRG) diffusely located in the dorsal medulla, which generates inspiratory ramp by the virtue of their autorhythmic property. They are mainly composed of NTS. The ventral respiratory groups located in the ventrolateral medulla control expiration. Another breathing center located in the ventrolateral medulla is Pre-Bötzinger complex (pre-BötC), which is formed by a groups of pacemaker neurons generating the rhythmic respiratory impulses. These groups of neurons compose the intrinsic periodic firing rate controlling the respiratory rhythm.

The apneustic center located in the reticular formation of lower pons controls the depth of inspiration by sending excitatory impulses into the DRG. The pneumotaxic center, also known as the pontine respiratory group (PRG), located in the dorsolateral part of the reticular formation in upper pons inhibits inspiration ramp and thus the inspiration duration and volume and, secondarily respiratory rate. The PRG is composed of Kölliker-Fuse nucleus and medial parabrachial nucleus. The expiration is passive response resulted from the diaphragm and intercostal muscles relaxation and elastic recoil of the lungs under physiological condition. The

ventrolateral medulla respiratory center is an overdrive center for high-level pulmonary ventilation, such as heavy exercise (West and West, 2012).

The breathing activity is controlled by both central and local manners. The central regulation consists of the involuntary and voluntary controls (Gallego et al., 2001). Involuntary control affecting respiratory centers in brainstem include 1) chemoreceptor reflexes; 2) impulses from stretch receptors of lungs; 3) impulses from Juxtacapillary (J) receptors of lungs; 4) impulses from irritant receptors of lungs; 5) impulses from proprioceptors; 6) impulses from thermoreceptors; 7) impulses from pain receptors; 8) cough reflex; 9) sneezing reflex; and 10) deglutition reflex.

Chemoreceptor reflex refers to the central and peripheral chemoreceptors that detect changes in chemical composition of the blood and cerebrospinal fluid. Peripheral chemoreceptors located in the carotid and aortic bodies, as introduced above, are sensitive to the change of partial pressure of O_2 in arterial blood. Hypoxia is the most potent stimulant for peripheral chemoreceptors. When the partial pressure of O_2 decreases, the chemoreceptors are activated and send impulses to both cardiac and inspiratory centers and stimulate them. Then the respiratory rate and force increase to compensate the lack of O_2. In addition to hypoxia, peripheral chemoreceptors also mildly respond to hypercapnia and increased H^+ concentration in the blood (Kumar and Prabhakar, 2012).

Central chemoreceptors act slowly but effectively. It has been reported that the central chemosensitive neurons are found in many brain regions including the ventral surface and midline of the medulla and dorsal areas of the brainstem. The ventral chemosensitive groups include the retrogtrapezoid nucleus (RTN), parapyramidal region of raphe nuclei, and ventral respiratory group. Dorsal chemosensitive groups contain NTS (Nattie and Li, 2002), LC

(Biancardi et al., 2008; Coates et al., 1993), and area postrema (AP) (Belegu et al., 1999). Norepinepherinergic neurons (NE-ergic) in the LC are inherently CO_2 sensitive, which will be further discussed below. Midline raphe nuclei in the medulla are also well accepted chemosensitive neurons (Bernard et al., 1996; Nattie and Li, 2001). These central chemoreceptors have several specific characteristics, 1) These neurons exhibit c-fos expression when whole-body animals are exposed to hypercapnia, which is a condition that excessive CO_2 level in the blood because of inadequate respiration (Okada et al., 2002; Sato et al., 1992). 2) The neuronal activities, including firing rate and membrane potential, can be stimulated or inhibited by the change of CO_2 or pH levels *in vitro* (Filosa et al., 2002; Okada et al., 2002). 3) Focal acidification *in vivo* increases ventilation (Bernard et al., 1996; Nattie and Li, 1996).

Different from peripheral chemoreceptors, central chemoreceptors are exquisitely sensitive to the concentration of H^+ of nearby cerebral spinal fluid that cannot cross the blood-brain barrier (BBB). Increased H^+ in the blood cannot stimulate the central chemoreceptors directly. But when CO_2 concentration is high in the blood stream, it crosses the BBB easily and enters the interstitial fluid or the cerebrospinal fluid of the brain. CO_2 combines with H_2O forming carbonic acid, which is unstable and immediately dissociates into H^+ and HCO_3^-. The increased H^+ then stimulates central chemoreceptors, which subsequently, send impulses into DRG to increase respiratory rate and force. This process washes out excess CO_2 and brings respiration back to normal (Nattie, 2006).

The sensing mechanism of these central chemoreceptors is achieved through CO_2/pH sensing molecules located in one or more of these receptors. These molecules can be stimulated by the change of CO_2/pH level, which in turn, affect the membrane excitability of the central chemoreceptors (Bayliss and Barrett, 2008; Filosa and Putnam, 2003; Jiang et al., 2001; Kersh et

al., 2009). Several identified CO_2/pH sensing molecules include inward rectifier K^+ (Kir) channels, TASK, gap junction channels, Ca^{2+}-activated non-selective cation (CAN) channels, voltage-gated Ca^{2+} channels, transient receptor potential (TRP) channels and proton-activated receptor GPR4. The primary function of the Kir channel is to modulate cellular excitability by inward rectification at voltages near the resting potential in various types of cells (Fang et al., 2005; Seifert et al., 2009). It has reported that Kir channels are found to mediate CO_2-induced neuronal response in the LC neurons and rostroventrolateral medulla (Pineda and Aghajanian, 1997; Schultz et al., 2003). High-level CO_2/pH inhibits channel activities, which in turn, leads to membrane depolarization and higher firing frequency. It remains unknown that which subtype of Kir channel plays the role in chemoreception in CNS, whether the Kir channels are necessary and sufficient for CO_2/pH chemoreception, and why the Kir channels are specifically utilized by certain groups of chemosensitive groups but not others. TWIK-related acid-sensitive K^+ (TASK) channels, TASK-1, and TASK-3, may be responsible for pH sensitivity in dorsal raphé and hypoglossal motor neurons (HNs) (Bayliss et al., 2001; Washburn et al., 2002). Another noticeable sensor is proton-activated receptor (GPR4) expressed in RTN, that senses protons generated by H_2CO_3 in the blood. Genetic knock-out of GRP4 disturbed acidosis-dependent activation of RTN whereas reintroduction of GPR4 into RTN restored RTN neuronal activities and ventilation in mice (Kumar et al., 2015).

When the lungs are overstretched, the stretch receptors expressed in the walls of bronchi transmit signals through the vagus nerves into the DRG to stop excess inspiration. This process is called the *Hering-Breuer inflation reflex*, which protectively prevents excess lung inflation. Besides, J receptor located in the wall of the alveoli that are stimulated by certain conditions like pulmonary edema, pulmonary congestion, pneumonia, and exposure to the exogenous and

endogenous chemicals like histamine and 5-HT. Stimulation of J receptor results in a reflex response apnea. Another type of receptors in the lungs is irritant receptors that also located on the wall of bronchi and bronchioles. They are stimulated by harmful chemicals, such as ammonia and sulfur dioxide and produce a cough and prolonged inspiration along with bronchospasm to prevent harmful chemicals from entering into the alveoli (Perez et al., 2015). Proprioceptors located in joints, muscles, and tendons, are sensitive to the change in the position of different parts of the body. They are stimulated during exercise and sends impulses to the cerebral cortex, which in turn, activate medullary respiratory centers causing hyperventilation (Proske and Gandevia, 2012). Thermoreceptors give a response to the change of body temperature. When they are stimulated, they send signals to the cerebral cortex, which in turn, stimulate the respiratory center leading to hyperventilation (Kozyreva and Simonova, 1998). Pain receptors responding to pain stimuli send impulses to the cerebral cortex, which in turn, stimulate respiratory center inducing hyperventilation (Borgbjerg et al., 1996). Cough reflex is a protective reflex caused by irritation of respiratory tract through the vagus nerve. Sneezing reflex is also a protective reflex occurs due to the irritation of nasal mucus membrane through olfactory receptors and trigeminal nerve endings (Sant'Ambrogio et al., 1995). Deglutition reflex occurs during swallowing of the food. The temporary arrest of the respiration during swallowing is called swallowing apnea or deglutition apnea, which prevents the food particles entering into the respiratory tract (Nishino, 1993).

The voluntary control of breathing originates in the motor cortex of frontal lobe of the cerebrum that sends action potentials along the corticospinal tracts to respiratory neurons in spinal cord bypassing medullary impact (McKay et al., 2003). The voluntary control, however, can be overridden by chemical factors, such as O_2, CO_2, and H^+ level.

Local control of respiration is automatic control independent of the CNS including adjustments of pulmonary blood perfusion and alveolar ventilation (Grant et al., 1976). Lung perfusion is controlled by vasomotor of arterioles supplying lungs areas according to the partial pressure of O_2. When O_2 is insufficient in a region of alveoli, local receptors in the capillaries can detect it, which in turn, induce vasoconstriction of arterioles reducing blood flow to this area and preventing wasted perfusion into poorly oxygenated alveoli. The adjustment of alveolar ventilation is completed by changing the size of bronchioles in response to alveolar PCO_2. For instance, decreased alveolar PCO_2 leads to local bronchodilation so that more air enters into the area allowing O_2 to reach alveoli and facilitating CO_2 removal (Hughes, 1975).

2.4.3 Chemosensitive NE-ergic neurons in the LC

In the CNS, neurotransmitters and neuromodulators act at different levels of the respiratory reflex to influence respiration comprehensively including the magnitude, frequency, duration, and regularity (Doi and Ramirez, 2008). The major neurotransmitters and neuromodulators include several categories: amino acids, peptides, gasotransmitters, purines, and monoamines. Amino acids consist of glutamate, aspartate, D-serine, γ-aminobutyric acid (GABA), and glycine. Peptides include somatostatin, substance P, cocaine, amphetamine, and opioid peptides. Gasotransmitters are NO, carbon monoxide (CO), and H_2S. Purines refer to ATP and adenosine. Monoamines, as one of the most important class of neuromodulators, is derived from aromatic amino acids, including DA, NE, epinephrine, and 5-HT. Among these monoamines, the first three are also known as catecholamine, and the last one is known as indoleamine. NE and adrenaline groups within the brainstem are numbered from caudal to rostral direction, which A1-A7 groups contain NE while C1-C3 are adrenergic groups. A5-A7 groups

are located in the pons and all other groups are located in the medulla (Pocock and Richards, 2006).

The central monoamines have multiple impacts on brain function, especially those related to autonomic regulation, such as respiration (Haxhiu et al., 2001; Zhao and Debski, 2005). Monoaminergic neurons modulate the basal breathing rhythm and chemosensitivity by widespread projection to respiratory nuclei in the brainstem. Notably, some of the monaminergic neurons are chemosensitive themselves, such as NE-ergic neurons in the LC, and 5-HT neurons in the raphé nuclei (Haxhiu et al., 2001).

NE plays an important role in the regulation of breathing activity. Normally, NE stabilizes respiratory network activity (Hilaire et al., 2004). Many studies have demonstrated that NE regulates respiration through modulating respiratory rhythmic generation neurons in the medulla, and the respiratory center in the CNS (Viemari et al., 2004; Viemari et al., 2005). NE exerts excitation and inhibition by acting on different α-adrenoceptors. The A6 LC group contains the majority of the central NE-ergic neuron and is the principle site of NE synthesis in the CNS. Application of α_1 receptor agonist to the LC neurons accelerates breathing frequency in brainstem-spinal cord preparations *in vitro*, whereas the application of α_1 receptor antagonist, prazosin, to A5 region in the pons seems to inhibit breathing activity (Hilaire et al., 2004). NE modulates respiratory rhythmic activity in both the preBötC (Viemari et al., 2013; Viemari and Ramirez, 2006) and the HNs (Parkis et al., 1995; Rukhadze and Kubin, 2007). The preBötC is a cluster interneurons located in the ventrolateral medulla that is essential for the generation of respiratory rhythm. The HNs located in causal brainstem innervates the tongue muscles, controlling tongue movements, food manipulation, and swallowing. Therefore, the innervation of the HNs plays a critical role in maintaining upper airway patency during breathing.

The LC is located in the posterior area of the rostral pons in the lateral floor of the fourth ventricle. The LC provides more than 70% of noradrenergic innervation in the CNS (Viemari, 2008). The projection of this nucleus reaches far and wide areas, including brainstem and spinal cord, cerebellum, cerebral cortex, hypothalamus, and amygdala, which influence many systemic functions and cognitive behaviors, such as cardiorespiratory activity, stress response, motor control, arousal status, and attention (Aston-Jones and Cohen, 2005). The NE-ergic neurons in the LC area have excitatory effect on most of the projected nuclei through adrenoceptors while these neurons receive both excitatory and inhibitory afferents, such as glutamate, GABA, opiate, 5-HT, and DA from some other brain regions (Samuels and Szabadi, 2008). The integrative innervation may lead LC neurons functioning differently, although the LC neurons are primarily homogeneous regarding morphology and neurochemical properties (Nestler et al., 1999).

The LC has been identified as a central chemoreceptor since the 1980s (Elam et al., 1981). When CO_2 level increases, the firing rate of the LC neurons in rat increases while the ventilation is enhanced in rats. This effect is independent of peripheral input. After that, more evidences were reported that the NE-ergic neurons in the LC area play a critical role in central chemoreception. Indeed, over 80% of the LC neurons were activated by hypercapnia or acidic pH (Putnam et al., 2004). The acidification of the LC region raises discharge frequency of phrenic nerves in cats, which accounts for ~30% of the overall response to hypercapnia (Coates et al., 1993). Selectively destroying NE-ergic neurons in the LC area significantly reduces the CO_2 induced the respiratory response in rats (Biancardi et al., 2008). Beside, considering the widespread projection from the LC neurons to other CO_2 sensitive sites, the LC neurons may also act as a relay center for further process of the afferent signals in response to the change of

CO_2/pH level in respiratory neural networks. Therefore, the chemosensitive NE-ergic neurons in the LC region is essential in the regulation of breathing activity.

2.4.4 Mecp2 and Rett Syndrome

Dysfunction of monoaminergic systems in the CNS is associated with several severe breathing disorders, such as Rett syndrome (RTT) (Viemari et al., 2005), congenital central hypoventilation syndrome (CCHS) (Gaultier, 2004) and sudden infant death syndrome (SIDS) (Hilaire, 2006). Among all of these disorders, the RTT as a neurodevelopmental disease is demonstrated to be related to an abnormality of NE level by overwhelming studies. RTT affects female children worldwide because of the genetic defect of a transcriptional regulator, Methyl CpG binding protein 2 (*MECP2*) (Neul et al., 2008). As an X-linked gene, *MECP2* encode for the MeCP2 protein, which has two major domains, the methyl- binding domain (MBD) and the transcriptional repression domain (TRD). The MBD directs the specific binding of MeCP2 to methylated DNA, and the TRD conducts a repressive function in gene transcription. In addition, MeCP2 may also act as a transcriptional activator that contributes to alternative gene splicing (Chahrour et al., 2008; Young et al., 2005). Mutation of MeCP2 always occurs in the MBD and TRD leading to RTT and other neurological diseases (Moretti and Zoghbi, 2006).

Because the LC region is the principle synthesis location of NE and project NE to other brain regions, the alteration of these neurons contributes to several symptoms in RTT, such as cognitive, motor, and autonomic dysfunction (Bengzon et al., 1999; Funk et al., 1994; Sparenborg and Gabriel, 1992). Breathing disorder may be one of the most severe symptoms including episodic breath-holding, apnea, respiratory irregularity, apneusis, Valsalva breathing, and air swallowing, etc (Ogier and Katz, 2008). Most of these breathing abnormalities during the development of RTT lead to further brain damage and even asphyxiation, and they are difficult

to be watched by parents of the patient. Consistent with the abnormal breathing, the defected neuronal properties of the LC neurons, hyperexcitability and decreased NE innervation have been found in RTT mouse models. Also, a reduced mRNA level of rate-limiting enzyme, tyrosine hydroxylase (TH), in catecholamine synthesis was detected in the pons of Mecp2-null male and heterozygous female mice (Taneja et al., 2009). Decreased TH protein staining level, the number of TH-expressing neurons in the LC area, and density of dendrites were seen in symptomatic Mecp2-null male mice (Roux et al., 2010). The application of exogenous NE or the NE uptake inhibitor improves respiratory rhythm, the number of TH-positive neurons in the medulla, and extends the lifespan of *Mecp2*$^{-/Y}$ mice (Berridge and Waterhouse, 2003; Roux et al., 2010; Taneja et al., 2009). Otherwise, the relationship between the deficiency in NE synthesis enzymes and the defects of the LC neuronal activities in RTT remain unclear.

2.4.5 Intervention of neuronal activities

In the recent decade, the rapid development of optogenetics, transgenic animal model, gene delivery strategies, and bioinformatics allows neuroscientists to go beyond observation of neurons with spatially selective and temporally precise manipulation of specific neuronal circuits and individual neurons with light stimulation *in vitro* and *in vivo*. By utilizing different light sensitive opsins, researchers can either stimulate or silence the specific neurons or circuits with light at a different wavelength as discussed above.

3 SIGNIFICANCE

The ANS innervates different parts of the body, including the cardiomyocytes, vascular smooth muscles, respiratory system and other internal organs to maintain the normal involuntary

function of the body. Although progress has been achieved in understanding the mechanisms and functions of the ANS, there are still many problems in health and disease remained unknown.

Uncontrolled excessive vascular hyporeactivity in septic shock is one of the critical unsolved problems. Septic shock is a server hypotension that occurs during sepsis, a whole-body inflammation. In the US, septic shock is the leading lethal symptom in ICU (Fitch and Gossage, 2002) that kills about 800,000 people annually (Goyette et al., 2004). The most characterized symptom of the septic shock is persistent vasodilation and hypotension despite clinical treatment with α-adrenergic receptor agonist or intravenous fluids. Indeed, the current clinical guideline to manage the hypotension in septic shock heavily relies on α-adrenoceptor agonists including NE, EPI, vasopressin, DA, and phenylephrine (PE) (Dellinger et al., 2013). However, these vasoconstrictors are ineffective to reverse the low blood pressure in septic shock. The irreversible hypotension leads to hypoperfusion to pivotal organs, tissue hypoxia, multiple organ failures, and death. Besides, the application of antibiotics causes bacterial cell lysis that induces a release of more septic pathogens to the blood stream and worsens the symptom (Lepper et al., 2002). The pathophysiology of the septic shock is not completely understood, especially the uncontrolled vasodilation throughout the body. Therefore, understanding the mechanism underlying the excessive hypotension in septic shock is significant and critical for an effective clinical management.

Another unsolved problem related to the cardiovascular system is the primary function of the endothelium, which has been studied for over a century. The endothelium is considered as the largest tissue in the body (Endemann and Schiffrin, 2004). It resembles the endocrine system in addition to being a barrier between blood and other tissues. The acknowledged function of the endothelium is to release vasoactive substances leading to vasoconstriction and vasodilation.

How the non-excitable ECs respond to direct activation per se and how they selectively release vasoactive substances are unclear. This is mainly because of limited accessibility to the endothelium. Therefore, effectively approach and test the endothelium is highly significant to understand their primary function.

Breathing activity in health and disease is another essential function regulated by the ANS, which has not been understood well. For example, the mechanism of the lethal breathing disorder in RTT remains unknown. With high disability rate and high unexpected death rate, RTT is a severe neurodevelopmental disorder (Kerr et al., 1997) that occurs one out of every 10,000 female worldwide. Indeed, over 25% of RTT patients die of unexplained causes (Kerr et al., 1997). This high sudden death rate is mostly attributed to the respiratory disorder, such as episodic apnea, hyperventilation, and air swallowing (Glaze, 2005). Although it does not lead to death in some patients, the breathing disorder may cause further brain damage, which in turn, contributes to multiple brain dysfunction. Meanwhile, the breathing abnormalities are difficult to be monitored and corrected in RTT patients, especially when the patients are little.

Although RTT has an equal prevalence of amyotrophic lateral sclerosis, Huntington's and cystic fibrosis, it is recognized only 25 years ago and poorly studies because of underfunding in comparison to other disorders. So far there is no cure beyond symptomatic and supportive, which is often ineffective. Therefore, understanding the cellular and molecular mechanism of breathing and breathing disorder in RTT is clinically significant. The results of the proposed experiments will enrich the database of mechanism and regulation in breathing under both physiological and pathological RTT conditions, and thereby improve the deterioration of neuronal dysfunction and decrease sudden death in RTT patients.

4 EXPERIMENTS

4.1 Animals

All animal procedures were conducted in accordance with the National Institutes of Health *Guidelines for the Care and Use of Laboratory Animals* and approved by the Georgia State University Institutional Animal Care and Use Committee.

4.1.1 Rats

Male Sprague-Dawley rats, ~300-400 g in body weight were purchased from Charles River Laboratories for mesenteric artery ring preparation and tension measurement.

4.1.2 Mice

4.1.2.1 Transgenic mice with ChR expression in endothelium

Transgenic cdh5-ChR mice with fluorescence labeled endothelium were generated by mating the *cdh5* promoter-driven Cre mice (B6.FVBTg(*Cdh*5-Cre)7Mlia/J, Stock No. 006137; Jackson Laboratory) and ChR-loxP mice (B6; 129S-Gt(ROSA)26Sor$^{tm32(CAG-COP4*H134R/EYFP)Hze/J}$, Stock No.012569; Jackson Laboratory).

4.1.2.2 Double transgenic RRT mice with ChR expression in LC

The following mouse lines were used for the breeding of double transgenic mice : TH::IRES-cre (Jackson Laboratory, stock number: 008601, strain name: B6.Cg-Tg(Th-cre)1Tmd/J) (Lindeberg et al., 2004), ChR-loxP, which is ChR-eYFP downstream of a floxed STOP cassette (Jackson Laboratory, stock number: 012569, strain name: B6;129S-Gt(ROSA)26Sor$^{tm32(CAG-COP4*H134R/EYFP)Hze/J}$) (Madisen et al., 2012), female heterozygous *Mecp2*$^{+/-}$ mice (Strain name: B6.129P2(C)-*Mecp* $^{tm1.1Bird/J}$; Stock number 003890) (Guy et al., 2001). The TH::IRES-cre mice were cross-bred with the ChR-loxP mice to produce single transgenic TH-ChR mice. The TH-ChR mice then were cross-bred with female *Mecp2*$^{+/-}$ mice to

generate double transgenic TH-ChR-*Mecp2*$^{-/Y}$. All experiments were performed in double transgenic hemizygous *Mecp2*-null males (TH-ChR-*Mecp2*$^{-/Y}$) and control males (TH-ChR).

4.1.2.3 Genotyping

Genotyping were done with PCR using punched ear or tail tissue and suggested primers from Jackson Laboratory.

4.2 Mesenteric arterial septic shock model preparation

Male Sprague–Dawley rats were anesthetized by inhalation of saturated isoflurane. Under sterilized condition, mesenteric arteries were dissected in ice-cold phosphate buffer solution (PBS) (containing in mM: 137.0 NaCl, 2.7, KCl, 10 Na_2HPO_4, 2 KH_2PO_4). The secondary and tertiary mesenteric arteries were cut into 6-8 rings of 3-4 mm in length. The rings were then transferred into the Dulbecco's Modified Eagle Medium (DMEM, Cellgro, Manassas, VA) with 4.5 g/L glucose, 10% fetal bovine serum (FBS) and 2% penicillin–streptomycin (Cellgro, Herndon, VA). The rings were cultured in the DMEM for 20 h in a CO_2 incubator at 37 °C with 5% CO_2. Before culture, 1 μg/ml LPS (from *Escherichia coli* 0127:B8, Sigma, St. Louis, MO) was added into one Petri dish with 3 rings, and another 3 rings in a separate Petri dish without LPS served as sham control. ED rings were also used in which the endothelium was removed by rolling the rings on tweezers for 4-5 times back and forth. The success of endothelium denudation was accepted if the ring lost > 80% relaxation to ACh (10^{-6} M) after PE (10^{-4} M) pre-contraction.

4.3 Blood vessel tension measurement

The cultured mesenteric artery rings were mounted on a 6-channel force-electricity transducer (i-DAQ-4 Data Acquisition System, Globaltown Microtech, Sarasota, FL) in 3 ml 37 °C water organ bath perfused with Krebs solution (containing in mM: 118.0 NaCl, 25.0

$NaHCO_3$, 3.6 KCl, 1.2 $MgSO_4$, 1.2 KH_2PO_4, 11.0 glucose, and 2.5 $CaCl_2$), gassed with 95% O_2 and 5% CO_2. Before the experiment, the rings were given a 0.4 g pre-load and equilibrated for 30 min. The vitality of control rings and septic model rings was confirmed with the vasoconstriction to PE at a dose from 10^{-9} to 10^{-4} M. The hyporeactivity to PE was accepted when the rings lost 80% contractility after LPS treatment in comparison to the sham control. Otherwise, the preparation was considered as a failure and rejected for further studies. Another criterion of variability of the ring preparation was the response to high dose KCl (60mM) at the end of each experiment, indicating the intact of contractile machinery of VSMs in both control and LPS-treated groups.

4.4 Histology of ChR labeled Endothelium in Multiple Organs

Heart and kidney from cdh5-ChR and wild type (WT) mice were fixed in 1% paraformaldehyde at room temperature for over 4 hrs and then dehydrated in 30% sucrose in PBS at 4 °C for 24 hrs. Fixed tissues were embedded in the Tissue-Tek OCT Compound (Andwin Scientific, Torrance, CA), and cut into 8-10 μm slices by using the Microm HM 550 Cryostats system (Thermo Scientific, PA). Dura mater was peeled off from the inner side of skull and mount on glassed slices directly. YFP fluorescence was detected with 514/527 nm (excitation/emission wavelength) filters under the microscope (Carl Zeiss, Gottingen, Germany).

4.5 Acute dissociation of ECs

ECs were acutely dissociated from aorta obtained from both cdh5-ChR and WT mice by enzymatic dissociation for 10-15 min at 37℃ using neutral protease (8 U/ml, obtained from Sigma, St. Louis, MO) and elastase (2 U/ml, Worthington, Lakewood, NJ, USA) mg/ml in the following digestion buffer (in mM): 138 NaCl, 5 KCl, 1.5 $MgCl_2$, 0.42 Na_2HPO_4, 0.44 NaH_2PO_4, 0.1 $CaCl_2$, 10 HEPES, 4.2 $NaHCO_3$ and 0.3% BSA. This was followed by a 1-2 min 37℃

incubation with collagenase type IA (120 U/ml, obtained from Sigma, St. Louis, MO). The tissue segments were then washed with digestion buffer and gently triturated with a fire-polished glass pipettes. The trituration solution containing ECs was dropped on a Petri dish coated with poly-L-lysine (Sigma, P8920) and easily identified via fluorescence microscopy.

4.6 Electrophysiology on acute dissociated ECs

Dissociated ECs were placed in the recording chamber and identified by YFP fluorescence using the same microscope set up as described under histology section. Whole-cell currents and membrane potentials of the dissociated ECs were recorded at room temperature in the voltage and current clamp mode, respective. Recorded signals were amplified with an Axopatch 200B amplifier (Molecular Devices, Union City, CA), digitized at 10 kHz, filtered at 2 kHz, and collected with the Clampex 10 data acquisition software (Molecular Devices, Union City, CA). The patch pipettes with the resistance of 4-6 MΩ were made with 1.2 mm borosilicate glass capillaries (Sutter Instrument CO., Novato, CA). The optical stimulation was delivered by using a xenon arc lamp with high-speed switcher (Lambda DG-4, Sutter Instruments, Novato, CA). The light source was connected to the incident-light illuminator port of the microscope, and passed through a 470 nm bandpass filter (~20 mW/mm^2). Light pulse trains were generated with the Digitimer D4030 pulse generator (Digitimer Ltd, Letchworth Garden City, UK). The solution applied to the bath contained (in mM) 130.0 NaCl, 10.0 KCl, 1.0 $MgCl_2$, 1.5 $CaCl_2$, 10.0 glucose, 10.0 HEPES, and 3.0 NaOH (pH 7.4). The internal (pipette) solution contained (in mM) 10.0 KCl, 133.0 K-gluconate, 5.0 EGTA, 5.0 glucose, 1.0 K_2-ATP, 0.5 Na-ADP, and 10.0 HEPES (pH 7.4), and the final Mg^{2+} concentration was adjusted to 1 mM using a $[Ca^{2+}]$ / $[Mg^{2+}]$ calculation software Maxchelator (Chris Patton, Stanford University, Pacific Grove, CA).

4.7 Langendorff Heart perfusion

Coronary circulation resistance was studied in the Langendorff isolated and perfused heart preparation. The heart and lungs were entirely removed from terminally euthanized mice, and placed in the ice-cold Krebs-Henseleit (KH) solution containing (in mM): 119 NaCl, 4.7 KCl, 2.5 $MgSO_4$, 2.5 $CaCl_2$, 10 glucose, 0.5 disodium EDTA, 25 $NaHCO_3$. All of pulmonary arteries and veins were tightened by cotton thread followed by removal of lungs. Then a cannula was inserted into the ascending aorta connected to the perfusion apparatus. This cannula is attached to the outflow of a reservoir containing an oxygenated KH solution, maintained at > ~35 °C, and continuously gassed with 5% CO_2 and 95% O_2. The perfusion solution was delivered to coronary arteries in the retrograde direction through the aorta. For each heart, the hydrostatic pressure (80 cm H_2O) and perfusion speed at 0.2 - 0.4 ml/min was determined with a calibrated roller pump (Syringe Pump, Farmingdale, NY, NE-300, NE-4000). The perfusate exited the coronary venous circulation through the coronary sinus in the open vena cava. The viability of the isolated and perfused heart was assessed by its spontaneous beating (> 250 beats/min), coronary vasoconstriction to 10-5 M PE and 60 mM KCl, and coronary vasodilation response to the β receptor agonist isoproterenol (Isop, 10^{-5} M).

4.8 Renal perfusion

Kidneys from both cdh5-ChR and WT mice were isolated, cannulated through the renal arteries, and perfused at a constant rate (0.2-0.4 ml/min at baseline) with KH solution at 37 °C as described above. The viability of the isolated and perfused kidneys was assessed by the renal vascular response to 10^{-5} M PE, 10^{-5} M Isop and 60 mM KCl.

4.9 Immunohistochemistry

Double transgenic mice, TH-ChR-*Mecp2*−/Y, at seven weeks of age were anesthetized with inhalation of saturated isoflurane and transcardially perfused with 0.9% saline and 4% (w/v) paraformaldehyde sequentially. Then the brain was removed, and the transverse pontine sections (30-40 μm) were cut from the brainstem on a cryostat (Leica, Wetzlar, Germany). Catecholaminergic neurons in both LC and medullary areas were labeled with anti-dopamine beta-hydroxylase (DBH, red) and anti-GFP (green) antibodies that also detect YFP. Briefly, the frozen sections were incubated with primary GFP/YFP monoclonal antibodies (1:400, life technologies) and anti-DBH (1:1000, sigma), followed by AF488 conjugate donkey anti-mouse (1:400, life technologies) and biotin conjugate goat anti-rabbit (1:400, Sigma) secondary antibodies. Finally, the slides were treated with Texas Red-conjugated antibody (1:400, Jackson ImmunoResearch), followed with dehydration. After mounted with 2,2'-Thiodiethanol (Sigma), the fluorescence images were taken with a LSM 510 Zeiss confocal microscope (Jena, Germany).

4.10 Plethysmograph

Breathing activity was recorded from conscious mice of 4-5 weeks old without anesthesia. The mice were kept in a plethysmograph chamber (~ 40 ml) with a same volume reference chamber connected to the same gas source. Mouse breathing signals were barometrically recorded continuously by measuring the pressure changes between the animal chamber and a reference chamber with a force-electricity transducer (PanaVise Products, Inc. Nevada). The chambers were constantly ventilated with air at a flow rate of 50 ml/min, and the animal was allowed to stay in the chamber for 10 min to adapt to the chamber environment before recording, followed by a recording of 20 minutes at room temperature. The signal was

collected and analyzed with Clampfit software (Molecular Devices). The variability of breathing frequency (f) was calculated as the division of standard deviation (SD) divided by arithmetic mean of breathing interval. All of the SD and mean values were measured from at least 200 successive breathing cycles. Apnea (apneas / h) was counted only if a breathing cycle was longer than two prior breathes.

4.11 Brain slice preparation and electrophysiology

Brain slices were prepared as described previously (Zhang et al., 2010). In brief, THChR-*Mecp2*$^{-/Y}$ and control mice at 4-6 week-old were anesthetized as described above. The brainstem was obtained rapidly and placed in an ice-cold, sucrose-rich artificial cerebrospinal fluid (sucrose aCSF) containing (in mM) 200 sucrose, 3 KCl, 2 $CaCl_2$, 2 $MgCl_2$, 26 $NaHCO_3$, 1. 25 NaH_2PO_4 and 10 D-glucose. The solution was bubbled with 95% O_2 and 5% CO_2 (pH 7.40). Transverse pontine sections (300 μm) containing the LC area and transverse medullary slices (200 μm) containing the HNs were obtained using a vibratome (1000 Plus, Vibratome, St. Louis, MO). The slices were transferred to normal aCSF in which the sucrose was substituted with 124 mM NaCl, allowed to recover at 33°C for 1 h, and then kept at room temperature before being used for recording.

One slice was transferred to a recording chamber that was perfused with oxygenated aCSF at a rate of 2 ml/min and maintained at 32–35°C. LC neurons were identified as described previously (Zhang et al., 2010). Whole-cell current clamp was performed in brain slices, and LC neurons were patched. Sutter pipette puller (Model P-97, Novato, CA) was used to pull the patch pipettes with a resistance of 3–5 MΩ. The pipette solution (in mM) contained 130 K-gluconate, 10 KCl, 10 HEPES, 2 Mg-ATP, 0.3 Na-GTP and 0.4 EGTA (pH 7. 3). The bath solution was normal aCSF bubbled with 95% O_2 and 5% CO_2 (pH 7. 40). In one set of experiments, α-

adrenoceptors were blocked by adding phentolamine (10 μM, Sigma) to the bath solution. Recorded signals were amplified with an Axopatch 200B amplifier (Molecular Devices, Union City, CA), digitized at 10 kHz, filtered at 1 kHz, and collected with the Clampex software. The temperature was maintained at 33°C during recording by a dual automatic temperature control (Warner Instruments).

4.12 Data Analysis

The relationship of contractile force with agent concentration was described using the Hill equation: $f = F_{Max}/[1 + (EC_{50}/x)h]$, where f is the contractile force, F_{Max} is the maximum contractile force, x is agent concentration, and h is the Hill coefficient.

Delayed excitation (DE) was described as the time delay of the first action potential at the end of each hyperpolarization command, which was fit with a Boltzmann equation as normalized $D = 1 /\{1 + exp [- (V - V_{½}) / k]\}$ where D is the delay period, V is the hyperpolarizing membrane potential, $V_{½}$ is the half-inactivation, and k is the slope factor.

Data are presented as the means ± S.E. The electrophysiological data were analyzed with Clampfit 10.3 software. Data are presented as means ± SE. Differences were evaluated using Student t-tests for a pair data, ANOVA for three groups or more, and Mann–Whitney U test for breathing activity. Statistical significance was accepted if when $P \leq 0.05$.

5 RESULT 1: INTERVENTION TO VASCULAR TONES IN LPS INDUCED VASODILATION MODEL

Published as **Shuang Zhang**, Ningren Cui, Shanshan Li, Lei Guo, Yang Wu, Daling Zhu and Chun Jiang. Interception of the endotoxin-induced arterial hyporeactivity to vasoconstrictors. ***Vascul Pharmacol***. 2014 Jul;62(1): 15-23.

Note: Elsevier, the publisher of Vascular Pharmacology, automatically granted the authors the permission for using article in a thesis and/or dissertation.

5.1 Abstract

Septic shock is a severe pathophysiologic condition characterized by vasodilation, hypotension, hypoperfusion, tissue hypoxia, multiple organ failures and death. It is unclear what causes the septic vasodilation that may result from general dysfunction of vascular smooth muscles (VSMs) or selective disruption of vasomotor balances in VSMs. The latter could be due to enhanced vasorelaxation and/or depressed vasoconstriction. Understanding these may lead to pharmacological interventions to septic vasodilation. Therefore, we performed studies in isolated and perfused mesenteric arterial rings. A 20-h exposure of the rings to LPS (1μg/ml) led to hyporeactivity to PE. However, the responses of the LPS-treated rings to high concentrations of KCl (60mM) and ATP remained comparable to control rings, suggesting that contractility of VSMs is retained. The hyporeactivity was marginally affected by atropine, indomethacin and L-NAME, suggesting that endothelium-dependent vasorelaxation does not play a major role. In addition to PE, the LPS-treated rings were hyporeactive to dopamine, histamine and angiotensin II. They showed intermediate hyporeactivity to the thromboxane-A2 receptor agonist U46619.

Little hyporeactivity to endothelin-1 (ET-1), serotonin (5-HT) and vasopressin was found. ET-1-induced vasoconstriction occurred without endothelium, whereas the effect of serotonin was endothelium dependent. Although rings were hyporeactive to some of the vasopressors, their vasoconstriction effects were significantly potentiated by PE co-application. Taken together, these data suggest that the endotoxin-induced vasodilation may not result from general dysfunction of VSMs, neither from the endothelium-dependent vasorelaxation. The promising vascular response to various vasoconstrictors found in this study warrants further investigations of therapeutic potentials of these agents.

5.2 Introduction

Approximately, 200,000 people die of severe sepsis or septic shock in the United States each year (Angus et al., 2001; Martin et al., 2003). Septic shock is characterized by excessive vasodilation and vascular hyporeactivity to vasoconstrictors, especially α-adrenergic receptor agonists. The vascular response handicaps therapeutic interventions to vascular tones, leading to uncontrolled hypotension, hypoperfusion, tissue hypoxia, multiple organ failures and death (Dellinger et al., 2013; Vincent et al., 2006). Even the application of antibiotics is hindered by the cascade of events, as the bacterial cell lysis induced by certain antibiotics leads to a release of more septic pathogens such as LPS from Gem-negative bacteria to the blood stream (Lepper et al., 2002). A treatment with LPS, also known as endotoxins, can produce most the features of the cascade of events in sepsis, including the vascular hyporeactivity to α-adrenergic activators (Medzhitov, 2001; O'Brien et al., 2001).

Interruption of the cascade of events in sepsis and endotoxemia relies on restoration or management of vascular responsiveness to vasopressors, while it is unclear how LPS causes the vascular hyporeactivity to vasoconstrictors. Such a vascular response may result from injuries to

VSMCs or the contractile machineries in the cells leading to dysfunction of VSM contractility; LPS may activate extensively the vasodilatory mechanisms disrupting the balance of vasoconstriction versus vasodilation; alternatively, the regulation of the VSM by certain vasoconstrictors may be impaired.

The understanding of how these potential cellular mechanisms contribute to the vascular hyporeactivity in endotoxemia may lead to novel therapeutic interventions to septic shock. With the belief, we performed these studies in isolated and perfused mesenteric arterial rings. Our results suggest that the vascular hyporeactivity does not seem to result from general injuries to VSM contractility, neither from the enhanced endothelium-dependent vasorelaxation. Remarkably, we found that the regulation of the VSM by certain vasopressors appeared to be disrupted. The disruption of vasoconstriction was selective for several vasoconstrictors. Surprisingly, none of the vasopressors recommended in the current guidelines for management of severe sepsis and septic shock produced effective vasoconstriction in our endotoxin model, while none of the three promising vasopressors, ET-1, 5-HT, and TXA_2 receptor agonist found in our study has been considered for the treatment of septic shock.

5.3 Results

5.3.1 Hyporeactivity of mesenteric arterial rings to PE with LPS treatment

LPS is known to cause systemic hypotension and the vascular hyporeactivity to circulating vasoconstrictors, both of which are characteristic symptoms of septic shock. Therefore, endotoxins are ideal for the study of the vascular hyporeactivity although it may not produce all clinical manifestations of sepsis (Deitch, 1998). To achieve reliable vascular hyporeactivity and to demonstrate unambiguously its reversibility, we chose to use a high concentration of LPS (1μg/ml) as described in our previous studies (Shi et al., 2010). An over-

night exposure of the isolated mesenteric rings to LPS caused clear hyporeactivity to PE (Fig. 5-1). In response to a graded increase in PE concentrations, the endothelium-intact (EI) rings cultured without LPS showed a concentration-dependent increase in contractility. An obvious increase in the contractile force was seen with 10^{-7} M PE, and the maximum vasoconstriction was reached at 10^{-5} M. In contrast, rings treated with LPS for the same period as for the sham control failed to respond to PE at 10^{-7} M, and 10^{-5} M PE produced only a transient vasoconstriction with the much smaller amplitude than that of the sham control rings (Fig. 5-1A). When the contractile force was plotted against PE concentration, the concentration dependence was observed in the control rings, which was described by the Hill equation with EC_{50} 7×10^{-7} M (h = 1.1). The force-concentration relationship curve shifted to the right in rings treated with LPS with EC_{50} 3.3×10^{-6} M (h = 1.6) (Fig. 5-1B). Meanwhile the mean maximal force response to PE was decreased from 0.74 g to 0.18 g (Fig. 5-1B). The difference in the contractile force at the concentration of 10^{-6} M PE was statistically significant between these two groups of rings ($P < 0.01$, n = 20). Therefore, the vascular hyporeactivity to PE was produced by LPS under our experimental condition.

5.3.2 The effect of LPS on contractile capability of the VSM

One potential mechanism for the vascular hyporeactivity is the injury to VSM contractile capability or even to the cell viability caused by endotoxins. To test the viability of VSM contractile machineries, vasoconstrictions were produced by high concentrations of K^+ and certain ionotropic receptor ligands that are independent of the conventional circulating vasoconstrictors and membrane receptors. Surprisingly, we found that rings treated with LPS responded to high concentration of KCl (60mM) very well, even though they showed marked hyporeactivity to PE and histamine, another vasoconstrictor inducing hyperactivity in LPS-

treated rings (Fig. 5-2A). There was no significant difference in contraction forces between these LPS-treated rings and the sham controls indeed ($P > 0.05$, $n = 12$) (Fig. 5-2C). Pinacidil was applied as a vasodilator to confirm the capability of vasodilation is maintained in both control and LPS-treated groups. In EI rings, the application of pinacidil (10^{-5} M) after vasoconstriction completely relaxed both control and endotoxin treated mesenteric arterial rings to the pre-contraction level, which was also observed with 1 μM ACh.

ATP is a natural ligand of the purine receptors including P2X and P2Y receptors. The P2X receptors are ionotropic and permeable to cations non-selectively. Previous studies have shown that ATP produces vasoconstriction by acting on the P2X receptors (Li et al., 2011). We found that the vasoconstrictive effect of ATP was not compromised by the LPS treatment. Instead, the LPS-treated rings showed even stronger vasoconstriction response to ATP than the control group (Fig. 5-2B,D). These results indicate that the constriction capability of the isolated mesenteric rings is still retained when the vascular hyporeactivity to PE has developed after the LPS treatment. Therefore, it is unlikely that the vascular hyporeactivity with LPS exposure results from injuries of the VSM cells or their contractile machineries.

5.3.3 Contribution of endothelium-dependent relaxation

Although vasodilation can be produced by several circulating hormones, they are either absent, or present in very low concentrations, in isolated mesenteric rings. Thus, the major vasodilators should be those originated from endothelial cells in our *in vitro* preparation. NO is a strong vasodilator released from the endothelium. A 10-min treatment of rings with the NO synthase inhibitor L-NAME (100 μM, 10 min) did not have any effect on the LPS-induced vascular hyporeactivity (Fig. 5-3A,B). Similar results were obtained with the cyclooxygenase inhibitor indomethacin (10 μM, 10 min) and the muscarinic receptor blocker atropine (0.5 μM,

10 min) (Fig. 5-3B), suggesting that the endothelium-dependent vasodilators do not play a major role in the LPS-induced vascular hyporeactivity. To strengthen these findings, experiments were performed in endothelium-denuded (ED) rings. Mechanical removal of endothelium followed by LPS exposure (1 μg/ml, 20 h) did not affect the hyporeactivity, although the ED rings had slightly higher contractile response to PE than EI rings with or without LPS treatment (Fig. 5-3C,D). In the ED rings, the PE induced vasoconstriction curve was shifted rightward with EC_{50} 5×10^{-7} M (h = 1.0) in control group compared to the LPS group with EC_{50} 3×10^{-6} M (h = 1.8, Table 5-1). The EC_{50} was not affected by removing endothelium when compared to those in EI rings. These results suggest that the endotoxin-induced vascular hyporeactivity cannot be attributed to the vasodilators released from the endothelium.

5.3.4 Responses of mesenteric arterial rings to various vasoconstrictors with endotoxin exposure

Several circulating hormones, neurotransmitters and local mediators have vasoconstriction effects. Some of the vasoconstrictors such as epinephrine and norepinephrine may be involved in the maintenance of basal vascular tones under normal conditions. An inadequate response of the VSM to these vasopressors may lead to hypotension and shock. Since several different cellular mechanisms can be activated by the vasoconstrictors to produce VSM contractions, it is possible that endotoxin exposure may cause the vascular hyporeactivity to some of the vasoconstrictors, and allow the mesenteric arteries to respond to the others. To test this possibility, we screened all known vasoconstrictors whose pharmacological agents are available commercially.

Similar to PE, histamine (Hist), Ang II, and dopamine produced much smaller, or not at all, vasoconstriction after LPS treatment, suggesting that the mesenteric rings become

hyporeactive to these vasoconstrictors. We compared the effect of all of vasoconstrictors in both control and endotoxin groups under the concentration at or near to their EC_{50} in LPS-treated group (Fig. 5-6C). EC_{50} and h value for dopamine, Hist, and Ang II are listed in Table 5-1.

ET-1 is a peptide vasoconstrictor that activates both endothelin A (ET_A) and endothelin B (ET_B) receptors (Rubanyi and Polokoff, 1994). Strikingly, we found that ET-1 remained to produce strong vasoconstriction in the mesenteric rings treated with LPS (Fig. 5-4A, B). When the contractility was compared between the LPS-treated rings and the sham controls, we found that LPS had no significant effect on the maximum contractile force although the EC_{50} increased from 5×10^{-8} M (h = 1.0) to 8×10^{-8} M (h = 1.2) (Fig. 5-4B; Fig. 5-6C,D). Endothelium had very little contribution to the ET-1 mediated vasoconstriction, as similar contractility was observed in ED rings with and without LPS treatment (Fig. 5-4C,D; Fig. 5-6C,D). In ED rings, EC_{50} of vasoconstriction curve induced by ET-1 increased from 5×10^{-8} M (h = 1.2) in control group to 1.4×10^{-7} M (h = 1.4) in LPS-treated group (Table 5-1). In addition, the mean maximal force in response to ET-1 was even increased slightly from 1.82 g in control group to 1.88 g in LPS-treated group (Fig. 5-4B).

5-HT is another vasoconstrictor acting on the resistant arteries of cerebral, coronary and pulmonary circulations (Faraci and Heistad, 1990; Lamping et al., 1989; MacLean et al., 2000). Rings with LPS treatment did not show obvious hyporeactivity to 5-HT (Fig. 5-5A). No significant changes in the maximum constriction were observed ($P > 0.05$) except a slight increase in EC_{50} from 9×10^{-7} M (h = 1.5) to 1.2×10^{-6} M (h = 1.6, Fig. 5-5B, Table 5-1). Unlike ET-1, the effect of 5-HT relied partially on endothelium, as ED rings showed a marked reduction in their contractility after LPS exposure (Fig. 5-5C,D; Fig. 5-6C,D). EC_{50} in control group was increased from 7×10^{-7} M (h = 1.3) to 1.4×10^{-6} M (h = 1.5, Fig. 5-5D, Table 5-1). No evident

hyporeactivity to arginine vasopressin (AVP) was found in LPS-treated rings (EC_{50} 2.5×10^{-9} M were same in both control and endotoxin groups, but h value was increased from 1.2 to 1.8). Meanwhile the maximal vasoconstriction in control rings was increased from 0.06 g to 0.09 g in LPS-treated rings. Nevertheless, further studies were not attempted because of AVP triggered weak vasoconstrictive effect (Fig. 5-6D).

The mesenteric ring response to the TXA_2 receptor agonist U46619 was retained by over 70% with an EC_{50} increase from 1.3×10^{-8} M (h = 1.2) to 4.5×10^{-8} M (h = 1.2) after LPS treatment (Fig. 5-6 A,B), suggesting an intermediate hyporeactivity to the TXA_2 receptor agonist.

5.3.5 Vascular reactivity to a combined application of PE with another vasoconstrictor

Since some of the vasoconstrictors were used together with PE in septic therapies, we also examined their joint effects on the vascular hyporeactivity. PE (10^{-9} to 10^{-4} M) was combined with ET-1 (10^{-10} to 10^{-6} M), 5-HT (10^{-9} to 10^{-4} M), dopamine (10^{-6} to 10^{-2} M), Hist (10^{-7} to 10^{-2} M), Ang II (10^{-9} to 10^{-5} M), AVP (10^{-9} to 10^{-5} M), U46619 (10^{-10} to 10^{-6} M). Each combination was applied to both control and LPS-treated rings. At the concentration for the maximal vasoconstriction under the control condition without LPS, the ring response to the combined application of the vasoconstrictors was smaller than, or similar to, the arithmetic addition of two individual responses to the vasoconstrictors (not shown). When the joint effects under endotoxin-induced conditions were compared with those of the control, interestingly, only Hist/PE showed significant hyporeactivity (Fig. 5-7), suggesting a potential approach to reverse the endotoxin-induced vascular hyporeactivity by a joint application of these vasoconstrictors with PE.

5.3.6 Vasoconstriction signaling pathways of ET-1 and 5-HT

ET-1 activates multiple signaling systems. To dissect the ET-1 signaling for mesenteric arterial constriction, responses of mesenteric rings to ET-1 were compared in the presence versus absence of various blockers targeting specific signaling pathway. There are two ET receptors, ET_AR and ET_BR. The ET-1 induced vasoconstriction was significantly suppressed by BQ-123, an ET_AR antagonist, but not by BQ-788, an ET_BR antagonist (Fig. 5-8A_1-A_3). The ET-1 signaling was inhibited by pertussis toxin suggesting the involvement of $G_{i/o}$ – AC – PKA signaling pathway (Fig. 5-8A_4). The ET-1 effects were mostly eliminated by the blocker of the phosphatidylcholine-specific phospholipase C (PC-PLC, D609) (Adibhatla et al., 2012) but not the blocker of inositol triphosphate specific phospholipase C (PI-PLC, U73122) (Zizzo et al., 2006). Interestingly, the protein kinase C inhibitor Calphostin C did not inhibit the ET-1 vasoconstriction (Fig. 5-8A_5-A_7). Also, the ROK pathway does not seem to be involved as the ROK inhibitor Y27632 had no significant effect on the ET-1 vasoconstriction (Fig. 5-8A_8).

Serotonin receptors 5-HT_{1B}R, 5-HT_{2A}R and 5-HT_{2B}R are the major serotonergic players in vasculatures (Monassier et al., 2010). These metabotropic receptors produce their functions by activating $G_{i/o}$ and $G_{q/11}$ proteins. Using a similar approach as above, we found all of these 5-HT receptors were involved in mesenteric constriction by activating $G_{i/o}$ – AC – PKA pathway and the $G_{q/11}$ – PC-PLC – Calphostin C-insensitive signaling pathway (Fig. 5-8B_1-B_6). In addition, the ROK pathway appeared to play a role that is known to be activated by 5-HT_{2A}R (Fig. 5-8B_7).

5.4 Discussion

Excessive vasodilations are seen in several types of shock, while most of them can be controlled by α–adrenoceptor agonists or intravenous fluids (Laffon et al., 1999). None of these treatments, however, is effective for septic shock. The systemic hypotension and vascular

hyporeactvity to α-adrenergic agonists are found in the rat model with LPS injection (Bermejo et al., 2003; Cena et al., 2010). The vascular hyporeactivity is likely due to 1) poor response of the resistance arteries to vasopressors, and 2) refractory hypotension that hinders the blood pressure response to intravenous fluid resuscitation. As a result, the uncontrolled vasodilation develops in sepsis, leading to hypotension, hypoperfusion, tissue hypoxia, acidosis, multiple organ dysfunction and death. These two events may link to each other, as the hyporeactivity to circulating vasopressors can drastically compromise the vasomotor function, impair the myogenic autoregulation of basal vessel tones, reduce blood perfusion to several vital organs including the heart, decrease cardiac output, and resist to intravenous infusion. Therefore, the hyporeactivity of resistance arteries to vasoconstrictors is a critical event in septic shock, and better therapeutical interventions to the hyporeactivity are needed for the management of septic shock.

The management of septic shock could be very challenging if septic pathogens caused injuries to VSM cells. This is not simply a theoretical concern because accumulating experimental evidence indicates that several apoptotic events are initiated by endotoxins in both VSMs and endothelium (van Geel et al., 2000). The consequences of VSM cell apoptosis would be the impairment of contractility and vasodilation. Indeed, the cell injury hypothesis has been proposed to explain the excessive vasodilation and hyporeactivity in sepsis (Bannerman and Goldblum, 2003; Tesse et al., 2005). In the present study, we have found that the contractile capability of mesenteric arterial rings does not seem to be disrupted in 20-h endotoxin exposure. In rings that showed clear hyporeactivity to PE, a high concentration of KCl (60 mM) produces vasoconstriction as strongly as in the control rings, indicating that the VSM contractility is not lost. Consistent with this finding, the P2X receptor ligand ATP produces a similar level of

vasoconstriction with or without LPS treatment. It is known that high concentrations of extracellular K^+ bring about depolarization, and raise intracellular Ca^{2+} to initiate filament sliding and muscle contraction, while the activation of P2X receptors tends to have similar effect to a smaller degree though (Bean, 1992; Ganitkevich and Isenberg, 1991). Therefore, it is very likely that the filament-based contractile machineries remain functional during a 20-h endotoxin exposure when severe hyporeactivity to PE takes place in mesenteric arteries. Thus there must be other underlying mechanisms for vascular hyporeactivity.

The maintenance of the VSM contractile capability in endotoxin exposure is an encouraging finding, which motivated us to examine potential regulatory mechanisms for the hyporeactivity. Our results suggest that the endotoxin-induced vascular hyporeactivity cannot be attributed to the vasodilators released by local endothelium. NO is a powerful vasodilator, which has been shown to play a role in septic vasodilation (Pedoto et al., 1998). In the presence of L-NAME, however, the mesenteric rings show clear hyporeactivity to PE, suggesting that locally released NO may not be a major player in the endotoxemic vascular hyporeactivity. Several prostaglandins have vasodilatory effects, including PGI_2, PGE_1 and PGE_2, arachidonic acid products catalyzed by COX (Mollace et al., 2005). Inhibition of COX1 and COX2 with the COX inhibitor indomethacin does not alleviate the endotoxemic hyporeactivity. The hyporeactivity is not affected by prevention of endothelium from being activated by muscarinic acetylcholine receptor using atropine either. Consistent with these observations, the hyporeactivity remains after the endothelium is mostly removed in ED rings. These results strongly suggest that the vascular hyporeactivity may not result from the release of vasodilators from the endothelium in local vasculature, although these results do not exclude the possibility that endothelium may be involved in the regulation of basal vascular tones as well as the vascular responses to exogenous

vasoactive substances in endotoxin exposure. In addition, by taking advantage of this ex vivo vascular model, our results may rule out the involvement of the circulating hormones and cellular metabolites in the LPS-induced vasodilation.

Our results indicate that several vasoconstrictors are spared from the hyporeactivity, although the mesenteric arteries are hyporeactive to many other vasoconstrictors after the rings were treated with LPS. ET-1 produces strong vasoconstriction that is barely affected by endotoxins. Endothelium is not critical for the ET-1 effect as no clear hyporeactivity is seen in ED rings treated with LPS. 5-HT is another strong vasoconstrictor for the mesenteric rings. Its vasoconstricting effect is not diminished by endotoxins when endothelium is intact. The rings are not hyporeactive to AVP either, although its effect on mesenteric vasoconstriction is only modest. In addition, the TXA_2 receptor agonist U46619 is capable of constricting the LPS-treated mesenteric rings by >70% of the control level in comparison to PE (10-20%). Agonists of the ET-1, 5-HT and TXA_2 receptors are commercially available (Green et al., 1981; Hassid, 1984; Watts, 2010) ; they have been widely studied in-vitro and in-vivo (Bender and Klabunde, 2007; Tanaka et al., 2004; Xie et al., 2012); their side-effects are largely known (Bonsi et al., 2007; Ohkubo et al., 1996; Prasanna et al., 2001); and their effective concentrations for vasoconstriction are relatively low (Matsumoto et al., 2010; Takata et al., 1999; Zhou et al., 2006). Therefore, it is possible that some of them may turn out to be effective vasoconstrictive agents for the treatment of septic shock if these novel findings in the present study are confirmed by further studies.

None of ET-1 and 5-HT has been used as effective vasoconstricting agents for septic shock. Currently, norepinephrine, phenylephrine, dopamine, epinephrine, and vasopressin (as well its analogue terlipressin) are the only five vasopressors available for the treatment of septic

shock in the United States (Ferguson-Myrthil, 2012). Serum ET-1 concentrations increase in sepsis (Huribal et al., 1995). A previous study has shown that the nonselective endothelin receptor antagonist TAK-044 improves renal hemodynamics and renal function in endotoxemia (Mitaka et al., 1999). Because of this finding, more attention has been paid to endothelin receptor antagonists (Gardiner et al., 2001; Krejci et al., 2003). 5-HT has been studied under normal condition as a vasoconstrictor, acting on the resistant arteries of cerebral, coronary and pulmonary circulations (Faraci and Heistad, 1990; Lamping et al., 1989; MacLean et al., 2000). Experimental evidence indicates that 5-HT plays a role in regulating pulmonary circulation in sepsis (Spapen and Vincken, 1992), and its effect on vasculatures relies on NO and endothelium (Mostefai et al., 2008). Our data suggest that in rat mesenteric arteries ET-1 acts on the G_i – AC – PKA and $G_{q/11}$ – PC-PLC – DAG – PKC signaling systems through ET_AR. In addition to these two signaling pathways, 5-HT seems to activate ROK through $5\text{-}HT_{1B}R$ leading to inhibition of the MLCP and vasoconstriction (Fig. 5-8C).

The demonstration that the mesenteric arteries are capable of maintaining their responses to certain vasoconstrictors in endotoxin exposure is promising, as this finding encourages further research for more potent and effective vasoconstrictors with little, or not at all, hyporeactivity in sepsis. As a result, new and more effective therapeutical modalities for the treatment of septic shock may be discovered. Therefore, our findings in the present study may have impact beyond the demonstration of a few vasoconstrictors effective for the control of endotoxin-induced vasodilation.

Since many of the natural counterparts of the vasoconstrictors that we have studied exist in the circulation or local tissues, they may play a role not only as an individual but also as combination. Also, multiple vasopressors have been used in combination to treat septic shock

(Meidan et al., 1992). Thus, we have examined the joint application of two vasoconstrictors in the control of endotoxin-induced vasodilation. The rings are hyporeactive to some vasoconstrictors such as PE, U46619, Hist and Ang II with LPS treatment. When the joint contractile forces are compared, the mesenteric arteries are hyporeactive only to Hist/PE, while all other vasoconstrictors produce vasoconstriction as potent as their effects on control rings. High dose dopamine induced vessel tissue damaged so that we did not perform the combination experiment on it. These results suggest that a joint application of these agents with PE may help to achieve better vasoconstriction in endotoxin exposure and perhaps sepsis as well.

In conclusion, three potential mechanisms have been studied in the endotoxin-induced vascular hyporeactivity. The vascular hyporeactivity does not seem to be a result of general injuries to VSM contractility, neither the endothelium-dependent vasorelaxation. Instead, the regulation of the VSM by certain vasoconstrictors appears to be disrupted, while the mesenteric arteries respond very well to other vasoconstrictors. The disruption of vascular responses to selective vasoconstrictors including α-adrenergic agonists used widely in the control of the excessive vasodilation in septic shock indicates the necessity to consider alternative agents for shock control, while the demonstration of the vasoconstriction to certain vasopressors in the present study suggests that the alternative therapeutic approaches are highly feasible.

5.5 Acknowledgement

This work was supported by the NIH (NS073875) and the American Heart Association (09GRNT2010037). SZ is a fellow of the Molecular Based Disease (MBD) program at Georgia State University.

Table 5.1 EC_{50} and h values for various vasoconstrictors.

	Control	LPS
PE	0.7 μM (1.1)	3.3 μM (1.6)
PE/ED	0.5 μM (1.0)	3.0 μM (1.8)
U46619	13 nM (1.2)	45 nM (1.2)
5-HT	0.9 μM (1.5)	1.2 μM (1.6)
5-HT/ED	0.9 μM (1.3)	1.4 μM (1.5)
ET-1	50 nM (1.0)	80 nM (1.2)
ET-1/ED	50 nM (1.2)	141 nM (1.4)
Histamine	1.3 mM (1.6)	3.0 mM (1.5)
AVP	2.5 nM (1.2)	2.5 nM (1.8)
Dopamine	0.6 mM (1.5)	2.0 mM (1.5)

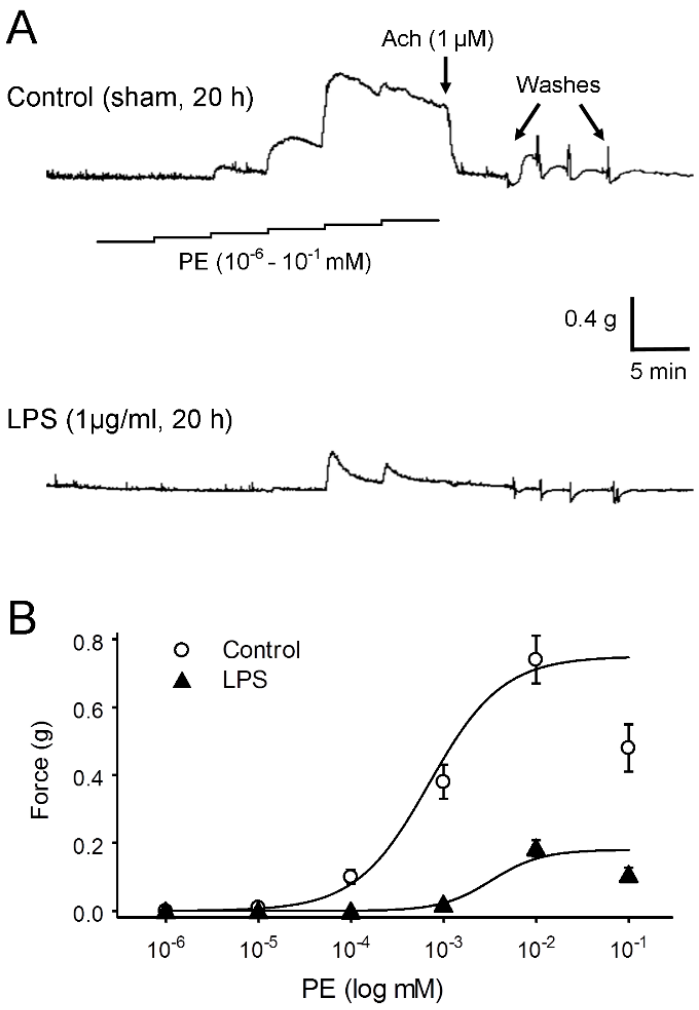

Figure 5-1 The hyporeactivity of mesenteric rings to α-adrenergic agonist in endotoxin exposure.

A*. Concentration-dependent vasoconstrictions are clearly seen in the control ring (upper) with PE (10^{-9} to 10^{-4} M). The same PE exposure had rather weak effect on the mesenteric ring treated with LPS (1μg/ml) overnight (lower). Both control and LPS-treated rings in each set comparative experiment, cultured in the same condition for 20 h except LPS, and treated in the same way during the experiment. Lower trace is sharing same label with the upper trace.* ***B****. The contractility of the rings is a function of PE concentrations. The relationship of the contractile force vs. PE concentration can be described by the Hill equation with* EC_{50} 7×10^{-7} *M and h 1.1 for the control group (n=12), and* 3.3×10^{-6} *M and h 1.6 for LPS treated rings (n=12), respectively. Data are presented as means ± S.E.*

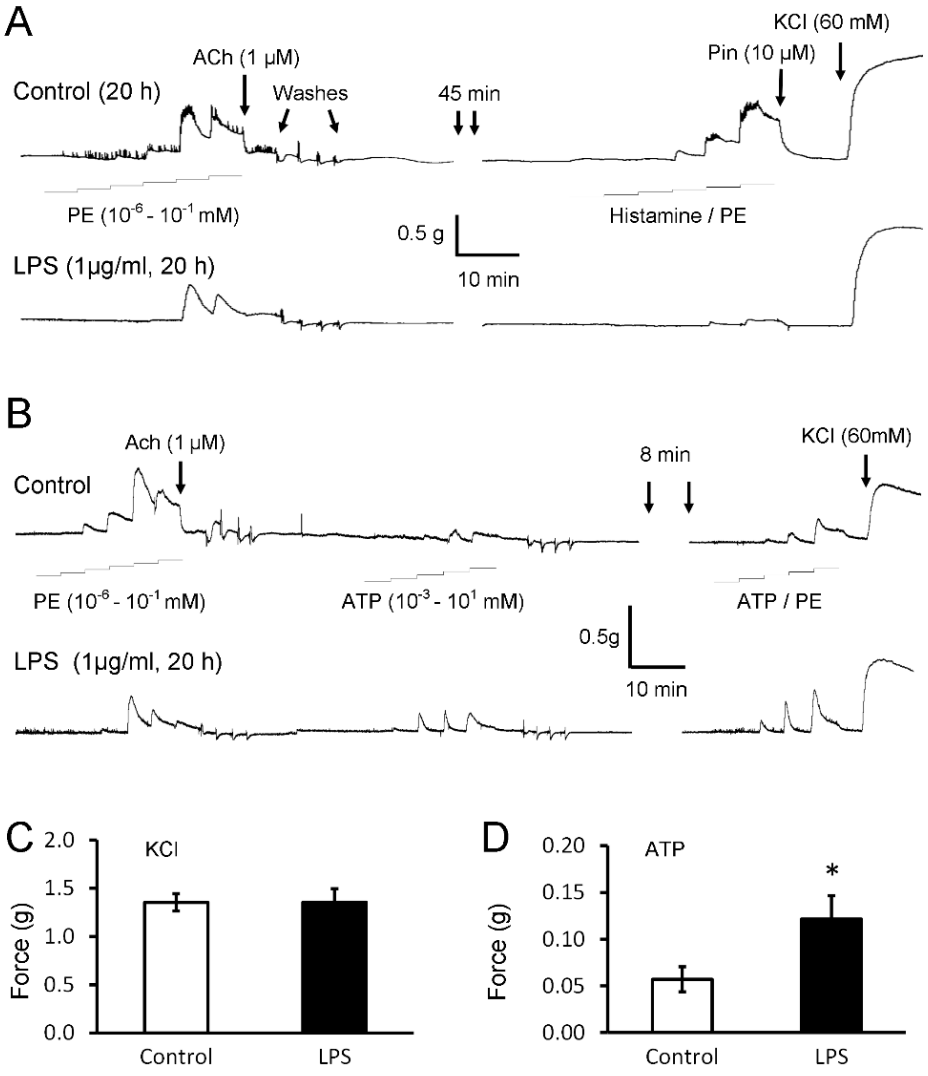

Figure 5-2 VSM contractile capability in endotoxin exposure.
A*. Parallel comparisons of the contractility of cultured mesenteric rings with and without LPS treatment. While the contractile force was developed well in the control ring (upper) in response to PE or histamine plus PE, the LPS-treated ring showed marked hyporesponsiveness to both vasoconstriction treatments (lower). Despite this, the LPS-treated ring responded to high concentration KCl (60 mM) as strongly as the control ring. Pin, pinacidil, a vasodilator. Note that there is a gap of 45 min between two arrows.* ***B****. Similar studies on the vasoconstriction effect of ATP, the natural agonist of P2X receptor. Although the LPS-treated ring was hyporeactive to PE, its vasoconstriction responses to ATP and KCl did not show any reductions. Instead, the contractility of the LPS-treated ring was slightly stronger than the control.* ***C****. Statistically, no significant difference in contractile forces was found between the LPS-treated and control rings ($P > 0.05$, $n = 12$).* ***D****. No clear reduction in contractile force in the LPS-treated rings in response to ATP (10^{-3} M) either ($P > 0.05$, $n = 6$).*

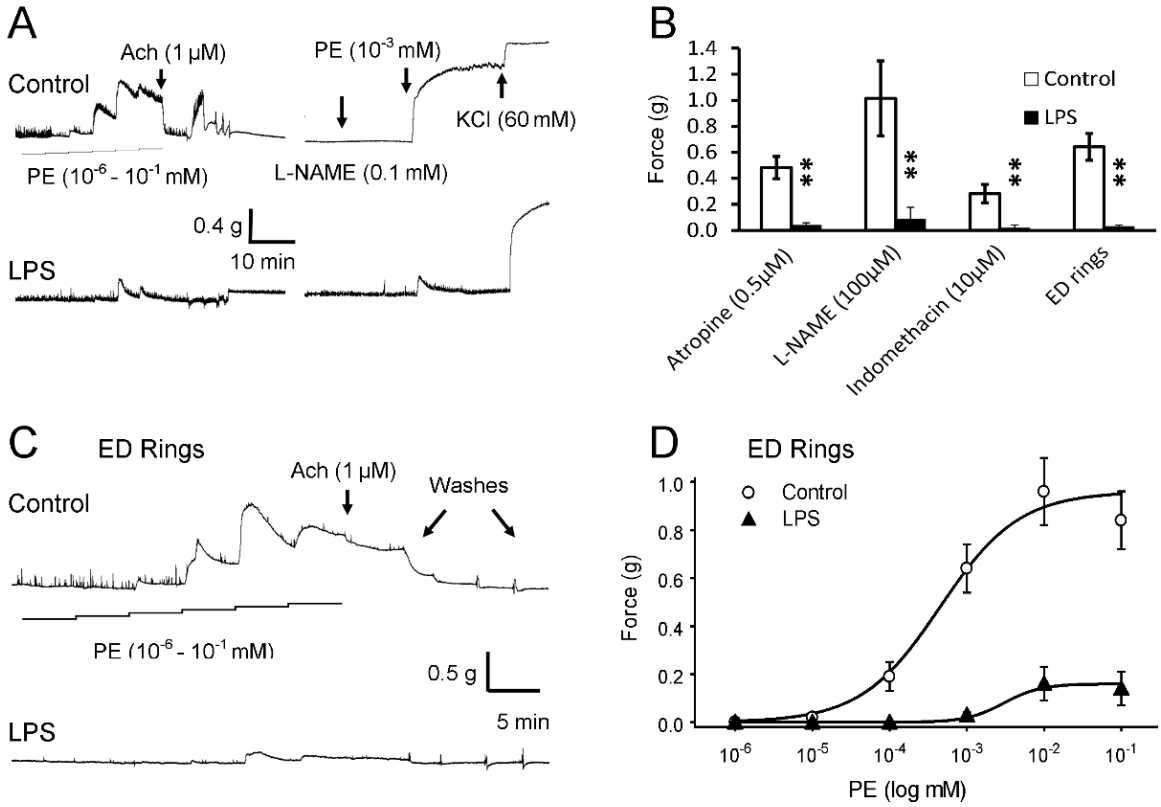

Figure 5-3 Endothelium-derived vasodilators.
***A**. A treatment with the NOS inhibitor L-NAME (100 µM) for 10 min did not eliminate mesenteric arterial hyporeactivity to PE. **B**. Significant reduction in the vascular response to PE was seen in the LPS-treated rings in the presence of L-NAME. The hyporeactivity existed in the presence of the muscarinic receptor antagonist atropine (0.5 µM) and the nonselective COX inhibitor indomethacin (10 µM) as well. **, P<0.01 (n =14 to 27). **C**. The mesenteric ring remained hyporeactive to PE after the endothelium was removed mostly as evidenced by the lack of response to Ach. **D**. The force-PE concentration relationships in the ED rings were comparable to those shown in Fig. 5-1B.*

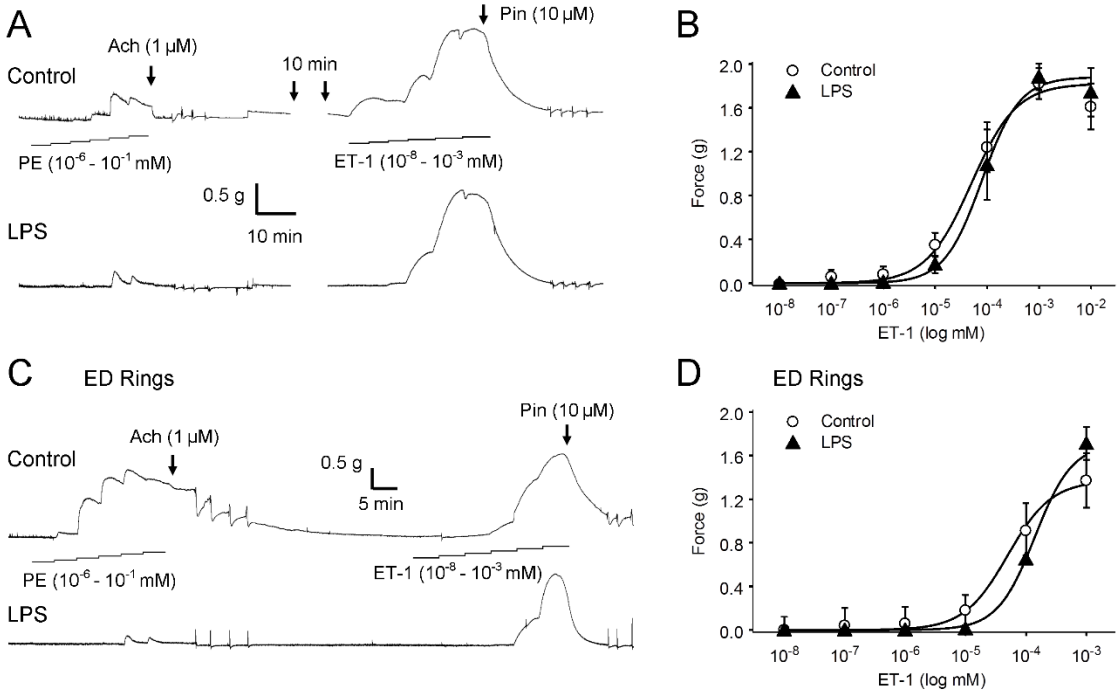

Figure 5-4 Lack of hyporesponsiveness to ET-1 in endotoxin exposure.
A. *In EI rings, ET-1 exposures produced strong vasoconstrictions in presence and absence of LPS in the culture media.* ***B****. Concentration-response curves of ET-1 in LPS-pretreated rings resemble that in control rings. See Table 1 for the* EC_{50} *and h values (n=6).* ***C,D****. Similar effects of ET-1 were observed in ED rings.*

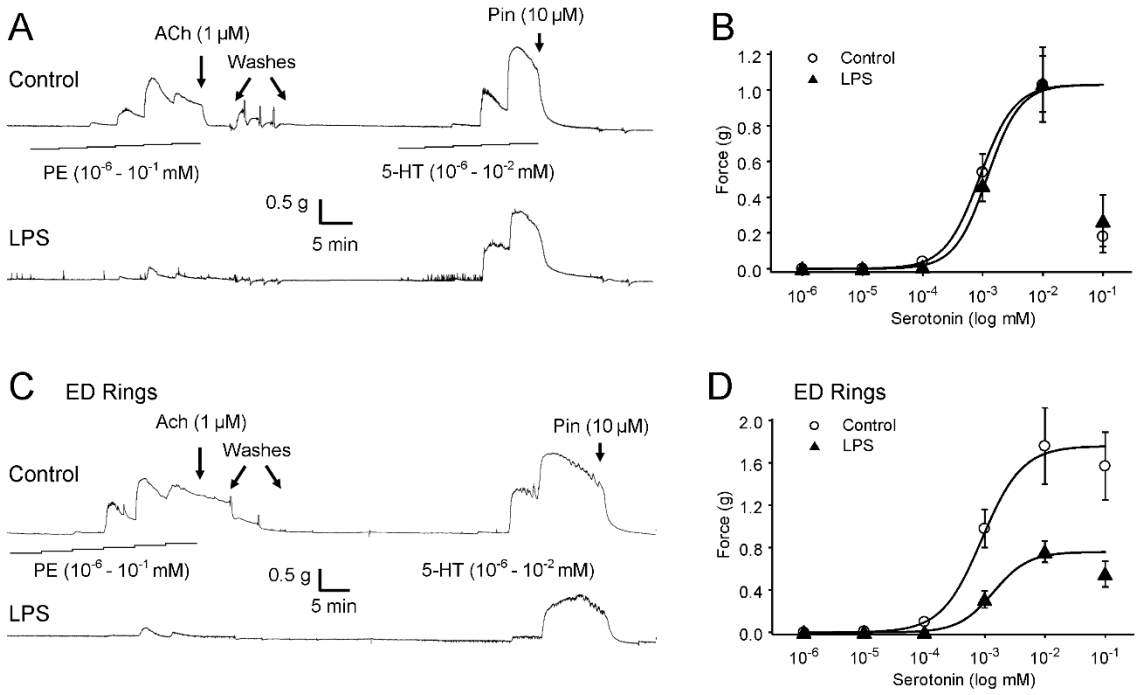

Figure 5-5 5-HT in the endotoxin-induced hyporeactivity.
A,B*. Concentration-dependent vasoconstrictions were found with 5-HT (10^{-9} to 10^{-5} M) with or without LPS treatment in EI rings.* ***C,D****. The contractility of mesenteric rings in response to 5-HT (10^{-9} to 10^{-5} M) was reduced after endothelium was removed from the rings before tissue culture. See Table 1 for the EC_{50} and h values.*

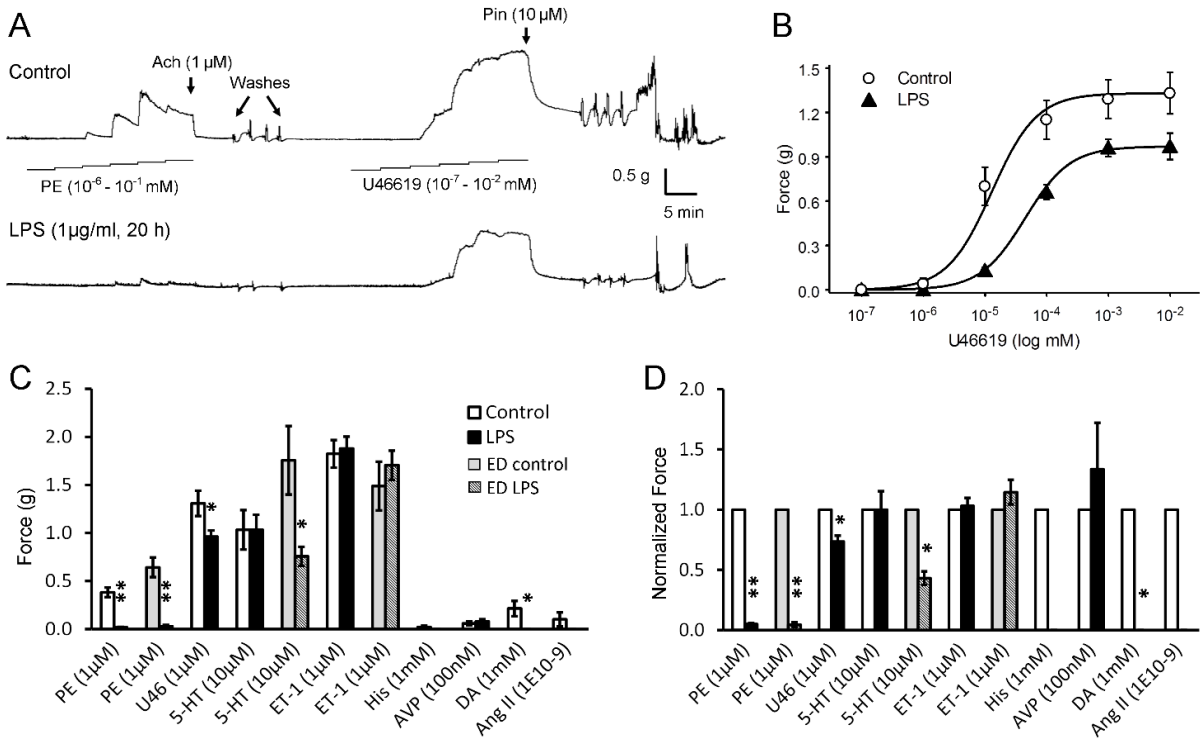

Figure 5-6 Responses of mesenteric rings to various vasoconstrictors.
A,B. *The ring responses to the TXA_2 receptor agonist U46619 (10^{-10} to 10^{-5} M) were reduced only moderately with the LPS treatment.* ***C****. The LPS exposure caused significant reductions in the vascular responses to PE, U46619, Hist, dopamine (DA) and Ang II in EI rings. The ED rings were significantly hyporeactive to 5-HT.* ***D****. Concentration-responses curves of AVP in control and LPS-treated groups. *, P<0.05; **, P<0.01 (n= 4 to 38).*

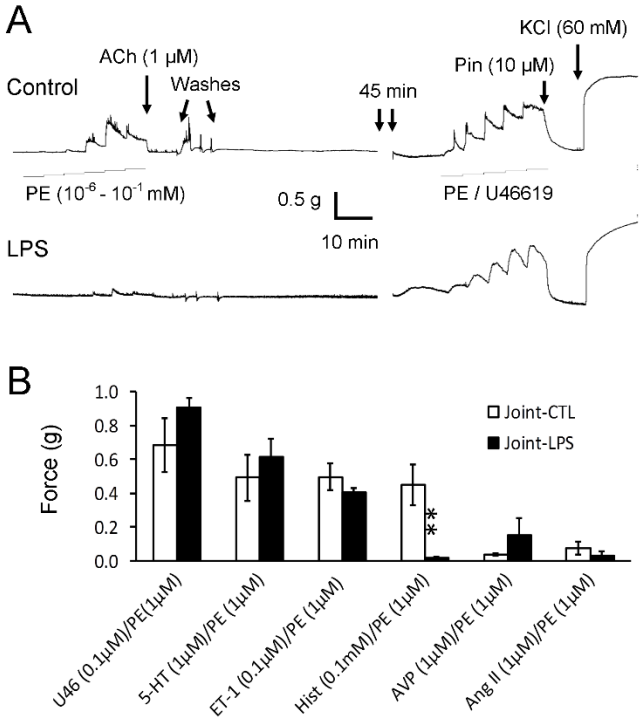

Figure 5-7 Effects of joint application of two vasopressors on the hyporeactivity.
A*. Although the LPS-treated ring was hyporeactive to PE, its responses to a joint application of U46619 (10^{-10} to 10^{-6}) and PE (10^{-9} to 10^{-5}) resembled those of the control ring.* ***B****. Comparison of the joint effect between the control and LPS group. Significant hyporeactivity was found only to the Hist/PE group.*

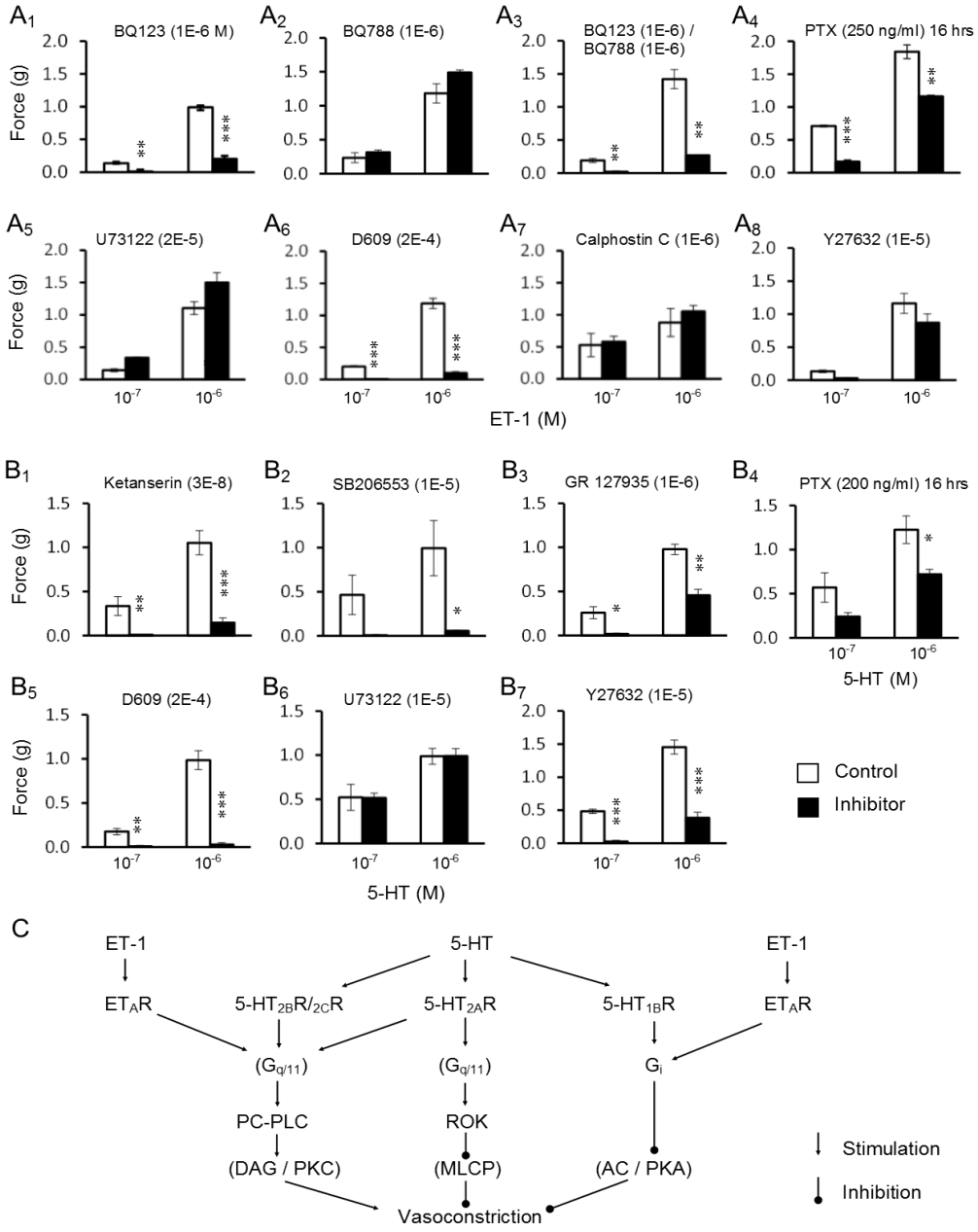

Figure 5-8 Dissection of cellular signaling systems for mesenteric constriction by ET-1 and 5-HT.

A_1-A_8. *Comparison of vasoconstriction in the presence versus absence of blockers. BQ-123, ET_AR antagonist; BQ-788, ET_BR antagonist; PTX, pertussis toxin, a $G_{i/o}$ protein inhibitor; D609, PC-PLC inhibitor; U73122, PI-PLC inhibitor; Y27332, Rho kinase inhibitor. Numbers in parentheses are inhibitor concentrations. B_1-B_7. Effect of blockade of 5-HT signaling on vasoconstriction. Ketanserin, 5-HT_{2A}R antagonist; SB206553, 5-$HT_{2B/2C}$R antagonist; GR127935, 5-HT_{1B}R antagonist.* ***C.*** *Schematic cellular signaling pathways for ET-1 and 5-HT in the mesenteric arterial smooth muscles. Players in parentheses are 2nd messengers that are commonly accepted in the pathway. All others are supported by our data.*

6 RESULT 2: OPTOGENETIC INTERVENTION TO THE VASCULAR ENDOTHELIUM

Published as **Shuang Zhang**, Ningren Cui, Yang Wu, Weiwei Zhong, Christopher M. Johnson, Jiang C. Vascul Pharmacol. Optogenetic intervention to the vascular endothelium. ***Vascul Pharmacol***. 2015 Nov;74: 122-9.

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6.1 Abstract

Endothelium lining the interior of cardiovascular system and most visceral organs plays an important role in vascular function. Its dysfunction occurs in some of the most challenging diseases. An important function of the endothelium is to release vasoactive substances that act on the smooth muscle to change vascular tones. Substance secretion from endocrine cells relies on membrane potentials and firing activity, while it is unclear whether the membrane potential regulates substance release from the ECs. Understanding of this requires selective intervention to membrane potentials of the endothelial cells *in situ*. Here we show a novel intervention to endothelial cells using the optogenetic approach. A strain of transgenic mice was developed with the Cre-loxP recombination system. These transgenic mice expressed channelrhodopsin (ChR) in endothelial cells driven by the vascular endothelial cadherin or *cdh*5 promoter. Linked in a tandem with YFP, the ChR expression was detected by YFP fluorescence in various endothelium-lining tissues and organs. The YFP fluorescence was observed in the lumen of

blood vessels and pericardium, but not in tissues beneath the endothelium lining. Optostimulation of dissociated endothelial cells evoked inward currents and depolarization. In the isolated and perfused heart, surprisingly, optostimulation of endothelial cells produced fast, robust, reproducible and long-lasting vasoconstriction that was not blocked by either ET-1A or TXA_2 receptor antagonist. Similar optical vasoconstriction was found in the isolated and perfused kidney. These results indicate that the optogenetics is an effective intervention to vascular endothelium where optostimulation produces vasoconstriction.

6.2 Introduction

Endothelium, a single layer of cell lining in the cardiovascular system, lymph vessels and several internal organs, plays a critical role in vascular function including substance exchange, vascular tone regulation, angiogenesis and thrombosis (Lerman and Zeiher, 2005). Endothelial dysfunction contributes to several cardiovascular diseases such as hypertension, shock, stroke and diabetic vascular complications (Endemann and Schiffrin, 2004).

The endothelium is a major tissue with approximately 10^{13} cells in an adult human (Cines et al., 1998), a number that is 100 times more than all neurons in the brain. The endothelium tissue not only is a mechanical barrier between blood and other tissues, but also resembles the endocrine system (Inagami et al., 1995). Indeed, a prominent function of ECs is to release vasoactive substances that act on the SM to produce vasoconstriction or vasodilation, maintaining a homeostatic state of vascular tones under different conditions. How the ECs selectively release vasodilators or vasoconstrictors is unclear.

Most endocrine cells are excitable, which secrete hormones and transmitters via depolarization and firing activity (Lowe et al., 1988). In contrast, ECs are nonexcitable. Whether the membrane potential regulates substance release from the ECs remains elusive. It is known

that the membrane potential can affect a number of ion channels, transporters, intracellular Ca^{2+}, intracellular pH, etc. Activity of these molecules can in turn change cellular functions. Therefore, it is reasonable to believe that depolarization can affect the endothelium function. If such an assumption is proven correct, then it would be possible to reveal whether the endothelial depolarization results in vasodilation or vasoconstriction. To address these issues, novel methods are needed to selectively intervene to the membrane potentials of ECs when they remain interacting with vascular SM *in situ*, which will help to understand EC function in vascular tone regulation, and may have impacts on therapeutic designs for several cardiovascular diseases.

The successful development of optogenetics in neuroscience research (Boyden et al., 2005; Nagel et al., 2005) provides a unique way to access the endothelial membrane potentials with light. Generally, the optogenetics is a combination of genetics, electrophysiology and optics to control well-defined events within specific cells of living tissue by expressing an opsin in the cells. This approach allows to control membrane potentials and excitability of the opsin-expressing cells, which has been achieved in various types of neurons (Williams and Deisseroth, 2013), glia (Figueiredo et al., 2011), myocardium (Arrenberg et al., 2010), skeletal muscle cells (Sakar et al., 2012) and the vascular smooth muscle (Wu et al., 2015).

To demonstrate whether the EC function can be manipulated by optogenetics, we developed a new strain of mice that expressed channelrhodopsin (ChR) in ECs. The expression of ChR was detected in the lumen of blood vessels from multiple organs. Characteristics of optoactivation of ECs, including membrane excitability and vasomotor reactivity, were studied in the heart and kidney using combined optostimulation and traditional physiological techniques. Surprisingly, our results indicated that optostimulation of ECs can produce fast, reproducible, long-lasting vasoconstriction that is comparable in strength to the popular vasoconstrictor PE.

6.3 Results

6.3.1 Generation of transgenic mice with ChR expression in ECs

To achieve endothelial expression of ChR, we took the advantage of two strains of commercially available mice, *cdh5* promoter-driven Cre mice (B6.FVB-Tg(*Cdh*5-Cre)7Mlia/J, Stock No. 006137; JaxMice) (Alva et al., 2006) and ChR-loxP mice (B6; 129S-Gt(ROSA)26Sor$^{tm32(CAG-COP4*H134R/EYFP)Hze}$/J, Stock No.012569; JaxMice) (Madisen et al., 2012), to generate a new strain of transgenic mice driven by the *cdh5* promoter. The *cdh*5 is gene encoding VE-cadherin, also known as cadherin 5 or CD144, expressed mainly in endothelium. The *cdh*5 promoter has been previously demonstrated in adult/quiescent endothelium (Gory et al., 1999; Hisatsune et al., 2005), and also successfully used to direct EC-specific expression of several genes (Liu et al., 2013). Therefore, we employed the homozygous *cdh*5-Cre mice to cross-bred with heterozygotous ChR-loxP mice. Fifty percentage of their offspring mice were tested positive for both Cre and ChR-loxP expression (Fig. 6-1B), suggesting that these cdh5-ChR mice have gained the expression of ChR as a result of the Cre-loxP mediated crossover.

6.3.2 Expression of ChR-eYFP in the endothelium

The expression of ChR was indicated by YFP fluorescence as it was fused to a tandem protein (Fig. 6-1A). Indeed, the Cre and ChR-loxP positive mice showed strong YFP fluorescence on the luminal side of the aorta, pulmonary artery and coronary artery (Fig. 6-2A,B). In the transverse section of the heart, YFP fluorescence was detected in the surface pericardium and interior blood vessels, while the myocardia did not show YFP fluorescence (Fig. 6-2C). A large number of blood vessels in the dura matter of the brain were YFP-positive (Fig. 6-2D). In the transverse section of the kidney, glomeruli, arcuate artery, and interlobular artery were also fluorescent in cdh5-ChR mice (Fig 6-2E). In mesentery membrane from cdh5-ChR,

both of vein and artery were clearly seen fluorescent (Fig. 6-2F). In contrast, such YFP fluorescence was not seen in any of these tissues obtained from the WT mice (Fig 6-2G-L).

6.3.3 Effects of ChR activation on membrane potentials and ionic currents in acutely dissociated ECs

The presence of YFP fluorescence in a variety of blood vessels suggests that ChR is expressed in ECs. To demonstrate that is indeed the case, we performed functional studies of ionic currents in acutely dissociated ECs. In voltage clamp, ECs from WT mice showed whole-cell currents that did not respond to optostimulation (Fig. 6-3A). In ECs obtained from cdh5-ChR mice, optostimulation at 10, 50 and 100 ms of blue light (470 nm) produced an inward current (Fig. 6-3B). The amplitude of this current was large at hyperpolarizing membrane potential, decreased with depolarization, and reversed its polarity when the membrane potentials were more positive than 0mV. The current showed almost no adaptation with long-lasting optostimulation (Fig. 6-3C). In I/V plot, the current showed a weak inward rectification with a reversal potential at -10mV (Fig. 6-3D). Activation of this current led to strong depolarization of the ECs, which averaged by -15.2 mV from baseline at -45.3 ± 11.6 mV (n=4) (Fig. 6-3E,F). The optical depolarization was also seen in longer time optostimulation up to 50 and 100 ms (Data are not shown).

6.3.4 Contraction of coronary arteries by optostimulation in isolated and perfused hearts

The large inward current and depolarization produced by optostimulation should have impacts on endothelial function and vascular tones. To reveal the functional consequences of the optostimulation, we studied the coronary circulation in the Langendorff heart preparation. The specificity of optostimulation was tested with both blue (470 ± 40 nm) and yellow light (602 ± 70 nm). Only blue light triggered significant vasoconstriction, but not yellow light (Fig. 6-4A,B).

When the heart was exposed to blue light covering either left or right coronary arteries on ventricle, the resistance of coronary circulating increased markedly with perfusion pressure changes by 23.5 ± 6.9 cm H_2O (n=5), indicating coronary vasoconstriction (Fig. 6-4C,E). The light beam covered main left coronary artery, left circumflex branch, left anterior descending branch, left marginal artery and great cardiac vein on the left side of ventricle. On the right ventricle surface, the light beams covered main coronary artery and major branches, including conus arteriosus branch, right anterior ventricular and right marginal arteries, and anterior and small cardiac veins. No obvious different responses were seen between the left and right optical approaches to the heart ventricle. The heart rate and pulse pressure were not affected by optostimulation (Fig. 6-4 C,G). The coronary vasoconstriction by optostimulation was nicely reproducible with repetitive light exposures, and showed no adaption with a long optostimulation up to 6 min (Fig. 6-4C). Interestingly, a prior exposure to PE (10^{-5} M) or Isop (10^{-5} M) largely diminished the coronary vasoconstriction by optostimulation, suggesting that they may share the same ionic mechanisms in the SM. In comparison, the optostimulation produced coronary vasoconstriction to the degree that was comparable to 10^{-5} M PE (26.0 ± 9.1 cm H_2O, n=4) and 60 mM KCl (24.2 ± 7.9 cm H_2O, n=4) with no significant difference ($P > 0.05$, Fig. 6-4C,E). The coronary vasoconstriction by optostimulation had fast time response with the onset and offset time to be ~ 10 sec (Fig 4F). Such coronary vasoconstriction was not found in WT heart when it was exposed to the same light stimulation (Fig. 6-4D).

6.3.5 Optical vasoconstriction in isolated and perfused kidney

Optostimulation also produced vasoconstriction in the renal circulation. When blue light was applied to the surface of the isolated and perfused kidney. In the kidney, repetitive optostimulations gave rise to repetitive increases in the perfusion pressure (13.9 ± 3.3 cm H_2O,

n=9) with no clear adaptions (Fig. 6-5A). The optostimulation-induced renal vasoconstriction was significantly reduced when vasoconstriction was induced by PE (10^{-5} M) (Fig 5A). It was partially recovered when the effect of PE largely declined (Fig. 6-5A). The onset and offset times of the optical vasoconstriction was similar to those in the heart (11-14 sec) (Fig. 6-5C). The optical vasoconstriction was reproducible without marked adaptation (Fig. 6-5A,D). Compared to the vasoconstriction produced by 10^{-5} M PE (110.0 ± 35.2 cm H_2O) and 60 mM KCl (84.9 ± 20.9cm H_2O), the optical vasoconstriction of the renal circulation was significantly smaller ($P < 0.001$, freedom=11 and 14, separately). In contrast, optostimulation did not induce any response in the circulation resistance of WT kidney (Fig. 6-5C).

6.3.6 Mechanism underlying optostimulaiton induced vasoconstriction

To address the underlying mechanism, two preeminent endothelium-derived vasoconstrictors, ET-1 and TXA_2 were tested in perfused hearts of cdh5-ChR mice. In the presence of the ET-1A receptor blocker BQ-123 (10^{-6} M), blue light produced the same levels of vasoconstriction (Fig. 6-6 A,B). The vasoconstriction by optostimulation was not attenuated with the TXA_2 receptor antagonist SQ-29548 (10^{-6} M) either (Fig. 6-6 C,D). Therefore, the optostimulation-induced vasoconstriction does not seem to be mediated by these two vasoconstrictors released from endothelium.

6.4 Discussion

To our knowledge this is the first report demonstrating the application of optogenetics to the endothelium tissue. By taking the advantage of Cre-LoxP recombination system, we created a new strain of mice that express ChR in ECs directed by the *cdh*5 promoter. In the cdh5-ChR mice, we observed YFP expression in ECs from various tissues and organs. Our functional

assays indicated that photo currents and depolarization can be produced in ECs, and optostimulation evokes vasoconstriction in the heart and the kidney.

Several tissue-specific promoters have previously been used to direct gene expression in ECs, such as Tie-1 and Tie-2-, PECAM, Flk-1, and SCL (Gothert et al., 2005; Gustafsson et al., 2001; Kisanuki et al., 2001; Licht et al., 2004; Terry et al., 1997). Although EC expression of desired genes are reported, unexpected expression has been found in these transgenic lines including hematopoietic lineages and other cell types (Alva et al., 2006). Such non-specific expression limits their transgenic applications, although they were widely used for gene deletions. Thus, more selective promoters are needed to generate transgenic models with specific expression in differentiated endothelium.

Cadherin-5 (Cdh5 or CD-144), a transmembrane glycoprotein, is an essential player in the morphogenesis of the vascular system, modulation of blood flow, vascular endothelial growth factor (VEGF) signaling, and vascular permeability in a variety of tissues (Carmeliet et al., 1999; Corada et al., 2001; Venkiteswaran et al., 2002). The *cdh*5 promoter has been characterized in adult/quiescent endothelium, and used to introduce specific gene expression in ECs (Alva et al., 2006). The success of EC-specific expression thus has led to the generation of mice with Cre recombinase expression in endothelium (Alva et al., 2006), which is now commercially available (Jackson lab). These mice have also been successfully used to modify gene expressions previously (Fioret et al., 2014; Guo et al., 2008). By taking the advantage of the *cdh*5-Cre mouse strain and another commercially available ChR-loxP strain, we have generated a new strain of mice. This strain of cdh5-ChR mice expresses ChR in a tandem with YFP. Fifty percent offspring (cdh5-ChR) of the mating pair of cdh5-Cre and ChR-loxP mice showed both

Cre (100 bp) and ChR-EYFP (212 bp) bands without any detectable physical and behavioral abnormalities.

Our morphological studies have shown that YFP fluorescence is detected in various tissues and organs. The YFP fluorescence is observed in the luminal lining of arteries including the aortic, pulmonary, coronary, renal, mesenteric and basilar arteries. It is also seen in renal, dura, skeletal muscle veins as well as pericardium and mesentery. Therefore, the cdh5-ChR mice appear to express ChR as expected.

These YFP positive ECs indeed express ChRs. Direct activation of these cationic channels with 470 nm blue light in acute dissociated ECs evokes large and long-lasting inward currents in voltage clamp with reversal potential at -10mV consistent to the cationic permeability of the ChR. The photo currents show moderate inward rectification in the I-V plot with no clear adaptation/inactivation in response to prolonged light exposure up to 100 ms. In current clamp, optostimulation produces depolarization of the ECs. These electrophysiological studies indicate that functional ChR is expressed in ECs obtained from the cdh5-ChR mice.

Consistent with the expression of ChR in ECs, we have found that the optostimulation increases perfusion resistance in coronary and renal circulation in isolated organs from the cdh5-ChR mice. This optostimulation is specific for blue light that activates ChR, and optical vasoconstriction is produced in organs obtained from cdh5-ChR mice but not from the WT. This suggests that the optical vasoconstriction is not produced by endogenous photo-sensitive mechanisms. It is known that in addition to endothelium, cdh5 is expressed in other cells including certain blood cells (Alva et al., 2006). The possibility of blood cell involvement may largely be ruled out in our in-vitro organ perfusion, because the blood cells are mostly removed in the preparations, and because the blood cells tend to release vasodilators rather

vasoconstrictors. This light-produced vasoconstriction in heart and kidney is fast, reproducible and long-lasting with no obvious decline in perfusion resistance in 6 min. This optical vasoconstriction in coronary circulation is as strong as the popular clinical vasoconstrictor PE at high concentration (10^{-5} M) and 60mM KCl. In the isolated kidney, the optical vasoconstriction is less potent than both of that produced by 10^{-5} M PE and 60 mM KCl, which is likely to be a result from the high expression level of α1 adrenoceptor and poor transparency of the organ.

In the heart, only can a part of coronary circulation (~ 30 % of surface area of the whole heart) be illuminated by the light beam during experiments. Despite that, optostimulation still produces potent vasoconstriction, which is comparable to that produced by 10^{-5} M PE or 60mM KCl in the heart. In addition to the robust response, this optogenetic method also provides a high temporal precision. The onset and offset time constant are short, within 11s in heart and 14s in kidney. This spatial and temporal performance of optostimulation on ECs in isolated and perfused organs seems to allow a selective access and manipulation of ECs with the optogenetic approach.

We have realized some limitations of the optostimulation in the perfused organs: 1) The tissue transparency is limited, especially in the isolated kidney. 2) Not all sections of blood vessels can be covered by the light beam with ~ 3 mm in diameter and 20 mW/mm^2 in strength. With the limited light penetration, therefore, the optostimulation seems to be effective only in the vessels on or near to the surface of the organ.

What produces the optical vasoconstriction is still unclear, which may involve several potentials mechanisms. Optostimulation may increase the release of endothelium-derived vasoconstrictors from ECs, such as ET-1, which is a peptide, while most peptide hormones and transmitters are packed in vesicles inside the cell. The vesicle release is a Ca^{2+}-dependent process

that usually occurs with depolarization. Other vasoconstrictors including TXA_2 and hydroxyeicosatetraenoic acids are lipid metabolites. Their productions are more important in their biological effects than the releasing mechanism. It is possible that some of their biosynthetic and degradative enzymes are affected by certain events associated with depolarization. Our results suggest that neither ET-1 nor TXA_2 seems to play a major role in optical vasoconstriction, as the optical vasoconstriction remains when their receptor antagonists are present in the perfusion solution.

A decreased release of endothelium-derived vasodilators from ECs may be attributable as well, including NO, prostacyclin, and 11,12-epoxyeicosatrienoic acids. The release and production of these endothelium-derived vasodilators are regulated by several endothelium-targeting substances such as acetylcholine, bradykinin and histamine. How these molecules are affected by membrane potentials, and whether a decreased release of the endothelium-derived vasodilators occurs with optostimulation are still not clear. K^+ is a potential player too, which can be released from ECs by optical depolarization, and subsequently produces depolarization of the SM. Another possibility is the gap junction between ECs and SM cells, with which the light induced EC depolarization may be coupled to the SM leading depolarization and vasoconstriction. Testing these possibilities requires systematic studies including screening potential contributors, pinpointing the critical players and elaborating their action mechanisms, Clearly this is a major undertaking that cannot be accomplished in the present study. Nevertheless, the demonstration of the feasibility of optoactivation of ECs in the present study appears to affirm a powerful approach for further investigation of the vasoconstriction produced by depolarization.

The application of optogenetics to endothelium may have several implications on future research and therapeutical agent tests. 1) This method may allow researchers to identify endothelium-derived substances *in situ* without introducing any receptor agonists/antagonists that tend to have widespread effects on vasculatures and nearby tissues. 2) The high temporal and spatial precision may provide a new way to approach local ECs lining the vasculature without systematical administration of drugs. 3) The local optostimulation-induced vasoconstriction may be helpful to manipulate the interaction among ECs, SM cells, and immune cells, as well as certain substances, including endothelium derived factors/mediators and cytokines. 4) The optical manipulation of ECs may be useful to control the EC dysfunction, vascular growth and inflammation that are known to participate in cardiovascular pathogenesis. 5) Beyond cardiovascular field, the accuracy in specific location of optical stimulation could be used to reveal the mechanism of neuron-pericyte-vasculature coupling in certain brain area, as neurons regulating homeostatic function may require constant blood supplies. 6) The activation of ECs may affect the permeability of the capillary endothelium (Frohlich, 2002), helping drugs to pass through certain tissue barriers such as the blood brain barrier and placenta barrier, which may have impact on drug delivery. We expect that all of these potential applications of the optical activation of ECs to develop and expand rapidly when further investigations take place.

In conclusion, the present study demonstrates a novel intervention to vascular ECs. This method is based on newly developed optogenetics and the availability to transgenic mice, and allows us to express selectively ChR in ECs. Positive ChR expression is identified with YFP fluorescence in the endothelial lining in various tissues. Optostimulation of the ECs that express ChR triggers large inward currents, leading to depolarization. In isolated and perfused organs, optostimulation produces vasoconstriction that is vigorous, reproducible and sustained. These

results indicate that the optogenetics in the endothelium seems to be a powerful intervention to the cardiovascular system.

6.5 Acknowledgment

This work was supported by the NIH grant R01-NS-073875. Shuang Zhang is a Molecular Basis of Disease (MBD) fellow of Georgia State University.

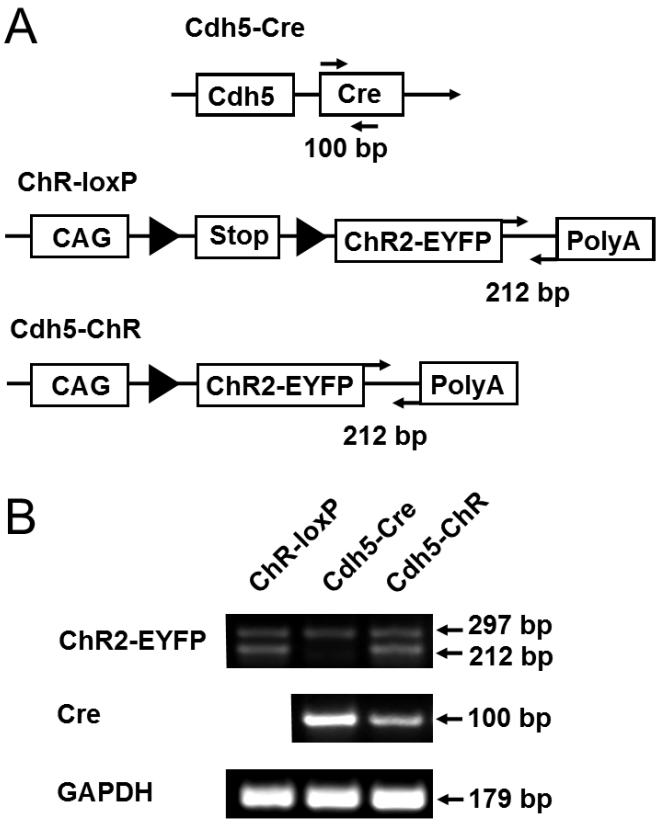

Figure 6-1 Generation of cdh5-ChR transgenic mice.
A. *Construction pattern of parental cdh5-Cre and ChR-loxP mice as well as their expected offspring that express both Cre and ChR2-eYFP. In the presence of Cre recombinase, the stop codon flanked by loxP (solid triangle) in the ChR-loxP mouse will be removed, resulting in expression of the ChR2-eYFP in a tandem driven by the CAG promoter in cdh5-ChR mice. Note that arrows indicate primers for genotyping with expected PCR fragment shown below.* ***B.*** *Primers targeted at the 3' UTR of ChR2-eYFP produced a 212 bp PCR product, and primers targeted at the Cre open-reading frame yielded a 100 bp. The presence of both 212 bp and 100 bp bands indicated positive Cre-loxP recombination producing the cdh5-ChR strain, while cdh5-Cre and ChR-loxP mice have only the 100 bp and 212 bp bands, respectively. Note that a ~300 bp non-specific band was found in all mice. Abbreviations: CAG, cytomegalovirus-immediate-early (CMV-IE) enhancer/chicken β-actin/rabbit β -globin hybrid promoter; PolyA, bovine growth hormone polyadenylation signal and flippase recognition target flanked phosphoglycerate kinase-Neo-polyA cassette; GAPDH, glyceraldehyde 3-phosphate dehydrogenase.*

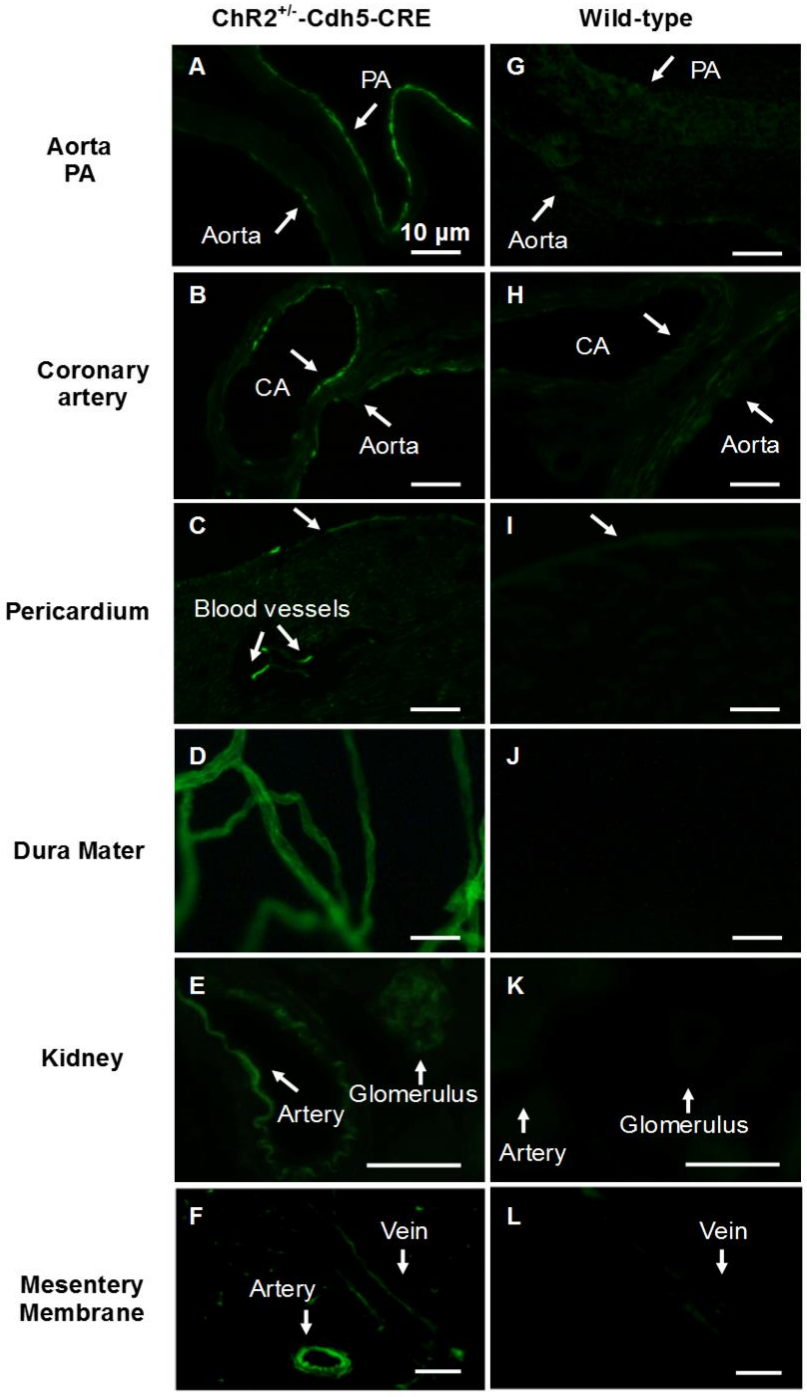

Figure 6-2 YFP fluorescence in various tissues and organs in cdh5-ChR (left column) and WT mice (right column).

In cdh5-ChR mice, strong fluorescence was seen on lumen side of aorta (A,B), pulmonary artery (PA) (A) and coronary artery (CA) (B). YFP fluorescence was also seen in pericardium (C), blood vessels in dura mater (D), renal arcuate artery (E), glomerulus (E), mesenteric artery (F) and mesenteric vein (F). In contrast, YFP fluorescence was not observed in these tissues and organs from WT mice (G-L). Scale bar is 10 µm.

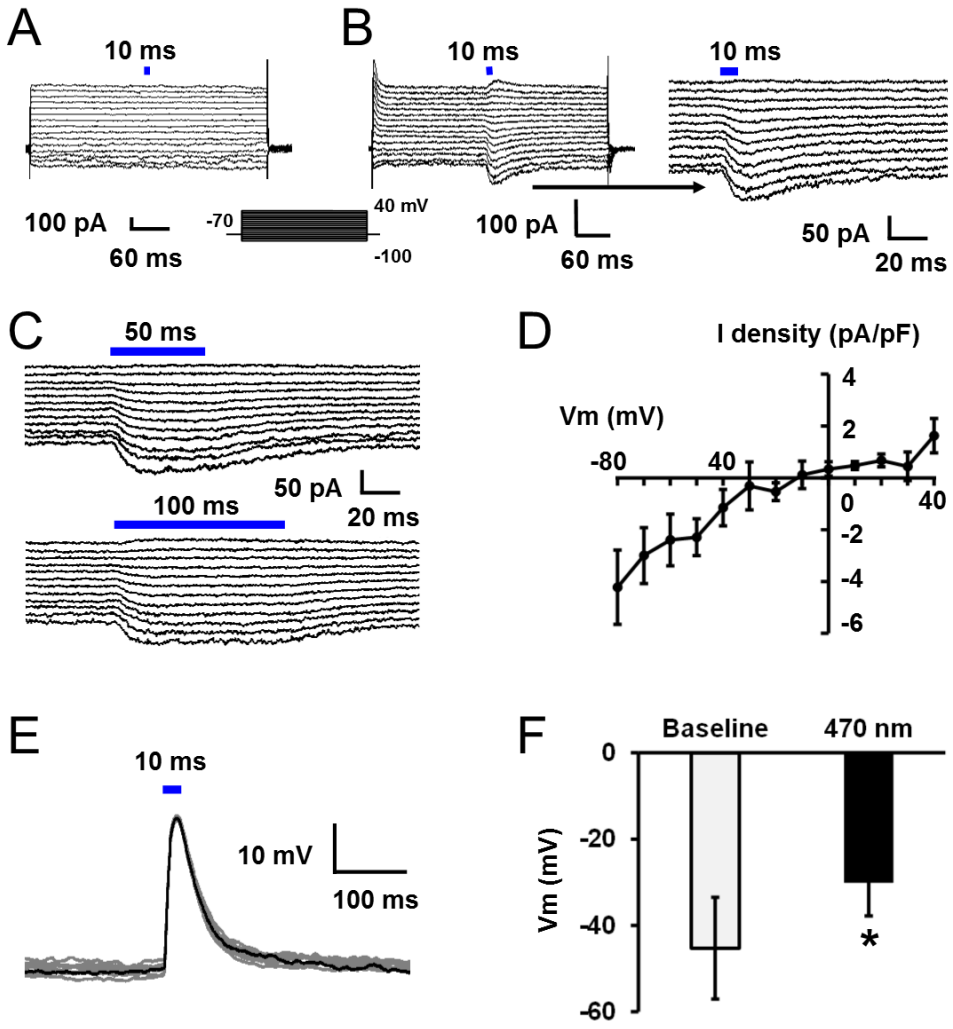

Figure 6-3 Optical excitation of acutely dissociated ECs from aorta.
A. *Whole-cell currents were recorded from an EC from a WT mouse in voltage clamp. Light stimulation (10 ms) did not produce any change of membrane currents. Steps of voltage commands (from -100 to 40 with a 10 mV increment) were applied to the cell at a holding potential of -70 mV.* ***B.*** *Optostimulation with 10 ms pulses of blue light (470 nm) to an EC from a cdh5-ChR mouse evoked inward currents. The amplitude of this current decreased with depolarization, and reversed its polarity when the membrane potentials were more positive than 0mV. The photo currents decayed slowly after turning off the light, which are better seen in the expanded display on the right panel.* ***C.*** *The inward photo currents responding to longer durations (50 and 100 ms) of blue light stimulations showed almost no reduction in current amplitudes.* ***D.*** *Current-voltage (I-V) relationship of the photo currents showed a reversal potential at -10 mV with moderate inward rectification.* ***E.*** *In current clamp, blue light stimulation produced strong depolarization in an EC from cdh-ChR mouse.* ***F****. Optostimulation produced significant depolarization. Data are presented as means ± SE (*, P<0.05, Student's t-test; n=4 cells).*

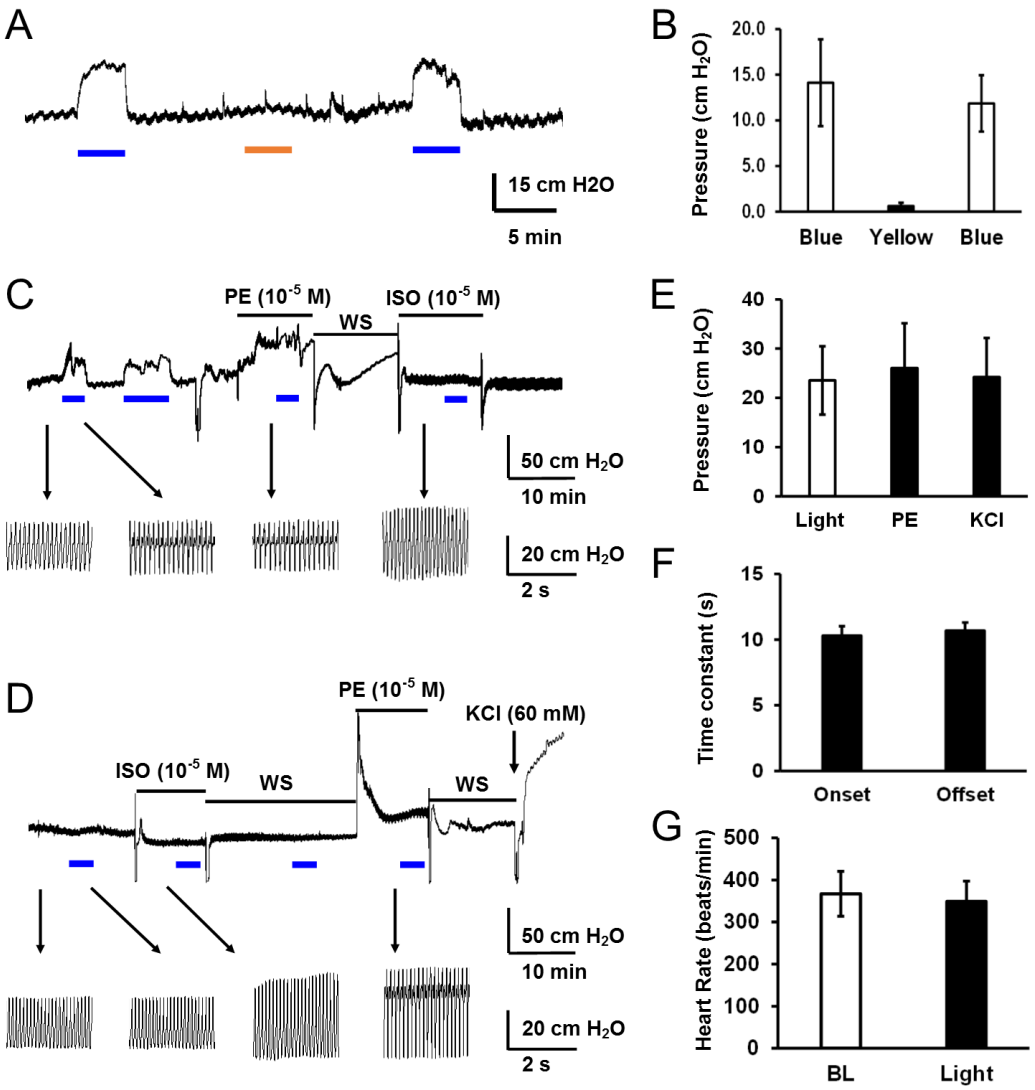

Figure 6-4 Optical vasoconstriction of coronary arteries by optostimulation.
A,B*. In isolated and perfused hearts from cdh5-ChR mice, significant coronary constriction was produced by blue light stimulation (470 nm), but not by yellow light (602 nm).* ***C.*** *Coronary constriction was produced by optostimulation followed by PE and isoproterenol (Isop) treatments. The optical coronary vasoconstriction was strong, long-lasting, reproducible with repetitive optostimulation, and did not cause evident changes in heart rate and pulse pressure. The latter both were augmented by Isop. Heart rate and pulse pressure were indicated by arrows toward the enlarged pattern on the bottom of the figure.* ***D.*** *Optostimulation did not produce any vasoconstriction in a heart from WT mouse.* ***E.*** *In the perfused heart from cdh5-ChR mouse, the optical vasoconstriction with ~20 mW/mm^2 blue light was competitive comparing to that produced by 10^{-5} M PE and 60 mM KCl.* ***F.*** *The optostimulation triggered fast response in heart perfusion with onset and offset time less than 11s.* ***G.*** *Optical vasoconstriction had no significant effect on heart rate.*

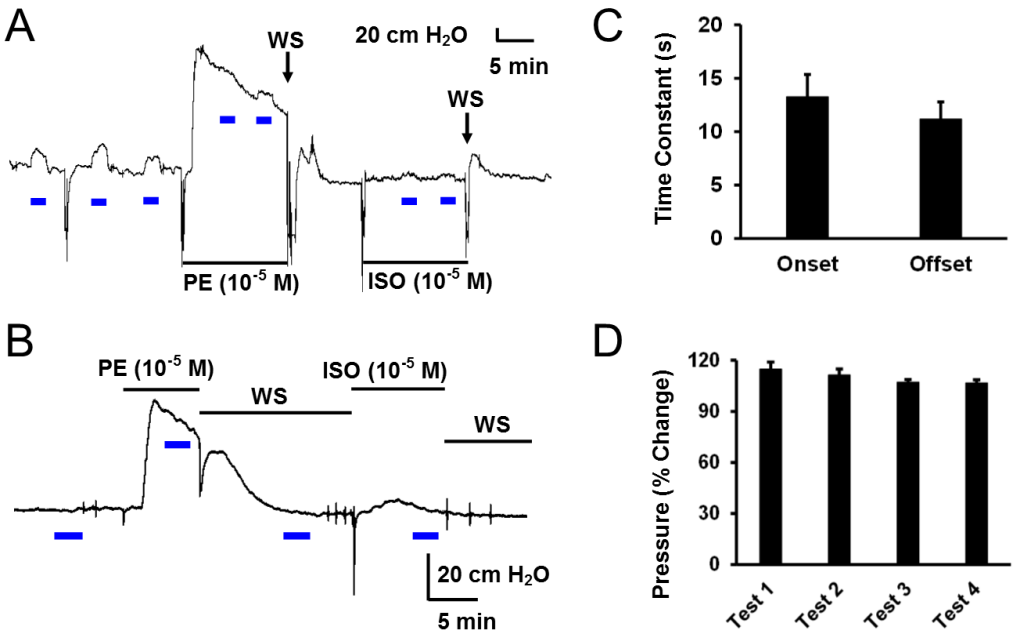

Figure 6-5 Optical vasoconstriction in isolated kidney.
A. *Blue light produced vasoconstriction in the kidney obtained from a cdh5-ChR mouse but not that from a WT mouse* ***(B)***. ***C.*** *The optostimulation has fast onset and offset (10-14s).* ***D.*** *Repetitive blue light stimulation produced up to 114.7% vasoconstriction in perfused kidney from cdh5-ChR mouse with slight decline in the amplitude. Blue light intensity is ~20 mW/mm^2 and stimulation duration is 2 min with 5 min interval.*

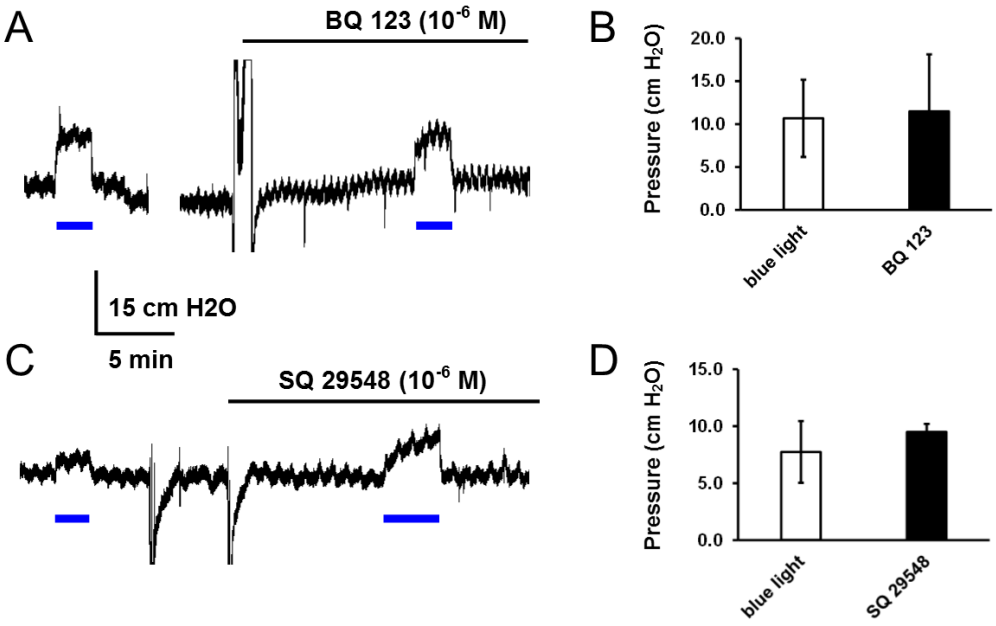

Figure 6-6 Effects of ET-1A and TXA2 receptor antagonists on optical vasoconstriction. ***A,B****. In isolated and perfused hearts from cdh5-ChR mice, coronary constriction was produced by blue light stimulation. The optical vasoconstriction remained in the presence of the ET-1A receptor antagonist BQ-123 (10^{-6} M).* ***C, D.*** *The optical vasoconstriction was not suppressed by the TXA_2 receptor antagonist SQ-29548 (10^{-6} M) either. Data are presented as means ± SE (n = 3 hearts, P > 0.05). Note that there is a gap of 10 min in A.*

7 RESULT 3: AN OPTOGENETIC MOUSE MODEL OF RETT SYNDROME TARGETING ON CATECHOLAMINERGIC NEURONS

Accepted as **Shuang Zhang**, Christopher M. Johnson, Ningren Cui, Hao Xing, Weiwei Zhong, Yang Wu, Chun Jiang. An optogenetic mouse model of Rett syndrome targeting on catecholaminergic neurons. ***J Neurosci Res***. 2016 April.

Note: Wiley, the publisher of Journal of Neuroscience Research, automatically granted the authors the permission for using article in a thesis and/or dissertation.

7.1 Abstract

Rett Syndrome (RTT) is a neurodevelopmental disorder affecting multiple functions, including the norepinephrine (NE) system. In the CNS, NE is mostly produced by neurons in the locus coeruleus (LC) where defects in intrinsic neuronal properties, NE biosynthetic enzymes, neuronal CO_2 sensitivity and synaptic currents have been reported in mouse models of RTT. The LC neurons in *Mecp2*-null mice show a high rate of spontaneous firing, while whether such hyperexcitability may increase or decrease the NE release from synapses is unknown. To activate the NE-ergic axonal terminals selectively, we generated an optogenetic mouse model of RTT where NE-ergic neuronal excitability can be manipulated with light. Using commercially available mouse breeders, we produced a new strain of double transgenic mice with *Mecp2* knockout and channelrhodopsin knock-in in catecholaminergic neurons. Several RTT-like phenotypes were found in the TH-ChR-*Mecp2*$^{-/Y}$ mice, including hypoactivity, low body weight, hind limb clasping, and breathing disorders. In brain slices, optostimulation produced depolarization and an increase in the firing rate of LC neurons from TH-ChR control mice. Optostimulation of presynaptic NE-ergic neurons augmented the firing rate of hypoglossal neurons in TH-ChR control mice, which was blocked by the α-adrenoceptor antagonist phentolamine. Such optostimulation of NE-ergic terminals had barely any effects on hypoglossal neurons from two to three TH-ChR-*Mecp2*$^{-/Y}$ mice, indicating that excessive excitation of presynaptic neurons does not benefit NE-ergic modulation in mice with *Mecp2* disruption. Also, these results demonstrate the feasibility to generate double transgenic mice for studies of RTT using commercially available mice, which are inexpensive, labor / time-efficient, and promising for cell-specific stimulation.

7.2 Significance Statement

RTT is a neurodevelopmental disease that occurs in 1 in 10,000 live-birth females globally. Over 25% of RTT patients die of unexplained causes, which is mostly attributable to their respiratory disorders and autonomic dysfunction involving the NE-ergic system in the brainstem. Thus, understanding the cellular mechanism for the abnormal NE-ergic system in RTT models is clinically significant. One characteristic feature of the NE-ergic defects is hyperexcitability in LC neurons, while the effect of the hyperexcitability on NE release is unclear. Here we created a double transgenic mouse model that allowed accesses to LC neuronal excitability with light. Our results suggest that the *Mecp2* disruption appears to impair NE release from axonal terminals, which cannot be compensated with extensive neuronal excitation.

7.3 Introduction

Rett Syndrome (RTT) is a neurodevelopmental disorder seen in 1 of every 10,000 female births. The disease affects multiple functions and systems including the norepinephrine (NE) system. As a result of the NE defect, people with RTT have breathing abnormalities and other autonomic dysfunctions that are also found in mouse models of RTT with *Mecp2* disruption (Julu et al., 2001). In the CNS, NE is mainly produced by neurons in the locus coeruleus (LC). These NE-ergic neurons in the LC project to the frontal cortex, hippocampus, cerebellum, spinal cord and multiple brainstem nuclei, brain regions that are involved in regulation of cognition, attention, anxiety, psychosis, motor control, and cardiorespiratory functions. Several previous studies have shown that LC neurons in *Mecp2*$^{-/Y}$ mice have decreased NE biosynthesis enzymes, defective intrinsic membrane properties, insufficient GABA synaptic inhibition, and increased excitability (Zhang et al., 2010; Zhong et al., 2015).

The NE content in the brainstem and whole-brain preparations is markedly reduced in *Mecp2*$^{-/Y}$ mice compared to the WT mice (Ide et al., 2005; Viemari et al., 2005). The NE metabolite 3-methoxy-4-hydroxyphenylethylene (MHPG) is low in the cerebrospinal fluid of RTT patients (Zoghbi et al., 1985). The decreases in NE content and NE metabolites occur earlier and appear more severe than changes in other neurotransmitters (Zoghbi et al., 1989). Application of exogenous NE or NE uptake inhibitors improves respiratory rhythm, increases the number of TH-positive neurons in the medulla, and extends the lifespan of *Mecp2*$^{-/Y}$ mice (Roux et al., 2007; Villard and Roux, 2006; Zhang et al., 2011b).

The defect in the NE system is associated with LC neuronal hyperexcitability or the increased firing rate, while their relationship is unclear. An increase in LC neuronal firing activity would theoretically enhance the NE release from presynaptic terminals, whereas the poor expression of TH and DBH may reduce NE biosynthesis in LC neurons hindering the effect of neuronal excitability. Although the increased LC neuronal excitability may be a compensatory response of LC neurons to the deficiency in NE production, whether such a process is indeed beneficial to the NE-ergic modulation remains to be demonstrated. The understanding of these problems relies on an animal model that carries the *Mecp2* defect and allows an access to the NE-ergic neuronal excitability. Therefore, we generate a new optogenetic mouse model of RTT by targeting on catecholaminergic neurons using commercially available mouse breeders, characterized several RTT-like phenotypes, and studied the NE-ergic neuronal modulation in brain slices.

7.4 Results

7.4.1 Generation of double transgenic mice

We took advantage of two strains of commercially available mice (TH-cre and ChR-loxP) and generated transgenic mice with ChR expression in catecholaminergic neurons. The heterozygous TH-ChR mice obtained were then used to cross-breed with heterozygous female *Mecp2*$^{+/-}$ mice. A half of their female offspring carrying ChR$^{-/-}$ was excluded for further studies. In the other half, mice with *Mecp2*$^{+/Y}$ and *Mecp2*$^{+/-}$ genotypes as well as ChR$^{+/-}$ were identified (Fig. 7-1A,B). The male TH-ChR-*Mecp2*$^{-/Y}$ and TH-ChR-*Mecp2*$^{+/Y}$ or TH-ChR mice were chosen as the experimental and control groups in the present study. In addition, a TH-ChR female was used as control as well.

In comparison to the littermate control TH-ChR mice 21.6 ± 1.4 g during age four to nine weeks old, the TH-ChR-*Mecp2*$^{-/Y}$ mice showed lower body weight 15.1 ± 0.9 g (P = 0.018 unpaired t-test). All double transgenic mice also exhibited hindlimb clasping, the characteristic features found in several mouse models of RTT at five weeks. The Fig. 7-1C was taken at week five. (Fig. 7-1C,D).

7.4.2 Morphological Evidence for YFP expression in LC neurons

YFP fluorescence was observed in LC neurons in brain slices obtained from either TH-ChR mice or TH-ChR-*Mecp2*$^{-/Y}$ mice (Fig. 7-1E). Immunocytochemical studies showed that nearly all YFP-positive neurons were also immunoreactive to DBH antibodies (Fig. 7-1F-G). YFP expression was clearly seen in other brainstem areas including area postrema (Fig. 7-1J,K), which can be identified in dark filed images (Fig. 7-1I).

7.4.3 Breathing Abnormality (f variation, apnea count)

Several RTT-like breathing abnormalities have been found in Mecp2-null mice, including high apnea rate and breathing frequency (f) variation (Viemari et al., 2005; Zhang et al., 2011b). Both of these breathing abnormalities were observed in double transgenic TH-ChR-*Mecp2*$^{-/Y}$ mice showing alternating periods of fast and slow respiratory frequency and a high rate of apnea (Fig. 7-2A). Such breathing abnormalities were not seen in TH-ChR mice (Fig. 7-2B). Statistical analysis of breathing f variation in controls 0.21 ± 0.03 vs. double transgenic mice 0.34 ± 0.03 (P = 0.003 unpaired t-test) and apnea counts 0 vs. double transgenic mice 75.5 (P<0.001, $U_{9,10}$ = 2.5, Mann-Whitney U test) in the same age mice indicated that both RTT-like symptoms were significantly higher in the TH-ChR-*Mecp2*$^{-/Y}$ mice than in the TH-ChR (Fig. 7-2C,D).

7.4.4 Electrophysiological Recording from LC neurons in TH-ChR-Mecp2$^{-/Y}$ mice

To confirm the functional expression of ChR in LC neurons, we patched these cells and exposed them to blue light (470nm) in brain slices *in vitro*. In whole-cell recording, these cells from the TH-ChR and TH-ChR-*Mecp2*$^{-/Y}$ mice displayed depolarization in response to a 10 ms optostimulation (Fig. 7-3A,B), and strong inward rectification (Fig. 7-3C). The depolarization amplitude increased with hyperpolarizing pulses and decreased with depolarizing pulses indicating that it was produced by non-selective cationic channels. Optostimulation also raised firing activity in spontaneously firing LC neurons, consistent with the ChR expression in the cell (Fig. 7-3D).

The LC cells from TH-ChR-*Mecp2*$^{-/Y}$ mice exhibited typical membrane properties of LC neurons in *Mecp2*-null mice. Delayed excitation (DE) was seen, which as one of most characteristic properties of LC neurons occurs as delayed action potentials following hyperpolarization (Fig. 7-4A). LC neurons in the TH-ChR-*Mecp2*$^{-/Y}$ mice had a $V_{½}$ of 81.4 ± 5.4 mV (n=4 cells/2 mice), which was not significantly different from *Mecp2*-null mice, 78.4 ± 1.8

mV (n=5 cells/3 mice) (P = 0.585 unpaired t-test, Fig. 7-4B,C). The slope factor of double transgenic mice is 4.4 ± 1.0, which had no significant difference compared to *Mecp2*-null mice at 3.4 ± 0.8 (P = 0.43, unpaired t-test, Fig. 7-4D). The input resistance of TH-ChR-*Mecp2*$^{-/Y}$ mice was 416.5 ± 46.3 MΩ (n=4 cells/2 mice) and 461.3 ± 46.18 MΩ (n=5 cells/3 mice) in *Mecp2*-null mice, which were not significantly different (P = 0.606 unpaired t-test, Fig. 7-4E). The Inward Rectification ratio of double transgenic mice 1.4 ± 0.1 was not significantly different from *Mecp2*-null mice 2.0 ± 0.4 (P = 0.166, unpaired t-test, Fig. 7-4F). The spontaneous firing frequency of TH-ChR-*Mecp2*$^{-/Y}$ mice was 4.3 ± 1.0 Hz (n=4 cells/2 mice) and 5.5 ± 0.8 Hz (n=5 cells/3 mice) (P = 0.361, unpaired t-test, Fig. 7-4G). The average resting membrane potential of TH-ChR-*Mecp2*$^{-/Y}$ mice was -43.7 ± 1. mV (n=4 cells/2 mice) compared to -41.9 ± 1.0 mV in *Mecp2*-null mice (n=5 cells/3 mouse) (P = 0.321, unpaired t-test, data not shown). Thus, our results indicated that the introduction of the exogenous ChR protein did not alter the membrane properties of LC cells in double transgenic mice.

7.4.5 Modulation of brain stem neurons by optostimulation of NE-ergic neurons

In our previous study, we have shown the postsynaptic modulation of hypoglossal motoneurons (HNs) by norepinephrine (Jin et al., 2013). These neurons may remain to be modulated in the medullary slice preparation as the NE-ergic neurons are found in area postrema (Viemari et al., 2005). To determine how optostimulation of NE-ergic cells expressing ChR modulates HNs, we recorded HNs while giving optostimulation to NE-ergic terminals. The HNs in TH-ChR mice (n=9 cells/2 mice) showed typical properties as shown in previous studies such as resting membrane potential -58.1 ± 1.2 mV, input resistance 56.4 ± 11.0 MΩ (n=9 cells/2 mice), whole-cell capacitance 63.1 ± 8.3 pF, and display of a sag potential in response to hyperpolarizing currents with an average of 18.3 ± 3.3 mV which was followed by post-inhibitory rebound (Fig.

7-5A). The sag was defined as the difference between the peak voltage during the current injection and the steady-state voltage. HNs did not show delayed excitation (Fig. 7-5B) as LC neurons, while displaying increased firing activity by each depolarizing pulse. The cells showed moderate spike frequency adaptation (SFA), which is described as Fp/Fs ratio (Fig. 7-5C,D,E). In response to the same depolarizing pulse, the firing rate of the first two action potentials at the peak state (Fp) 48.5 ± 6.0 Hz was significantly higher than that at steady state (Fs) 34.4 ± 2.2 Hz (P = 0.043, paired t-test, Fig. 7-5F)

HNs were typically silent at rest in brain slices except for one cell. Therefore, we measured evoked firing activity of HNs by giving a depolarizing pulse at a level slightly above the firing threshold. Firing activity of HNs was measured at baseline, during light stimulation, and without light stimulation as washout. Light stimulation consisting of 20ms blue light pulses at 10 Hz increased the evoked firing activity, which returned to baseline levels during washout when the light was off (Fig. 7-6A, B). On average, the firing activity of these HNs in control TH-ChR mice increased from baseline 8.4 ± 1.9 Hz (n=9 cells/2 mice) to 12.3 ± 1.5 Hz during light stimulation, which was significantly increased (P = 0.003, paired t-test, Fig. 7-7C). Although optostimulation augmented HN firing activity, the light did not produce any visible depolarization.

In our previous study, we have also shown that NE modulates HNs through postsynaptic α-adrenoceptors. To determine if the effects of light stimulation were mediated through α-adrenoceptors, HNs from control TH-ChR mice were measured during light stimulation in the presence of the α-adrenoceptor antagonist phentolamine (10 μM). Light stimulation failed to increase firing activity in the presence of phentolamine. The evoked firing rate was 9.1 ± 3.3 Hz at baseline vs. 9.1 ± 3.5 Hz (n=4 cells/1 mouse) during optostimulation (Fig. 7-6C,D, Fig. 7-7D, P = 0.985 paired t-test).

7.4.6 Modulation of brain stem neurons by optostimulation of NE-ergic neurons in TH-ChR-Mecp2$^{-/Y}$ Mice

The *Mecp2* disruption in mice causes reductions in NE content (Roux et al., 2008). To show how the NE-ergic synaptic terminals respond to optostimulation, we measured evoked firing activity of HNs before, during and after light stimulation of presynaptic NE-ergic terminals as mentioned above. The evoked firing rate of HNs from double transgenic mice was 10.2 ± 2.9 Hz (n=6 cells/2 mice) at baseline, which was not significantly different from controls (n=9 cells/2 mice, P = 0.908, unpaired t-test). Optostimulation augmented the firing rate of the HNs in double transgenic mice modestly to 9.5 ± 3.7 Hz (Fig. 7-7A,B), which was not significantly different from the baseline level (P = 0.595, paired t-test, Fig. 7-7E). In comparison to the TH-ChR neurons (Fig. 7-7E), the average response of firing rate to light stimulation was significantly lower in TH-ChR-*Mecp2*$^{-/Y}$ mice (-0.7 ± 1.6 Hz, n=6 cells/2 mice) than in control mice (3.8 ± 0.9 Hz, n=9 cells/2 mice; P = 0.01, unpaired t-test).

7.5 Discussion

We have shown the generation of a new double transgenic RTT mouse model with ChR-eYFP expression in catecholaminergic neurons in this study. TH is a rate-limiting enzyme for catecholamine synthesis. The TH promoter has been previously used to direct α-synuclein expression in dopaminergic neurons to generate Parkinson's disease model (Daher et al., 2009; Richfield et al., 2002; Tofaris et al., 2006) and GFP expression in NE-ergic neurons (Lammel et al., 2015; Wang et al., 2014). Consistently, our results indicate that ChR-eYFP is expressed in LC neurons showing YFP fluorescence and functional ChR activity in our TH-ChR mice.

The mouse strain with the loxP ChR-eYFP was used in this study. The loxP ChR-eYFP cassette was constructed in the ROSA26 locus known to be constitutively active, which is

consistent with our finding that the heterozygous mice express ChR-eYFP. This as well as the fact that the ROSA26 locus is located on chromosome 6, and the *Mecp2* is on the X chromosome favors the generation of double transgenic mice. Indeed, we have successfully obtained the TH-ChR-*Mecp2*$^{-/Y}$ double transgenic mice with extensive mouse breeding and step-by-step genotyping. The reasons for using the male mouse model are that 1) the *Mecp2*$^{-/Y}$ males offer a completely *Mecp2*-null condition that is not always available in *Mecp2*$^{+/-}$ females owing to uncontrolled X-chromosome inactivation; 2) the male *Mecp2*$^{-/Y}$ mice have been widely used as a model of RTT, and the results obtained from this model can be easily compared and evaluated with existing literature.

The TH-ChR-*Mecp2*$^{-/Y}$ mice exhibit typical phenotypes seen in other mouse models of RTT, including hypoactivity, low body weight, short life span, and hind limb clasping. They also show clear breathing disorders, including significant higher apnea counts and more frequent breathing f variation than the TH-ChR control. Also similar to the existing mouse models of RTT are the high infant fatality rate and short lifespan (not shown). Nevertheless, our breeding strategy allows production of the TH-ChR-*Mecp2*$^{-/Y}$ mice roughly in the same successful rate as other mouse models of RTT that we have used.

The chance to get male double transgenic mice based on our breeding scheme is 12.5%. We realized that the availability of the double transgenic mice limited the power analysis. It took three to four months to generate F1 generation of double transgenic mice and the average litter size was approximately 6 with 12.5% mutation ratio. This ratio can be enhanced to ~25% by crossbreeding male TH-ChR and female TH-ChR-*Mecp2*$^{-/+}$ mice. The production rate and sustainable generation of double transgenic mice suggest that these mice may provide another way

to study RTT by targeting on specific neurons in central nervous system or certain tissues in peripheral system as well.

Immunohistochemistry identification of catecholaminergic neurons with anti-YFP antibodies in both pons and medulla were clearly seen while these neurons are exhibiting endogenous genetically engineered fluorescence (Fig. 7-2). Further evaluation of ChR expression indicates that ChR is fully functional in LC neurons in double transgenic mice, producing depolarization and firing activity in the cells and NE-ergic modulation with blue light stimulation. The knock-in of ChR in LC neurons did not alter intrinsic properties of LC neurons as well. Therefore, this new strain of double transgenic mice, TH-ChR-*Mecp2*$^{-/Y}$, seems to allow access to LC neurons with high specificity as well as excellent temporal and spatial resolutions.

One group of brainstem neurons targeted by NE modulation is the HNs. NE augments their firing activity by pre- and post-synaptic mechanisms (Jin et al., 2013). Such an NE-ergic modulation is defective in *Mecp2*-null mice, suggesting that HNs are ideal for testing NE modulation in our TH-ChR-*Mecp2*$^{-/Y}$ mice. However, the hypoglossal nucleus is located remotely from the LC, making the joined brain slice studies of these two types of neurons very difficult, if possible. Because of the existence of local NE-ergic neurons in the close vicinity of the hypoglossal nucleus and because of the possibility of optostimulation of axonal terminals of NE-ergic neurons, we have tested the NE-ergic modulation of HNs by optostimulation of NE release in this study. In TH-ChR mice, the HNs firing activity is clearly augmented with optostimulation. Such an effect was not seen in the presence of the non-selective α-adrenoceptors antagonist phentolamine, indicating that HNs are modulated by NE through α-adrenoceptors. Notably, the HNs became silent during washout, which seems due to residual NE modulation that is suppressed by phentolamine.

In TH-ChR-*Mecp2*$^{-/Y}$ mice, we have found that the augmentation of HN firing activity by optostimulation of NE-ergic neurons is drastically impaired. Indeed, optostimulation did not show any significant effect on the HN firing activity. Previous studies indicate that the *Mecp2* disruption causes defects in NE biosynthesis (Viemari et al., 2005). Thus, the impaired NE-ergic modulation of HNs is likely to be caused by a decrease in NE content at synaptic terminals, a reduction in NE release from NE-ergic synapses, or both. In the presence of these defects, optoexcitation of NE-ergic neurons and axonal terminals apparently does not help to improve the NE release. Because the strong optostimulation used in the present study fails to augment NE-ergic modulation, an increase in spontaneous firing of the NE-ergic neurons may not benefit much to the NE release either. Instead, the excessive neuronal excitability might even worsen the NE-ergic modulation by perhaps depleting NE in presynaptic terminals. Therefore, LC neuronal hyperexcitability appears harmful to the NE-ergic modulation under the condition of *Mecp2* disruption.

7.6 Acknowledgement

This work was supported by the NIH (NS073875). Shuang Zhang is a Molecular Basis of Disease (MBD) fellow of Georgia State University.

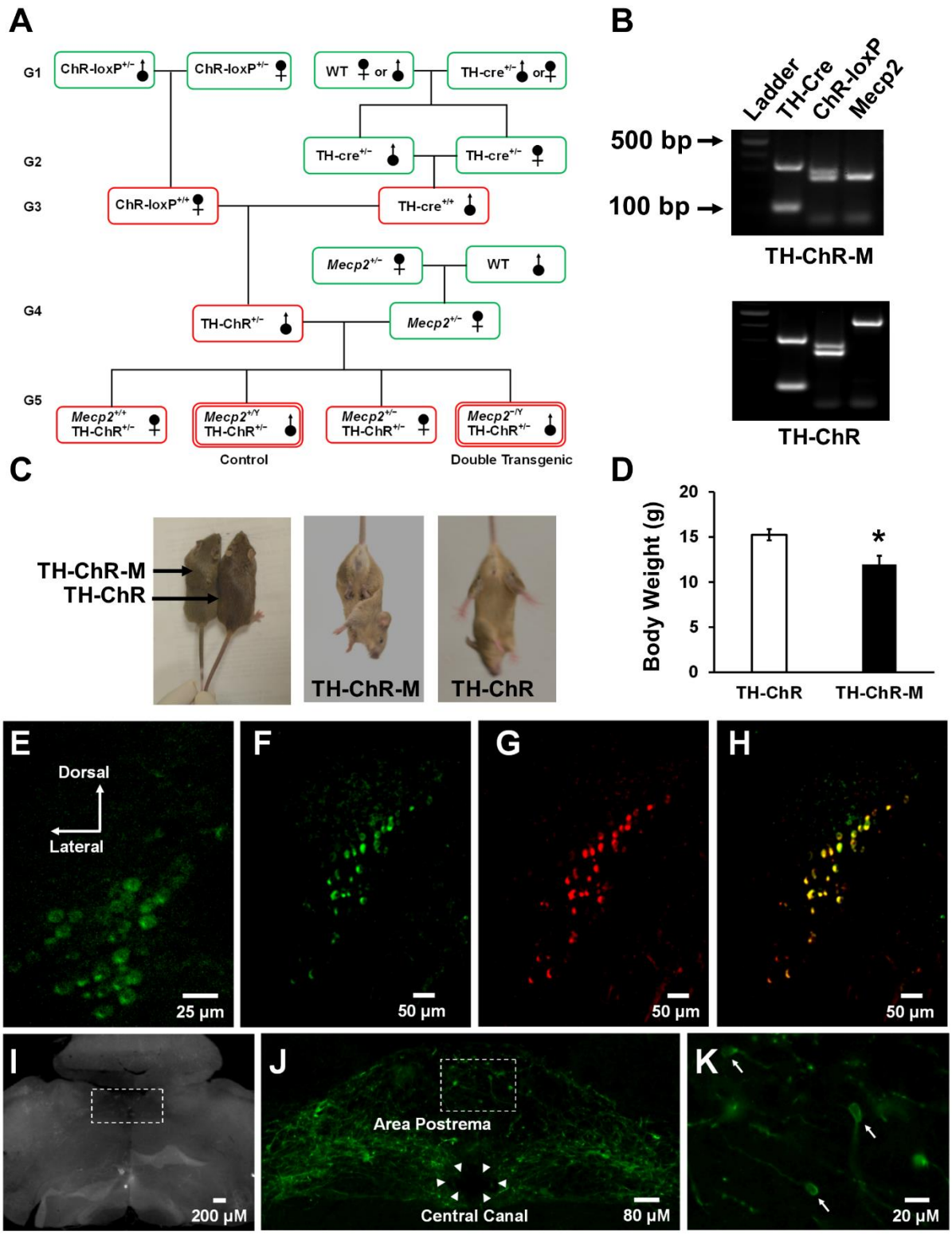

Figure 7-1 Generation of double transgenic RTT mice model, TH-ChR-Mecp2$^{-/Y}$.

A. *Breeding paradigm to generate double transgenic mice. Green, mouse strains available commercially; red, mice generated be cross-breeding.* ***B.*** *Genotyping of transgenic TH-ChR-Mecp2$^{-/Y}$ and TH-ChR mice. TH-Cre expression: internal positive control = 324 bp, transgene = ~100 bp (this assay does not distinguish heterozygous from homozygous transgenic animals); ChR-loxP expression: mutant = 253 bp, heterozygote = 253 and 297 bp, wild type = 297 bp; Mecp2 expression: mutant = 240 bp, wild type = 465 bp.* ***C.*** *Photographs of TH-ChR-M and TH-ChR mice at five weeks old. RTT-like phenotypes were observed in the double transgenic mice, including hind limb clasping and low body weight.* ***D.*** *Significant lower body weight was found in TH-ChR-M mice (n=11 animals) when compared with littermate TH-ChR mice (n=11 animals) during postnatal week four to nine. Data are presented as mean ± SEM.* ***E.*** *YFP fluorescence in LC neurons obtained from a double transgenic TH-ChR-M mouse.* ***F-H.*** *Most DBH-immunoreactive LC neurons showed YFP in a TH-ChR mouse. LC neurons were detected with anti-GFP antibodies (F) and anti-DBH antibodies (G). The overlay of F and G indicates most LC neurons express YFP (H).* ***I.*** *Non-fluorescent images of sections in J and K.* ***J,K.*** *In the medulla oblongata, catecholaminergic neurons were labeled with anti-GFP antibodies in area postrema. Arrow heads, the central canal; arrows, cell bodies of catecholaminergic neurons. Note that J is obtained from the boxed area in I, and K is acquired from the boxed area in J.*

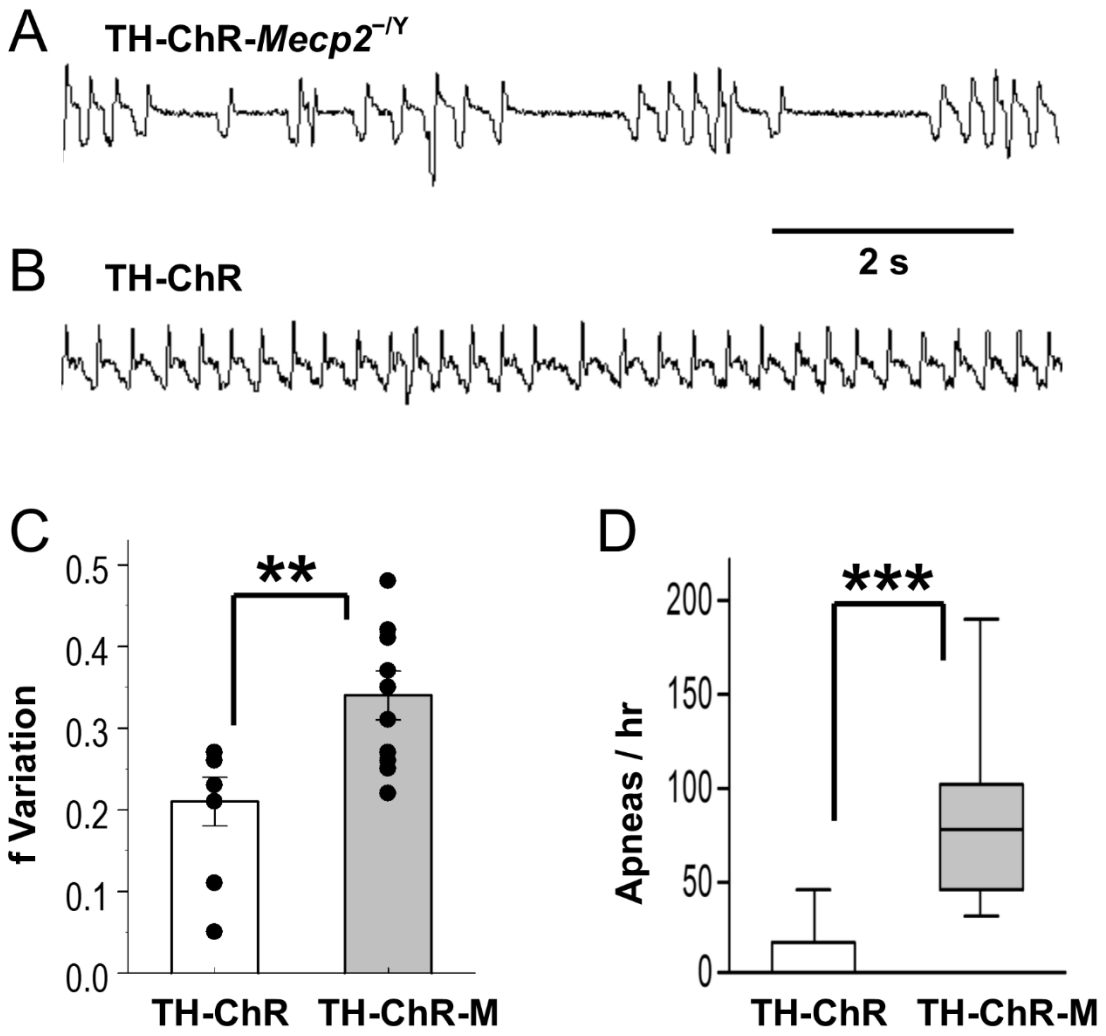

Figure 7-2 Breathing activity of TH-ChR-Mecp2$^{-/Y}$ and TH-ChR mice measured in plethysmography.

A,B. *Real-time trace of breathing activity of a double transgenic (upper) and control mice (lower). Multiple apneas can be identified in double transgenic mice but not in control mice.* ***C,D.*** *Statistically, the double transgenic mice (TH-ChR-M) exhibited significantly larger breathing frequency (f) variability and more apnea counts than the control mice. *, P<0.05; **, P<0.01, ***, P<0.001 (Mann-Whitney U test for apnea count and Student's t-test for f variation).*

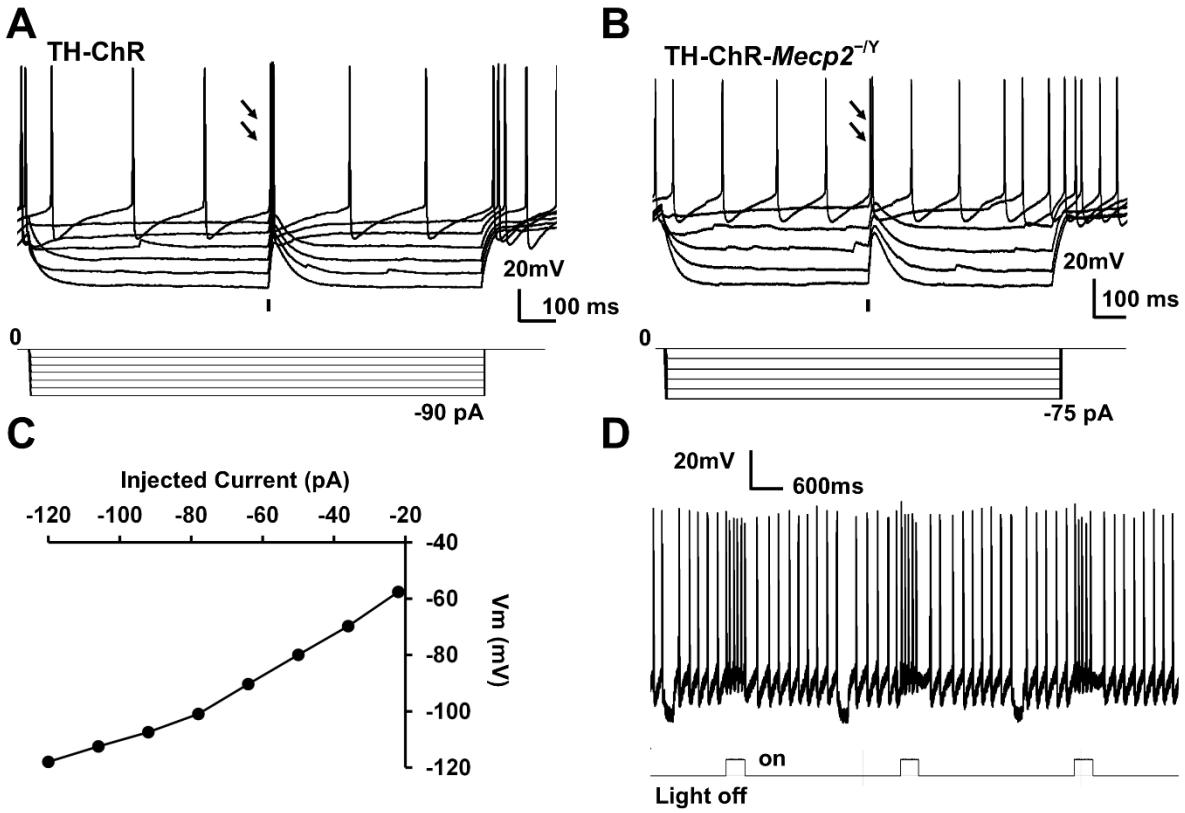

Figure 7-3 Optostimulation of LC neurons.
A. *Light-evoked depolarization in an LC neuron from a TH-ChR mouse by 10 ms blue light pulses (470 nm) as indicated by the black bar. The light-evoked depolarization is larger with hyperpolarization. Arrows indicate action potentials evoked by optostimulation.* ***B.*** *Light-evoked depolarization an LC neuron from a TH-ChR-Mecp2$^{-/Y}$ mouse by 10 ms blue light pulses (470 nm) as indicated by the black bar. Arrows indicate action potentials evoked by optostimulation (n=3 cells/1 mouse).* ***C.*** *I-V plot of a LC neuron from a TH-ChR-Mecp2$^{-/Y}$ mouse.* ***D.*** *Optostimulation enhanced firing activity of an LC neuron with high fidelity from a TH-ChR-Mecp2$^{-/Y}$ mouse.*

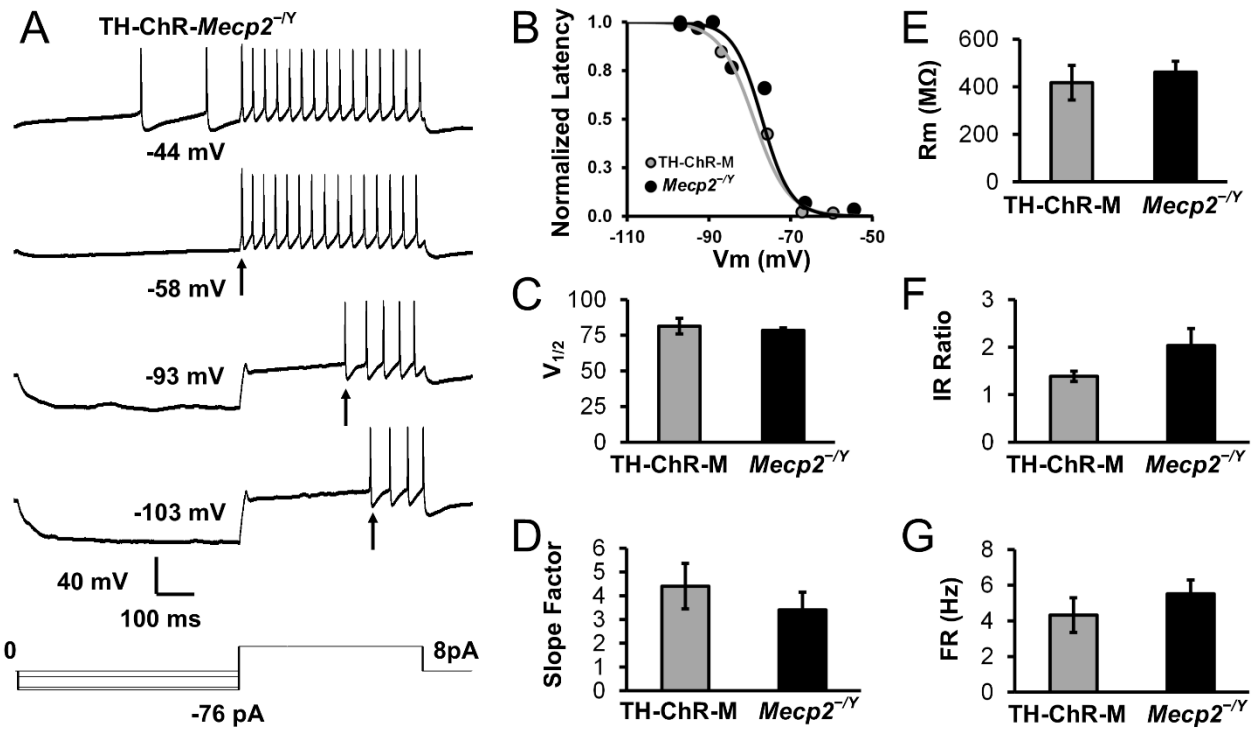

Figure 7-4 Electrophysiological properties of LC neurons.
A. *Whole cell current clamp recording from an LC neuron TH-ChR-Mecp2$^{-/Y}$ (TH-ChR-M) mice. The LC neuron showed delayed excitation (DE) with a series of hyperpolarizing steps followed by a depolarizing pulse. Step increases in hyperpolarization elongated the delay of the first spike (indicated by arrows).* ***B.*** *The relationship between action potential delays and the conditioning membrane potentials was fitted with the Boltzmann equations in TH-ChR-M and Mecp2-null mice and plotted.* ***C-D.*** *There was no difference in the $V_{½}$ and slope factor between TH-ChR-M mice (n=4 cells/2 mice) and Mecp2-null (n=5 cells/3 mice).* ***E-G.*** *There was no significant difference in input resistance (P = 0.606 unpaired t-test), inward rectification (P = 0.166, unpaired t-test), and firing rate (P = 0.361, unpaired t-test) between TH-ChR-M mice (n=4 cells/2 mice) and Mecp2-null (n=5 cells/3 mice).*

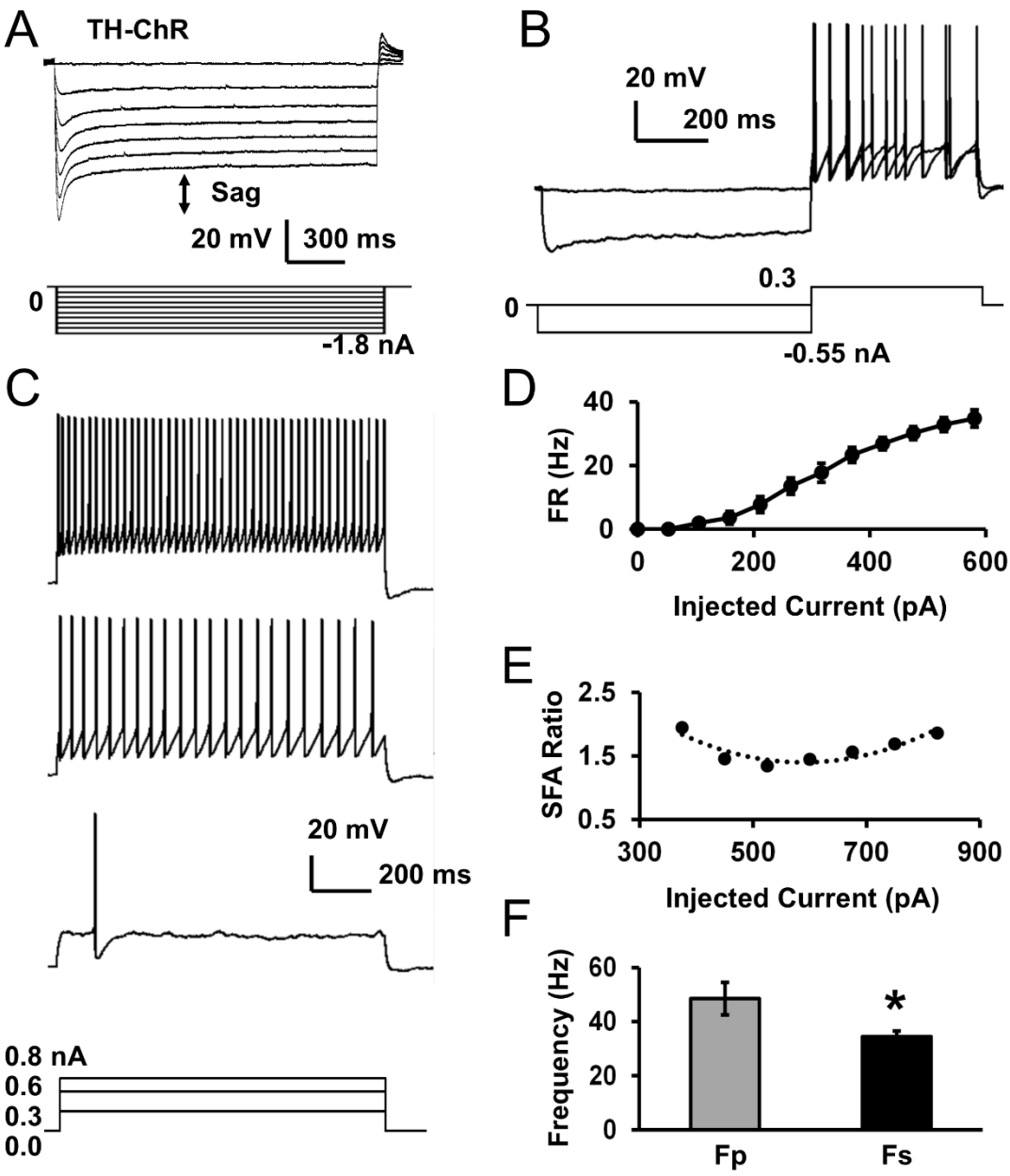

Figure 7-5 Electrophysiological properties of hypoglossal neurons (HNs) from TH-ChR mice. ***A.*** *An HN was recorded in current clamp. The cell showed typical sag potential described as the voltage difference between the peak and steady-state voltages in response to steps of hyperpolarizing pulses (indicated with the arrow). The cell also displayed a post-inhibitory rebound of depolarization, which is the difference between the baseline and rebound voltage, after termination of hyperpolarizing pulses.* ***B.*** *The HN did not show DE.* ***C.*** *Increased firing activity and SFA was seen in the cell with depolarization currents.* ***D.*** *The average firing rate was calculated and graphed with depolarizing current injections, n=9 cells/2 mice.* ***E.*** *A moderate SFA ratio was seen in the HN.* ***F.*** *Fp and Fs were compared, n=9 cells/2 mice. Data are presented as mean ± SEM (*, P<0.05; Student's t-test).*

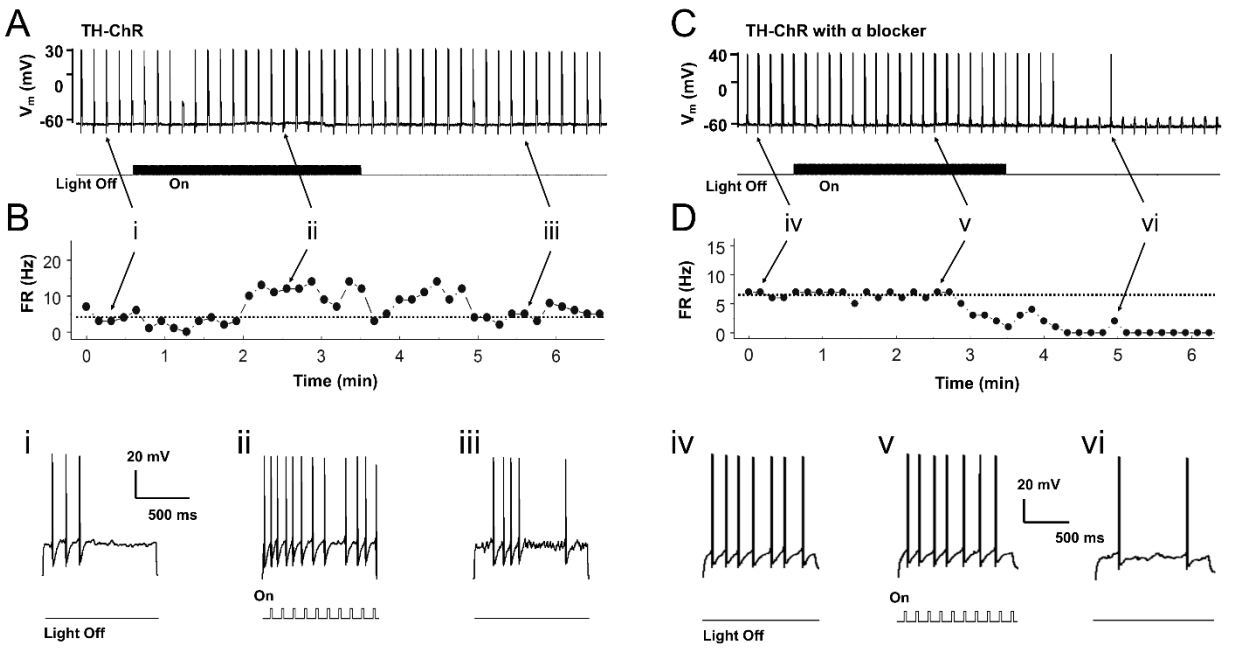

Figure 7-6 The response of hypoglossal neurons to NE-dependent optostimulation in TH-ChR mice.

A. *Evoked firing activity of an HN was measured during baseline, optostimulation and light-off recovery.* ***B.*** *Measurement of instantaneous firing rate (FR) of the HN.* ***C,D.*** *Firing activity of another HN recorded in the presence of α-adrenoceptors blocker phentolamine (10 µM). Recording traces in i through vi were obtained from their corresponding sites above with expansion.*

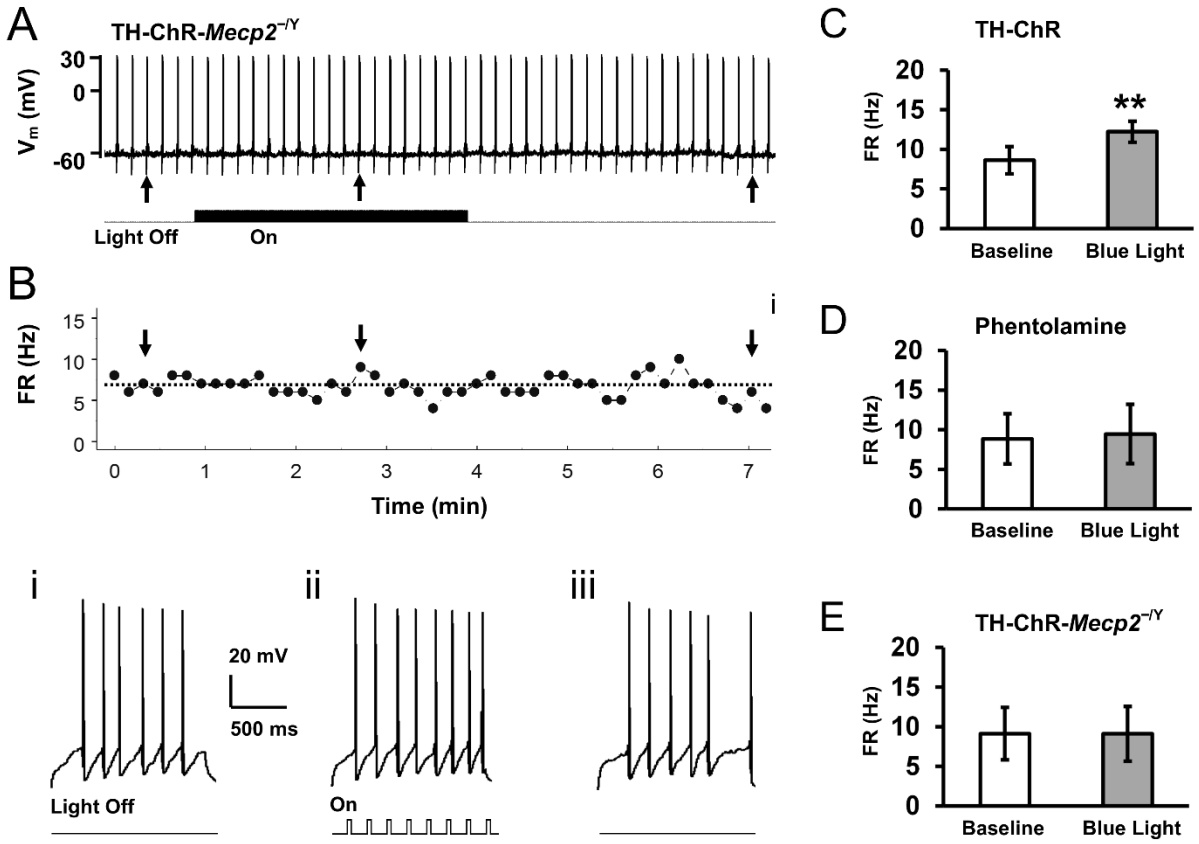

Figure 7-7 The NE-dependent optostimulation has no effect on hypoglossal neurons in TH-ChR-Mecp2$^{-/Y}$ mice.

A. *Evoked firing activity of an HN from a TH-ChR-Mecp2$^{-/Y}$ mouse.* ***B.*** *The instantaneous firing rate of the HN. Recording traces in i through iii were obtained from their corresponding sites above with expansion.* ***C.*** *Optical NE-ergic stimulation augmented firing activity of HNs in TH-ChR mice, n=9 cells/2 mice. Data are presented as mean ± SE (**, P<0.01; Student's t-test).* ***D.*** *The same optical NE-ergic stimulation failed to enhance firing activity in TH-ChR mice when phentolamine (10 µM) was added to the perfusion solution (P>0.05, n=4 cells/1 mouse).* ***E.*** *The NE-dependent optostimulation had no significant effect on firing in TH-ChR-Mecp2$^{-/Y}$ mice (P>0.05, n=6 cells/2 mice).*

8 GENERAL DISCUSSIONS

8.1 Dysfunction of the ANS

Dysfunction of the ANS has severe impacts on multiple organs and systems. The mechanisms of the dysfunction remain unknown. Thus, we utilized drastic and innovative interventions to the ANS, such as the application of endotoxin, optogenetics, and transgenic mice, and made several new findings.

One of the life-threatening dysfunction of the ANS is the irreversible hypotension and vascular hyporeactivity in septic shock. To understand mechanisms, we reproduced hyporeactivity of the mesenteric arteries with the treatment of endotoxin LPS overnight *in vitro* and screened out a few vasoactive agents that have vasoconstriction effects. Three candidates, ET-1, 5-HT, and vasopressin, are found effective, which have never been used for clinical management for septic shock. Besides, U46619, an agonist of the TXA_2 receptor, can partially reverse the hyporeactivity. In the system, these vasopressors may play a role not only as an individual but also as a combination with other vasoconstrictors. Indeed, we have found that the vasoconstriction effects of these agents are significantly potentiated by co-application with PE.

Our results suggest that the endotoxin-induced vascular hyporeactivity is not attributed to the damage of VSM in the early phase (first 24 hours) of septic shock, neither to the over-released vasodilators from endothelium. The main reason for the septic hypotension seems to be caused by vascular hyporeactivity to α adrenoceptors that are unfortunately the main targets in the current therapeutic management of septic shock. Although the underlying mechanism for the vascular hyporeactivity is still elusive, our dissections of intracellular signaling pathways revealed that ET-1 acts on ET_AR and works through G_i – AC – PKA and $G_{q/11}$ – PC – PLC – DAG – PKC signaling pathway, while 5-HT activates ROK through $5\text{-}HT_{1B}R$ leading to

inhibition of the myosin light-chain phosphatase (MLCP) and vasoconstriction. Since some of these signaling pathways are shared by the α adrenoceptor system, it is likely that the dysfunction of the α adrenoceptor system may occur in upstream pathways. Taken together, this pharmaceutical research may provide new and effective therapeutical intervention of septic shock.

The endothelium regulates vascular homeostasis by releasing a variety of vasoactive substances including vasoconstrictors and vasodilators, while how the endothelial activation affects vascular tones is unknown. Current belief is vasodilation, as the overall function of the endothelium in vasomotor is vasodilation through the NO-dependent signaling pathway. This inner layer system we worked on is located inside of blood vessels. Therefore, it was a challenge to choose an effective intervention method to activate ECs selectively without affecting the VMS. To address this, we generated transgenic mice with ChR expression in the ECs throughout the whole body persistently in a life-long time scale. Optostimulation on dissected EC evoked depolarization and vasoconstriction in perfused heart and kidney. This is the first direct evidence demonstrating that activation of EC leads to vasoconstriction. Also, our results indicated that such opto-vasoconstriction is fast, reproducible, long-lasting that is comparable in strength to the popular vasoconstrictor PE. Meanwhile, we performed another study applying optogenetics in VSMC and demonstrated that optostimulation in VSMC induces significant vasoconstriction that is fast, reproducible, light intensity-dependent, and comparable in strength to popular vasoconstrictors (Wu et al., 2015).

These studies open a new avenue to applying optogenetics in peripheral system, especially cardiovascular system, by passing potential inference from adjacent tissues, even as close as VSMC and ECs are in blood vessels. With the successful application of optogenetics in

peripheral system, we were confident to dig further with effective intervention in the CNS. We chose the breathing control as it is the most important function of the ANS within the brain. Dysfunction of breathing in certain genetic diseases, such as RTT. NE production and projection play a critical role in the regulation of breathing in the CNS while NE synthesis is deficient in both RTT mouse model and patients. Also, the primary area for NE production is located in LC, where neurons are hyperexcitable. Such neuronal hyperexcitability may be a compensatory response to the inadequate NE, or an adverse manifestation of the disease. To understand whether the increased LC neuronal excitability improves NE modulation, we generated a new strain of double transgenic mice with *Mecp2* null and ChR expression in LC neurons. The TH-ChR-*Mecp2*$^{-/Y}$ mice exhibit typical phenotypes seen in other mouse models of RTT including hypoactivity, low body weight, short life span, and hind limb clasping. They also show clear breathing disorders, including significant higher apnea counts and more frequent breathing f variation than the TH-ChR control. We found that the NE-ergic modulation of hypoglossal neurons is impaired in the double transgenic mice but not in the control mice. The defected NE projection is not improved with optostimulation, suggesting that LC neuronal hyperexcitability does not seem beneficial to the NE modulation in RTT. Instead, the neuronal hyperexcitability may worsen the homeostatic status of local neuronal networks in the LC.

8.2 Effective interventions in the ANS

By using innovative and/or drastic interventions, this dissertation addressed several unsolved problems as to function and dysfunction of the ANS. It is worth noting that the interventions in this dissertation are innovative, effective, robust and specific in terms of targeting at each tissue or cell type. These all have shown potentials in addressing basic research questions and clinical phenomena.

This dissertation employed an *in vitro* disease model in which mesenteric arteries were cultured with or without endotoxin or LPS for >20 hours. The blood pressure is mainly regulated by resistance vessels, such as the mesenteric arteries. Thus, this model is ideal to study the hyporeactivity in vasculature at the early stage of septic shock. Besides, this *in vitro* tissue model ensures the specific study on the vascular reactivity by ruling out the interference from immune molecules. Our study revealed the potential underlying mechanism of hypotension in septic shock and the effective vasoconstrictors ET-1 and 5-HT that can reverse the excessive vasodilation. This is significant for clinical management of septic shock and severe sepsis. The advantage of this *in vitro* tissue disease model is that it is free from circulation environment, which is also a drawback because the impact of certain natural circulating molecules was missing. Therefore, according to the specific research purpose, disease and target tissue/organs, the interventions need to be well-designed to gain the optimal results.

The *in vitro* study must be tested *in vivo* eventually with living animal models, which enables closer approximation to a human response. Therefore, animal models have been widely used in exploration and characterization of disease pathophysiology, target identification, and evaluation of novel therapeutic management. The laboratory animal disease models can be categorized into five types, induced disease models, spontaneous disease models, transgenic disease models, negative disease models, and orphan disease models (Hau and Van Hoosier, 2003).

The induced models are generated from healthy animals in which the condition to be studied is experimentally induced either through surgical and/or genetic modifications or chemical injections. Theoretically, the induced model is the only category that allows a free choice of species. Indeed, the etiology of a disease in an induced animal model is usually different from the

corresponding disease in the human. The spontaneous animal models have naturally occurring genetic variants and/or mutations. Many strains with inherited disorders modeling similar conditions in the human have been conserved and characterized, such as the nude mice with a genetic mutation that causes a deteriorated or absent thymus that has been used as metastasis models. The majority of the spontaneous models are mice and rats. The spontaneous models are often isomorphic, which means the animal's symptoms or anatomy are similar to those in humans. (Hau and Van Hoosier, 2003).

The transgenic disease models may be the most important type of animal disease model, which emerged along with the rapid development of genetic engineering and embryo manipulation technology during the past decade. Mouse are the most important and available resource for the development of the transgenic model by far (Hau and Van Hoosier, 2003). Indeed, transgenic models are the induced models carrying inserted DNA (known-in) or deleted DNA (knock-out) in the genome. Transgenic animals are commonly produced with following procedures, 1) germline modifications of gamete; 2) microinjection of DNA or gene constructs into zygotes; 3) incorporating modified cells into later stage embryos. The resultant embryos will be matured to term in recipient female. The advantage of transgenic disease models is significant because many more animal models for human diseases have been developed through this technology (Pinkert, 2003). However, this model has drawbacks: 1) Many physiological functions are controlled by more than one gene so that it requires research to identify the contribution of multiple genes to normal and disease conditions. 2) The deletion or insertion of DNA from/into animal genome may result in unpredictable outcomes including pathological interferences and animal well-being, especially the first generation of animal produced. 3) The selection, breeding, and cloning of transgenic lines remain challenging and can be inaccurate in some cases. To overcome these

problems, the recent completion of the mouse and human genome maps can provide remarkable clues for the generation of new homologous animal models with both genotype and phenotype of the human.

The negative animal models are strains fail to react to a disease or particular stimulus that are mainly used to study the mechanisms of disease resistance. Combining with transgenic technology, the negative animal models become increasingly important, such as a generation of novel transgenic mouse strains failed to develop certain diseases. The orphan models are the opposite of negative models. They refer to the animals in which a functional disorder occurs naturally in a non-human species. The negative models become the induced models when a similar disease is recognized in humans later on (Hau and Van Hoosier, 2003).

The criteria to choose the appropriate animal disease model for the study involve research factors, animal factors, and physical factors. Generally, the research factors include the considerations of analogue animal species of the human diseases, transferability of the result information between the animal models and humans, repeatable and verifiable data gained from the animal models, ethical implications, number of the animal needed, and customary practice with or without justification can be applied to the animal models, and existing literatures support. The animal care factors include cost, availability, housing, husbandry, and stress factors of the animal strains. Besides, genetic background, adaptability to experimental manipulation, size, life span, age, sex, progeny need, and special features of the animals are also need to be considered in term of animal factor aspect. Last but not the least, the physical and environmental criteria need to be evaluated including ecological consequences, hazardous components, and environmental influences.

Optogenetics is a novel combined intervention of physical light stimulation and transgenic animal model that emerged over the last ten years. By employing this effective intervention, we investigated and revealed some unknown mechanisms related to function and dysfunction of vasomotor and respiration.

The optogenetics was originally invented by Boris Zemelman and Gero Miesenböck and applied in testing neuronal activities *in vitro* and *in vivo* by Dr. Deissroth and his group members since 2005 (Boyden et al., 2005). After that, the application of optogenetics has been limited within the CNS for next 5 years (Aravanis et al., 2007; Deisseroth et al., 2006; Zhang et al., 2006). Until 2010, the application of optogenetics was extended in cardiomyocytes for the first time (Knollmann, 2010).

At that time, our lab was concentrating on the underlying mechanisms and potential treatment for certain cardiovascular diseases, such as septic shock, by using dramatic endotoxin intervention. The major difficulty we met was to evaluate the VSMCs and ECs respectively and specifically. The innovative optogenetic technology inspired us to apply it into the peripheral vasculature so that we can adapt the spatiotemporal accuracy. We chose cross-bred commercially available breeders to generate transgenic mice, cdh5-ChR, which has fluorescence labeling in endothelium. The advantage of this strain is the persistent and extensive expression of ChR, economic cost and commercial availability.

It is important to select effective ways to manipulate and read out of the optical response of the ECs bypassing interference from the VSMC. We creatively modified and used Langendorff heart perfusion and kidney perfusion to evaluate the endothelium with light stimulation. The endothelium is composed of a single layer ECs inside of blood vessel, so the heart and kidney perfusion allows better access of light to surface coronary arteries and renal vasculature without

damage comparing to vascular rings preparation. Besides, the organ perfusion preparation provides a superior level mimicking physiological condition *in vivo*. The light stimulation was also evaluated with single EC using patch clamp, and our results indicated that direct stimulation of the EC induces cell depolarization, which is consistent with our observation in organ perfusion that light stimulation of endothelium causes vasoconstriction. This new finding uncovered the unknown function of ECs, which is different form conventional understanding that ECs are mainly responsible for the vasodilation. Taken together, modified optogenetics technology in our lab effectively open a new realm of application of optogenetics in peripheral system.

After gaining successful experience from the first peripheral optogenetics study, we decided to further develop this powerful intervention to understand more function and dysfunction in the ANS, such as respiration, which extensively involves both the CNS and peripheral systems. The etiology of the breathing disorder in RTT has been one of the major focuses in our lab. Consistent with breathing abnormality occurs in RTT patients and mouse models, NE synthesis and projection are found disrupted. In the CNS, over 70% NE is synthesized in LC area (Viemari, 2008), and most of the neurons located in LC area are found defective with hyperexcitability (Biancardi et al., 2008). However, the causal relationship between disruption of NE synthesis and the defection of LC neurons is unknown. Therefore, based on widely accepted *MECP2* gene knockout RTT mouse model, we knocked in light sensitive opsin ChR2 in the NEegic neurons to generate a new double transgenic RTT mouse model, TH-ChR-*Mecp2*$^{-/Y}$, by mating male TH-ChR and female *Mecp2*$^{-/+}$ mice. Indeed, an alternative approach to insert ChR is to inject virus carrying ChR-loxP segment into LC area in *Mecp2*$^{-/Y}$ mouse model, or vice versa. The drawbacks of this approach are 1) gene expression of opsin diminishes over 4-5 weeks, 2) the invasive

injection procedure, 3) potentially unexpected damage to the CNS with multiple injections for etiology study of the RTT because it is a neurodevelopmental disease.

The new double transgenic mice exhibited all of the phenotypes of other mice models of RTT. Electrophysiology was performed in HNs on brain slices that receive NE projection from LC neurons. We found NE projection is disrupted in double transgenic RTT mice comparing to control TH-ChR mice, which indicate that it is feasible to evaluate neuronal modulation in RTT animal model with the optogenetic tool. To furtherly evaluate the etiology of breathing disorder in RTT, it would be ideal to insert optical fiber into LC region and observe the development of the disease with and without light stimulation. In addition, the *in vivo* test would provide an effective intervention to study the function and dysfunction of specific nuclei in neurodevelopmental diseases and potential clinical treatment.

REFERENCES

Adrogue, H.E., and Adrogue, H.J. (2001). Acid-base physiology. Respiratory care *46*, 328-341.

Alva, J.A., Zovein, A.C., Monvoisin, A., Murphy, T., Salazar, A., Harvey, N.L., Carmeliet, P., and Iruela-Arispe, M.L. (2006). VE-Cadherin-Cre-recombinase transgenic mouse: a tool for lineage analysis and gene deletion in endothelial cells. Dev Dyn *235*, 759-767.

Andrea, J.E., and Walsh, M.P. (1992). Protein kinase C of smooth muscle. Hypertension *20*, 585-595.

Angus, D.C., Linde-Zwirble, W.T., Lidicker, J., Clermont, G., Carcillo, J., and Pinsky, M.R. (2001). Epidemiology of severe sepsis in the United States: analysis of incidence, outcome, and associated costs of care. Crit Care Med *29*, 1303-1310.

Aravanis, A.M., Wang, L.P., Zhang, F., Meltzer, L.A., Mogri, M.Z., Schneider, M.B., and Deisseroth, K. (2007). An optical neural interface: in vivo control of rodent motor cortex with integrated fiberoptic and optogenetic technology. J Neural Eng *4*, S143-156.

Arrenberg, A.B., Stainier, D.Y., Baier, H., and Huisken, J. (2010). Optogenetic control of cardiac function. Science *330*, 971-974.

Arrigo, L., and Avanzino, G.L. (1979). [Current developments on the physiology of the cardiac atrial pacemaker an excitation conduction of the left atrium]. Archivio di fisiologia *71*, 25-66.

Aston-Jones, G., and Cohen, J.D. (2005). Adaptive gain and the role of the locus coeruleus-norepinephrine system in optimal performance. J Comp Neurol *493*, 99-110.

Atasoy, D., Aponte, Y., Su, H.H., and Sternson, S.M. (2008). A FLEX switch targets Channelrhodopsin-2 to multiple cell types for imaging and long-range circuit mapping. J Neurosci *28*, 7025-7030.

Atherton, A., and Born, G.V. (1972). Quantitative investigations of the adhesiveness of circulating polymorphonuclear leucocytes to blood vessel walls. J Physiol *222*, 447-474.

Bannerman, D.D., and Goldblum, S.E. (2003). Mechanisms of bacterial lipopolysaccharide-induced endothelial apoptosis. American journal of physiology Lung cellular and molecular physiology *284*, L899-914.

Barlow, R.S., and White, R.E. (1998). Hydrogen peroxide relaxes porcine coronary arteries by stimulating BKCa channel activity. Am J Physiol *275*, H1283-1289.

Bauersachs, J., Popp, R., Hecker, M., Sauer, E., Fleming, I., and Busse, R. (1996). Nitric oxide attenuates the release of endothelium-derived hyperpolarizing factor. Circulation *94*, 3341-3347.

Bayliss, D.A., and Barrett, P.Q. (2008). Emerging roles for two-pore-domain potassium channels and their potential therapeutic impact. Trends in pharmacological sciences *29*, 566-575.

Bayliss, D.A., Talley, E.M., Sirois, J.E., and Lei, Q. (2001). TASK-1 is a highly modulated pH-sensitive 'leak' K(+) channel expressed in brainstem respiratory neurons. Respir Physiol *129*, 159-174.

Bean, B.P. (1992). Pharmacology and electrophysiology of ATP-activated ion channels. Trends in pharmacological sciences *13*, 87-90.

Belegu, R., Hadziefendic, S., Dreshaj, I.A., Haxhiu, M.A., and Martin, R.J. (1999). CO2-induced c-fos expression in medullary neurons during early development. Respir Physiol *117*, 13-28.

Belvisi, M.G., Stretton, C.D., Yacoub, M., and Barnes, P.J. (1992). Nitric oxide is the endogenous neurotransmitter of bronchodilator nerves in humans. European journal of pharmacology *210*, 221-222.

Bender, S.B., and Klabunde, R.E. (2007). Altered role of smooth muscle endothelin receptors in coronary endothelin-1 and alpha1-adrenoceptor-mediated vasoconstriction in Type 2 diabetes. Am J Physiol Heart Circ Physiol *293*, H2281-2288.

Bengzon, J., Hansson, S.R., Hoffman, B.J., and Lindvall, O. (1999). Regulation of norepinephrine transporter and tyrosine hydroxylase mRNAs after kainic acid-induced seizures. Brain Res *842*, 239-242.

Bermejo, A., Zarzuelo, A., and Duarte, J. (2003). In vivo vascular effects of genistein on a rat model of septic shock induced by lipopolysaccharide. Journal of cardiovascular pharmacology *42*, 329-338.

Bernard, D.G., Li, A., and Nattie, E.E. (1996). Evidence for central chemoreception in the midline raphe. J Appl Physiol (1985) *80*, 108-115.

Berridge, C.W., and Waterhouse, B.D. (2003). The locus coeruleus-noradrenergic system: modulation of behavioral state and state-dependent cognitive processes. Brain Res Brain Res Rev *42*, 33-84.

Bhakta, G., Mitra, S., and Maitra, A. (2005). DNA encapsulated magnesium and manganous phosphate nanoparticles: potential non-viral vectors for gene delivery. Biomaterials *26*, 2157-2163.

Biancardi, V., Bicego, K.C., Almeida, M.C., and Gargaglioni, L.H. (2008). Locus coeruleus noradrenergic neurons and CO2 drive to breathing. Pflugers Arch *455*, 1119-1128.

Bishop, V.S., Peterson, D.F., and Horwitz, L.D. (1976). Factors influencing cardiac performance. International review of physiology *9*, 239-273.

Bohm, F., and Pernow, J. (2007). The importance of endothelin-1 for vascular dysfunction in cardiovascular disease. Cardiovascular research *76*, 8-18.

Bonsi, P., Cuomo, D., Ding, J., Sciamanna, G., Ulrich, S., Tscherter, A., Bernardi, G., Surmeier, D.J., and Pisani, A. (2007). Endogenous serotonin excites striatal cholinergic interneurons via the activation of 5-HT 2C, 5-HT6, and 5-HT7 serotonin receptors: implications for extrapyramidal side effects of serotonin reuptake inhibitors. Neuropsychopharmacology : official publication of the American College of Neuropsychopharmacology *32*, 1840-1854.

Borgbjerg, F.M., Nielsen, K., and Franks, J. (1996). Experimental pain stimulates respiration and attenuates morphine-induced respiratory depression: a controlled study in human volunteers. Pain *64*, 123-128.

Boron, W.F., and Boulpaep, E.L. (2009). Medical physiology : a cellular and molecular approach, 2nd edn (Philadelphia, PA: Saunders/Elsevier).

Boulanger, C., and Luscher, T.F. (1990). Release of endothelin from the porcine aorta. Inhibition by endothelium-derived nitric oxide. J Clin Invest *85*, 587-590.

Boussif, O., Lezoualc'h, F., Zanta, M.A., Mergny, M.D., Scherman, D., Demeneix, B., and Behr, J.P. (1995). A versatile vector for gene and oligonucleotide transfer into cells in culture and in vivo: polyethylenimine. Proc Natl Acad Sci U S A *92*, 7297-7301.

Boyden, E.S. (2011). A history of optogenetics: the development of tools for controlling brain circuits with light. F1000 Biol Rep *3*, 11.

Boyden, E.S., Zhang, F., Bamberg, E., Nagel, G., and Deisseroth, K. (2005). Millisecond-timescale, genetically targeted optical control of neural activity. Nat Neurosci *8*, 1263-1268.

Brayden, J.E., and Nelson, M.T. (1992). Regulation of arterial tone by activation of calcium-dependent potassium channels. Science *256*, 532-535.

Britt, J.P., McDevitt, R.A., and Bonci, A. (2012). Use of channelrhodopsin for activation of CNS neurons. Current protocols in neuroscience / editorial board, Jacqueline N Crawley [et al] *Chapter 2*, Unit2 16.

Bruegmann, T., Malan, D., Hesse, M., Beiert, T., Fuegemann, C.J., Fleischmann, B.K., and Sasse, P. (2010). Optogenetic control of heart muscle in vitro and in vivo. Nat Methods *7*, 897-900.

Bulbring, E., and Tomita, T. (1987). Catecholamine action on smooth muscle. Pharmacological reviews *39*, 49-96.

Busse, R., Edwards, G., Feletou, M., Fleming, I., Vanhoutte, P.M., and Weston, A.H. (2002). EDHF: bringing the concepts together. Trends in pharmacological sciences *23*, 374-380.

Cardin, J.A., Carlen, M., Meletis, K., Knoblich, U., Zhang, F., Deisseroth, K., Tsai, L.H., and Moore, C.I. (2010). Targeted optogenetic stimulation and recording of neurons in vivo using cell-type-specific expression of Channelrhodopsin-2. Nature protocols *5*, 247-254.

Carette, B. (1999). Noradrenergic responses of neurones in the mediolateral part of the lateral septum: alpha1-adrenergic depolarization and rhythmic bursting activities, and alpha2-adrenergic hyperpolarization from guinea pig brain slices. Brain research bulletin *48*, 263-276.

Carmeliet, P., Lampugnani, M.G., Moons, L., Breviario, F., Compernolle, V., Bono, F., Balconi, G., Spagnuolo, R., Oosthuyse, B., Dewerchin, M., *et al.* (1999). Targeted deficiency or cytosolic truncation of the VE-cadherin gene in mice impairs VEGF-mediated endothelial survival and angiogenesis. Cell *98*, 147-157.

Caulfield, M.P., and Birdsall, N.J. (1998). International Union of Pharmacology. XVII. Classification of muscarinic acetylcholine receptors. Pharmacological reviews *50*, 279-290.

CDC (2013). Deaths: Final Data for 2013.

Cena, J.J., Lalu, M.M., Cho, W.J., Chow, A.K., Bagdan, M.L., Daniel, E.E., Castro, M.M., and Schulz, R. (2010). Inhibition of matrix metalloproteinase activity in vivo protects against vascular hyporeactivity in endotoxemia. Am J Physiol Heart Circ Physiol *298*, H45-51.

Chahrour, M., Jung, S.Y., Shaw, C., Zhou, X., Wong, S.T., Qin, J., and Zoghbi, H.Y. (2008). MeCP2, a key contributor to neurological disease, activates and represses transcription. Science *320*, 1224-1229.

Chen, G., Suzuki, H., and Weston, A.H. (1988). Acetylcholine releases endothelium-derived hyperpolarizing factor and EDRF from rat blood vessels. Br J Pharmacol *95*, 1165-1174.

Chow, B.Y., Han, X., Dobry, A.S., Qian, X., Chuong, A.S., Li, M., Henninger, M.A., Belfort, G.M., Lin, Y., Monahan, P.E., *et al.* (2010). High-performance genetically targetable optical neural silencing by light-driven proton pumps. Nature *463*, 98-102.

Cines, D.B., Pollak, E.S., Buck, C.A., Loscalzo, J., Zimmerman, G.A., McEver, R.P., Pober, J.S., Wick, T.M., Konkle, B.A., Schwartz, B.S., *et al.* (1998). Endothelial cells in physiology and in the pathophysiology of vascular disorders. Blood *91*, 3527-3561.

Clark, R.K. (2005). Anatomy and physiology : understanding the human body (Sudbury, Mass.: Jones and Bartlett Publishers).

Coates, E.L., Li, A., and Nattie, E.E. (1993). Widespread sites of brain stem ventilatory chemoreceptors. J Appl Physiol (1985) *75*, 5-14.

Corada, M., Liao, F., Lindgren, M., Lampugnani, M.G., Breviario, F., Frank, R., Muller, W.A., Hicklin, D.J., Bohlen, P., and Dejana, E. (2001). Monoclonal antibodies directed to different regions of vascular endothelial cadherin extracellular domain affect adhesion and clustering of the protein and modulate endothelial permeability. Blood *97*, 1679-1684.

Corson, M.A., James, N.L., Latta, S.E., Nerem, R.M., Berk, B.C., and Harrison, D.G. (1996). Phosphorylation of endothelial nitric oxide synthase in response to fluid shear stress. Circ Res *79*, 984-991.

Crews, J.K., Murphy, J.G., and Khalil, R.A. (1999). Gender differences in Ca(2+) entry mechanisms of vasoconstriction in Wistar-Kyoto and spontaneously hypertensive rats. Hypertension *34*, 931-936.

Critchley, H.D., and Harrison, N.A. (2013). Visceral influences on brain and behavior. Neuron *77*, 624-638.

Daher, J.P., Ying, M., Banerjee, R., McDonald, R.S., Hahn, M.D., Yang, L., Flint Beal, M., Thomas, B., Dawson, V.L., Dawson, T.M., *et al.* (2009). Conditional transgenic mice expressing C-terminally truncated human alpha-synuclein (alphaSyn119) exhibit reduced striatal dopamine without loss of nigrostriatal pathway dopaminergic neurons. Molecular neurodegeneration *4*, 34.

Dampney, R.A. (1994). Functional organization of central pathways regulating the cardiovascular system. Physiol Rev *74*, 323-364.

Deanfield, J., Donald, A., Ferri, C., Giannattasio, C., Halcox, J., Halligan, S., Lerman, A., Mancia, G., Oliver, J.J., Pessina, A.C., *et al.* (2005). Endothelial function and dysfunction. Part I: Methodological issues for assessment in the different vascular beds: a statement by the Working Group on Endothelin and Endothelial Factors of the European Society of Hypertension. Journal of hypertension *23*, 7-17.

Deisseroth, K., Feng, G., Majewska, A.K., Miesenbock, G., Ting, A., and Schnitzer, M.J. (2006). Next-generation optical technologies for illuminating genetically targeted brain circuits. J Neurosci *26*, 10380-10386.

Deitch, E.A. (1998). Animal models of sepsis and shock: a review and lessons learned. Shock *9*, 1-11.

Dellinger, R.P., Levy, M.M., Rhodes, A., Annane, D., Gerlach, H., Opal, S.M., Sevransky, J.E., Sprung, C.L., Douglas, I.S., Jaeschke, R., *et al.* (2013). Surviving sepsis campaign: international guidelines for management of severe sepsis and septic shock: 2012. Crit Care Med *41*, 580-637.

Diep, Q.N., Amiri, F., Touyz, R.M., Cohn, J.S., Endemann, D., Neves, M.F., and Schiffrin, E.L. (2002). PPARalpha activator effects on Ang II-induced vascular oxidative stress and inflammation. Hypertension *40*, 866-871.

Doi, A., and Ramirez, J.M. (2008). Neuromodulation and the orchestration of the respiratory rhythm. Respir Physiol Neurobiol *164*, 96-104.

Drake, R.L., Vogl, W., Mitchell, A.W.M., and Gray, H. (2005). Gray's anatomy for students (Philadelphia: Elsevier/Churchill Livingstone).

Dunser, M.W., Mayr, A.J., Ulmer, H., Knotzer, H., Sumann, G., Pajk, W., Friesenecker, B., and Hasibeder, W.R. (2003). Arginine vasopressin in advanced vasodilatory shock: a prospective, randomized, controlled study. Circulation *107*, 2313-2319.

Ebert, T.J., and Stowe, D.F. (1996). Neural and endothelial control of the peripheral circulation--implications for anesthesia: Part I. Neural control of the peripheral vasculature. Journal of cardiothoracic and vascular anesthesia *10*, 147-158.

Edwards, G., Dora, K.A., Gardener, M.J., Garland, C.J., and Weston, A.H. (1998). K+ is an endothelium-derived hyperpolarizing factor in rat arteries. Nature *396*, 269-272.

Edwards, G., Feletou, M., and Weston, A.H. (2010). Endothelium-derived hyperpolarising factors and associated pathways: a synopsis. Pflugers Arch *459*, 863-879.

Eisen, D., Planatscher, H., Hardie, D.B., Kraushaar, U., Pynn, C.J., Stoll, D., Borchers, C., Joos, T.O., and Poetz, O. (2013). G protein-coupled receptor quantification using peptide group-specific enrichment combined with internal peptide standard reporter calibration. Journal of proteomics *90*, 85-95.

Elam, M., Yao, T., Thoren, P., and Svensson, T.H. (1981). Hypercapnia and hypoxia: chemoreceptor-mediated control of locus coeruleus neurons and splanchnic, sympathetic nerves. Brain Res *222*, 373-381.

Ellis, A., and Triggle, C.R. (2003). Endothelium-derived reactive oxygen species: their relationship to endothelium-dependent hyperpolarization and vascular tone. Can J Physiol Pharmacol *81*, 1013-1028.

Emerson, G.G., and Segal, S.S. (2000). Electrical coupling between endothelial cells and smooth muscle cells in hamster feed arteries: role in vasomotor control. Circ Res *87*, 474-479.

Endemann, D.H., and Schiffrin, E.L. (2004). Endothelial dysfunction. Journal of the American Society of Nephrology : JASN *15*, 1983-1992.

Esper, R.J., Nordaby, R.A., Vilarino, J.O., Paragano, A., Cacharron, J.L., and Machado, R.A. (2006). Endothelial dysfunction: a comprehensive appraisal. Cardiovascular diabetology *5*, 4.

Fang, Y., Schram, G., Romanenko, V.G., Shi, C., Conti, L., Vandenberg, C.A., Davies, P.F., Nattel, S., and Levitan, I. (2005). Functional expression of Kir2.x in human aortic endothelial cells: the dominant role of Kir2.2. Am J Physiol Cell Physiol *289*, C1134-1144.

Faraci, F.M., and Heistad, D.D. (1990). Regulation of large cerebral arteries and cerebral microvascular pressure. Circ Res *66*, 8-17.

Feigl, E.O. (1983). Coronary physiology. Physiol Rev *63*, 1-205.

Feletou, M. (2011). In The Endothelium: Part 1: Multiple Functions of the Endothelial Cells-Focus on Endothelium-Derived Vasoactive Mediators (San Rafael (CA)).

Felgner, P.L., Gadek, T.R., Holm, M., Roman, R., Chan, H.W., Wenz, M., Northrop, J.P., Ringold, G.M., and Danielsen, M. (1987). Lipofection: a highly efficient, lipid-mediated DNA-transfection procedure. Proc Natl Acad Sci U S A *84*, 7413-7417.

Fenno, L., Yizhar, O., and Deisseroth, K. (2011). The development and application of optogenetics. Annu Rev Neurosci *34*, 389-412.

Ferguson-Myrthil, N. (2012). Vasopressor use in adult patients. Cardiology in review *20*, 153-158.

Figueiredo, M., Lane, S., Tang, F., Liu, B.H., Hewinson, J., Marina, N., Kasymov, V., Souslova, E.A., Chudakov, D.M., Gourine, A.V., *et al.* (2011). Optogenetic experimentation on astrocytes. Experimental physiology *96*, 40-50.

Filosa, J.A., Dean, J.B., and Putnam, R.W. (2002). Role of intracellular and extracellular pH in the chemosensitive response of rat locus coeruleus neurones. J Physiol *541*, 493-509.

Filosa, J.A., and Putnam, R.W. (2003). Multiple targets of chemosensitive signaling in locus coeruleus neurons: role of K+ and Ca2+ channels. Am J Physiol Cell Physiol *284*, C145-155.

Fioret, B.A., Heimfeld, J.D., Paik, D.T., and Hatzopoulos, A.K. (2014). Endothelial cells contribute to generation of adult ventricular myocytes during cardiac homeostasis. Cell reports *8*, 229-241.

Fisslthaler, B., Popp, R., Kiss, L., Potente, M., Harder, D.R., Fleming, I., and Busse, R. (1999). Cytochrome P450 2C is an EDHF synthase in coronary arteries. Nature *401*, 493-497.

Fitch, S.J., and Gossage, J.R. (2002). Optimal management of septic shock. Rapid recognition and institution of therapy are crucial. Postgraduate medicine *111*, 53-56, 59-60, 63-54 passim.

Fitch, W.M., and Yasunobu, K.T. (1975). Phylogenies from amino acid sequences aligned with gaps: the problem of gap weighting. Journal of molecular evolution *5*, 1-24.

Forstermann, U., Closs, E.I., Pollock, J.S., Nakane, M., Schwarz, P., Gath, I., and Kleinert, H. (1994). Nitric oxide synthase isozymes. Characterization, purification, molecular cloning, and functions. Hypertension *23*, 1121-1131.

Forstermann, U., and Munzel, T. (2006). Endothelial nitric oxide synthase in vascular disease: from marvel to menace. Circulation *113*, 1708-1714.

Forstermann, U., and Sessa, W.C. (2012). Nitric oxide synthases: regulation and function. European heart journal *33*, 829-837, 837a-837d.

Frohlich, E. (2002). [Structure and function of blood-tissue barriers]. Deutsche medizinische Wochenschrift *127*, 2629-2634.

Funk, G.D., Smith, J.C., and Feldman, J.L. (1994). Development of thyrotropin-releasing hormone and norepinephrine potentiation of inspiratory-related hypoglossal motoneuron discharge in neonatal and juvenile mice in vitro. J Neurophysiol *72*, 2538-2541.

Gallego, J., Nsegbe, E., and Durand, E. (2001). Learning in respiratory control. Behavior modification *25*, 495-512.

Ganitkevich, V.Y., and Isenberg, G. (1991). Depolarization-mediated intracellular calcium transients in isolated smooth muscle cells of guinea-pig urinary bladder. J Physiol *435*, 187-205.

Gardiner, S.M., March, J.E., Kemp, P.A., and Bennett, T. (2001). Effects of the novel selective endothelin ET(A) receptor antagonist, SB 234551, on the cardiovascular responses to endotoxaemia in conscious rats. Br J Pharmacol *133*, 1371-1377.

Garrett, R., Grisham, C.M., and Sabat, M. (2013). Biochemistry, 5th edn (Australia ; United Kingdom: Brooks/Cole Cengage Learning).

Gaultier, C. (2004). Genes and genetics in respiratory control. Paediatric respiratory reviews *5*, 166-172.

Gebremedhin, D., Lange, A.R., Lowry, T.F., Taheri, M.R., Birks, E.K., Hudetz, A.G., Narayanan, J., Falck, J.R., Okamoto, H., Roman, R.J., *et al.* (2000). Production of 20-HETE and its role in autoregulation of cerebral blood flow. Circ Res *87*, 60-65.

Glaze, D.G. (2005). Neurophysiology of Rett syndrome. Journal of child neurology *20*, 740-746.

Gong, M.C., Fujihara, H., Somlyo, A.V., and Somlyo, A.P. (1997). Translocation of rhoA associated with Ca2+ sensitization of smooth muscle. J Biol Chem *272*, 10704-10709.

Gordan, R., Gwathmey, J.K., and Xie, L.H. (2015). Autonomic and endocrine control of cardiovascular function. World journal of cardiology *7*, 204-214.

Gory, S., Vernet, M., Laurent, M., Dejana, E., Dalmon, J., and Huber, P. (1999). The vascular endothelial-cadherin promoter directs endothelial-specific expression in transgenic mice. Blood *93*, 184-192.

Gothert, J.R., Gustin, S.E., Hall, M.A., Green, A.R., Gottgens, B., Izon, D.J., and Begley, C.G. (2005). In vivo fate-tracing studies using the Scl stem cell enhancer: embryonic hematopoietic stem cells significantly contribute to adult hematopoiesis. Blood *105*, 2724-2732.

Govers, R., and Rabelink, T.J. (2001). Cellular regulation of endothelial nitric oxide synthase. American journal of physiology Renal physiology *280*, F193-206.

Goyette, R.E., Key, N.S., and Ely, E.W. (2004). Hematologic changes in sepsis and their therapeutic implications. Seminars in respiratory and critical care medicine *25*, 645-659.

Graham, R.M., Perez, D.M., Hwa, J., and Piascik, M.T. (1996). alpha 1-adrenergic receptor subtypes. Molecular structure, function, and signaling. Circ Res *78*, 737-749.

Grant, B.J., Davies, E.E., Jones, H.A., and Hughes, J.M. (1976). Local regulation of pulmonary blood flow and ventilation-perfusion ratios in the coatimundi. Journal of applied physiology *40*, 216-228.

Green, A.R., Hall, J.E., and Rees, A.R. (1981). A behavioural and biochemical study in rats of 5-hydroxytryptamine receptor agonists and antagonists, with observations on structure-activity requirements for the agonists. Br J Pharmacol *73*, 703-719.

Griendling, K.K., and FitzGerald, G.A. (2003). Oxidative stress and cardiovascular injury: Part I: basic mechanisms and in vivo monitoring of ROS. Circulation *108*, 1912-1916.

Griendling, K.K., Rittenhouse, S.E., Brock, T.A., Ekstein, L.S., Gimbrone, M.A., Jr., and Alexander, R.W. (1986). Sustained diacylglycerol formation from inositol phospholipids in angiotensin II-stimulated vascular smooth muscle cells. J Biol Chem *261*, 5901-5906.

Groves, P., Kurz, S., Just, H., and Drexler, H. (1995). Role of endogenous bradykinin in human coronary vasomotor control. Circulation *92*, 3424-3430.

Gryglewski, R. (1983). Prostacyclin--experimental and clinical approach. Advances in prostaglandin, thromboxane, and leukotriene research *11*, 457-461.

Guilluy, C., Sauzeau, V., Rolli-Derkinderen, M., Guerin, P., Sagan, C., Pacaud, P., and Loirand, G. (2005). Inhibition of RhoA/Rho kinase pathway is involved in the beneficial effect of sildenafil on pulmonary hypertension. Br J Pharmacol *146*, 1010-1018.

Guimaraes, S., and Moura, D. (2001). Vascular adrenoceptors: an update. Pharmacological reviews *53*, 319-356.

Guo, W., Lasky, J.L., Chang, C.J., Mosessian, S., Lewis, X., Xiao, Y., Yeh, J.E., Chen, J.Y., Iruela-Arispe, M.L., Varella-Garcia, M., *et al.* (2008). Multi-genetic events collaboratively contribute to Pten-null leukaemia stem-cell formation. Nature *453*, 529-533.

Gustafsson, E., Brakebusch, C., Hietanen, K., and Fassler, R. (2001). Tie-1-directed expression of Cre recombinase in endothelial cells of embryoid bodies and transgenic mice. Journal of cell science *114*, 671-676.

Halushka, P.V., Mais, D.E., Mayeux, P.R., and Morinelli, T.A. (1989). Thromboxane, prostaglandin and leukotriene receptors. Annu Rev Pharmacol Toxicol *29*, 213-239.

Han, X. (2012). In vivo application of optogenetics for neural circuit analysis. ACS chemical neuroscience *3*, 577-584.

Han, X., and Boyden, E.S. (2007). Multiple-color optical activation, silencing, and desynchronization of neural activity, with single-spike temporal resolution. PLoS One *2*, e299.

Harvey, R.D., and Belevych, A.E. (2003). Muscarinic regulation of cardiac ion channels. Br J Pharmacol *139*, 1074-1084.

Hassid, A. (1984). Stimulation of prostacyclin synthesis by thromboxane A2-like prostaglandin endoperoxide analogues in cultured vascular smooth muscle cells. Biochem Biophys Res Commun *123*, 21-26.

Hau, J., and Van Hoosier, G.L. (2003). Handbook of laboratory animal science, 2nd edn (Boca Raton ; London: CRC Press).

Hauser, B., Radermacher, P., Thiemermann, C., and Matejovic, M. (2004). Nitric oxide, bacteria, and host defense in sepsis: who needs what? Shock *22*, 588-590.

Haxhiu, M.A., Tolentino-Silva, F., Pete, G., Kc, P., and Mack, S.O. (2001). Monoaminergic neurons, chemosensation and arousal. Respir Physiol *129*, 191-209.

Heppner, T.J., Werner, M.E., Nausch, B., Vial, C., Evans, R.J., and Nelson, M.T. (2009). Nerve-evoked purinergic signalling suppresses action potentials, Ca2+ flashes and contractility evoked by muscarinic receptor activation in mouse urinary bladder smooth muscle. J Physiol *587*, 5275-5288.

Herrmann, J., and Lerman, A. (2001). The endothelium: dysfunction and beyond. J Nucl Cardiol *8*, 197-206.

Hertsens, T. (2005). An overview of laser diode characteristics: Application note.

Hilaire, G. (2006). Endogenous noradrenaline affects the maturation and function of the respiratory network: possible implication for SIDS. Autonomic neuroscience : basic & clinical *126-127*, 320-331.

Hilaire, G., Viemari, J.C., Coulon, P., Simonneau, M., and Bevengut, M. (2004). Modulation of the respiratory rhythm generator by the pontine noradrenergic A5 and A6 groups in rodents. Respir Physiol Neurobiol *143*, 187-197.

Hill-Eubanks, D.C., Werner, M.E., Heppner, T.J., and Nelson, M.T. (2011). Calcium signaling in smooth muscle. Cold Spring Harbor perspectives in biology *3*, a004549.

Hisatsune, H., Matsumura, K., Ogawa, M., Uemura, A., Kondo, N., Yamashita, J.K., Katsuta, H., Nishikawa, S., Chiba, T., and Nishikawa, S. (2005). High level of endothelial cell-specific gene expression by a combination of the 5' flanking region and the 5' half of the first intron of the VE-cadherin gene. Blood *105*, 4657-4663.

Hollenberg, S.M. (2007). Vasopressor support in septic shock. Chest *132*, 1678-1687.

Hughes, J.M. (1975). Lung gas tensions and active regulation of ventilation/perfusion ratios in health and disease. British journal of diseases of the chest *69*, 153-170.

Hulme, E.C., Birdsall, N.J., and Buckley, N.J. (1990). Muscarinic receptor subtypes. Annu Rev Pharmacol Toxicol *30*, 633-673.

Huribal, M., Cunningham, M.E., D'Aiuto, M.L., Pleban, W.E., and McMillen, M.A. (1995). Endothelin levels in patients with burns covering more than 20% body surface area. The Journal of burn care & rehabilitation *16*, 23-26.

Hyvelin, J.M., O'Connor, C., and McLoughlin, P. (2004). Effect of changes in pH on wall tension in isolated rat pulmonary artery: role of the RhoA/Rho-kinase pathway. American journal of physiology Lung cellular and molecular physiology *287*, L673-684.

Ide, S., Itoh, M., and Goto, Y. (2005). Defect in normal developmental increase of the brain biogenic amine concentrations in the mecp2-null mouse. Neurosci Lett *386*, 14-17.

Inagami, T., Naruse, M., and Hoover, R. (1995). Endothelium as an endocrine organ. Annual review of physiology *57*, 171-189.

Inoue, A., Yanagisawa, M., Kimura, S., Kasuya, Y., Miyauchi, T., Goto, K., and Masaki, T. (1989). The human endothelin family: three structurally and pharmacologically distinct isopeptides predicted by three separate genes. Proc Natl Acad Sci U S A *86*, 2863-2867.

Irion, S., Luche, H., Gadue, P., Fehling, H.J., Kennedy, M., and Keller, G. (2007). Identification and targeting of the ROSA26 locus in human embryonic stem cells. Nature biotechnology *25*, 1477-1482.

Irisawa, H., Brown, H.F., and Giles, W. (1993). Cardiac pacemaking in the sinoatrial node. Physiol Rev *73*, 197-227.

Ishizuka, T., Kakuda, M., Araki, R., and Yawo, H. (2006). Kinetic evaluation of photosensitivity in genetically engineered neurons expressing green algae light-gated channels. Neurosci Res *54*, 85-94.

Ito, A., Shimokawa, H., Nakaike, R., Fukai, T., Sakata, M., Takayanagi, T., Egashira, K., and Takeshita, A. (1994). Role of protein kinase C-mediated pathway in the pathogenesis of coronary artery spasm in a swine model. Circulation *90*, 2425-2431.

Jespersen, B., Tykocki, N.R., Watts, S.W., and Cobbett, P.J. (2015). Measurement of smooth muscle function in the isolated tissue bath-applications to pharmacology research. Journal of visualized experiments : JoVE, 52324.

Jia, Z., Valiunas, V., Lu, Z., Bien, H., Liu, H., Wang, H.Z., Rosati, B., Brink, P.R., Cohen, I.S., and Entcheva, E. (2011). Stimulating cardiac muscle by light: cardiac optogenetics by cell delivery. Circ Arrhythm Electrophysiol *4*, 753-760.

Jiang, C., Xu, H., Cui, N., and Wu, J. (2001). An alternative approach to the identification of respiratory central chemoreceptors in the brainstem. Respir Physiol *129*, 141-157.

Jin, X.T., Cui, N., Zhong, W., Jin, X., Wu, Z., and Jiang, C. (2013). Pre- and postsynaptic modulations of hypoglossal motoneurons by alpha-adrenoceptor activation in wild-type and Mecp2(-/Y) mice. Am J Physiol Cell Physiol *305*, C1080-1090.

Johnson, J.A., and Liggett, S.B. (2011). Cardiovascular pharmacogenomics of adrenergic receptor signaling: clinical implications and future directions. Clinical pharmacology and therapeutics *89*, 366-378.

Joyner, M.J., Nauss, L.A., Warner, M.A., and Warner, D.O. (1992). Sympathetic modulation of blood flow and O2 uptake in rhythmically contracting human forearm muscles. Am J Physiol *263*, H1078-1083.

Julu, P.O., Kerr, A.M., Apartopoulos, F., Al-Rawas, S., Engerstrom, I.W., Engerstrom, L., Jamal, G.A., and Hansen, S. (2001). Characterisation of breathing and associated central autonomic dysfunction in the Rett disorder. Archives of disease in childhood *85*, 29-37.

Kadamur, G., and Ross, E.M. (2013). Mammalian phospholipase C. Annual review of physiology *75*, 127-154.

Kam, N.W., Liu, Z., and Dai, H. (2005). Functionalization of carbon nanotubes via cleavable disulfide bonds for efficient intracellular delivery of siRNA and potent gene silencing. J Am Chem Soc *127*, 12492-12493.

Kenakin, T.P. (1984). The classification of drugs and drug receptors in isolated tissues. Pharmacological reviews *36*, 165-222.

Kerr, A.M., Armstrong, D.D., Prescott, R.J., Doyle, D., and Kearney, D.L. (1997). Rett syndrome: analysis of deaths in the British survey. European child & adolescent psychiatry *6 Suppl 1*, 71-74.

Kersh, A.E., Hartzler, L.K., Havlin, K., Hubbell, B.B., Nanagas, V., Kalra, A., Chua, J., Whitesell, R., Ritucci, N.A., Dean, J.B., *et al.* (2009). pH regulating transporters in neurons from various chemosensitive brainstem regions in neonatal rats. American journal of physiology Regulatory, integrative and comparative physiology *297*, R1409-1420.

Khalil, R.A. (2010). Regulation of vascular smooth muscle function (San Rafael, Calif.: Morgan & Claypool Life Sciences).

Khalil, R.A., Menice, C.B., Wang, C.L., and Morgan, K.G. (1995). Phosphotyrosine-dependent targeting of mitogen-activated protein kinase in differentiated contractile vascular cells. Circ Res *76*, 1101-1108.

Khazaei, M., Moien-Afshari, F., and Laher, I. (2008). Vascular endothelial function in health and diseases. Pathophysiology : the official journal of the International Society for Pathophysiology / ISP *15*, 49-67.

Kiely, D.G., Cargill, R.I., Struthers, A.D., and Lipworth, B.J. (1997). Cardiopulmonary effects of endothelin-1 in man. Cardiovascular research *33*, 378-386.

Kim, H.R., Appel, S., Vetterkind, S., Gangopadhyay, S.S., and Morgan, K.G. (2008). Smooth muscle signalling pathways in health and disease. Journal of cellular and molecular medicine *12*, 2165-2180.

Kisanuki, Y.Y., Hammer, R.E., Miyazaki, J., Williams, S.C., Richardson, J.A., and Yanagisawa, M. (2001). Tie2-Cre transgenic mice: a new model for endothelial cell-lineage analysis in vivo. Developmental biology *230*, 230-242.

Kizub, I.V., Pavlova, O.O., Johnson, C.D., Soloviev, A.I., and Zholos, A.V. (2010). Rho kinase and protein kinase C involvement in vascular smooth muscle myofilament calcium sensitization in arteries from diabetic rats. Br J Pharmacol *159*, 1724-1731.

Kneuer, C., Sameti, M., Bakowsky, U., Schiestel, T., Schirra, H., Schmidt, H., and Lehr, C.M. (2000). A nonviral DNA delivery system based on surface modified silica-nanoparticles can efficiently transfect cells in vitro. Bioconjug Chem *11*, 926-932.

Knollmann, B.C. (2010). Pacing lightly: optogenetics gets to the heart. Nat Methods *7*, 889-891.

Koppenol, W.H., Moreno, J.J., Pryor, W.A., Ischiropoulos, H., and Beckman, J.S. (1992). Peroxynitrite, a cloaked oxidant formed by nitric oxide and superoxide. Chem Res Toxicol *5*, 834-842.

Kozasa, T., Jiang, X., Hart, M.J., Sternweis, P.M., Singer, W.D., Gilman, A.G., Bollag, G., and Sternweis, P.C. (1998). p115 RhoGEF, a GTPase activating protein for Galpha12 and Galpha13. Science *280*, 2109-2111.

Kozyreva, T.V., and Simonova, T.G. (1998). [Modulating effect of peripheral thermoreceptors on human respiration]. Vestnik Rossiiskoi akademii meditsinskikh nauk / Rossiiskaia akademiia meditsinskikh nauk, 14-18.

Krejci, V., Hiltebrand, L.B., Erni, D., and Sigurdsson, G.H. (2003). Endothelin receptor antagonist bosentan improves microcirculatory blood flow in splanchnic organs in septic shock. Crit Care Med *31*, 203-210.

Kumar, N.N., Velic, A., Soliz, J., Shi, Y., Li, K., Wang, S., Weaver, J.L., Sen, J., Abbott, S.B., Lazarenko, R.M., *et al.* (2015). PHYSIOLOGY. Regulation of breathing by CO(2) requires the proton-activated receptor GPR4 in retrotrapezoid nucleus neurons. Science *348*, 1255-1260.

Kumar, P., and Prabhakar, N.R. (2012). Peripheral chemoreceptors: function and plasticity of the carotid body. Comprehensive Physiology *2*, 141-219.

Laffon, M., Lu, L.N., Modelska, K., Matthay, M.A., and Pittet, J.F. (1999). alpha-adrenergic blockade restores normal fluid transport capacity of alveolar epithelium after hemorrhagic shock. Am J Physiol *277*, L760-768.

Lammel, S., Steinberg, E.E., Foldy, C., Wall, N.R., Beier, K., Luo, L., and Malenka, R.C. (2015). Diversity of transgenic mouse models for selective targeting of midbrain dopamine neurons. Neuron *85*, 429-438.

Lamping, K.G., Kanatsuka, H., Eastham, C.L., Chilian, W.M., and Marcus, M.L. (1989). Nonuniform vasomotor responses of the coronary microcirculation to serotonin and vasopressin. Circ Res *65*, 343-351.

Landmesser, U., Dikalov, S., Price, S.R., McCann, L., Fukai, T., Holland, S.M., Mitch, W.E., and Harrison, D.G. (2003). Oxidation of tetrahydrobiopterin leads to uncoupling of endothelial cell nitric oxide synthase in hypertension. J Clin Invest *111*, 1201-1209.

Lanyi, J.K., Duschl, A., Hatfield, G.W., May, K., and Oesterhelt, D. (1990). The primary structure of a halorhodopsin from Natronobacterium pharaonis. Structural, functional and evolutionary implications for bacterial rhodopsins and halorhodopsins. J Biol Chem *265*, 1253-1260.

Laporte, R., Hui, A., and Laher, I. (2004). Pharmacological modulation of sarcoplasmic reticulum function in smooth muscle. Pharmacological reviews *56*, 439-513.

Laragh, J.H., and Brenner, B.M. (1995). Hypertension : pathophysiology, diagnosis, and management, 2nd edn (New York: Raven Press).

Lepper, P.M., Held, T.K., Schneider, E.M., Bolke, E., Gerlach, H., and Trautmann, M. (2002). Clinical implications of antibiotic-induced endotoxin release in septic shock. Intensive Care Med *28*, 824-833.

Lerman, A., and Zeiher, A.M. (2005). Endothelial function: cardiac events. Circulation *111*, 363-368.

Li, L., Jia, Z.H., Chen, C., Wei, C., Han, J.K., Wu, Y.L., and Ren, L.M. (2011). Physiological significance of P2X receptor-mediated vasoconstriction in five different types of arteries in rats. Purinergic signalling *7*, 221-229.

Licht, A.H., Raab, S., Hofmann, U., and Breier, G. (2004). Endothelium-specific Cre recombinase activity in flk-1-Cre transgenic mice. Dev Dyn *229*, 312-318.

Lin, J.Y. (2011). A user's guide to channelrhodopsin variants: features, limitations and future developments. Experimental physiology *96*, 19-25.

Lin, J.Y., Lin, M.Z., Steinbach, P., and Tsien, R.Y. (2009). Characterization of engineered channelrhodopsin variants with improved properties and kinetics. Biophys J *96*, 1803-1814.

Liu, S., Zhang, Y., Moayeri, M., Liu, J., Crown, D., Fattah, R.J., Wein, A.N., Yu, Z.X., Finkel, T., and Leppla, S.H. (2013). Key tissue targets responsible for anthrax-toxin-induced lethality. Nature *501*, 63-68.

Louis, S.F., and Zahradka, P. (2010). Vascular smooth muscle cell motility: From migration to invasion. Experimental and clinical cardiology *15*, e75-85.

Lowe, A.W., Madeddu, L., and Kelly, R.B. (1988). Endocrine secretory granules and neuronal synaptic vesicles have three integral membrane proteins in common. The Journal of cell biology *106*, 51-59.

MacLean, M.R., Herve, P., Eddahibi, S., and Adnot, S. (2000). 5-hydroxytryptamine and the pulmonary circulation: receptors, transporters and relevance to pulmonary arterial hypertension. Br J Pharmacol *131*, 161-168.

Madisen, L., Mao, T., Koch, H., Zhuo, J.M., Berenyi, A., Fujisawa, S., Hsu, Y.W., Garcia, A.J., 3rd, Gu, X., Zanella, S., *et al.* (2012). A toolbox of Cre-dependent optogenetic transgenic mice for light-induced activation and silencing. Nat Neurosci *15*, 793-802.

Makita, K., Falck, J.R., and Capdevila, J.H. (1996). Cytochrome P450, the arachidonic acid cascade, and hypertension: new vistas for an old enzyme system. FASEB J *10*, 1456-1463.

Mangoni, M.E., and Nargeot, J. (2008). Genesis and regulation of the heart automaticity. Physiol Rev *88*, 919-982.

Martin, G.S., Mannino, D.M., Eaton, S., and Moss, M. (2003). The epidemiology of sepsis in the United States from 1979 through 2000. N Engl J Med *348*, 1546-1554.

Matsumoto, T., Kobayashi, T., Ishida, K., Taguchi, K., and Kamata, K. (2010). Enhancement of mesenteric artery contraction to 5-HT depends on Rho kinase and Src kinase pathways in the ob/ob mouse model of type 2 diabetes. Br J Pharmacol *160*, 1092-1104.

McBride, H.M., Neuspiel, M., and Wasiak, S. (2006). Mitochondria: more than just a powerhouse. Current biology : CB *16*, R551-560.

McKay, L.C., Evans, K.C., Frackowiak, R.S., and Corfield, D.R. (2003). Neural correlates of voluntary breathing in humans. J Appl Physiol (1985) *95*, 1170-1178.

Medzhitov, R. (2001). Toll-like receptors and innate immunity. Nature reviews Immunology *1*, 135-145.

Meidan, R., Altstein, M., and Girsh, E. (1992). Biosynthesis and release of oxytocin by granulosa cells derived from preovulatory bovine follicles: effects of forskolin and insulin-like growth factor-I. Biology of reproduction *46*, 715-720.

Michiels, C. (2003). Endothelial cell functions. J Cell Physiol *196*, 430-443.

Milstien, S., and Katusic, Z. (1999). Oxidation of tetrahydrobiopterin by peroxynitrite: implications for vascular endothelial function. Biochem Biophys Res Commun *263*, 681-684.

Minneman, K.P., Pittman, R.N., and Molinoff, P.B. (1981). Beta-adrenergic receptor subtypes: properties, distribution, and regulation. Annu Rev Neurosci *4*, 419-461.

Mitchell, J.A., and Warner, T.D. (2006). COX isoforms in the cardiovascular system: understanding the activities of non-steroidal anti-inflammatory drugs. Nat Rev Drug Discov *5*, 75-86.

Molinoff, P.B. (1984). Alpha- and beta-adrenergic receptor subtypes properties, distribution and regulation. Drugs *28 Suppl 2*, 1-15.

Mollace, V., Muscoli, C., Masini, E., Cuzzocrea, S., and Salvemini, D. (2005). Modulation of prostaglandin biosynthesis by nitric oxide and nitric oxide donors. Pharmacological reviews *57*, 217-252.

Monassier, L., Laplante, M.A., Ayadi, T., Doly, S., and Maroteaux, L. (2010). Contribution of gene-modified mice and rats to our understanding of the cardiovascular pharmacology of serotonin. Pharmacology & therapeutics *128*, 559-567.

Moncada, S., Gryglewski, R., Bunting, S., and Vane, J.R. (1976). An enzyme isolated from arteries transforms prostaglandin endoperoxides to an unstable substance that inhibits platelet aggregation. Nature *263*, 663-665.

Moncada, S., Palmer, R.M., and Higgs, E.A. (1991). Nitric oxide: physiology, pathophysiology, and pharmacology. Pharmacological reviews *43*, 109-142.

Moretti, P., and Zoghbi, H.Y. (2006). MeCP2 dysfunction in Rett syndrome and related disorders. Curr Opin Genet Dev *16*, 276-281.

Mostefai, H.A., Meziani, F., Mastronardi, M.L., Agouni, A., Heymes, C., Sargentini, C., Asfar, P., Martinez, M.C., and Andriantsitohaina, R. (2008). Circulating microparticles from patients with septic shock exert protective role in vascular function. Am J Respir Crit Care Med *178*, 1148-1155.

Nagel, G., Brauner, M., Liewald, J.F., Adeishvili, N., Bamberg, E., and Gottschalk, A. (2005). Light activation of channelrhodopsin-2 in excitable cells of Caenorhabditis elegans triggers rapid behavioral responses. Current biology : CB *15*, 2279-2284.

Nagel, G., Ollig, D., Fuhrmann, M., Kateriya, S., Musti, A.M., Bamberg, E., and Hegemann, P. (2002). Channelrhodopsin-1: a light-gated proton channel in green algae. Science *296*, 2395-2398.

Nagel, G., Szellas, T., Huhn, W., Kateriya, S., Adeishvili, N., Berthold, P., Ollig, D., Hegemann, P., and Bamberg, E. (2003). Channelrhodopsin-2, a directly light-gated cation-selective membrane channel. Proc Natl Acad Sci U S A *100*, 13940-13945.

Nattie, E. (2006). Why do we have both peripheral and central chemoreceptors? J Appl Physiol (1985) *100*, 9-10.

Nattie, E.E., and Li, A. (1996). Central chemoreception in the region of the ventral respiratory group in the rat. J Appl Physiol (1985) *81*, 1987-1995.

Nattie, E.E., and Li, A. (2001). CO2 dialysis in the medullary raphe of the rat increases ventilation in sleep. J Appl Physiol (1985) *90*, 1247-1257.

Nattie, E.E., and Li, A. (2002). CO2 dialysis in nucleus tractus solitarius region of rat increases ventilation in sleep and wakefulness. J Appl Physiol (1985) *92*, 2119-2130.

Nestler, E.J., Alreja, M., and Aghajanian, G.K. (1999). Molecular control of locus coeruleus neurotransmission. Biological psychiatry *46*, 1131-1139.

Neul, J.L., Fang, P., Barrish, J., Lane, J., Caeg, E.B., Smith, E.O., Zoghbi, H., Percy, A., and Glaze, D.G. (2008). Specific mutations in methyl-CpG-binding protein 2 confer different severity in Rett syndrome. Neurology *70*, 1313-1321.

Nishikawa, M., and Huang, L. (2001). Nonviral vectors in the new millennium: delivery barriers in gene transfer. Hum Gene Ther *12*, 861-870.

Nishikawa, Y., Stepp, D.W., and Chilian, W.M. (2000). Nitric oxide exerts feedback inhibition on EDHF-induced coronary arteriolar dilation in vivo. Am J Physiol Heart Circ Physiol *279*, H459-465.

Nishimura, J., Khalil, R.A., Drenth, J.P., and van Breemen, C. (1990). Evidence for increased myofilament Ca2+ sensitivity in norepinephrine-activated vascular smooth muscle. Am J Physiol *259*, H2-8.

Nishino, T. (1993). Swallowing as a protective reflex for the upper respiratory tract. Anesthesiology *79*, 588-601.

Noback, C.R., Demarest, R.J., Ruggiero, D.A., Strominger, N.L., and SpringerLink (Online service) (2005). The Human Nervous System Structure and Function (Totowa, NJ: Humana Press Inc.,).

Noda, M., Yasuda-Fukazawa, C., Moriishi, K., Kato, T., Okuda, T., Kurokawa, K., and Takuwa, Y. (1995). Involvement of rho in GTP gamma S-induced enhancement of phosphorylation of 20 kDa myosin light chain in vascular smooth muscle cells: inhibition of phosphatase activity. FEBS letters *367*, 246-250.

O'Brien, A.J., Wilson, A.J., Sibbald, R., Singer, M., and Clapp, L.H. (2001). Temporal variation in endotoxin-induced vascular hyporeactivity in a rat mesenteric artery organ culture model. Br J Pharmacol *133*, 351-360.

Ogier, M., and Katz, D.M. (2008). Breathing dysfunction in Rett syndrome: understanding epigenetic regulation of the respiratory network. Respir Physiol Neurobiol *164*, 55-63.

Ohkubo, S., Nakahata, N., and Ohizumi, Y. (1996). Thromboxane A2-mediated shape change: independent of Gq-phospholipase C--Ca2+ pathway in rabbit platelets. Br J Pharmacol *117*, 1095-1104.

Okada, Y., Chen, Z., Jiang, W., Kuwana, S., and Eldridge, F.L. (2002). Anatomical arrangement of hypercapnia-activated cells in the superficial ventral medulla of rats. J Appl Physiol (1985) *93*, 427-439.

Olivetti, G., Cigola, E., Maestri, R., Corradi, D., Lagrasta, C., Gambert, S.R., and Anversa, P. (1996). Aging, cardiac hypertrophy and ischemic cardiomyopathy do not affect the proportion of mononucleated and multinucleated myocytes in the human heart. Journal of molecular and cellular cardiology *28*, 1463-1477.

Ozawa, S., and Sand, O. (1986). Electrophysiology of excitable endocrine cells. Physiol Rev *66*, 887-952.

Pablo Huidobro-Toro, J., and Veronica Donoso, M. (2004). Sympathetic co-transmission: the coordinated action of ATP and noradrenaline and their modulation by neuropeptide Y in human vascular neuroeffector junctions. European journal of pharmacology *500*, 27-35.

Palmer, R.M., Ferrige, A.G., and Moncada, S. (1987). Nitric oxide release accounts for the biological activity of endothelium-derived relaxing factor. Nature *327*, 524-526.

Parente, L., and Perretti, M. (2003). Advances in the pathophysiology of constitutive and inducible cyclooxygenases: two enzymes in the spotlight. Biochem Pharmacol *65*, 153-159.

Parkis, M.A., Bayliss, D.A., and Berger, A.J. (1995). Actions of norepinephrine on rat hypoglossal motoneurons. J Neurophysiol *74*, 1911-1919.

Paton, J.F., Deuchars, J., Ahmad, Z., Wong, L.F., Murphy, D., and Kasparov, S. (2001). Adenoviral vector demonstrates that angiotensin II-induced depression of the cardiac baroreflex is mediated by endothelial nitric oxide synthase in the nucleus tractus solitarii of the rat. J Physiol *531*, 445-458.

Pedoto, A., Tassiopoulos, A.K., Oler, A., McGraw, D.J., Hoffmann, S.P., Camporesi, E.M., and Hakim, T.S. (1998). Treatment of septic shock in rats with nitric oxide synthase inhibitors and inhaled nitric oxide. Crit Care Med *26*, 2021-2028.

Perez, C.M., Hazari, M.S., and Farraj, A.K. (2015). Role of autonomic reflex arcs in cardiovascular responses to air pollution exposure. Cardiovascular toxicology *15*, 69-78.

Pernow, J., Kaijser, L., Lundberg, J.M., and Ahlborg, G. (1996). Comparable potent coronary constrictor effects of endothelin-1 and big endothelin-1 in humans. Circulation *94*, 2077-2082.

Pineda, J., and Aghajanian, G.K. (1997). Carbon dioxide regulates the tonic activity of locus coeruleus neurons by modulating a proton- and polyamine-sensitive inward rectifier potassium current. Neuroscience *77*, 723-743.

Pinkert, C.A. (2003). Transgenic animal technology: alternatives in genotyping and phenotyping. Comparative medicine *53*, 126-139.

Pocock, G., and Richards, C.D. (2006). Human physiology : the basis of medicine, 3rd edn (Oxford ; New York: Oxford University Press).

Porth, C., Matfin, G., and Porth, C. (2009). Pathophysiology : concepts of altered health states, 8th edn (Philadelphia: Wolters Kluwer Health/Lippincott Williams & Wilkins).

Prasanna, G., Dibas, A., Hulet, C., and Yorio, T. (2001). Inhibition of Na(+)/K(+)-atpase by endothelin-1 in human nonpigmented ciliary epithelial cells. J Pharmacol Exp Ther *296*, 966-971.

Pratt, P.F., Li, P., Hillard, C.J., Kurian, J., and Campbell, W.B. (2001). Endothelium-independent, ouabain-sensitive relaxation of bovine coronary arteries by EETs. Am J Physiol Heart Circ Physiol *280*, H1113-1121.

Proske, U., and Gandevia, S.C. (2012). The proprioceptive senses: their roles in signaling body shape, body position and movement, and muscle force. Physiol Rev *92*, 1651-1697.

Putnam, R.W., Filosa, J.A., and Ritucci, N.A. (2004). Cellular mechanisms involved in CO(2) and acid signaling in chemosensitive neurons. Am J Physiol Cell Physiol *287*, C1493-1526.

Rajagopalan, S., Kurz, S., Munzel, T., Tarpey, M., Freeman, B.A., Griendling, K.K., and Harrison, D.G. (1996). Angiotensin II-mediated hypertension in the rat increases vascular superoxide production via membrane NADH/NADPH oxidase activation. Contribution to alterations of vasomotor tone. J Clin Invest *97*, 1916-1923.

Richfield, E.K., Thiruchelvam, M.J., Cory-Slechta, D.A., Wuertzer, C., Gainetdinov, R.R., Caron, M.G., Di Monte, D.A., and Federoff, H.J. (2002). Behavioral and neurochemical effects of wild-type and mutated human alpha-synuclein in transgenic mice. Experimental neurology *175*, 35-48.

Roux, J.C., Dura, E., Moncla, A., Mancini, J., and Villard, L. (2007). Treatment with desipramine improves breathing and survival in a mouse model for Rett syndrome. The European journal of neuroscience *25*, 1915-1922.

Roux, J.C., Dura, E., and Villard, L. (2008). Tyrosine hydroxylase deficit in the chemoafferent and the sympathoadrenergic pathways of the Mecp2 deficient mouse. Neurosci Lett *447*, 82-86.

Roux, J.C., Panayotis, N., Dura, E., and Villard, L. (2010). Progressive noradrenergic deficits in the locus coeruleus of Mecp2 deficient mice. J Neurosci Res *88*, 1500-1509.

Roy, I., Mitra, S., Maitra, A., and Mozumdar, S. (2003). Calcium phosphate nanoparticles as novel non-viral vectors for targeted gene delivery. Int J Pharm *250*, 25-33.

Roy, I., Ohulchanskyy, T.Y., Bharali, D.J., Pudavar, H.E., Mistretta, R.A., Kaur, N., and Prasad, P.N. (2005). Optical tracking of organically modified silica nanoparticles as DNA carriers: a nonviral, nanomedicine approach for gene delivery. Proc Natl Acad Sci U S A *102*, 279-284.

Rubio, R., and Berne, R.M. (1975). Regulation of coronary blood flow. Progress in cardiovascular diseases *18*, 105-122.

Rukhadze, I., and Kubin, L. (2007). Mesopontine cholinergic projections to the hypoglossal motor nucleus. Neurosci Lett *413*, 121-125.

Sakar, M.S., Neal, D., Boudou, T., Borochin, M.A., Li, Y., Weiss, R., Kamm, R.D., Chen, C.S., and Asada, H.H. (2012). Formation and optogenetic control of engineered 3D skeletal muscle bioactuators. Lab on a chip *12*, 4976-4985.

Salamanca, D.A., and Khalil, R.A. (2005). Protein kinase C isoforms as specific targets for modulation of vascular smooth muscle function in hypertension. Biochem Pharmacol *70*, 1537-1547.

Salem, A.K., Searson, P.C., and Leong, K.W. (2003). Multifunctional nanorods for gene delivery. Nat Mater *2*, 668-671.

Samuels, E.R., and Szabadi, E. (2008). Functional neuroanatomy of the noradrenergic locus coeruleus: its roles in the regulation of arousal and autonomic function part I: principles of functional organisation. Current neuropharmacology *6*, 235-253.

Sant'Ambrogio, G., Tsubone, H., and Sant'Ambrogio, F.B. (1995). Sensory information from the upper airway: role in the control of breathing. Respir Physiol *102*, 1-16.

Sato, M., Severinghaus, J.W., and Basbaum, A.I. (1992). Medullary CO2 chemoreceptor neuron identification by c-fos immunocytochemistry. J Appl Physiol (1985) *73*, 96-100.

Schmidt, R.F., and Thews, G. (1989). Human physiology, 2nd completely rev. edn (Berlin ; New York: Springer-Verlag).

Schultz, J.H., Czachurski, J., Volk, T., Ehmke, H., and Seller, H. (2003). Central sympathetic chemosensitivity and Kir1 potassium channels in the cat. Brain Res *963*, 113-120.

Seifert, G., Huttmann, K., Binder, D.K., Hartmann, C., Wyczynski, A., Neusch, C., and Steinhauser, C. (2009). Analysis of astroglial K+ channel expression in the developing hippocampus reveals a predominant role of the Kir4.1 subunit. J Neurosci *29*, 7474-7488.

Seko, T., Ito, M., Kureishi, Y., Okamoto, R., Moriki, N., Onishi, K., Isaka, N., Hartshorne, D.J., and Nakano, T. (2003). Activation of RhoA and inhibition of myosin phosphatase as important components in hypertension in vascular smooth muscle. Circ Res *92*, 411-418.

Severs, N.J. (2000). The cardiac muscle cell. BioEssays : news and reviews in molecular, cellular and developmental biology *22*, 188-199.

Shi, W., Cui, N., Wu, Z., Yang, Y., Zhang, S., Gai, H., Zhu, D., and Jiang, C. (2010). Lipopolysaccharides up-regulate Kir6.1/SUR2B channel expression and enhance vascular KATP channel activity via NF-kappaB-dependent signaling. J Biol Chem *285*, 3021-3029.

Smith, G.C., Coleman, R.A., and McGrath, J.C. (1994). Characterization of dilator prostanoid receptors in the fetal rabbit ductus arteriosus. J Pharmacol Exp Ther *271*, 390-396.

Smyth, S.S., McEver, R.P., Weyrich, A.S., Morrell, C.N., Hoffman, M.R., Arepally, G.M., French, P.A., Dauerman, H.L., Becker, R.C., and Platelet Colloquium, P. (2009). Platelet functions beyond hemostasis. J Thromb Haemost *7*, 1759-1766.

Somlyo, A.P., and Somlyo, A.V. (2003). Ca2+ sensitivity of smooth muscle and nonmuscle myosin II: modulated by G proteins, kinases, and myosin phosphatase. Physiol Rev *83*, 1325-1358.

Spapen, H., and Vincken, W. (1992). Pulmonary arterial hypertension in sepsis and the adult respiratory distress syndrome. Acta clinica Belgica *47*, 30-41.

Sparenborg, S., and Gabriel, M. (1992). Local norepinephrine depletion and learning-related neuronal activity in cingulate cortex and anterior thalamus of rabbits. Experimental brain research *92*, 267-285.

Sparks, H.V., and Rooke, T.W. (1987). Essentials of cardiovascular physiology (Minneapolis: University of Minnesota Press).

Stankevicius, E., Kevelaitis, E., Vainorius, E., and Simonsen, U. (2003). [Role of nitric oxide and other endothelium-derived factors]. Medicina (Kaunas) *39*, 333-341.

Stern, D.M., Esposito, C., Gerlach, H., Gerlach, M., Ryan, J., Handley, D., and Nawroth, P. (1991). Endothelium and regulation of coagulation. Diabetes Care *14*, 160-166.

Sugiyama, T., Yoshizumi, M., Takaku, F., Urabe, H., Tsukakoshi, M., Kasuya, T., and Yazaki, Y. (1986). The elevation of the cytoplasmic calcium ions in vascular smooth muscle cells in SHR--measurement of the free calcium ions in single living cells by lasermicrofluorospectrometry. Biochem Biophys Res Commun *141*, 340-345.

Suzuki, Y.J., and Ford, G.D. (1992). Superoxide stimulates IP3-induced Ca2+ release from vascular smooth muscle sarcoplasmic reticulum. Am J Physiol *262*, H114-116.

Taddei, S., Ghiadoni, L., Virdis, A., Versari, D., and Salvetti, A. (2003). Mechanisms of endothelial dysfunction: clinical significance and preventive non-pharmacological therapeutic strategies. Current pharmaceutical design *9*, 2385-2402.

Takagi, Y., Hirata, Y., Takata, S., Yoshimi, H., Fukuda, Y., Fujita, T., and Hidaka, H. (1988). Effects of protein kinase inhibitors on growth factor-stimulated DNA synthesis in cultured rat vascular smooth muscle cells. Atherosclerosis *74*, 227-230.

Takahashi, T., Forsythe, I.D., Tsujimoto, T., Barnes-Davies, M., and Onodera, K. (1996). Presynaptic calcium current modulation by a metabotropic glutamate receptor. Science *274*, 594-597.

Takashima, S., Sugimoto, N., Takuwa, N., Okamoto, Y., Yoshioka, K., Takamura, M., Takata, S., Kaneko, S., and Takuwa, Y. (2008). G12/13 and Gq mediate S1P2-induced inhibition of Rac and migration in vascular smooth muscle in a manner dependent on Rho but not Rho kinase. Cardiovascular research *79*, 689-697.

Takata, Y., Nishimura, Y., Maeda, H., and Yokoyama, M. (1999). Phospholipase A2 augments contraction and intracellular calcium mobilization through thromboxane A2 in bovine tracheal smooth muscle. The European respiratory journal *14*, 396-404.

Takemoto, M., Sun, J., Hiroki, J., Shimokawa, H., and Liao, J.K. (2002). Rho-kinase mediates hypoxia-induced downregulation of endothelial nitric oxide synthase. Circulation *106*, 57-62.

Tamaki, H. (2007). Glucose-stimulated cAMP-protein kinase A pathway in yeast Saccharomyces cerevisiae. Journal of bioscience and bioengineering *104*, 245-250.

Tanaka, H., Homma, K., White, H.D., Yanagida, T., and Ikebe, M. (2008). Smooth muscle myosin phosphorylated at single head shows sustained mechanical activity. J Biol Chem *283*, 15611-15618.

Tanaka, K.A., Szlam, F., Katori, N., Tsuda, A., and Levy, J.H. (2004). In vitro effects of antihypertensive drugs on thromboxane agonist (U46619)-induced vasoconstriction in human internal mammary artery. British journal of anaesthesia *93*, 257-262.

Taneja, P., Ogier, M., Brooks-Harris, G., Schmid, D.A., Katz, D.M., and Nelson, S.B. (2009). Pathophysiology of locus ceruleus neurons in a mouse model of Rett syndrome. J Neurosci *29*, 12187-12195.

Taniyama, Y., and Griendling, K.K. (2003). Reactive oxygen species in the vasculature: molecular and cellular mechanisms. Hypertension *42*, 1075-1081.

Taylor, E.W., Jordan, D., and Coote, J.H. (1999). Central control of the cardiovascular and respiratory systems and their interactions in vertebrates. Physiol Rev *79*, 855-916.

Taylor, S.S., Kim, C., Cheng, C.Y., Brown, S.H., Wu, J., and Kannan, N. (2008). Signaling through cAMP and cAMP-dependent protein kinase: diverse strategies for drug design. Biochimica et biophysica acta *1784*, 16-26.

Terry, R.W., Kwee, L., Baldwin, H.S., and Labow, M.A. (1997). Cre-mediated generation of a VCAM-1 null allele in transgenic mice. Transgenic research *6*, 349-356.

Tesse, A., Martinez, M.C., Hugel, B., Chalupsky, K., Muller, C.D., Meziani, F., Mitolo-Chieppa, D., Freyssinet, J.M., and Andriantsitohaina, R. (2005). Upregulation of proinflammatory proteins

through NF-kappaB pathway by shed membrane microparticles results in vascular hyporeactivity. Arterioscler Thromb Vasc Biol *25*, 2522-2527.

Thomas, G.D. (2011). Neural control of the circulation. Advances in physiology education *35*, 28-32.

Thomas, W.G., Thekkumkara, T.J., and Baker, K.M. (1996). Cardiac effects of AII. AT1A receptor signaling, desensitization, and internalization. Advances in experimental medicine and biology *396*, 59-69.

Tofaris, G.K., Garcia Reitbock, P., Humby, T., Lambourne, S.L., O'Connell, M., Ghetti, B., Gossage, H., Emson, P.C., Wilkinson, L.S., Goedert, M., *et al.* (2006). Pathological changes in dopaminergic nerve cells of the substantia nigra and olfactory bulb in mice transgenic for truncated human alpha-synuclein(1-120): implications for Lewy body disorders. J Neurosci *26*, 3942-3950.

Tran, Q.K., and Watanabe, H. (2006). Calcium signalling in the endothelium. Handbook of experimental pharmacology, 145-187.

Triposkiadis, F., Karayannis, G., Giamouzis, G., Skoularigis, J., Louridas, G., and Butler, J. (2009). The sympathetic nervous system in heart failure physiology, pathophysiology, and clinical implications. J Am Coll Cardiol *54*, 1747-1762.

Tune, J.D. (2014). Coronary Circulation. In Colloquium Series on Integrated Systems Physiology: From Molecule to Function (San Rafael: Biota Publishing), pp. 1 online resource (191 pages).

Uehata, M., Ishizaki, T., Satoh, H., Ono, T., Kawahara, T., Morishita, T., Tamakawa, H., Yamagami, K., Inui, J., Maekawa, M., *et al.* (1997). Calcium sensitization of smooth muscle mediated by a Rho-associated protein kinase in hypertension. Nature *389*, 990-994.

van Geel, P.P., Pinto, Y.M., Voors, A.A., Buikema, H., Oosterga, M., Crijns, H.J., and van Gilst, W.H. (2000). Angiotensin II type 1 receptor A1166C gene polymorphism is associated with an increased response to angiotensin II in human arteries. Hypertension *35*, 717-721.

Vanhoutte, P.M., Feletou, M., and Taddei, S. (2005). Endothelium-dependent contractions in hypertension. Br J Pharmacol *144*, 449-458.

Venkiteswaran, K., Xiao, K., Summers, S., Calkins, C.C., Vincent, P.A., Pumiglia, K., and Kowalczyk, A.P. (2002). Regulation of endothelial barrier function and growth by VE-cadherin, plakoglobin, and beta-catenin. Am J Physiol Cell Physiol *283*, C811-821.

Viemari, J.C. (2008). Noradrenergic modulation of the respiratory neural network. Respir Physiol Neurobiol *164*, 123-130.

Viemari, J.C., Bevengut, M., Burnet, H., Coulon, P., Pequignot, J.M., Tiveron, M.C., and Hilaire, G. (2004). Phox2a gene, A6 neurons, and noradrenaline are essential for development of normal respiratory rhythm in mice. J Neurosci *24*, 928-937.

Viemari, J.C., Garcia, A.J., 3rd, Doi, A., Elsen, G., and Ramirez, J.M. (2013). beta-Noradrenergic receptor activation specifically modulates the generation of sighs in vivo and in vitro. Front Neural Circuits *7*, 179.

Viemari, J.C., and Ramirez, J.M. (2006). Norepinephrine differentially modulates different types of respiratory pacemaker and nonpacemaker neurons. J Neurophysiol *95*, 2070-2082.

Viemari, J.C., Roux, J.C., Tryba, A.K., Saywell, V., Burnet, H., Pena, F., Zanella, S., Bevengut, M., Barthelemy-Requin, M., Herzing, L.B., *et al.* (2005). Mecp2 deficiency disrupts norepinephrine and respiratory systems in mice. J Neurosci *25*, 11521-11530.

Villar, I.C., Francis, S., Webb, A., Hobbs, A.J., and Ahluwalia, A. (2006). Novel aspects of endothelium-dependent regulation of vascular tone. Kidney Int *70*, 840-853.

Villard, L., and Roux, J.C. (2006). [Noradrenaline deficiency as the origin of respiratory disorders in Rett syndrome an animal model]. Medecine sciences : M/S *22*, 81-83.

Vincent, J.L., Sakr, Y., Sprung, C.L., Ranieri, V.M., Reinhart, K., Gerlach, H., Moreno, R., Carlet, J., Le Gall, J.R., Payen, D., *et al.* (2006). Sepsis in European intensive care units: results of the SOAP study. Crit Care Med *34*, 344-353.

Wakade, A.R., and Wakade, T.D. (1983). Contribution of nicotinic and muscarinic receptors in the secretion of catecholamines evoked by endogenous and exogenous acetylcholine. Neuroscience *10*, 973-978.

Wang, X., Pinol, R.A., Byrne, P., and Mendelowitz, D. (2014). Optogenetic stimulation of locus ceruleus neurons augments inhibitory transmission to parasympathetic cardiac vagal neurons via activation of brainstem alpha1 and beta1 receptors. J Neurosci *34*, 6182-6189.

Warden, M.R., Cardin, J.A., and Deisseroth, K. (2014). Optical neural interfaces. Annual review of biomedical engineering *16*, 103-129.

Washburn, C.P., Sirois, J.E., Talley, E.M., Guyenet, P.G., and Bayliss, D.A. (2002). Serotonergic raphe neurons express TASK channel transcripts and a TASK-like pH- and halothane-sensitive K+ conductance. J Neurosci *22*, 1256-1265.

Watts, S.W. (2010). Endothelin receptors: what's new and what do we need to know? American journal of physiology Regulatory, integrative and comparative physiology *298*, R254-260.

West, J.B., and West, J.B. (2012). Pulmonary pathophysiology : the essentials, 8th edn (Philadelphia: Wolters Kluwer/Lippincott Williams & Wilkins Health).

Wettschureck, N., and Offermanns, S. (2005). Mammalian G proteins and their cell type specific functions. Physiol Rev *85*, 1159-1204.

Williams, S.C., and Deisseroth, K. (2013). Optogenetics. Proc Natl Acad Sci U S A *110*, 16287.

Woodsome, T.P., Eto, M., Everett, A., Brautigan, D.L., and Kitazawa, T. (2001). Expression of CPI-17 and myosin phosphatase correlates with Ca(2+) sensitivity of protein kinase C-induced contraction in rabbit smooth muscle. J Physiol *535*, 553-564.

Wu, Y., Huang, A., Sun, D., Falck, J.R., Koller, A., and Kaley, G. (2001). Gender-specific compensation for the lack of NO in the mediation of flow-induced arteriolar dilation. Am J Physiol Heart Circ Physiol *280*, H2456-2461.

Wu, Y., Li, S.S., Jin, X., Cui, N., Zhang, S., and Jiang, C. (2015). Optogenetic approach for functional assays of the cardiovascular system by light activation of the vascular smooth muscle. Vascular pharmacology *71*, 192-200.

Xiao, S., Wagner, L., Schmidt, R.J., and Baylis, C. (2001). Circulating endothelial nitric oxide synthase inhibitory factor in some patients with chronic renal disease. Kidney Int *59*, 1466-1472.

Xie, D.J., Uta, D., Feng, P.Y., Wakita, M., Shin, M.C., Furue, H., and Yoshimura, M. (2012). Identification of 5-HT receptor subtypes enhancing inhibitory transmission in the rat spinal dorsal horn in vitro. Molecular pain *8*, 58.

Young, J.I., Hong, E.P., Castle, J.C., Crespo-Barreto, J., Bowman, A.B., Rose, M.F., Kang, D., Richman, R., Johnson, J.M., Berget, S., *et al.* (2005). Regulation of RNA splicing by the methylation-dependent transcriptional repressor methyl-CpG binding protein 2. Proc Natl Acad Sci U S A *102*, 17551-17558.

Zeng, H., and Madisen, L. (2012). Mouse transgenic approaches in optogenetics. Progress in brain research *196*, 193-213.

Zhang, F., Vierock, J., Yizhar, O., Fenno, L.E., Tsunoda, S., Kianianmomeni, A., Prigge, M., Berndt, A., Cushman, J., Polle, J., *et al.* (2011a). The microbial opsin family of optogenetic tools. Cell *147*, 1446-1457.

Zhang, F., Wang, L.P., Boyden, E.S., and Deisseroth, K. (2006). Channelrhodopsin-2 and optical control of excitable cells. Nat Methods *3*, 785-792.

Zhang, S., Cui, N., Wu, Y., Zhong, W., Johnson, C.M., and Jiang, C. (2015). Optogenetic intervention to the vascular endothelium. Vascular pharmacology *74*, 122-129.

Zhang, X., Su, J., Cui, N., Gai, H., Wu, Z., and Jiang, C. (2011b). The disruption of central CO2 chemosensitivity in a mouse model of Rett syndrome. Am J Physiol Cell Physiol *301*, C729-738.

Zhang, X., Su, J., Rojas, A., and Jiang, C. (2010). Pontine norepinephrine defects in Mecp2-null mice involve deficient expression of dopamine beta-hydroxylase but not a loss of catecholaminergic neurons. Biochem Biophys Res Commun *394*, 285-290.

Zhao, B., and Debski, E.A. (2005). Serotonergic reticular formation cells in Rana pipiens: categorization, development, and tectal projections. J Comp Neurol *487*, 441-456.

Zheng, B., Sage, M., Sheppeard, E.A., Jurecic, V., and Bradley, A. (2000). Engineering mouse chromosomes with Cre-loxP: range, efficiency, and somatic applications. Molecular and cellular biology *20*, 648-655.

Zhong, W., Cui, N., Jin, X., Oginsky, M.F., Wu, Y., Zhang, S., Bondy, B., Johnson, C.M., and Jiang, C. (2015). Methyl CpG Binding Protein 2 Gene Disruption Augments Tonic Currents of gamma-Aminobutyric Acid Receptors in Locus Coeruleus Neurons: IMPACT ON NEURONAL EXCITABILITY AND BREATHING. J Biol Chem *290*, 18400-18411.

Zhou, Y., Mitra, S., Varadharaj, S., Parinandi, N., Zweier, J.L., and Flavahan, N.A. (2006). Increased expression of cyclooxygenase-2 mediates enhanced contraction to endothelin ETA receptor stimulation in endothelial nitric oxide synthase knockout mice. Circ Res *98*, 1439-1445.

Zizzo, M.G., Mule, F., and Serio, R. (2006). Mechanisms underlying hyperpolarization evoked by P2Y receptor activation in mouse distal colon. European journal of pharmacology *544*, 174-180.

Zoghbi, H.Y., Milstien, S., Butler, I.J., Smith, E.O., Kaufman, S., Glaze, D.G., and Percy, A.K. (1989). Cerebrospinal fluid biogenic amines and biopterin in Rett syndrome. Annals of neurology *25*, 56-60.

Zoghbi, H.Y., Percy, A.K., Glaze, D.G., Butler, I.J., and Riccardi, V.M. (1985). Reduction of biogenic amine levels in the Rett syndrome. N Engl J Med *313*, 921-924.

Zou, M.H., Cohen, R., and Ullrich, V. (2004). Peroxynitrite and vascular endothelial dysfunction in diabetes mellitus. Endothelium : journal of endothelial cell research *11*, 89-97.

Adrogue, H.E., and Adrogue, H.J. (2001). Acid-base physiology. Respiratory care *46*, 328-341.

Alva, J.A., Zovein, A.C., Monvoisin, A., Murphy, T., Salazar, A., Harvey, N.L., Carmeliet, P., and Iruela-Arispe, M.L. (2006). VE-Cadherin-Cre-recombinase transgenic mouse: a tool for lineage analysis and gene deletion in endothelial cells. Dev Dyn *235*, 759-767.

Amir, R.E., Van den Veyver, I.B., Wan, M., Tran, C.Q., Francke, U., and Zoghbi, H.Y. (1999). Rett syndrome is caused by mutations in X-linked MECP2, encoding methyl-CpG-binding protein 2. Nature genetics *23*, 185-188.

Andrea, J.E., and Walsh, M.P. (1992). Protein kinase C of smooth muscle. Hypertension *20*, 585-595.

Angus, D.C., Linde-Zwirble, W.T., Lidicker, J., Clermont, G., Carcillo, J., and Pinsky, M.R. (2001). Epidemiology of severe sepsis in the United States: analysis of incidence, outcome, and associated costs of care. Crit Care Med *29*, 1303-1310.

Aravanis, A.M., Wang, L.P., Zhang, F., Meltzer, L.A., Mogri, M.Z., Schneider, M.B., and Deisseroth, K. (2007). An optical neural interface: in vivo control of rodent motor cortex with integrated fiberoptic and optogenetic technology. J Neural Eng *4*, S143-156.

Arrenberg, A.B., Stainier, D.Y., Baier, H., and Huisken, J. (2010). Optogenetic control of cardiac function. Science *330*, 971-974.

Arrigo, L., and Avanzino, G.L. (1979). [Current developments on the physiology of the cardiac atrial pacemaker an excitation conduction of the left atrium]. Archivio di fisiologia *71*, 25-66.

Aston-Jones, G., and Cohen, J.D. (2005). Adaptive gain and the role of the locus coeruleus-norepinephrine system in optimal performance. J Comp Neurol *493*, 99-110.

Atasoy, D., Aponte, Y., Su, H.H., and Sternson, S.M. (2008). A FLEX switch targets Channelrhodopsin-2 to multiple cell types for imaging and long-range circuit mapping. J Neurosci *28*, 7025-7030.

Atherton, A., and Born, G.V. (1972). Quantitative investigations of the adhesiveness of circulating polymorphonuclear leucocytes to blood vessel walls. J Physiol *222*, 447-474.

Bannerman, D.D., and Goldblum, S.E. (2003). Mechanisms of bacterial lipopolysaccharide-induced endothelial apoptosis. American journal of physiology Lung cellular and molecular physiology *284*, L899-914.

Barlow, R.S., and White, R.E. (1998). Hydrogen peroxide relaxes porcine coronary arteries by stimulating BKCa channel activity. Am J Physiol *275*, H1283-1289.

Bauersachs, J., Popp, R., Hecker, M., Sauer, E., Fleming, I., and Busse, R. (1996). Nitric oxide attenuates the release of endothelium-derived hyperpolarizing factor. Circulation *94*, 3341-3347.

Bayliss, D.A., and Barrett, P.Q. (2008). Emerging roles for two-pore-domain potassium channels and their potential therapeutic impact. Trends in pharmacological sciences *29*, 566-575.

Bayliss, D.A., Talley, E.M., Sirois, J.E., and Lei, Q. (2001). TASK-1 is a highly modulated pH-sensitive 'leak' K(+) channel expressed in brainstem respiratory neurons. Respir Physiol *129*, 159-174.

Bean, B.P. (1992). Pharmacology and electrophysiology of ATP-activated ion channels. Trends in pharmacological sciences *13*, 87-90.

Belegu, R., Hadziefendic, S., Dreshaj, I.A., Haxhiu, M.A., and Martin, R.J. (1999). CO2-induced c-fos expression in medullary neurons during early development. Respir Physiol *117*, 13-28.

Belvisi, M.G., Stretton, C.D., Yacoub, M., and Barnes, P.J. (1992). Nitric oxide is the endogenous neurotransmitter of bronchodilator nerves in humans. European journal of pharmacology *210*, 221-222.

Bender, S.B., and Klabunde, R.E. (2007). Altered role of smooth muscle endothelin receptors in coronary endothelin-1 and alpha1-adrenoceptor-mediated vasoconstriction in Type 2 diabetes. Am J Physiol Heart Circ Physiol *293*, H2281-2288.

Bengzon, J., Hansson, S.R., Hoffman, B.J., and Lindvall, O. (1999). Regulation of norepinephrine transporter and tyrosine hydroxylase mRNAs after kainic acid-induced seizures. Brain Res *842*, 239-242.

Bermejo, A., Zarzuelo, A., and Duarte, J. (2003). In vivo vascular effects of genistein on a rat model of septic shock induced by lipopolysaccharide. Journal of cardiovascular pharmacology *42*, 329-338.

Bernard, D.G., Li, A., and Nattie, E.E. (1996). Evidence for central chemoreception in the midline raphe. J Appl Physiol (1985) *80*, 108-115.

Berridge, C.W., and Waterhouse, B.D. (2003). The locus coeruleus-noradrenergic system: modulation of behavioral state and state-dependent cognitive processes. Brain Res Brain Res Rev *42*, 33-84.

Bhakta, G., Mitra, S., and Maitra, A. (2005). DNA encapsulated magnesium and manganous phosphate nanoparticles: potential non-viral vectors for gene delivery. Biomaterials *26*, 2157-2163.

Biancardi, V., Bicego, K.C., Almeida, M.C., and Gargaglioni, L.H. (2008). Locus coeruleus noradrenergic neurons and CO2 drive to breathing. Pflugers Arch *455*, 1119-1128.

Bishop, V.S., Peterson, D.F., and Horwitz, L.D. (1976). Factors influencing cardiac performance. International review of physiology *9*, 239-273.

Bohm, F., and Pernow, J. (2007). The importance of endothelin-1 for vascular dysfunction in cardiovascular disease. Cardiovascular research *76*, 8-18.

Bonsi, P., Cuomo, D., Ding, J., Sciamanna, G., Ulrich, S., Tscherter, A., Bernardi, G., Surmeier, D.J., and Pisani, A. (2007). Endogenous serotonin excites striatal cholinergic interneurons via the activation of 5-HT 2C, 5-HT6, and 5-HT7 serotonin receptors: implications for extrapyramidal side effects of serotonin reuptake inhibitors. Neuropsychopharmacology : official publication of the American College of Neuropsychopharmacology *32*, 1840-1854.

Borgbjerg, F.M., Nielsen, K., and Franks, J. (1996). Experimental pain stimulates respiration and attenuates morphine-induced respiratory depression: a controlled study in human volunteers. Pain *64*, 123-128.

Boron, W.F., and Boulpaep, E.L. (2009). Medical physiology : a cellular and molecular approach, 2nd edn (Philadelphia, PA: Saunders/Elsevier).

Boulanger, C., and Luscher, T.F. (1990). Release of endothelin from the porcine aorta. Inhibition by endothelium-derived nitric oxide. J Clin Invest *85*, 587-590.

Boussif, O., Lezoualc'h, F., Zanta, M.A., Mergny, M.D., Scherman, D., Demeneix, B., and Behr, J.P. (1995). A versatile vector for gene and oligonucleotide transfer into cells in culture and in vivo: polyethylenimine. Proc Natl Acad Sci U S A *92*, 7297-7301.

Boyden, E.S. (2011). A history of optogenetics: the development of tools for controlling brain circuits with light. F1000 Biol Rep *3*, 11.

Boyden, E.S., Zhang, F., Bamberg, E., Nagel, G., and Deisseroth, K. (2005). Millisecond-timescale, genetically targeted optical control of neural activity. Nat Neurosci *8*, 1263-1268.

Brayden, J.E., and Nelson, M.T. (1992). Regulation of arterial tone by activation of calcium-dependent potassium channels. Science *256*, 532-535.

Britt, J.P., McDevitt, R.A., and Bonci, A. (2012). Use of channelrhodopsin for activation of CNS neurons. Current protocols in neuroscience / editorial board, Jacqueline N Crawley [et al] *Chapter 2*, Unit2 16.

Bruegmann, T., Malan, D., Hesse, M., Beiert, T., Fuegemann, C.J., Fleischmann, B.K., and Sasse, P. (2010). Optogenetic control of heart muscle in vitro and in vivo. Nat Methods *7*, 897-900.

Bulbring, E., and Tomita, T. (1987). Catecholamine action on smooth muscle. Pharmacological reviews *39*, 49-96.

Busse, R., Edwards, G., Feletou, M., Fleming, I., Vanhoutte, P.M., and Weston, A.H. (2002). EDHF: bringing the concepts together. Trends in pharmacological sciences *23*, 374-380.

Cardin, J.A., Carlen, M., Meletis, K., Knoblich, U., Zhang, F., Deisseroth, K., Tsai, L.H., and Moore, C.I. (2010). Targeted optogenetic stimulation and recording of neurons in vivo using cell-type-specific expression of Channelrhodopsin-2. Nature protocols *5*, 247-254.

Carette, B. (1999). Noradrenergic responses of neurones in the mediolateral part of the lateral septum: alpha1-adrenergic depolarization and rhythmic bursting activities, and alpha2-adrenergic hyperpolarization from guinea pig brain slices. Brain research bulletin *48*, 263-276.

Carmeliet, P., Lampugnani, M.G., Moons, L., Breviario, F., Compernolle, V., Bono, F., Balconi, G., Spagnuolo, R., Oosthuyse, B., Dewerchin, M., *et al.* (1999). Targeted deficiency or cytosolic truncation of the VE-cadherin gene in mice impairs VEGF-mediated endothelial survival and angiogenesis. Cell *98*, 147-157.

Caulfield, M.P., and Birdsall, N.J. (1998). International Union of Pharmacology. XVII. Classification of muscarinic acetylcholine receptors. Pharmacological reviews *50*, 279-290.

CDC (2013). Deaths: Final Data for 2013.

Cena, J.J., Lalu, M.M., Cho, W.J., Chow, A.K., Bagdan, M.L., Daniel, E.E., Castro, M.M., and Schulz, R. (2010). Inhibition of matrix metalloproteinase activity in vivo protects against vascular hyporeactivity in endotoxemia. Am J Physiol Heart Circ Physiol *298*, H45-51.

Chahrour, M., Jung, S.Y., Shaw, C., Zhou, X., Wong, S.T., Qin, J., and Zoghbi, H.Y. (2008). MeCP2, a key contributor to neurological disease, activates and represses transcription. Science *320*, 1224-1229.

Chen, G., Suzuki, H., and Weston, A.H. (1988). Acetylcholine releases endothelium-derived hyperpolarizing factor and EDRF from rat blood vessels. Br J Pharmacol *95*, 1165-1174.

Chow, B.Y., Han, X., Dobry, A.S., Qian, X., Chuong, A.S., Li, M., Henninger, M.A., Belfort, G.M., Lin, Y., Monahan, P.E., *et al.* (2010). High-performance genetically targetable optical neural silencing by light-driven proton pumps. Nature *463*, 98-102.

Cines, D.B., Pollak, E.S., Buck, C.A., Loscalzo, J., Zimmerman, G.A., McEver, R.P., Pober, J.S., Wick, T.M., Konkle, B.A., Schwartz, B.S., *et al.* (1998). Endothelial cells in physiology and in the pathophysiology of vascular disorders. Blood *91*, 3527-3561.

Clark, R.K. (2005). Anatomy and physiology : understanding the human body (Sudbury, Mass.: Jones and Bartlett Publishers).

Coates, E.L., Li, A., and Nattie, E.E. (1993). Widespread sites of brain stem ventilatory chemoreceptors. J Appl Physiol (1985) *75*, 5-14.

Corada, M., Liao, F., Lindgren, M., Lampugnani, M.G., Breviario, F., Frank, R., Muller, W.A., Hicklin, D.J., Bohlen, P., and Dejana, E. (2001). Monoclonal antibodies directed to different regions of vascular endothelial cadherin extracellular domain affect adhesion and clustering of the protein and modulate endothelial permeability. Blood *97*, 1679-1684.

Corson, M.A., James, N.L., Latta, S.E., Nerem, R.M., Berk, B.C., and Harrison, D.G. (1996). Phosphorylation of endothelial nitric oxide synthase in response to fluid shear stress. Circ Res *79*, 984-991.

Crews, J.K., Murphy, J.G., and Khalil, R.A. (1999). Gender differences in Ca(2+) entry mechanisms of vasoconstriction in Wistar-Kyoto and spontaneously hypertensive rats. Hypertension *34*, 931-936.

Critchley, H.D., and Harrison, N.A. (2013). Visceral influences on brain and behavior. Neuron *77*, 624-638.

Daher, J.P., Ying, M., Banerjee, R., McDonald, R.S., Hahn, M.D., Yang, L., Flint Beal, M., Thomas, B., Dawson, V.L., Dawson, T.M., *et al.* (2009). Conditional transgenic mice expressing C-terminally truncated human alpha-synuclein (alphaSyn119) exhibit reduced striatal dopamine without loss of nigrostriatal pathway dopaminergic neurons. Molecular neurodegeneration *4*, 34.

Dampney, R.A. (1994). Functional organization of central pathways regulating the cardiovascular system. Physiol Rev *74*, 323-364.

Deanfield, J., Donald, A., Ferri, C., Giannattasio, C., Halcox, J., Halligan, S., Lerman, A., Mancia, G., Oliver, J.J., Pessina, A.C., *et al.* (2005). Endothelial function and dysfunction. Part I: Methodological issues for assessment in the different vascular beds: a statement by the Working Group on Endothelin and Endothelial Factors of the European Society of Hypertension. Journal of hypertension *23*, 7-17.

Deisseroth, K., Feng, G., Majewska, A.K., Miesenbock, G., Ting, A., and Schnitzer, M.J. (2006). Next-generation optical technologies for illuminating genetically targeted brain circuits. J Neurosci *26*, 10380-10386.

Deitch, E.A. (1998). Animal models of sepsis and shock: a review and lessons learned. Shock *9*, 1-11.

Dellinger, R.P., Levy, M.M., Rhodes, A., Annane, D., Gerlach, H., Opal, S.M., Sevransky, J.E., Sprung, C.L., Douglas, I.S., Jaeschke, R., *et al.* (2013). Surviving sepsis campaign: international guidelines for management of severe sepsis and septic shock: 2012. Crit Care Med *41*, 580-637.

Diep, Q.N., Amiri, F., Touyz, R.M., Cohn, J.S., Endemann, D., Neves, M.F., and Schiffrin, E.L. (2002). PPARalpha activator effects on Ang II-induced vascular oxidative stress and inflammation. Hypertension *40*, 866-871.

Doi, A., and Ramirez, J.M. (2008). Neuromodulation and the orchestration of the respiratory rhythm. Respir Physiol Neurobiol *164*, 96-104.

Drake, R.L., Vogl, W., Mitchell, A.W.M., and Gray, H. (2005). Gray's anatomy for students (Philadelphia: Elsevier/Churchill Livingstone).

Dunser, M.W., Mayr, A.J., Ulmer, H., Knotzer, H., Sumann, G., Pajk, W., Friesenecker, B., and Hasibeder, W.R. (2003). Arginine vasopressin in advanced vasodilatory shock: a prospective, randomized, controlled study. Circulation *107*, 2313-2319.

Ebert, T.J., and Stowe, D.F. (1996). Neural and endothelial control of the peripheral circulation--implications for anesthesia: Part I. Neural control of the peripheral vasculature. Journal of cardiothoracic and vascular anesthesia *10*, 147-158.

Edwards, G., Dora, K.A., Gardener, M.J., Garland, C.J., and Weston, A.H. (1998). K+ is an endothelium-derived hyperpolarizing factor in rat arteries. Nature *396*, 269-272.

Edwards, G., Feletou, M., and Weston, A.H. (2010). Endothelium-derived hyperpolarising factors and associated pathways: a synopsis. Pflugers Arch *459*, 863-879.

Eisen, D., Planatscher, H., Hardie, D.B., Kraushaar, U., Pynn, C.J., Stoll, D., Borchers, C., Joos, T.O., and Poetz, O. (2013). G protein-coupled receptor quantification using peptide group-specific enrichment combined with internal peptide standard reporter calibration. Journal of proteomics *90*, 85-95.

Elam, M., Yao, T., Thoren, P., and Svensson, T.H. (1981). Hypercapnia and hypoxia: chemoreceptor-mediated control of locus coeruleus neurons and splanchnic, sympathetic nerves. Brain Res *222*, 373-381.

Ellis, A., and Triggle, C.R. (2003). Endothelium-derived reactive oxygen species: their relationship to endothelium-dependent hyperpolarization and vascular tone. Can J Physiol Pharmacol *81*, 1013-1028.

Emerson, G.G., and Segal, S.S. (2000). Electrical coupling between endothelial cells and smooth muscle cells in hamster feed arteries: role in vasomotor control. Circ Res *87*, 474-479.

Endemann, D.H., and Schiffrin, E.L. (2004). Endothelial dysfunction. Journal of the American Society of Nephrology : JASN *15*, 1983-1992.

Esper, R.J., Nordaby, R.A., Vilarino, J.O., Paragano, A., Cacharron, J.L., and Machado, R.A. (2006). Endothelial dysfunction: a comprehensive appraisal. Cardiovascular diabetology *5*, 4.

Fang, Y., Schram, G., Romanenko, V.G., Shi, C., Conti, L., Vandenberg, C.A., Davies, P.F., Nattel, S., and Levitan, I. (2005). Functional expression of Kir2.x in human aortic endothelial cells: the dominant role of Kir2.2. Am J Physiol Cell Physiol *289*, C1134-1144.

Faraci, F.M., and Heistad, D.D. (1990). Regulation of large cerebral arteries and cerebral microvascular pressure. Circ Res *66*, 8-17.

Feigl, E.O. (1983). Coronary physiology. Physiol Rev *63*, 1-205.

Feletou, M. (2011). In The Endothelium: Part 1: Multiple Functions of the Endothelial Cells-Focus on Endothelium-Derived Vasoactive Mediators (San Rafael (CA)).

Felgner, P.L., Gadek, T.R., Holm, M., Roman, R., Chan, H.W., Wenz, M., Northrop, J.P., Ringold, G.M., and Danielsen, M. (1987). Lipofection: a highly efficient, lipid-mediated DNA-transfection procedure. Proc Natl Acad Sci U S A *84*, 7413-7417.

Fenno, L., Yizhar, O., and Deisseroth, K. (2011). The development and application of optogenetics. Annu Rev Neurosci *34*, 389-412.

Ferguson-Myrthil, N. (2012). Vasopressor use in adult patients. Cardiology in review *20*, 153-158.

Figueiredo, M., Lane, S., Tang, F., Liu, B.H., Hewinson, J., Marina, N., Kasymov, V., Souslova, E.A., Chudakov, D.M., Gourine, A.V., *et al.* (2011). Optogenetic experimentation on astrocytes. Experimental physiology *96*, 40-50.

Filosa, J.A., Dean, J.B., and Putnam, R.W. (2002). Role of intracellular and extracellular pH in the chemosensitive response of rat locus coeruleus neurones. J Physiol *541*, 493-509.

Filosa, J.A., and Putnam, R.W. (2003). Multiple targets of chemosensitive signaling in locus coeruleus neurons: role of K+ and Ca2+ channels. Am J Physiol Cell Physiol *284*, C145-155.

Fioret, B.A., Heimfeld, J.D., Paik, D.T., and Hatzopoulos, A.K. (2014). Endothelial cells contribute to generation of adult ventricular myocytes during cardiac homeostasis. Cell reports *8*, 229-241.

Fisslthaler, B., Popp, R., Kiss, L., Potente, M., Harder, D.R., Fleming, I., and Busse, R. (1999). Cytochrome P450 2C is an EDHF synthase in coronary arteries. Nature *401*, 493-497.

Fitch, S.J., and Gossage, J.R. (2002). Optimal management of septic shock. Rapid recognition and institution of therapy are crucial. Postgraduate medicine *111*, 53-56, 59-60, 63-54 passim.

Fitch, W.M., and Yasunobu, K.T. (1975). Phylogenies from amino acid sequences aligned with gaps: the problem of gap weighting. Journal of molecular evolution *5*, 1-24.

Forstermann, U., Closs, E.I., Pollock, J.S., Nakane, M., Schwarz, P., Gath, I., and Kleinert, H. (1994). Nitric oxide synthase isozymes. Characterization, purification, molecular cloning, and functions. Hypertension *23*, 1121-1131.

Forstermann, U., and Munzel, T. (2006). Endothelial nitric oxide synthase in vascular disease: from marvel to menace. Circulation *113*, 1708-1714.

Forstermann, U., and Sessa, W.C. (2012). Nitric oxide synthases: regulation and function. European heart journal *33*, 829-837, 837a-837d.

Frohlich, E. (2002). [Structure and function of blood-tissue barriers]. Deutsche medizinische Wochenschrift *127*, 2629-2634.

Funk, G.D., Smith, J.C., and Feldman, J.L. (1994). Development of thyrotropin-releasing hormone and norepinephrine potentiation of inspiratory-related hypoglossal motoneuron discharge in neonatal and juvenile mice in vitro. J Neurophysiol *72*, 2538-2541.

Gallego, J., Nsegbe, E., and Durand, E. (2001). Learning in respiratory control. Behavior modification *25*, 495-512.

Ganitkevich, V.Y., and Isenberg, G. (1991). Depolarization-mediated intracellular calcium transients in isolated smooth muscle cells of guinea-pig urinary bladder. J Physiol *435*, 187-205.

Gardiner, S.M., March, J.E., Kemp, P.A., and Bennett, T. (2001). Effects of the novel selective endothelin ET(A) receptor antagonist, SB 234551, on the cardiovascular responses to endotoxaemia in conscious rats. Br J Pharmacol *133*, 1371-1377.

Garrett, R., Grisham, C.M., and Sabat, M. (2013). Biochemistry, 5th edn (Australia ; United Kingdom: Brooks/Cole Cengage Learning).

Gaultier, C. (2004). Genes and genetics in respiratory control. Paediatric respiratory reviews *5*, 166-172.

Gebremedhin, D., Lange, A.R., Lowry, T.F., Taheri, M.R., Birks, E.K., Hudetz, A.G., Narayanan, J., Falck, J.R., Okamoto, H., Roman, R.J., *et al.* (2000). Production of 20-HETE and its role in autoregulation of cerebral blood flow. Circ Res *87*, 60-65.

Glaze, D.G. (2005). Neurophysiology of Rett syndrome. Journal of child neurology *20*, 740-746.

Gong, M.C., Fujihara, H., Somlyo, A.V., and Somlyo, A.P. (1997). Translocation of rhoA associated with Ca2+ sensitization of smooth muscle. J Biol Chem *272*, 10704-10709.

Gordan, R., Gwathmey, J.K., and Xie, L.H. (2015). Autonomic and endocrine control of cardiovascular function. World journal of cardiology *7*, 204-214.

Gory, S., Vernet, M., Laurent, M., Dejana, E., Dalmon, J., and Huber, P. (1999). The vascular endothelial-cadherin promoter directs endothelial-specific expression in transgenic mice. Blood *93*, 184-192.

Gothert, J.R., Gustin, S.E., Hall, M.A., Green, A.R., Gottgens, B., Izon, D.J., and Begley, C.G. (2005). In vivo fate-tracing studies using the Scl stem cell enhancer: embryonic hematopoietic stem cells significantly contribute to adult hematopoiesis. Blood *105*, 2724-2732.

Govers, R., and Rabelink, T.J. (2001). Cellular regulation of endothelial nitric oxide synthase. American journal of physiology Renal physiology *280*, F193-206.

Goyette, R.E., Key, N.S., and Ely, E.W. (2004). Hematologic changes in sepsis and their therapeutic implications. Seminars in respiratory and critical care medicine *25*, 645-659.

Graham, R.M., Perez, D.M., Hwa, J., and Piascik, M.T. (1996). alpha 1-adrenergic receptor subtypes. Molecular structure, function, and signaling. Circ Res *78*, 737-749.

Grant, B.J., Davies, E.E., Jones, H.A., and Hughes, J.M. (1976). Local regulation of pulmonary blood flow and ventilation-perfusion ratios in the coatimundi. Journal of applied physiology *40*, 216-228.

Green, A.R., Hall, J.E., and Rees, A.R. (1981). A behavioural and biochemical study in rats of 5-hydroxytryptamine receptor agonists and antagonists, with observations on structure-activity requirements for the agonists. Br J Pharmacol *73*, 703-719.

Griendling, K.K., and FitzGerald, G.A. (2003). Oxidative stress and cardiovascular injury: Part I: basic mechanisms and in vivo monitoring of ROS. Circulation *108*, 1912-1916.

Griendling, K.K., Rittenhouse, S.E., Brock, T.A., Ekstein, L.S., Gimbrone, M.A., Jr., and Alexander, R.W. (1986). Sustained diacylglycerol formation from inositol phospholipids in angiotensin II-stimulated vascular smooth muscle cells. J Biol Chem *261*, 5901-5906.

Groves, P., Kurz, S., Just, H., and Drexler, H. (1995). Role of endogenous bradykinin in human coronary vasomotor control. Circulation *92*, 3424-3430.

Gryglewski, R. (1983). Prostacyclin--experimental and clinical approach. Advances in prostaglandin, thromboxane, and leukotriene research *11*, 457-461.

Guilluy, C., Sauzeau, V., Rolli-Derkinderen, M., Guerin, P., Sagan, C., Pacaud, P., and Loirand, G. (2005). Inhibition of RhoA/Rho kinase pathway is involved in the beneficial effect of sildenafil on pulmonary hypertension. Br J Pharmacol *146*, 1010-1018.

Guimaraes, S., and Moura, D. (2001). Vascular adrenoceptors: an update. Pharmacological reviews *53*, 319-356.

Guo, W., Lasky, J.L., Chang, C.J., Mosessian, S., Lewis, X., Xiao, Y., Yeh, J.E., Chen, J.Y., Iruela-Arispe, M.L., Varella-Garcia, M., *et al.* (2008). Multi-genetic events collaboratively contribute to Pten-null leukaemia stem-cell formation. Nature *453*, 529-533.

Gustafsson, E., Brakebusch, C., Hietanen, K., and Fassler, R. (2001). Tie-1-directed expression of Cre recombinase in endothelial cells of embryoid bodies and transgenic mice. Journal of cell science *114*, 671-676.

Halushka, P.V., Mais, D.E., Mayeux, P.R., and Morinelli, T.A. (1989). Thromboxane, prostaglandin and leukotriene receptors. Annu Rev Pharmacol Toxicol *29*, 213-239.

Han, X. (2012). In vivo application of optogenetics for neural circuit analysis. ACS chemical neuroscience *3*, 577-584.

Han, X., and Boyden, E.S. (2007). Multiple-color optical activation, silencing, and desynchronization of neural activity, with single-spike temporal resolution. PLoS One *2*, e299.

Harvey, R.D., and Belevych, A.E. (2003). Muscarinic regulation of cardiac ion channels. Br J Pharmacol *139*, 1074-1084.

Hassid, A. (1984). Stimulation of prostacyclin synthesis by thromboxane A2-like prostaglandin endoperoxide analogues in cultured vascular smooth muscle cells. Biochem Biophys Res Commun *123*, 21-26.

Hau, J., and Van Hoosier, G.L. (2003). Handbook of laboratory animal science, 2nd edn (Boca Raton ; London: CRC Press).

Hauser, B., Radermacher, P., Thiemermann, C., and Matejovic, M. (2004). Nitric oxide, bacteria, and host defense in sepsis: who needs what? Shock *22*, 588-590.

Haxhiu, M.A., Tolentino-Silva, F., Pete, G., Kc, P., and Mack, S.O. (2001). Monoaminergic neurons, chemosensation and arousal. Respir Physiol *129*, 191-209.

Heppner, T.J., Werner, M.E., Nausch, B., Vial, C., Evans, R.J., and Nelson, M.T. (2009). Nerve-evoked purinergic signalling suppresses action potentials, Ca2+ flashes and contractility evoked by muscarinic receptor activation in mouse urinary bladder smooth muscle. J Physiol *587*, 5275-5288.

Herrmann, J., and Lerman, A. (2001). The endothelium: dysfunction and beyond. J Nucl Cardiol *8*, 197-206.

Hertsens, T. (2005). An overview of laser diode characteristics: Application note.

Hilaire, G. (2006). Endogenous noradrenaline affects the maturation and function of the respiratory network: possible implication for SIDS. Autonomic neuroscience : basic & clinical *126-127*, 320-331.

Hilaire, G., Viemari, J.C., Coulon, P., Simonneau, M., and Bevengut, M. (2004). Modulation of the respiratory rhythm generator by the pontine noradrenergic A5 and A6 groups in rodents. Respir Physiol Neurobiol *143*, 187-197.

Hill-Eubanks, D.C., Werner, M.E., Heppner, T.J., and Nelson, M.T. (2011). Calcium signaling in smooth muscle. Cold Spring Harbor perspectives in biology *3*, a004549.

Hisatsune, H., Matsumura, K., Ogawa, M., Uemura, A., Kondo, N., Yamashita, J.K., Katsuta, H., Nishikawa, S., Chiba, T., and Nishikawa, S. (2005). High level of endothelial cell-specific gene expression by a combination of the 5' flanking region and the 5' half of the first intron of the VE-cadherin gene. Blood *105*, 4657-4663.

Hollenberg, S.M. (2007). Vasopressor support in septic shock. Chest *132*, 1678-1687.

Hughes, J.M. (1975). Lung gas tensions and active regulation of ventilation/perfusion ratios in health and disease. British journal of diseases of the chest *69*, 153-170.

Hulme, E.C., Birdsall, N.J., and Buckley, N.J. (1990). Muscarinic receptor subtypes. Annu Rev Pharmacol Toxicol *30*, 633-673.

Huribal, M., Cunningham, M.E., D'Aiuto, M.L., Pleban, W.E., and McMillen, M.A. (1995). Endothelin levels in patients with burns covering more than 20% body surface area. The Journal of burn care & rehabilitation *16*, 23-26.

Hyvelin, J.M., O'Connor, C., and McLoughlin, P. (2004). Effect of changes in pH on wall tension in isolated rat pulmonary artery: role of the RhoA/Rho-kinase pathway. American journal of physiology Lung cellular and molecular physiology *287*, L673-684.

Ide, S., Itoh, M., and Goto, Y. (2005). Defect in normal developmental increase of the brain biogenic amine concentrations in the mecp2-null mouse. Neurosci Lett *386*, 14-17.

Inagami, T., Naruse, M., and Hoover, R. (1995). Endothelium as an endocrine organ. Annual review of physiology *57*, 171-189.

Inoue, A., Yanagisawa, M., Kimura, S., Kasuya, Y., Miyauchi, T., Goto, K., and Masaki, T. (1989). The human endothelin family: three structurally and pharmacologically distinct isopeptides predicted by three separate genes. Proc Natl Acad Sci U S A *86*, 2863-2867.

Irion, S., Luche, H., Gadue, P., Fehling, H.J., Kennedy, M., and Keller, G. (2007). Identification and targeting of the ROSA26 locus in human embryonic stem cells. Nature biotechnology *25*, 1477-1482.

Irisawa, H., Brown, H.F., and Giles, W. (1993). Cardiac pacemaking in the sinoatrial node. Physiol Rev *73*, 197-227.

Ishizuka, T., Kakuda, M., Araki, R., and Yawo, H. (2006). Kinetic evaluation of photosensitivity in genetically engineered neurons expressing green algae light-gated channels. Neurosci Res *54*, 85-94.

Ito, A., Shimokawa, H., Nakaike, R., Fukai, T., Sakata, M., Takayanagi, T., Egashira, K., and Takeshita, A. (1994). Role of protein kinase C-mediated pathway in the pathogenesis of coronary artery spasm in a swine model. Circulation *90*, 2425-2431.

Jespersen, B., Tykocki, N.R., Watts, S.W., and Cobbett, P.J. (2015). Measurement of smooth muscle function in the isolated tissue bath-applications to pharmacology research. Journal of visualized experiments : JoVE, 52324.

Jia, Z., Valiunas, V., Lu, Z., Bien, H., Liu, H., Wang, H.Z., Rosati, B., Brink, P.R., Cohen, I.S., and Entcheva, E. (2011). Stimulating cardiac muscle by light: cardiac optogenetics by cell delivery. Circ Arrhythm Electrophysiol *4*, 753-760.

Jiang, C., Xu, H., Cui, N., and Wu, J. (2001). An alternative approach to the identification of respiratory central chemoreceptors in the brainstem. Respir Physiol *129*, 141-157.

Jin, X.T., Cui, N., Zhong, W., Jin, X., Wu, Z., and Jiang, C. (2013). Pre- and postsynaptic modulations of hypoglossal motoneurons by alpha-adrenoceptor activation in wild-type and Mecp2(-/Y) mice. Am J Physiol Cell Physiol *305*, C1080-1090.

Johnson, J.A., and Liggett, S.B. (2011). Cardiovascular pharmacogenomics of adrenergic receptor signaling: clinical implications and future directions. Clinical pharmacology and therapeutics *89*, 366-378.

Joyner, M.J., Nauss, L.A., Warner, M.A., and Warner, D.O. (1992). Sympathetic modulation of blood flow and O2 uptake in rhythmically contracting human forearm muscles. Am J Physiol *263*, H1078-1083.

Julu, P.O., Kerr, A.M., Apartopoulos, F., Al-Rawas, S., Engerstrom, I.W., Engerstrom, L., Jamal, G.A., and Hansen, S. (2001). Characterisation of breathing and associated central autonomic dysfunction in the Rett disorder. Archives of disease in childhood *85*, 29-37.

Kadamur, G., and Ross, E.M. (2013). Mammalian phospholipase C. Annual review of physiology *75*, 127-154.

Kam, N.W., Liu, Z., and Dai, H. (2005). Functionalization of carbon nanotubes via cleavable disulfide bonds for efficient intracellular delivery of siRNA and potent gene silencing. J Am Chem Soc *127*, 12492-12493.

Kenakin, T.P. (1984). The classification of drugs and drug receptors in isolated tissues. Pharmacological reviews *36*, 165-222.

Kerr, A.M., Armstrong, D.D., Prescott, R.J., Doyle, D., and Kearney, D.L. (1997). Rett syndrome: analysis of deaths in the British survey. European child & adolescent psychiatry *6 Suppl 1*, 71-74.

Kersh, A.E., Hartzler, L.K., Havlin, K., Hubbell, B.B., Nanagas, V., Kalra, A., Chua, J., Whitesell, R., Ritucci, N.A., Dean, J.B., *et al.* (2009). pH regulating transporters in neurons from various chemosensitive brainstem regions in neonatal rats. American journal of physiology Regulatory, integrative and comparative physiology *297*, R1409-1420.

Khalil, R.A. (2010). Regulation of vascular smooth muscle function (San Rafael, Calif.: Morgan & Claypool Life Sciences).

Khalil, R.A., Menice, C.B., Wang, C.L., and Morgan, K.G. (1995). Phosphotyrosine-dependent targeting of mitogen-activated protein kinase in differentiated contractile vascular cells. Circ Res *76*, 1101-1108.

Khazaei, M., Moien-Afshari, F., and Laher, I. (2008). Vascular endothelial function in health and diseases. Pathophysiology : the official journal of the International Society for Pathophysiology / ISP *15*, 49-67.

Kiely, D.G., Cargill, R.I., Struthers, A.D., and Lipworth, B.J. (1997). Cardiopulmonary effects of endothelin-1 in man. Cardiovascular research *33*, 378-386.

Kim, H.R., Appel, S., Vetterkind, S., Gangopadhyay, S.S., and Morgan, K.G. (2008). Smooth muscle signalling pathways in health and disease. Journal of cellular and molecular medicine *12*, 2165-2180.

Kisanuki, Y.Y., Hammer, R.E., Miyazaki, J., Williams, S.C., Richardson, J.A., and Yanagisawa, M. (2001). Tie2-Cre transgenic mice: a new model for endothelial cell-lineage analysis in vivo. Developmental biology *230*, 230-242.

Kizub, I.V., Pavlova, O.O., Johnson, C.D., Soloviev, A.I., and Zholos, A.V. (2010). Rho kinase and protein kinase C involvement in vascular smooth muscle myofilament calcium sensitization in arteries from diabetic rats. Br J Pharmacol *159*, 1724-1731.

Kneuer, C., Sameti, M., Bakowsky, U., Schiestel, T., Schirra, H., Schmidt, H., and Lehr, C.M. (2000). A nonviral DNA delivery system based on surface modified silica-nanoparticles can efficiently transfect cells in vitro. Bioconjug Chem *11*, 926-932.

Knollmann, B.C. (2010). Pacing lightly: optogenetics gets to the heart. Nat Methods *7*, 889-891.

Koppenol, W.H., Moreno, J.J., Pryor, W.A., Ischiropoulos, H., and Beckman, J.S. (1992). Peroxynitrite, a cloaked oxidant formed by nitric oxide and superoxide. Chem Res Toxicol *5*, 834-842.

Kozasa, T., Jiang, X., Hart, M.J., Sternweis, P.M., Singer, W.D., Gilman, A.G., Bollag, G., and Sternweis, P.C. (1998). p115 RhoGEF, a GTPase activating protein for Galpha12 and Galpha13. Science *280*, 2109-2111.

Kozyreva, T.V., and Simonova, T.G. (1998). [Modulating effect of peripheral thermoreceptors on human respiration]. Vestnik Rossiiskoi akademii meditsinskikh nauk / Rossiiskaia akademiia meditsinskikh nauk, 14-18.

Krejci, V., Hiltebrand, L.B., Erni, D., and Sigurdsson, G.H. (2003). Endothelin receptor antagonist bosentan improves microcirculatory blood flow in splanchnic organs in septic shock. Crit Care Med *31*, 203-210.

Kumar, N.N., Velic, A., Soliz, J., Shi, Y., Li, K., Wang, S., Weaver, J.L., Sen, J., Abbott, S.B., Lazarenko, R.M., *et al.* (2015). PHYSIOLOGY. Regulation of breathing by CO(2) requires the proton-activated receptor GPR4 in retrotrapezoid nucleus neurons. Science *348*, 1255-1260.

Kumar, P., and Prabhakar, N.R. (2012). Peripheral chemoreceptors: function and plasticity of the carotid body. Comprehensive Physiology *2*, 141-219.

Laffon, M., Lu, L.N., Modelska, K., Matthay, M.A., and Pittet, J.F. (1999). alpha-adrenergic blockade restores normal fluid transport capacity of alveolar epithelium after hemorrhagic shock. Am J Physiol *277*, L760-768.

Lammel, S., Steinberg, E.E., Foldy, C., Wall, N.R., Beier, K., Luo, L., and Malenka, R.C. (2015). Diversity of transgenic mouse models for selective targeting of midbrain dopamine neurons. Neuron *85*, 429-438.

Lamping, K.G., Kanatsuka, H., Eastham, C.L., Chilian, W.M., and Marcus, M.L. (1989). Nonuniform vasomotor responses of the coronary microcirculation to serotonin and vasopressin. Circ Res *65*, 343-351.

Landmesser, U., Dikalov, S., Price, S.R., McCann, L., Fukai, T., Holland, S.M., Mitch, W.E., and Harrison, D.G. (2003). Oxidation of tetrahydrobiopterin leads to uncoupling of endothelial cell nitric oxide synthase in hypertension. J Clin Invest *111*, 1201-1209.

Lanyi, J.K., Duschl, A., Hatfield, G.W., May, K., and Oesterhelt, D. (1990). The primary structure of a halorhodopsin from Natronobacterium pharaonis. Structural, functional and evolutionary implications for bacterial rhodopsins and halorhodopsins. J Biol Chem *265*, 1253-1260.

Laporte, R., Hui, A., and Laher, I. (2004). Pharmacological modulation of sarcoplasmic reticulum function in smooth muscle. Pharmacological reviews *56*, 439-513.

Laragh, J.H., and Brenner, B.M. (1995). Hypertension : pathophysiology, diagnosis, and management, 2nd edn (New York: Raven Press).

Lepper, P.M., Held, T.K., Schneider, E.M., Bolke, E., Gerlach, H., and Trautmann, M. (2002). Clinical implications of antibiotic-induced endotoxin release in septic shock. Intensive Care Med *28*, 824-833.

Lerman, A., and Zeiher, A.M. (2005). Endothelial function: cardiac events. Circulation *111*, 363-368.

Li, L., Jia, Z.H., Chen, C., Wei, C., Han, J.K., Wu, Y.L., and Ren, L.M. (2011). Physiological significance of P2X receptor-mediated vasoconstriction in five different types of arteries in rats. Purinergic signalling *7*, 221-229.

Licht, A.H., Raab, S., Hofmann, U., and Breier, G. (2004). Endothelium-specific Cre recombinase activity in flk-1-Cre transgenic mice. Dev Dyn *229*, 312-318.

Lin, J.Y. (2011). A user's guide to channelrhodopsin variants: features, limitations and future developments. Experimental physiology *96*, 19-25.

Lin, J.Y., Lin, M.Z., Steinbach, P., and Tsien, R.Y. (2009). Characterization of engineered channelrhodopsin variants with improved properties and kinetics. Biophys J *96*, 1803-1814.

Liu, S., Zhang, Y., Moayeri, M., Liu, J., Crown, D., Fattah, R.J., Wein, A.N., Yu, Z.X., Finkel, T., and Leppla, S.H. (2013). Key tissue targets responsible for anthrax-toxin-induced lethality. Nature *501*, 63-68.

Louis, S.F., and Zahradka, P. (2010). Vascular smooth muscle cell motility: From migration to invasion. Experimental and clinical cardiology *15*, e75-85.

Lowe, A.W., Madeddu, L., and Kelly, R.B. (1988). Endocrine secretory granules and neuronal synaptic vesicles have three integral membrane proteins in common. The Journal of cell biology *106*, 51-59.

MacLean, M.R., Herve, P., Eddahibi, S., and Adnot, S. (2000). 5-hydroxytryptamine and the pulmonary circulation: receptors, transporters and relevance to pulmonary arterial hypertension. Br J Pharmacol *131*, 161-168.

Madisen, L., Mao, T., Koch, H., Zhuo, J.M., Berenyi, A., Fujisawa, S., Hsu, Y.W., Garcia, A.J., 3rd, Gu, X., Zanella, S., *et al.* (2012). A toolbox of Cre-dependent optogenetic transgenic mice for light-induced activation and silencing. Nat Neurosci *15*, 793-802.

Makita, K., Falck, J.R., and Capdevila, J.H. (1996). Cytochrome P450, the arachidonic acid cascade, and hypertension: new vistas for an old enzyme system. FASEB J *10*, 1456-1463.

Mangoni, M.E., and Nargeot, J. (2008). Genesis and regulation of the heart automaticity. Physiol Rev *88*, 919-982.

Martin, G.S., Mannino, D.M., Eaton, S., and Moss, M. (2003). The epidemiology of sepsis in the United States from 1979 through 2000. N Engl J Med *348*, 1546-1554.

Matsumoto, T., Kobayashi, T., Ishida, K., Taguchi, K., and Kamata, K. (2010). Enhancement of mesenteric artery contraction to 5-HT depends on Rho kinase and Src kinase pathways in the ob/ob mouse model of type 2 diabetes. Br J Pharmacol *160*, 1092-1104.

McBride, H.M., Neuspiel, M., and Wasiak, S. (2006). Mitochondria: more than just a powerhouse. Current biology : CB *16*, R551-560.

McKay, L.C., Evans, K.C., Frackowiak, R.S., and Corfield, D.R. (2003). Neural correlates of voluntary breathing in humans. J Appl Physiol (1985) *95*, 1170-1178.

Medzhitov, R. (2001). Toll-like receptors and innate immunity. Nature reviews Immunology *1*, 135-145.

Meidan, R., Altstein, M., and Girsh, E. (1992). Biosynthesis and release of oxytocin by granulosa cells derived from preovulatory bovine follicles: effects of forskolin and insulin-like growth factor-I. Biology of reproduction *46*, 715-720.

Michiels, C. (2003). Endothelial cell functions. J Cell Physiol *196*, 430-443.

Milstien, S., and Katusic, Z. (1999). Oxidation of tetrahydrobiopterin by peroxynitrite: implications for vascular endothelial function. Biochem Biophys Res Commun *263*, 681-684.

Minneman, K.P., Pittman, R.N., and Molinoff, P.B. (1981). Beta-adrenergic receptor subtypes: properties, distribution, and regulation. Annu Rev Neurosci *4*, 419-461.

Mitchell, J.A., and Warner, T.D. (2006). COX isoforms in the cardiovascular system: understanding the activities of non-steroidal anti-inflammatory drugs. Nat Rev Drug Discov *5*, 75-86.

Molinoff, P.B. (1984). Alpha- and beta-adrenergic receptor subtypes properties, distribution and regulation. Drugs *28 Suppl 2*, 1-15.

Mollace, V., Muscoli, C., Masini, E., Cuzzocrea, S., and Salvemini, D. (2005). Modulation of prostaglandin biosynthesis by nitric oxide and nitric oxide donors. Pharmacological reviews *57*, 217-252.

Monassier, L., Laplante, M.A., Ayadi, T., Doly, S., and Maroteaux, L. (2010). Contribution of gene-modified mice and rats to our understanding of the cardiovascular pharmacology of serotonin. Pharmacology & therapeutics *128*, 559-567.

Moncada, S., Gryglewski, R., Bunting, S., and Vane, J.R. (1976). An enzyme isolated from arteries transforms prostaglandin endoperoxides to an unstable substance that inhibits platelet aggregation. Nature *263*, 663-665.

Moncada, S., Palmer, R.M., and Higgs, E.A. (1991). Nitric oxide: physiology, pathophysiology, and pharmacology. Pharmacological reviews *43*, 109-142.

Moretti, P., and Zoghbi, H.Y. (2006). MeCP2 dysfunction in Rett syndrome and related disorders. Curr Opin Genet Dev *16*, 276-281.

Mostefai, H.A., Meziani, F., Mastronardi, M.L., Agouni, A., Heymes, C., Sargentini, C., Asfar, P., Martinez, M.C., and Andriantsitohaina, R. (2008). Circulating microparticles from patients with septic shock exert protective role in vascular function. Am J Respir Crit Care Med *178*, 1148-1155.

Nagel, G., Brauner, M., Liewald, J.F., Adeishvili, N., Bamberg, E., and Gottschalk, A. (2005). Light activation of channelrhodopsin-2 in excitable cells of Caenorhabditis elegans triggers rapid behavioral responses. Current biology : CB *15*, 2279-2284.

Nagel, G., Ollig, D., Fuhrmann, M., Kateriya, S., Musti, A.M., Bamberg, E., and Hegemann, P. (2002). Channelrhodopsin-1: a light-gated proton channel in green algae. Science *296*, 2395-2398.

Nagel, G., Szellas, T., Huhn, W., Kateriya, S., Adeishvili, N., Berthold, P., Ollig, D., Hegemann, P., and Bamberg, E. (2003). Channelrhodopsin-2, a directly light-gated cation-selective membrane channel. Proc Natl Acad Sci U S A *100*, 13940-13945.

Nattie, E. (2006). Why do we have both peripheral and central chemoreceptors? J Appl Physiol (1985) *100*, 9-10.

Nattie, E.E., and Li, A. (1996). Central chemoreception in the region of the ventral respiratory group in the rat. J Appl Physiol (1985) *81*, 1987-1995.

Nattie, E.E., and Li, A. (2001). CO2 dialysis in the medullary raphe of the rat increases ventilation in sleep. J Appl Physiol (1985) *90*, 1247-1257.

Nattie, E.E., and Li, A. (2002). CO2 dialysis in nucleus tractus solitarius region of rat increases ventilation in sleep and wakefulness. J Appl Physiol (1985) *92*, 2119-2130.

Nestler, E.J., Alreja, M., and Aghajanian, G.K. (1999). Molecular control of locus coeruleus neurotransmission. Biological psychiatry *46*, 1131-1139.

Neul, J.L., Fang, P., Barrish, J., Lane, J., Caeg, E.B., Smith, E.O., Zoghbi, H., Percy, A., and Glaze, D.G. (2008). Specific mutations in methyl-CpG-binding protein 2 confer different severity in Rett syndrome. Neurology *70*, 1313-1321.

Nishikawa, M., and Huang, L. (2001). Nonviral vectors in the new millennium: delivery barriers in gene transfer. Hum Gene Ther *12*, 861-870.

Nishikawa, Y., Stepp, D.W., and Chilian, W.M. (2000). Nitric oxide exerts feedback inhibition on EDHF-induced coronary arteriolar dilation in vivo. Am J Physiol Heart Circ Physiol *279*, H459-465.

Nishimura, J., Khalil, R.A., Drenth, J.P., and van Breemen, C. (1990). Evidence for increased myofilament Ca2+ sensitivity in norepinephrine-activated vascular smooth muscle. Am J Physiol *259*, H2-8.

Nishino, T. (1993). Swallowing as a protective reflex for the upper respiratory tract. Anesthesiology *79*, 588-601.

Noback, C.R., Demarest, R.J., Ruggiero, D.A., Strominger, N.L., and SpringerLink (Online service) (2005). The Human Nervous System Structure and Function (Totowa, NJ: Humana Press Inc.,).

Noda, M., Yasuda-Fukazawa, C., Moriishi, K., Kato, T., Okuda, T., Kurokawa, K., and Takuwa, Y. (1995). Involvement of rho in GTP gamma S-induced enhancement of phosphorylation of 20 kDa myosin light chain in vascular smooth muscle cells: inhibition of phosphatase activity. FEBS letters *367*, 246-250.

O'Brien, A.J., Wilson, A.J., Sibbald, R., Singer, M., and Clapp, L.H. (2001). Temporal variation in endotoxin-induced vascular hyporeactivity in a rat mesenteric artery organ culture model. Br J Pharmacol *133*, 351-360.

Ogier, M., and Katz, D.M. (2008). Breathing dysfunction in Rett syndrome: understanding epigenetic regulation of the respiratory network. Respir Physiol Neurobiol *164*, 55-63.

Ohkubo, S., Nakahata, N., and Ohizumi, Y. (1996). Thromboxane A2-mediated shape change: independent of Gq-phospholipase C--Ca2+ pathway in rabbit platelets. Br J Pharmacol *117*, 1095-1104.

Okada, Y., Chen, Z., Jiang, W., Kuwana, S., and Eldridge, F.L. (2002). Anatomical arrangement of hypercapnia-activated cells in the superficial ventral medulla of rats. J Appl Physiol (1985) *93*, 427-439.

Olivetti, G., Cigola, E., Maestri, R., Corradi, D., Lagrasta, C., Gambert, S.R., and Anversa, P. (1996). Aging, cardiac hypertrophy and ischemic cardiomyopathy do not affect the proportion of mononucleated and multinucleated myocytes in the human heart. Journal of molecular and cellular cardiology *28*, 1463-1477.

Ozawa, S., and Sand, O. (1986). Electrophysiology of excitable endocrine cells. Physiol Rev *66*, 887-952.

Pablo Huidobro-Toro, J., and Veronica Donoso, M. (2004). Sympathetic co-transmission: the coordinated action of ATP and noradrenaline and their modulation by neuropeptide Y in human vascular neuroeffector junctions. European journal of pharmacology *500*, 27-35.

Palmer, R.M., Ferrige, A.G., and Moncada, S. (1987). Nitric oxide release accounts for the biological activity of endothelium-derived relaxing factor. Nature *327*, 524-526.

Parente, L., and Perretti, M. (2003). Advances in the pathophysiology of constitutive and inducible cyclooxygenases: two enzymes in the spotlight. Biochem Pharmacol *65*, 153-159.

Parkis, M.A., Bayliss, D.A., and Berger, A.J. (1995). Actions of norepinephrine on rat hypoglossal motoneurons. J Neurophysiol *74*, 1911-1919.

Paton, J.F., Deuchars, J., Ahmad, Z., Wong, L.F., Murphy, D., and Kasparov, S. (2001). Adenoviral vector demonstrates that angiotensin II-induced depression of the cardiac baroreflex is mediated by endothelial nitric oxide synthase in the nucleus tractus solitarii of the rat. J Physiol *531*, 445-458.

Pedoto, A., Tassiopoulos, A.K., Oler, A., McGraw, D.J., Hoffmann, S.P., Camporesi, E.M., and Hakim, T.S. (1998). Treatment of septic shock in rats with nitric oxide synthase inhibitors and inhaled nitric oxide. Crit Care Med *26*, 2021-2028.

Perez, C.M., Hazari, M.S., and Farraj, A.K. (2015). Role of autonomic reflex arcs in cardiovascular responses to air pollution exposure. Cardiovascular toxicology *15*, 69-78.

Pernow, J., Kaijser, L., Lundberg, J.M., and Ahlborg, G. (1996). Comparable potent coronary constrictor effects of endothelin-1 and big endothelin-1 in humans. Circulation *94*, 2077-2082.

Pineda, J., and Aghajanian, G.K. (1997). Carbon dioxide regulates the tonic activity of locus coeruleus neurons by modulating a proton- and polyamine-sensitive inward rectifier potassium current. Neuroscience *77*, 723-743.

Pinkert, C.A. (2003). Transgenic animal technology: alternatives in genotyping and phenotyping. Comparative medicine *53*, 126-139.

Pocock, G., and Richards, C.D. (2006). Human physiology : the basis of medicine, 3rd edn (Oxford ; New York: Oxford University Press).

Porth, C., Matfin, G., and Porth, C. (2009). Pathophysiology : concepts of altered health states, 8th edn (Philadelphia: Wolters Kluwer Health/Lippincott Williams & Wilkins).

Prasanna, G., Dibas, A., Hulet, C., and Yorio, T. (2001). Inhibition of Na(+)/K(+)-atpase by endothelin-1 in human nonpigmented ciliary epithelial cells. J Pharmacol Exp Ther *296*, 966-971.

Pratt, P.F., Li, P., Hillard, C.J., Kurian, J., and Campbell, W.B. (2001). Endothelium-independent, ouabain-sensitive relaxation of bovine coronary arteries by EETs. Am J Physiol Heart Circ Physiol *280*, H1113-1121.

Proske, U., and Gandevia, S.C. (2012). The proprioceptive senses: their roles in signaling body shape, body position and movement, and muscle force. Physiol Rev *92*, 1651-1697.

Putnam, R.W., Filosa, J.A., and Ritucci, N.A. (2004). Cellular mechanisms involved in CO(2) and acid signaling in chemosensitive neurons. Am J Physiol Cell Physiol *287*, C1493-1526.

Rajagopalan, S., Kurz, S., Munzel, T., Tarpey, M., Freeman, B.A., Griendling, K.K., and Harrison, D.G. (1996). Angiotensin II-mediated hypertension in the rat increases vascular superoxide production via membrane NADH/NADPH oxidase activation. Contribution to alterations of vasomotor tone. J Clin Invest *97*, 1916-1923.

Richfield, E.K., Thiruchelvam, M.J., Cory-Slechta, D.A., Wuertzer, C., Gainetdinov, R.R., Caron, M.G., Di Monte, D.A., and Federoff, H.J. (2002). Behavioral and neurochemical effects of wild-type and mutated human alpha-synuclein in transgenic mice. Experimental neurology *175*, 35-48.

Roux, J.C., Dura, E., Moncla, A., Mancini, J., and Villard, L. (2007). Treatment with desipramine improves breathing and survival in a mouse model for Rett syndrome. The European journal of neuroscience *25*, 1915-1922.

Roux, J.C., Panayotis, N., Dura, E., and Villard, L. (2010). Progressive noradrenergic deficits in the locus coeruleus of Mecp2 deficient mice. J Neurosci Res *88*, 1500-1509.

Roy, I., Mitra, S., Maitra, A., and Mozumdar, S. (2003). Calcium phosphate nanoparticles as novel non-viral vectors for targeted gene delivery. Int J Pharm *250*, 25-33.

Roy, I., Ohulchanskyy, T.Y., Bharali, D.J., Pudavar, H.E., Mistretta, R.A., Kaur, N., and Prasad, P.N. (2005). Optical tracking of organically modified silica nanoparticles as DNA carriers: a nonviral, nanomedicine approach for gene delivery. Proc Natl Acad Sci U S A *102*, 279-284.

Rubio, R., and Berne, R.M. (1975). Regulation of coronary blood flow. Progress in cardiovascular diseases *18*, 105-122.

Rukhadze, I., and Kubin, L. (2007). Mesopontine cholinergic projections to the hypoglossal motor nucleus. Neurosci Lett *413*, 121-125.

Sakar, M.S., Neal, D., Boudou, T., Borochin, M.A., Li, Y., Weiss, R., Kamm, R.D., Chen, C.S., and Asada, H.H. (2012). Formation and optogenetic control of engineered 3D skeletal muscle bioactuators. Lab on a chip *12*, 4976-4985.

Salamanca, D.A., and Khalil, R.A. (2005). Protein kinase C isoforms as specific targets for modulation of vascular smooth muscle function in hypertension. Biochem Pharmacol *70*, 1537-1547.

Salem, A.K., Searson, P.C., and Leong, K.W. (2003). Multifunctional nanorods for gene delivery. Nat Mater *2*, 668-671.

Samuels, E.R., and Szabadi, E. (2008). Functional neuroanatomy of the noradrenergic locus coeruleus: its roles in the regulation of arousal and autonomic function part I: principles of functional organisation. Current neuropharmacology *6*, 235-253.

Sant'Ambrogio, G., Tsubone, H., and Sant'Ambrogio, F.B. (1995). Sensory information from the upper airway: role in the control of breathing. Respir Physiol *102*, 1-16.

Sato, M., Severinghaus, J.W., and Basbaum, A.I. (1992). Medullary CO2 chemoreceptor neuron identification by c-fos immunocytochemistry. J Appl Physiol (1985) *73*, 96-100.

Schmidt, R.F., and Thews, G. (1989). Human physiology, 2nd completely rev. edn (Berlin ; New York: Springer-Verlag).

Schultz, J.H., Czachurski, J., Volk, T., Ehmke, H., and Seller, H. (2003). Central sympathetic chemosensitivity and Kir1 potassium channels in the cat. Brain Res *963*, 113-120.

Seifert, G., Huttmann, K., Binder, D.K., Hartmann, C., Wyczynski, A., Neusch, C., and Steinhauser, C. (2009). Analysis of astroglial K+ channel expression in the developing hippocampus reveals a predominant role of the Kir4.1 subunit. J Neurosci *29*, 7474-7488.

Seko, T., Ito, M., Kureishi, Y., Okamoto, R., Moriki, N., Onishi, K., Isaka, N., Hartshorne, D.J., and Nakano, T. (2003). Activation of RhoA and inhibition of myosin phosphatase as important components in hypertension in vascular smooth muscle. Circ Res *92*, 411-418.

Severs, N.J. (2000). The cardiac muscle cell. BioEssays : news and reviews in molecular, cellular and developmental biology *22*, 188-199.

Shi, W., Cui, N., Wu, Z., Yang, Y., Zhang, S., Gai, H., Zhu, D., and Jiang, C. (2010). Lipopolysaccharides up-regulate Kir6.1/SUR2B channel expression and enhance vascular KATP channel activity via NF-kappaB-dependent signaling. J Biol Chem *285*, 3021-3029.

Smith, G.C., Coleman, R.A., and McGrath, J.C. (1994). Characterization of dilator prostanoid receptors in the fetal rabbit ductus arteriosus. J Pharmacol Exp Ther *271*, 390-396.

Smyth, S.S., McEver, R.P., Weyrich, A.S., Morrell, C.N., Hoffman, M.R., Arepally, G.M., French, P.A., Dauerman, H.L., Becker, R.C., and Platelet Colloquium, P. (2009). Platelet functions beyond hemostasis. J Thromb Haemost *7*, 1759-1766.

Somlyo, A.P., and Somlyo, A.V. (2003). Ca2+ sensitivity of smooth muscle and nonmuscle myosin II: modulated by G proteins, kinases, and myosin phosphatase. Physiol Rev *83*, 1325-1358.

Spapen, H., and Vincken, W. (1992). Pulmonary arterial hypertension in sepsis and the adult respiratory distress syndrome. Acta clinica Belgica *47*, 30-41.

Sparenborg, S., and Gabriel, M. (1992). Local norepinephrine depletion and learning-related neuronal activity in cingulate cortex and anterior thalamus of rabbits. Experimental brain research *92*, 267-285.

Sparks, H.V., and Rooke, T.W. (1987). Essentials of cardiovascular physiology (Minneapolis: University of Minnesota Press).

Stankevicius, E., Kevelaitis, E., Vainorius, E., and Simonsen, U. (2003). [Role of nitric oxide and other endothelium-derived factors]. Medicina (Kaunas) *39*, 333-341.

Stern, D.M., Esposito, C., Gerlach, H., Gerlach, M., Ryan, J., Handley, D., and Nawroth, P. (1991). Endothelium and regulation of coagulation. Diabetes Care *14*, 160-166.

Sugiyama, T., Yoshizumi, M., Takaku, F., Urabe, H., Tsukakoshi, M., Kasuya, T., and Yazaki, Y. (1986). The elevation of the cytoplasmic calcium ions in vascular smooth muscle cells in SHR--measurement of the free calcium ions in single living cells by lasermicrofluorospectrometry. Biochem Biophys Res Commun *141*, 340-345.

Suzuki, Y.J., and Ford, G.D. (1992). Superoxide stimulates IP3-induced Ca2+ release from vascular smooth muscle sarcoplasmic reticulum. Am J Physiol *262*, H114-116.

Taddei, S., Ghiadoni, L., Virdis, A., Versari, D., and Salvetti, A. (2003). Mechanisms of endothelial dysfunction: clinical significance and preventive non-pharmacological therapeutic strategies. Current pharmaceutical design *9*, 2385-2402.

Takagi, Y., Hirata, Y., Takata, S., Yoshimi, H., Fukuda, Y., Fujita, T., and Hidaka, H. (1988). Effects of protein kinase inhibitors on growth factor-stimulated DNA synthesis in cultured rat vascular smooth muscle cells. Atherosclerosis *74*, 227-230.

Takahashi, T., Forsythe, I.D., Tsujimoto, T., Barnes-Davies, M., and Onodera, K. (1996). Presynaptic calcium current modulation by a metabotropic glutamate receptor. Science *274*, 594-597.

Takashima, S., Sugimoto, N., Takuwa, N., Okamoto, Y., Yoshioka, K., Takamura, M., Takata, S., Kaneko, S., and Takuwa, Y. (2008). G12/13 and Gq mediate S1P2-induced inhibition of Rac

and migration in vascular smooth muscle in a manner dependent on Rho but not Rho kinase. Cardiovascular research *79*, 689-697.

Takata, Y., Nishimura, Y., Maeda, H., and Yokoyama, M. (1999). Phospholipase A2 augments contraction and intracellular calcium mobilization through thromboxane A2 in bovine tracheal smooth muscle. The European respiratory journal *14*, 396-404.

Takemoto, M., Sun, J., Hiroki, J., Shimokawa, H., and Liao, J.K. (2002). Rho-kinase mediates hypoxia-induced downregulation of endothelial nitric oxide synthase. Circulation *106*, 57-62.

Tamaki, H. (2007). Glucose-stimulated cAMP-protein kinase A pathway in yeast Saccharomyces cerevisiae. Journal of bioscience and bioengineering *104*, 245-250.

Tanaka, H., Homma, K., White, H.D., Yanagida, T., and Ikebe, M. (2008). Smooth muscle myosin phosphorylated at single head shows sustained mechanical activity. J Biol Chem *283*, 15611-15618.

Tanaka, K.A., Szlam, F., Katori, N., Tsuda, A., and Levy, J.H. (2004). In vitro effects of antihypertensive drugs on thromboxane agonist (U46619)-induced vasoconstriction in human internal mammary artery. British journal of anaesthesia *93*, 257-262.

Taneja, P., Ogier, M., Brooks-Harris, G., Schmid, D.A., Katz, D.M., and Nelson, S.B. (2009). Pathophysiology of locus ceruleus neurons in a mouse model of Rett syndrome. J Neurosci *29*, 12187-12195.

Taniyama, Y., and Griendling, K.K. (2003). Reactive oxygen species in the vasculature: molecular and cellular mechanisms. Hypertension *42*, 1075-1081.

Taylor, E.W., Jordan, D., and Coote, J.H. (1999). Central control of the cardiovascular and respiratory systems and their interactions in vertebrates. Physiol Rev *79*, 855-916.

Taylor, S.S., Kim, C., Cheng, C.Y., Brown, S.H., Wu, J., and Kannan, N. (2008). Signaling through cAMP and cAMP-dependent protein kinase: diverse strategies for drug design. Biochimica et biophysica acta *1784*, 16-26.

Terry, R.W., Kwee, L., Baldwin, H.S., and Labow, M.A. (1997). Cre-mediated generation of a VCAM-1 null allele in transgenic mice. Transgenic research *6*, 349-356.

Tesse, A., Martinez, M.C., Hugel, B., Chalupsky, K., Muller, C.D., Meziani, F., Mitolo-Chieppa, D., Freyssinet, J.M., and Andriantsitohaina, R. (2005). Upregulation of proinflammatory proteins through NF-kappaB pathway by shed membrane microparticles results in vascular hyporeactivity. Arterioscler Thromb Vasc Biol *25*, 2522-2527.

Thomas, G.D. (2011). Neural control of the circulation. Advances in physiology education *35*, 28-32.

Thomas, W.G., Thekkumkara, T.J., and Baker, K.M. (1996). Cardiac effects of AII. AT1A receptor signaling, desensitization, and internalization. Advances in experimental medicine and biology *396*, 59-69.

Tofaris, G.K., Garcia Reitbock, P., Humby, T., Lambourne, S.L., O'Connell, M., Ghetti, B., Gossage, H., Emson, P.C., Wilkinson, L.S., Goedert, M., *et al.* (2006). Pathological changes in dopaminergic nerve cells of the substantia nigra and olfactory bulb in mice transgenic for truncated human alpha-synuclein(1-120): implications for Lewy body disorders. J Neurosci *26*, 3942-3950.

Tran, Q.K., and Watanabe, H. (2006). Calcium signalling in the endothelium. Handbook of experimental pharmacology, 145-187.

Triposkiadis, F., Karayannis, G., Giamouzis, G., Skoularigis, J., Louridas, G., and Butler, J. (2009). The sympathetic nervous system in heart failure physiology, pathophysiology, and clinical implications. J Am Coll Cardiol *54*, 1747-1762.

Tune, J.D. (2014). Coronary Circulation. In Colloquium Series on Integrated Systems Physiology: From Molecule to Function (San Rafael: Biota Publishing), pp. 1 online resource (191 pages).

Uehata, M., Ishizaki, T., Satoh, H., Ono, T., Kawahara, T., Morishita, T., Tamakawa, H., Yamagami, K., Inui, J., Maekawa, M., *et al.* (1997). Calcium sensitization of smooth muscle mediated by a Rho-associated protein kinase in hypertension. Nature *389*, 990-994.

van Geel, P.P., Pinto, Y.M., Voors, A.A., Buikema, H., Oosterga, M., Crijns, H.J., and van Gilst, W.H. (2000). Angiotensin II type 1 receptor A1166C gene polymorphism is associated with an increased response to angiotensin II in human arteries. Hypertension *35*, 717-721.

Vanhoutte, P.M., Feletou, M., and Taddei, S. (2005). Endothelium-dependent contractions in hypertension. Br J Pharmacol *144*, 449-458.

Venkiteswaran, K., Xiao, K., Summers, S., Calkins, C.C., Vincent, P.A., Pumiglia, K., and Kowalczyk, A.P. (2002). Regulation of endothelial barrier function and growth by VE-cadherin, plakoglobin, and beta-catenin. Am J Physiol Cell Physiol *283*, C811-821.

Viemari, J.C. (2008). Noradrenergic modulation of the respiratory neural network. Respir Physiol Neurobiol *164*, 123-130.

Viemari, J.C., Bevengut, M., Burnet, H., Coulon, P., Pequignot, J.M., Tiveron, M.C., and Hilaire, G. (2004). Phox2a gene, A6 neurons, and noradrenaline are essential for development of normal respiratory rhythm in mice. J Neurosci *24*, 928-937.

Viemari, J.C., Garcia, A.J., 3rd, Doi, A., Elsen, G., and Ramirez, J.M. (2013). beta-Noradrenergic receptor activation specifically modulates the generation of sighs in vivo and in vitro. Front Neural Circuits *7*, 179.

Viemari, J.C., and Ramirez, J.M. (2006). Norepinephrine differentially modulates different types of respiratory pacemaker and nonpacemaker neurons. J Neurophysiol *95*, 2070-2082.

Viemari, J.C., Roux, J.C., Tryba, A.K., Saywell, V., Burnet, H., Pena, F., Zanella, S., Bevengut, M., Barthelemy-Requin, M., Herzing, L.B., *et al.* (2005). Mecp2 deficiency disrupts norepinephrine and respiratory systems in mice. J Neurosci *25*, 11521-11530.

Villar, I.C., Francis, S., Webb, A., Hobbs, A.J., and Ahluwalia, A. (2006). Novel aspects of endothelium-dependent regulation of vascular tone. Kidney Int *70*, 840-853.

Villard, L., and Roux, J.C. (2006). [Noradrenaline deficiency as the origin of respiratory disorders in Rett syndrome an animal model]. Medecine sciences : M/S *22*, 81-83.

Vincent, J.L., Sakr, Y., Sprung, C.L., Ranieri, V.M., Reinhart, K., Gerlach, H., Moreno, R., Carlet, J., Le Gall, J.R., Payen, D., *et al.* (2006). Sepsis in European intensive care units: results of the SOAP study. Crit Care Med *34*, 344-353.

Wakade, A.R., and Wakade, T.D. (1983). Contribution of nicotinic and muscarinic receptors in the secretion of catecholamines evoked by endogenous and exogenous acetylcholine. Neuroscience *10*, 973-978.

Wang, X., Pinol, R.A., Byrne, P., and Mendelowitz, D. (2014). Optogenetic stimulation of locus ceruleus neurons augments inhibitory transmission to parasympathetic cardiac vagal neurons via activation of brainstem alpha1 and beta1 receptors. J Neurosci *34*, 6182-6189.

Warden, M.R., Cardin, J.A., and Deisseroth, K. (2014). Optical neural interfaces. Annual review of biomedical engineering *16*, 103-129.

Washburn, C.P., Sirois, J.E., Talley, E.M., Guyenet, P.G., and Bayliss, D.A. (2002). Serotonergic raphe neurons express TASK channel transcripts and a TASK-like pH- and halothane-sensitive K+ conductance. J Neurosci *22*, 1256-1265.

Watts, S.W. (2010). Endothelin receptors: what's new and what do we need to know? American journal of physiology Regulatory, integrative and comparative physiology *298*, R254-260.

West, J.B., and West, J.B. (2012). Pulmonary pathophysiology : the essentials, 8th edn (Philadelphia: Wolters Kluwer/Lippincott Williams & Wilkins Health).

Wettschureck, N., and Offermanns, S. (2005). Mammalian G proteins and their cell type specific functions. Physiol Rev *85*, 1159-1204.

Williams, S.C., and Deisseroth, K. (2013). Optogenetics. Proc Natl Acad Sci U S A *110*, 16287.

Woodsome, T.P., Eto, M., Everett, A., Brautigan, D.L., and Kitazawa, T. (2001). Expression of CPI-17 and myosin phosphatase correlates with Ca(2+) sensitivity of protein kinase C-induced contraction in rabbit smooth muscle. J Physiol *535*, 553-564.

Wu, Y., Huang, A., Sun, D., Falck, J.R., Koller, A., and Kaley, G. (2001). Gender-specific compensation for the lack of NO in the mediation of flow-induced arteriolar dilation. Am J Physiol Heart Circ Physiol *280*, H2456-2461.

Wu, Y., Li, S.S., Jin, X., Cui, N., Zhang, S., and Jiang, C. (2015). Optogenetic approach for functional assays of the cardiovascular system by light activation of the vascular smooth muscle. Vascular pharmacology *71*, 192-200.

Xiao, S., Wagner, L., Schmidt, R.J., and Baylis, C. (2001). Circulating endothelial nitric oxide synthase inhibitory factor in some patients with chronic renal disease. Kidney Int *59*, 1466-1472.

Xie, D.J., Uta, D., Feng, P.Y., Wakita, M., Shin, M.C., Furue, H., and Yoshimura, M. (2012). Identification of 5-HT receptor subtypes enhancing inhibitory transmission in the rat spinal dorsal horn in vitro. Molecular pain *8*, 58.

Young, J.I., Hong, E.P., Castle, J.C., Crespo-Barreto, J., Bowman, A.B., Rose, M.F., Kang, D., Richman, R., Johnson, J.M., Berget, S., *et al.* (2005). Regulation of RNA splicing by the methylation-dependent transcriptional repressor methyl-CpG binding protein 2. Proc Natl Acad Sci U S A *102*, 17551-17558.

Zeng, H., and Madisen, L. (2012). Mouse transgenic approaches in optogenetics. Progress in brain research *196*, 193-213.

Zhang, F., Vierock, J., Yizhar, O., Fenno, L.E., Tsunoda, S., Kianianmomeni, A., Prigge, M., Berndt, A., Cushman, J., Polle, J., *et al.* (2011a). The microbial opsin family of optogenetic tools. Cell *147*, 1446-1457.

Zhang, F., Wang, L.P., Boyden, E.S., and Deisseroth, K. (2006). Channelrhodopsin-2 and optical control of excitable cells. Nat Methods *3*, 785-792.

Zhang, S., Cui, N., Wu, Y., Zhong, W., Johnson, C.M., and Jiang, C. (2015). Optogenetic intervention to the vascular endothelium. Vascular pharmacology *74*, 122-129.

Zhang, X., Cui, N., Wu, Z., Su, J., Tadepalli, J.S., Sekizar, S., and Jiang, C. (2010a). Intrinsic membrane properties of locus coeruleus neurons in Mecp2-null mice. Am J Physiol Cell Physiol *298*, C635-646.

Zhang, X., Su, J., Cui, N., Gai, H., Wu, Z., and Jiang, C. (2011b). The disruption of central CO2 chemosensitivity in a mouse model of Rett syndrome. Am J Physiol Cell Physiol *301*, C729-738.

Zhang, X., Su, J., Rojas, A., and Jiang, C. (2010b). Pontine norepinephrine defects in Mecp2-null mice involve deficient expression of dopamine beta-hydroxylase but not a loss of catecholaminergic neurons. Biochem Biophys Res Commun *394*, 285-290.

Zhao, B., and Debski, E.A. (2005). Serotonergic reticular formation cells in Rana pipiens: categorization, development, and tectal projections. J Comp Neurol *487*, 441-456.

Zheng, B., Sage, M., Sheppeard, E.A., Jurecic, V., and Bradley, A. (2000). Engineering mouse chromosomes with Cre-loxP: range, efficiency, and somatic applications. Molecular and cellular biology *20*, 648-655.

Zhong, W., Cui, N., Jin, X., Oginsky, M.F., Wu, Y., Zhang, S., Bondy, B., Johnson, C.M., and Jiang, C. (2015). Methyl CpG Binding Protein 2 Gene Disruption Augments Tonic Currents of gamma-Aminobutyric Acid Receptors in Locus Coeruleus Neurons: IMPACT ON NEURONAL EXCITABILITY AND BREATHING. J Biol Chem *290*, 18400-18411.

Zhou, Y., Mitra, S., Varadharaj, S., Parinandi, N., Zweier, J.L., and Flavahan, N.A. (2006). Increased expression of cyclooxygenase-2 mediates enhanced contraction to endothelin ETA receptor stimulation in endothelial nitric oxide synthase knockout mice. Circ Res *98*, 1439-1445.

Zizzo, M.G., Mule, F., and Serio, R. (2006). Mechanisms underlying hyperpolarization evoked by P2Y receptor activation in mouse distal colon. European journal of pharmacology *544*, 174-180.

Zoghbi, H.Y., Milstien, S., Butler, I.J., Smith, E.O., Kaufman, S., Glaze, D.G., and Percy, A.K. (1989). Cerebrospinal fluid biogenic amines and biopterin in Rett syndrome. Annals of neurology *25*, 56-60.

Zoghbi, H.Y., Percy, A.K., Glaze, D.G., Butler, I.J., and Riccardi, V.M. (1985). Reduction of biogenic amine levels in the Rett syndrome. N Engl J Med *313*, 921-924.

Zou, M.H., Cohen, R., and Ullrich, V. (2004). Peroxynitrite and vascular endothelial dysfunction in diabetes mellitus. Endothelium : journal of endothelial cell research *11*, 89-97.

APPENDICES: PUBLICATIONS

1. Zhang S, Cui N, Wu Y, Zhong W, Johnson CM, Jiang C. Optogenetic intervention to the vascular endothelium. *Vascul Pharmacol.* 2015 Nov;64:122-9.

2. Zhang S, Cui N, Li S, Guo L, Wu Y, Zhu D, Jiang C. Interception of the endotoxin-induced arterial hyporeactivity to vasoconstrictors. *Vascul Pharmacol*. 2014 Jul; 62(1):15-23.

3. Shuang Zhang, Christopher M. Johnson, Ningren Cui, Yang Wu, Hao Xing, Weiwei Zhong, Chun Jiang. An optogenetic mouse model of Rett syndrome targeting on catecholaminergic neurons. *J Neurosci Res*. 2016 Apr [In Press].

4. Jin X, Li S, Bondy B, Zhong W, Oginsky MF, Wu Y, Johnson CM, Zhang S, Cui N, Jiang C. Identification of a group of GABAergic neurons in the dorsomedial area of the locus coeruleus. *PLos One.* 2016 Jan 19;11(1):e0146470.

5. Zhong W, Cui N, Jin X, Oginsky MF, Wu Y, Zhang S, Bondy B, Johnson CM, Jiang C. *J Biol Chem.* 2015 Jul; 290(30): 18400-11.

6. Wu Y, Li SS, Jin X, Cui N, Zhang S, Jiang C. Optogenetic approach for functional assays of the cardiovascular system by light activation of the vascular smooth muscle. *Vascul Pharmacol.* 2015 Aug; 71: 192-200.

7. Li SS, Cui N, Yang Y, Trower TC, Wei YM, Zhang S, Jin X, Jiang C. Impairments of the vascular KATP channel imposes fatal susceptibility to experimental diabetes due to multi-organ Injuries. *J Cell Physiol.* 2015 Dec; 230(12): 2915-26.

8. Yang Y, Li S, Konduru AS, Zhang S, Trower TC, Shi W, Cui N, Yu L, Wang Y, Zhu D, Jiang C. Prolonged exposure to methylglyoxal causes disruption of vascular KATP channel by mRNA instability. *Am J Physiol: Cell Physiol*. 2012 Nov; 303(10): C1045-54.

9. Jin X, Yu L, Wu Y, Zhang S, Shi Z, Chen X, Yang Y, Zhang X, Jiang C. S-Glutathionylation

underscores the modulation of the heteromeric Kir4.1-Kir5.1 channel in oxidative stress. *J Physiol*. 2012 Nov; 590(Pt 21): 5335-48.

10. Yang Y, Shi W, Chen X, Cui N, Konduru AS, Shi Y, Trower TC, Zhang S, Jiang C. Molecular basis and structural insight of vascular K(ATP) channel gating by S-glutathionylation. *J Biol Chem.* 2011 Mar 18; 286(11):9298-307.