NEURONAL FIRING PROPERTIES IN SYMPATHETIC GANGLION NEURONS IN HYPERTENSION

By

Xiaohong Wang

A DISSERTATION

Submitted to
Michigan State University
in partial fulfillment of the requirements
for the degree of

Physiology-Doctor of Philosophy

2014

ABSTRACT

NEURONAL FIRING PROPERTIES IN SYMPATHETIC GANGLION NEURONS IN HYPERTENSION

By

Xiaohong Wang

The activity of both sympathetic neurons in the central nervous system and peripheral sympathetic nerves that innervate the heart and blood vessels is elevated in hypertension. However it is unclear about the roles of sympathetic ganglia, which integrate central sympathetic signals and send their signals to cardiovascular end organs via sympathetic nerve. The celiac ganglion (CG) plays an important function in regulating blood pressure by the controlling splanchnic circulation containing 30% of total blood volume. Reactive oxygen species (ROS) are elevated in CG in hypertension. Ion channels, essential factors for neuronal firing, are possible targets for ROS. The purpose of this study is to find if neuronal firing and ion channels are modulated in hypertension and if ROS are related to changes in neuronal firing and ion channels in hypertension.

Neuronal firing patterns were explored in dissociated CG neurons from DOCA-salt hypertensive rats. In response to sustained suprathreshold current injection, more phasic neurons, fewer adaptive neurons and tonic neurons were shown in dissociated CG neurons from hypertensive rats. Moreover, the firing frequencies of tonic neurons were significantly lower in hypertensive rats compared to

matched normotensive rats. Furthermore several K^+ currents were found different in CG from the same animal model. Delay rectifier K^+ current (I_{KV}), big conductance Ca^{2+}-activated K^+ current (I_{BK}) and A-type K^+ current (I_A) were significantly lower in hypertensive rats compared to normotensive rats. The contribution of the decreased K^+ currents to neuronal firing changes in hypertension is confirmed by using specific K^+ channel blockers.

H_2O_2, one type of ROS, was applied to dissociated CG neurons. H_2O_2 caused more accommodating firing by converting tonic neurons to phasic/adaptive and adaptive neurons to phasic in response to sustained suprathreshold current injection. Neuronal firing frequency was also decreased by H_2O_2. All the changes in neuronal firing by H_2O_2 are similar to those differences between normotensive and hypertensive rats. Moreover H_2O_2 mimicked some of the reductions of K currents in hypertension by decreasing I_A and I_{BK}.

In conclusion, the changes in distribution of different neuronal firing patterns, associated with attenuated K^+ currents, are contributed by elevated ROS in CG neurons from hypertensive rats. The electrophysiological changes in CG might contribute to the increased sympathetic activity in DOCA-salt hypertensive rats.

ACKNOWLEDGEMENTS

I am sincerely thankful to my advisor, David Kreulen, who gives me enormous helps, enthusiasm and broad knowledge. Whenever I felt I could not achieve a goal, he always told me “you can do it, Xiaohong”. With his patient and motivational guidance I overcame many difficulties and finished my dissertation writing. I could not have imagined finishing my Ph.D study without his mentoring.

Besides my advisor, I would also like to thank the rest of my committee members: Drs. Bruce Uhal, James Galligan, Stephen Schneider, Steven Heidemann. Thank you for all your encouragement, suggestions and continuous supports.

Furthermore, I would like to thank the director of Physiology department, Dr. Arthur Weber for offering me the opportunity to study in the program and your many supports.

I am indebted to my many fellow labmates for their helps and camaraderie: Xiaoling Dai, Jolene Zheng, Erica Wehrwein, Xian Cao, Anna Wright, Lindsay Parker, Mohammad Esfahanian, Casey Henley, Timothy Houchin, Amit Shah.

I would also like to show my gratitude and thanks to my friends in the neighborhood laboratories. I could not finish my project without their helps: Xiaochun Bian, Hui Xu, Wei Li, Tracy Walker, Wen-hsin Ku and all the researchers working in Dr. galligan Dr. Fink and Dr. Watts’s lab. I would like to thank specially to Xiaochun Bian who taught me patch clamp which is the major technique for my research.

Last but not the least, I would like to give thanks to my family: my parents Zhenying Wang and Xiulan Chen, my husband Hui Wang and my two angels Timothy and Angelina. Thank my parents for giving birth to me and supporting me throughout my life. Thank my husband for the love and supports. Thank my angels for growing healthily and smiling to me everyday.

TABLE OF CONTENTS

LIST OF TABLES ix

LIST OF FIGURES x

LIST OF ABBREVIATIONS xii

CHAPTER 1: GENERAL INTRODUCTION 1
Hypertension 2
- **Introduction of hypertension** 2
- **Hypertension animal models** 3
- **Characteristics of the DOCA-salt hypertensive rat** 6

Sympathetic nervous system and hypertension 9
- **SNS and BP regulation** 9
- **Role of SNS in hypertension** 10
- **Structure of SNS** 11
- **Sympathetic neurons in the CNS and hypertension** 11
- **Sympathetic nerve activity and hypertension** 13

Sympathetic ganglia and hypertension 14
- **The structure of sympathetic ganglia** 14
- **Signal transmission in sympathetic ganglia** 15
- **Neurotransmission of sympathetic ganglia in hypertension** 16
- **Neuronal membrane firing properties of sympathetic postganglionic neurons** 17
- **Celiac ganglion and hypertension** 19

Ion channels and hypertension 20
- **Ion channels and neuronal firing** 20
- **K^+ and Ca^{2+} channel currents in CG neurons** 21
- **K^+ channels and neuronal firing properties** 22

Reactive Oxygen Species (ROS) and hypertension 24
- **Introduction** 24
- **Elevated ROS in hypertension** 25
- **Oxidation function of ROS on regulation of ion channels** 26
- **ROS and neuronal firing properties in hypertension** 27

Summary 28
BIBLIOGRAPHY 31

CHAPTER 2: NEURONAL MEMBRANE FIRING PROPERTIES OF DISSOCIATED CELIAC GANGLION NEURONS IN DOCA-SALT HYPERTENSIVE RATS 43
- **Abstract** 44
- **Introduction** 45

Materials and Methods **46**
Animals **46**
Tissure collection **47**
Cell culture **48**
Whole-cell patch clamp **49**
Data acquisition **50**
Statistical analysis **50**
Results **51**
Discussion **56**
APPENDIX **59**
BIBLIOGRAPHY **80**

CHAPTER 3: CALCIUM AND POTASSIUM CHANNEL CURRENTS OF DISSOCIATED CELIAC GANGLION NEURONS IN DOCA-SALT HYPERTENSIVE RATS **83**
Abstract **84**
Introduction **85**
Materials and Methods **87**
Animals **87**
Tissure collection **87**
Cell culture **87**
Whole-cell patch clamp **87**
Data acquisition **88**
Statistical analysis **89**
Results **89**
Discussion **92**
APPENDIX **96**
BIBLIOGRAPHY **111**

CHAPTER 4: EFFECTS OF HYDROGEN PEROXIDE ON NEURONAL FIRING AND POTASSIUM CHANNEL CURRENTS OF DISSOCIATED RAT CELIAC GANGLION NEURONS **115**
Abstract **116**
Introduction **117**
Materials and Methods **119**
Animals **119**
Tissure collection **119**
Cell culture **119**
Whole-cell patch clamp **119**
Statistical analysis **120**
Results **120**
Discussion **127**
APPENDIX **131**
BIBLIOGRAPHY **157**

CHAPTER 5: CONCLUSION AND DISCUSSION 160
The Sympathetic nervous system in hypertension 162
Increased sympathetic activity in hypertension 162
The sympathetic nervous system in DOCA-salt hypertensive rats 163
Predicting the possible roles of altered firing properties in CG neurons from hypertensive rats 164
***Neuronal firing pattern is a changeable firing property* 165**
***Neurotransmission in sympathetic ganglia* 166**
***Predicting the significance of alterations in firing properties of CG neurons for hypertension* 167**
Change in K^+ currents and neurotransmitter release in hypertension 169
Changes in the neuronal firing properties of SCG neurons in other animal models of hypertension 172
Neuronal firing properties and ROS 173
K^+ currents and neuronal firing 173
ROS and neuronal firing in the SNS 175
H_2O_2 in sympathetic ganglia and hypertension 177
Factors that may contribute the observed changes in neuronal firing properties and elevated ROS levels in CGs from DOCA-salt hypertensive rats 179
Increased central sympathetic drive 179
Upregulation of ET-B receptor 181
Other possible factors 182
Vascular neurons in the CG 183
Substructure of the CG 183
Identification of vascular neurons 184
Conclusion and perspectives 185
APPENDIX 188
BIBLIOGRAPHY 191

LIST OF TABLES

Table 2-1: Electrical membrane properties of dissociated CG neurons from NT and HT rats 55

Table 2-2: Single AP properties in response to sustained current injection of CG neurons in NT and HT rats 60

Table 2-3: Afterhyperpolarizaion of single AP of CG neurons in NT and HT rats 60

Table 4-1: The effects of H_2O_2 on electrical parameters of the first AP in the neuronal firing by a sustained depolarizing current injection 125

Table 4-2: The effects of H_2O_2 on membrane electrical parameters of a single AP .. 125

Table 5-1: Summaries of the differences between HT and NT rats and the changes by H_2O_2 in CG neuronal firing properties and K^+ channel current amplitudes of CG neurons 173

LIST OF FIGURES

Figure 2-1: Neuronal firing patterns of celiac neurons from NT and HT rats 56

Figure 2-2: The characteristics of firing frequency change in response to a sustained current injection in CG neurons..... 58

Figure 2-3: The effect of TEA on outward K^+ currents 58

Figure 2-4: Effect of TEA on neuronal firing of dissociated CG neurons 61

Figure 2-5: Effect of paxilline on outward K^+ currents 65

Figure 2-6: Effect of paxilline on neuronal firing of dissociated CG neurons 66

Figure 2-7: Effect of 4-AP on neuronal firing of dissociated CG neurons 67

Figure 3-1: Sustained outward K^+ currents of CG neurons in HT and NT rats..... 84

Figure 3-2: I_{KV} and I_{BK} of CG neurons in HT and NT rats..... 87

Figure 3-3: I_A of CG neurons in HT and NT rats 90

Figure 3-4: I_m of CG neurons in HT and NT rats..... 92

Figure 3-5: I_{Ca} of celiac neurons in HT and NT rats..... 94

Figure 4-1: Effects of H_2O_2 on firing patterns of dissociated CG neurons..... 118

Figure 4-2: Effects of H_2O_2 on firing frequency in dissociated CG neurons 122

Figure 4-3: Effects of H_2O_2 on sustained outward K^+ currents in dissociated CG neurons..... 126

Figure 4-4: The effects of H_2O_2 on transient outward K^+ current..... 129

Figure 4-5: The effects of H_2O_2 on activation and inactivation transient outward K^+ current..... 130

F**igure 4-6:** The effects of H_2O_2 on activation and inactivation transient outward K^+ current in presence of intracellular catalase..... 132

F**igure 5-1:** Prediction of the role of altered firing properties of CG neurons in HT..... 156

F**igure 5-2:** A diagram illustrating the possible role of CG neurons in sympathetic neurotransmission in DOCA-salt hypertensive rats..... 174

LIST OF ABBREVIATIONS

4-AP	4-Aminopyridine
AHP	afterhyperpolarization
AP	action potential
APs	action potentials
BK K^+ channel	large-conductance calcium-activated K^+ channel
BP	blood pressure
CG	celiac ganglion
DOCA	deoxycorticosterone acetate
DOCA-salt	deoxycorticosterone acetate–salt
f_i	instantaneous firing frequency
f_{1st}	first instantaneous firing frequency
f_{last}	last instantaneous firing frequency
H_2O_2	hydrogen peroxide
I_A	A-type K^+ channel current
I_{BK}	large-conductance calcium-activated K^+ channel current
I_{KCa}	calcium-activated K^+ channel current
I_{Kv}	delayed rectifier K^+ channel current
I_m	M-type K^+ channel current
I_r	rheobasic current
I_{SK}	small-conductance calcium-activated K^+ channel current

NADPH	nicotinamide adenine dinucleotide phosphate
NE	norepinephrine
$O_2^{\bullet-}$	superoxide anion
OH•	hydroxyl radical
R_a	access resistance
R_{in}	input resistance
RMP	resting membrane potential
ROS	reactive oxygen species
SCG	Superior cervical ganglia
SK K^+ channel	small-conductance calcium-activated K^+ channel
SNA	sympathetic nerve activity
SNS	sympathetic nervous system
TEA	Tetraethylammonium
TTX	tetrodotoxin
SHR	spontaneous hypertensive rat
CAP	compound action potential

CHAPTER 1

GENERAL INTRODUCTION

Hypertension

Introduction of hypertension

Hypertension is a chronic disease defined as a persistent elevation of BP (blood pressure) (systolic BP ≥ 140 mmHg and/or diastolic BP ≥ 90 mmHg). The blood pressure (BP) is the force applied by blood to the walls of arteries when the heart pumps blood into the circulatory system and it is precisely regulated within a normal physiological range that is systolic BP within 90-120 mmHg and diastolic BP within 60-80 mmHg. Prehypertension is the early form of hypertension and is categorized by a systolic BP of 120–139 mmHg and/or diastolic BP of 80–89 mmHg. People with prehypertension are likely to develop hypertension in the future. The underlying mechanisms of hypertension are still not well understood. Currently there is no curative treatment for hypertension; hypertension treatments primarily involve controlling BP with lifestyle changes and taking antihypertensive medications. However, the treatments are not always very effective. BP can be maintained at less than 140/90 mmHg in only 64% of hypertensive patients using antihypertensive medications (Chobanian *et al.*, 2003). Some patients are not even compliant with their hypertensive drug treatment, and hypertension is a very prevalent problem. According to the National Health and Nutrition Examination Surveys (1999-2006), 30.5% of American adults have hypertension and 28% of American adults have prehypertension. Therefore, approximately two-thirds of hypertensive patients, that is 20% of American adults, are still suffering from uncontrolled hypertension (Chobanian *et al.*, 2003).

Hypertension increases the risks of many life-threatening diseases such as heart attack, heart failure, stroke, and renal failure (Wang *et al.*, 2005b). The World Health

Organization reported that high BP (systolic BP >115mm Hg) is responsible for 62% of cerebrovascular disease and 49% of ischemic heart disease, including heart attacks in 2002. Persistently high BP leads to many cardiovascular problems such as ventricular hypertrophy, heart failure, vascular hypertrophy and atherosclerosis. Vascular changes in specific organs are the underlying causes for many diseases such as heart attack, stroke, dementia, renal failure and retina damage.

There are two types of hypertension: primary (essential) hypertension and secondary hypertension. Secondary hypertension is high blood pressure that has a known etiology such as renovascular disease, renal failure, pheochromocytoma, or aldosteronism. Alternatively approximately 95% of hypertension cases have complex, multifactorial causes, and this is called essential hypertension (Carretero & Oparil, 2000). A variety of factors play a role in the development of essential hypertension such as the renin-angiotensin system, the sympathetic nervous system (SNS), high salt intake, obesity and insulin resistance (Beevers *et al.*, 2001). This thesis explores the role of the SNS in essential hypertension.

Hypertension animal models

A spectrum of experimental animal models of hypertension has been developed by utilizing different etiological factors of hypertension such as excessive salt intake, hyperactive renin-angiotensin-aldosterone system and genetic factors (Badyal & Dadhich, 2003). However, there is no animal model that mimics every characteristic of human hypertension. Each animal model is valuable for understanding hypertension by endowing some etiological and pathological changes observed in hypertensive patients. The most commonly used animal model is the rat, which was also used in my studies.

No one specific animal model is preferred for hypertension research studies; therefore the selection of the animal model primarily depends on the characteristics of hypertension the researchers would like to focus on. I am interested in the role of SNS in hypertension. Three hypertensive rat models with elevated SNS activity are described below.

Spontaneously hypertensive rats (SHR): The spontaneously hypertensive rat (SHR) has an established hypertension trait, and it was obtained by intentionally breeding Wistar-Kyoto rats with high BP (Dornas & Silva, 2011). It is an animal model that reflects a genetic predisposition to hypertension. However, this model mimics only a rare subtype of human hypertension, which is a primary hypertension inherited in a Mendelian fashion. Furthermore, the kidney is found to be involved in the development of this type of hypertension (Rettig *et al.*, 1993), and SNS activation is exhibited in this animal model (Lundin *et al.*, 1984).

Renovascular hypertensive rats: In these hypertensive rats, one or both renal arteries are constricted. Common procedures to create this model include applying a U-shaped clip around the renal artery or performing a ligature around the blood supply to the kidney. Renal artery constriction leads to kidney ischemia and reduced blood flow to the glomeruli, which stimulates the release of renin from the juxtaglomerular renin-secreting granular cells. Renin enters the blood and activates the peripheral renin-angiotensin-aldosterone system to increase BP. In addition to the typical heart and vascular changes associated with hypertension, renal sympathetic nerve activity is elevated in this animal model (Han *et al.*, 2011).

DOCA-salt hypertensive rats: Deoxycorticosterone acetate (DOCA)-salt

hypertensive rats are produced by surgical uninephrectomy followed by the administration of a DOCA pellet and excess dietary salt. The etiology of hypertension is understood to some extent in this rat model. As a mineralocorticoid, DOCA increases Na^+ and water retention and K^+ secretion in the kidney. Excess dietary salt further increases the Na^+ level in the presence of DOCA. The plasma Na^+ level is elevated in DOCA-treated rats (Friedman & Tanaka, 1987). The hypertension developed in this rat model mimics the type of hypertension observed in African-American hypertensive patients. For example, both are resistant to renin-angiotensin inhibiting treatment but are sensitive to calcium channel blockers (Pinto *et al.*, 1998). This rat model exhibits increased secretion of vasopressin, impaired endothelium dependent relaxation and increased sympathetic nerve activity (Watson R.E. & Dipette D.J., 2008).

Although all three models are appropriate for these studies because they exhibit increased sympathetic activity, the DOCA-salt hypertensive rat, was chosen for three main reasons. First, SHR is representative of such a rare type of human hypertension, while DOCA-salt hypertensive rats represent African-American hypertensive patients. The only studies that have targeted the function of sympathetic ganglia have been performed in SHR (Magee & Schofield, 1992;Magee & Schofield, 1994). It is valuable to explore the changes in these ganglia in DOCA-salt hypertensive rats, which mimic some characteristics of human hypertension that are different in SHR rats. Second, sympathetic ganglia are rarely studied in renovascular hypertensive rats. There is limited background information for this animal model. Third, our lab worked with three other labs as a group to study hypertension using DOCA-salt hypertensive rats, which makes my study more convenient and reliable. One important finding from our group

involved celiac ganglion (CG), the largest prevertebral sympathetic ganglion. Reactive oxygen species (ROS) levels and NADPH oxidase activity levels are elevated in CG of hypertensive rats (Cao *et al.*, 2007;Dai *et al.*, 2004a). Removing the CG reduced the elevated BP and decreased vascular capacitance(Kandlikar & Fink, 2011). However the functional changes in CG neurons in hypertension remain unknown. My study focuses on the electrophysiological changes in CG neurons in DOCA-salt hypertensive rats.

Characteristics of the DOCA-salt hypertensive rat

DOCA-salt hypertensive rats exhibit the characteristic changes in a hypertension animal model. Several changes related to this study are introduced below:

High salt intake: The DOCA-salt hypertensive rat is a salt related hypertensive animal model. Salt intake is related to hypertension, even though the underlying mechanism is not clear (O'Donaughy & Brooks, 2006). High salt intake is a common problem worldwide. The average salt intake around the world is higher than 6 g per day, and it is higher than 12 g per day in Eastern Europe and Asia. However, the American Heart Association recommends a salt intake of 1.5 g per day (Appel *et al.*, 2011). High salt intake is associated with hypertension as shown by a meta-analysis of studies published from 1966-2008 (Strazzullo *et al.*, 2009). Guyton sets a model to explain the relationship between renal sodium excretion and BP (the pressure–natriuresis relationship)(Guyton, 1991). Sodium retention increases blood volume, which elevates BP via increased cardiac output. Eventually, increased tissue perfusion leads to elevated peripheral resistance, which normalizes the increased cardiac output. One study suggested that high salt intake may be related to increased sympathetic activity (Toal & Leenen, 1987).

Low renin: Na^+ intake is inversely related to renin secretion. Increased Na^+ absorption leads to low renin levels in DOCA-salt hypertensive rats. This phenomenon differentiates this animal model from other models with increased renin such as the renovascular hypertensive model. Renin is secreted from the juxtaglomerular cells of the kidney, and it converts angiotensinogen to angiotensin I, which is further converted to angiotensin II in the lung. Angiotensin II is a potent vasoconstrictor; therefore, angiotensin II is not the etiological factor for DOCA-salt hypertensive rats. The hypertension treatments are guided by the plasma renin activity of hypertensive patients. The patients with low plasma renin activity are treated with natriuretic volume mediated therapy instead of antirenin system therapy (Olson *et al.*, 2012).

Endothelin-1: Endothelin-1(ET-1) is released from endothelial cells as a potent vasoconstrictor. Enhanced ET-1 activity is thought to be involved in the pathogenesis of the DOCA-salt hypertensive rat (Schiffrin, 2001). Sustained increased BP is obtained by chronically infusing ET-1 in the normotensive rat (Mortensen *et al.*, 1990). More ET-1 is found in the aorta and superior mesenteric arteries from DOCA-salt hypertensive rats (Wang *et al.*, 2005a). Increased venous tone plays an important role in the development of hypertension in this animal model (Fink *et al.*, 2000). The ET-1 content is shown to be higher in veins than in arteries (Wang *et al.*, 2005a), and the venoconstriction of ET-1 is mediated by superoxide, which is one type of reactive oxygen species (ROS) (Li *et al.*, 2003). Other than its vasoconstriction effects, ET-1 also affects the sympathetic nervous system. Intracerebroventricular injection of ET-1 causes increased BP, which is abolished by blocking sympathetic ganglion (Matsumura *et al.*, 1991). ET-1 is also related to elevated ROS levels in sympathetic ganglia through its up-regulated ETB

receptor in this hypertensive rat (Dai *et al.*, 2004b). However, the mechanism by which ET-1 affects neuronal functions in the sympathetic nervous system remains unknown.

Reactive Oxygen Species: Increased levels of ROS are involved in the pathophysiology of hypertension. They contribute to a wide range of dysfunctions in hypertension such as changes in endothelial cells, vascular cells, the renin-angiotensin system, the kidney, and the central and peripheral sympathetic nervous systems (Rodrigo *et al.*, 2011). ROS levels are also found to be elevated in hypertensive patients (Sagar *et al.*, 1992). Antioxidative therapies are effective in controlling BP in hypertensive patients (Dovinova *et al.*, 2009). Interestingly, increased ROS is found to be related to elevated sympathetic activity (Koepke *et al.*, 1987) and high salt intake (Koga *et al.*, 2008) in hypertensive animal models. Our lab reports that ROS are elevated in sympathetic ganglia from DOCA-salt hypertensive rats (Dai *et al.*, 2004b). One of the objectives of this study is to explore if the elevated ROS levels are associated with altered sympathetic ganglionic neuronal activities in this animal model.

Sympathetic nervous system: Sympathetic activity is elevated in DOCA-salt hypertensive rats (this will be discussed in more detail below). The cardiovascular system is innervated by SNS, which regulates the heartbeat and vasoconstriction in a moment-to-moment manner at rest and in active states. Sympathetic activity is elevated in hypertensive patients (Schlaich *et al.*, 2004). Blocking SNS reduces the BP to within the normal range in some hypertensive patients (Chou *et al.*, 2005).

In summary, many factors contributing to essential hypertension are exhibited in DOCA-salt hypertensive rats. My study further clarifies the roles of SNS in this animal model.

Sympathetic nervous system and hypertension

The autonomic nervous system, which is part of the peripheral nervous system that controls visceral functions below the level of consciousness, consists of the sympathetic nervous system (SNS), the parasympathetic nervous system and the enteric nervous system. The SNS is often referred to as the “fight or flight” component of the autonomic nervous system. The physiological effects of sympathetic activation are balanced with actions of the parasympathetic nervous system, which is responsible for “rest and digest”. The SNS is involved in both short-term and long-term BP regulation by controlling cardiovascular and kidney functions.

SNS and BP regulation

BP varies with cardiac output and peripheral resistance. Elevated sympathetic activity augments cardiac output through increased heart rate and stroke volume, and it would also increases peripheral resistance by enhancing arterial constriction. In addition to influencing the heart, arterial circulation and blood viscosity, the SNS also densely innervates the veins (Li *et al.*, 2010). The elevated sympathetic activity augments venous constriction, which moves more blood to the heart and arterial circulation. Blood redistribution generated by venous constriction is related to hypertension development (Fink, 2009). Venous constriction is considered to be an important contributor in long-term BP regulation, and the SNS plays an important role in the neural regulation of BP. First, blood vessels are constantly activated by the SNS and remain under some degree of constant constriction (Malpas, 2010). By rapidly regulating the level of SNS activity, the degree of vasoconstriction in blood vessels is altered. Therefore, the SNS is

necessary to maintain normal vascular function, not just under extreme conditions such as disease status. Second, the SNS precisely regulates cardiac output by increasing the cardiac rate, the cardiac electric impulse conduction rate and cardiac contractility. Third, the SNS also extensively controls the kidney, which is an important organ that controls BP by regulating extracellular fluid volume, the renin-angiotensin aldosterone system and its vascular constriction (Navar, 2005). Many negative-feedback reflexes, such as the baroreflex, are activated to bring the sympathetic activity back within normal limits to maintain the BP; however, the elevated SNS activity contributes to the initiation and maintenance of hypertension.

Role of SNS in hypertension

The probability of developing hypertension is increased by chronically elevating the sympathetic activity level in both animals and humans (Joyner *et al.*, 2008). Recent research indicates that the SNS is involved in both short-term and long-term BP regulation, the latter of which is more related to hypertension. Some factors make it difficult to measure sympathetic activity in hypertension. First, the baseline sympathetic activity varies in different individuals. The range of muscle sympathetic nerve activities exhibited in hypertensive patients overlaps with the range exhibited in normotensive patients (Joyner *et al.*, 2008). Second, many counter-effective factors, such as vasodilating factors, overshadow the effects of SNS in hypertension development. However, extensive research findings demonstrate the important role of the SNS in hypertension development. For example, some hypertensive patients exhibit elevated SNS activity (Fink, 2009). High sympathetic activity is related to aging, sedentary lifestyle and oxidative stress, all of which are important factors that contribute to the

development of hypertension (Bell *et al.*, 2001;Koepke *et al.*, 1987). Other than changing cardiovascular function, the chronic elevation of sympathetic activity in hypertension also causes many other pathological changes such as vascular inflammation (Fisher & Paton, 2011), which is a long-term pathological change related to hypertension.

Structure of the SNS

The neurons of the SNS include the cardiovascular center of the brainstem, sympathetic preganglionic neurons in the intermediolateral column of the thoracic and lumbar spinal cord, and the sympathetic postganglionic neurons in the sympathetic ganglia. The neurons in the brain stem send signals to sympathetic preganglionic neurons. Then, the sympathetic postganglionic neurons integrate and modify the signals sent from the sympathetic preganglionic neurons. Acetylcholine is the primary neurotransmitter released from preganglionic neurons to postganglionic neurons. Finally, the sympathetic postganglionic nerves innervate their target organs by releasing neurotransmitters such as norepinephrine (NE), ATP and neuropeptides (Gibbins, 1995). The elevated sympathetic activity in hypertension may be caused by dysfunctions of any part of the SNS including the central and peripheral SNS. Different parts of the SNS are introduced below.

Sympathetic neurons in the CNS and hypertension

Brain sympathetic neurons are located primarily in the rostral ventrolateral medulla (RVLM), the hypothalamus and the nucleus of the solitary tract (NTS) in the brainstem. In addition, cortical, limbic, and midbrain regions communicate with the brainstem and play a role in modulating sympathetic activity (Gray *et al.*, 2009;Madden

& Sved, 2003). The brain neurons also receive afferent signals from baroreceptors, chemoreceptors and renal afferent nerves to maintain a stable BP. The central SNS is involved in long-term BP regulation even though its mechanism is not fully understood (Guyenet, 2006). Baseline sympathetic activity is controlled by the neurons from the RVLM, the hypothalamus and the NTS (Dampney, 1994). Depletion of most C1 neurons in the RVLM lowers the baseline arterial pressure. Although it is not clear if the brain encodes a BP set point, the C1 neurons in the RVLM show spontaneous firing, which may be responsible for the continuous firing in sympathetic efferent nerves. The NTS is the integrative center for baroreflex, whose reset is involved in BP regulation. The NTS also contains neurons expressing a high concentration of mineralocorticoid receptors (Geerling *et al.*, 2006), which may be involved in the generation of DOCA-salt hypertensive rats. It is well accepted that the sympathetic neuronal activity in the CNS is enhanced in hypertension. The spontaneous firing rate of neurons in the hypothalamus is higher in spontaneously hypertensive rats compared to matched normotensive rats (Shonis & Waldrop, 1993). The activity of neurons in the RVLM is increased in several hypertension animal models (Sved *et al.*, 2003). Increased norepinephrine release from subcortical brain regions is found in patients with essential hypertension (Ferrier *et al.*, 1993). In DOCA-salt hypertensive rats, the cholinergic activity of RVLM neurons is enhanced (Kubo *et al.*, 1996) and the firing frequency of the sympathetic preganglionic nerve is elevated (Iriuchijima *et al.*, 1975). The high osmolality in the brain may cause high central sympathetic activity in this animal model. The BP is lowered when central osmolality is decreased by bilateral intracarotid infusion of hypotonic fluid (O'Donaughy *et al.*, 2006).

Sympathetic nerve activity and hypertension

Sympathetic activity (or the activity of the SNS) is reflected as sympathetic nerve activity (SNA), which is the activity of the postganglionic sympathetic nerve. The postganglionic sympathetic nerve, also called the sympathetic nerve, is the final output of the SNS, and it passes signals and releases neurotransmitters to target organs directly. Each sympathetic nerve is composed of thousands of axons emanating from postganglionic neurons. The total activity of all the axons in the sympathetic nerve can be recorded *in vivo* as multi-unit bursts via implanted electrodes in muscle and skin nerve fascicles from animals or human subjects (Mano *et al.*, 2006). Single axon activities are also detectable using a single unit approach (Macefield *et al.*, 2002). Increasing clinical evidence indicates that hypertension is associated with increased SNA, and elevated SNA is found in hypertensive patients (Anderson *et al.*, 1989;Schlaich *et al.*, 2004). Denervation of renal sympathetic nerves attenuate the increased BP response to DOCA in DOCA-hypertensive rats (Jacob *et al.*, 2005). The elevated renal sympathetic nerve activity is alleviated by TEMPOL, a superoxide scavenger in DOCA-salt hypertensive rats (Xu *et al.*, 2004). In addition, the activity of the sympathetic nerves innervating the heart is elevated in this animal model (Lee *et al.*, 2012). According to the organization of the SNS, the SNA may be modulated at the central, ganglionic or postganglionic nerve levels. These findings demonstrate that the central SNS activity and postganglionic nerve activity are elevated in hypertension. It is unclear how postganglionic neurons located in sympathetic ganglia modify the increased tonic central drive and if postganglionic neurons contribute or dampen the elevated SNS in hypertensive animal model. In this study, we will focus on determining

this mechanism.

Sympathetic ganglia and hypertension

The structure of sympathetic ganglia

Sympathetic ganglia are composed of paravertebral and prevertebral sympathetic ganglia. Paravertebral sympathetic ganglia lie bilaterally along the vertebral column from C1 to S2, and the neurons in these ganglia mainly innervate the body wall and extremities (Kreulen, 2005). Prevertebral sympathetic ganglia lie along the ventral part of the aorta, which include a celiac ganglion, a superior mesenteric ganglion, and an inferior mesenteric ganglion. They control sympathetic outflow to the vasculature, gastrointestinal tract, and other organs in abdominal and pelvic cavities (Szurszewski, 1981;Kreulen, 2005). Compared to paravertebral sympathetic ganglia, prevertebral sympathetic ganglia act as relatively sophisticated integrative centers. Prevertebral sympathetic neurons receive synaptic input from both peripheral sensory nerves and central preganglionic nerves, whereas paravertebral sympathetic neurons receive input simply from central preganglionic nerves (Crowcroft & Szurszewski, 1971;McLachlan & Meckler, 1989). This study focuses on the celiac ganglion (CG), the largest prevertebral sympathetic ganglion that surrounds the celiac artery. It is composed of two large irregularly shaped masses joined by a group of fibers or by a small mass of ganglionic tissues. The CG receives stimulation from the greater and lesser splanchnic nerves, the inferior celiac nerve and the superior celiac nerve and sends out signals to many secondary plexuses to control abdominal visceral organs(Kreulen & Szurszewski, 1979).

Signal transmission in sympathetic ganglia

As a neurotransmission site from the central to peripheral nervous system, sympathetic ganglia are not simple relay centers. The signals from the CNS are processed and integrated in sympathetic ganglia, and postganglionic neurons in sympathetic ganglia are involved in generating the bursts firing pattern similar to the postganglionic sympathetic nerves (McAllen & Malpas, 1997), which is different from continuous firing witnessed in preganglionic nerves.

Sympathetic postganglionic neurons in sympathetic ganglia receive synaptic inputs from sympathetic preganglionic nerves and innervate target organs through sympathetic postganglionic nerves, which are also called sympathetic nerves. Acetylcholine released from preganglionic nerves acts at nicotinic receptors on postganglionic neurons to generate fast synaptic currents. Other co-transmitters such as neuropeptides and nitric oxide are also released from preganglionic neurons to modulate the excitability of postganglionic neurons (Gibbins, 1995). This signal transmission in sympathetic ganglia is not just a simple one-to-one relay. For example, one postganglionic neuron accepts the summation of inputs from many preganglionic nerves, and one neuron in prevertebral sympathetic ganglia could also receive inputs from neurons from the enteric plexus (Kreulen & Szurszewski, 1979). The postganglionic neuron integrates and modulates the input signals and sends its electrical firing outputs through sympathetic postganglionic nerves to target organs. Postganglionic neurons receive both strong and weak inputs. One strong input is usually suprathreshold and can generate an AP in the postganglionic neuron; however, the functions of weak inputs remain unclear (McLachlan, 2003). Moreover,

postganglionic neurons are not identical in anatomy and can be identified by expressing different functional molecules such as tyrosine hydroxylase, neuropeptide Y, and vasoactive intestinal peptide. Preganglionic neurons with the same functional molecules tend to cluster in the sympathetic ganglia (Murphy *et al.*, 1998), which indicates that the signal transmission in sympathetic ganglia may have different function-specific pathways.

Neurotransmission of sympathetic ganglia in hypertension

Signal transmission functions of sympathetic ganglionic neurons are tested *in vitro* in hypertension animal models. The sympathetic ganglion is dissected out to eliminate the effects from central sympathetic neurons. Neurotransmission is studied by applying electric stimulation on the preganglionic nerve. The ganglion tested is the superior cervical ganglia (SCG), which is a paravertebral sympathetic ganglion in the cervical level and is associated with cerebral circulation. A compound action potential (CAP) is recorded in the postganglionic nerve in response to artificial supramaximal preganglionic nerve stimulation. The amplitudes of CAPs are significantly higher in SCG from ouabain-dependent hypertensive rats compared to corresponding normotensive rats (Aileru *et al.*, 2001). This elevated CAP reflects altered membrane electrical activity and/or increased transmitter release from preganglionic nerve terminals in the SCG. Further studies were performed on the SCG in spontaneous hypertensive rats (SHR). The excitatory postsynaptic currents (EPSCs) were recorded in postganglionic neurons in response to artificial preganglionic nerve stimulation. EPSCs are significantly higher in the SCG from SHR rats compared to corresponding normotensive rats (Magee & Schofield, 1994). These data indicate that the neurotransmitter release from

preganglionic nerves may be enhanced in the SCG from SHR rats. To test the membrane firing properties of the SCG neuron, an artificial suprathrethold current was injected into the cultured SCG neuron. The neuronal firing patterns, in response to the current injection, are different between neurons from SHR rats and neurons from corresponding normotensive rats (Yarowsky & Weinreich, 1985). In DOCA-salt hypertensive rats, sympathetic preganglionic nerve activity is elevated (Iriuchijima *et al.*, 1975); however, changes in the neurotransmission of sympathetic ganglion in this animal model remain unclear. This study focuses on the membrane firing properties of CG neurons in DOCA-salt hypertensive rats and their corresponding control rats.

Neuronal membrane firing properties of sympathetic postganglionic neurons

Many studies have been conducted on membrane firing properties of sympathetic postganglionic neurons using the technique of injecting a sustained suprathreshold depolarizing current to dissociated neurons *in vitro*. Two basic electrophysiological types, called phasic neurons and tonic neurons, were identified. In response to the sustained suprathreshold depolarizing currents, phasic neurons exhibit a phasic (rapidly adapting) firing pattern, while tonic neurons display tonic (slowly adapting) firing patterns (Cassell *et al.*, 1986;Wang & McKinnon, 1995). Phasic neurons fire only several APs in the beginning of suprathreshold current injection, and tonic neurons fire continuously during the current injection. The distribution of phasic and tonic neurons varies between different sympathetic ganglia (Cassell *et al.*, 1986). In the rat CG, 42% of neurons are phasic and 58% are tonic, whereas all the neurons in the rat SCG are phasic (Wang & McKinnon, 1995). No correlation exists between the

tonic/phasic firing patterns regarding the locations of neurons in the CG (Kreulen & Szurszewski, 1979).

The functional roles of different firing patterns remain unclear; however they may be related to the differences between sympathetic prevertebral and paravertebral ganglia because the different distributions of firing patterns are exhibited between CG and SCG neurons. Additionally, the phasic and tonic firing properties are thought to be permanent properties for specific neurons. Phasic neurons are referred to as vascular neurons (Cassell *et al.*, 1986); however, more research showed that the firing patterns may be modified by chemicals or physiological conditions. Nerve growth factor is a neurotrophin and it changes the firing patterns of sympathetic neurons (Luther & Birren, 2009). In the hypertensive animal, the distribution is also modulated in sympathetic paravertebral ganglionic neurons. More tonic neurons are present in the SCG in spontaneously hypertensive rats in response to a sustained depolarizing current compared to normotensive controls (Jubelin & Kannan, 1990). Therefore, it is accepted that neuronal firing patterns are dynamic and not a permanent property of the sympathetic ganglionic neuron.

In a sympathetic ganglion, the postganglionic neurons are surrounded by many satellite cells and receive signals from preganglionic nerves and other afferent nerves, which make it difficult to study the membrane firing properties of postganglionic neurons in an intact sympathetic ganglion. Postganglionic neurons can be separated from other parts of the SNS by enzymatic dissociation, which was used in this study. APs were generated by injecting an artificial suprathreshold current in these neurons using the patch-clamp technique. This technique allows us to explore how the postganglionic

neurons process electrical input information. The artificial injected current does not reflect the actual current changes by preganglionic neurotransmitter releases in a postganglionic neuron, which is a limit of this technique. It is necessary to combine the *in vitro* data with other *in vivo* data to clarify the roles of postganglionic neurons in hypertension.

Celiac ganglion and hypertension

The SCG has been studied extensively in hypertension primarily because the neurons are more easily dissected and more viable for cell culture compared to other sympathetic ganglia. However, fewer studies have been conducted on the prevertebral sympathetic ganglia. The CG is the largest prevertebral sympathetic ganglion, and it controls splanchnic circulation and other functions of abdominal visceral organs. Splanchnic circulation transports blood to and from abdominal organs, and 30% of total body blood volume is contributed by splanchnic circulation. Changes in the levels of venous constriction of splanchnic circulation lead to blood redistribution from venous to arterial circulation (King *et al.*, 2007). Peripheral resistance is also increased by arterial contraction in splanchnic circulation, and this increased peripheral vascular resistance in the artery and redistribution of circulating blood will contribute to elevated BP. Furthermore, decreased splanchnic vascular resistance lowers the BP of hypertensive rats (Mishra *et al.*, 2010), and surgical removal of CG significantly lowers the high BP in the sarafotoxin 6c-induced hypertensive rats (Li *et al.*, 2008).

It has also been shown that the reactive oxygen species (ROS) level is elevated in sympathetic ganglia of sarafotoxin 6c-induced hypertensive rats. Our lab also found that the ROS level is elevated in sympathetic ganglia from DOCA-salt hypertensive rats

(Dai *et al.*, 2004b). The activity of NADPH (nicotinamide adenine dinucleotide phosphate) oxidase, a ROS-producing enzyme, is significantly elevated in the CG from DOCA-salt hypertensive rats (Cao *et al.*, 2007). This study overcame the technical difficulties involved in the dissection and dissociation of those ganglia. The electrophysiological activities of a single CG neuron were explored to further elucidate the role of sympathetic ganglia in hypertension.

Overall, the CG is an important hypertension contributing sympathetic ganglion that may be modulated in DOCA-salt hypertensive rats. Its neuronal membrane properties were tested in this study, and its elevated ROS level is one factor that is involved in this firing property change. The next two sections will review ion channels and ROS.

Ion channels and hypertension

Ion channels and neuronal firing

Ion channels are the fundamental elements involved in the neuronal membrane firing properties. Every action potential (AP) is generated by the precise opening or closing of different ion channels. The AP generated in a CG neuron is composed of a depolarization phase, a repolarization phase and an afterhyperpolarization (AHP) phase (Cassell *et al.*, 1986). Each phase of an AP, the firing pattern and the firing frequency are determined by the arrangement and the status of different ion channels. Voltage-gated Na^+, Ca^{2+}, K^+ and Cl^- channels are present in sympathetic postganglionic neurons. The presence and function of K^+ and Ca^{2+} channels determine the firing patterns of sympathetic neurons in response to depolarizing current injection. Specific

K^+ channel blockers decrease AP repolarization rate and afterhyperpolarization amplitude in SCG neurons (Marsh & Brown, 1991). Ca^{2+} channels along with Ca^{2+} activated K^+ channels and ryanodine receptors also involve in modulating AP repolarization and afterhyperpolarization in sympathetic ganglionic neurons (Akita & Kuba, 2000). In this study, K^+ and Ca^{2+} channel currents were examined in CG neurons from normotensive and hypertensive rats. Specific K^+ channel blockers were also applied to CG neurons to clarify the roles of specific K^+ channel currents in changing neuronal firing patterns and firing frequencies.

K^+ and Ca^{2+} channel currents in CG neurons

Four types of K^+ channel currents including delayed rectifier K^+ channel current (I_{Kv}), calcium-activated K^+ channel current (I_{KCa}), M-type K^+ channel current (I_M) and A-type K^+ channel current (I_A) have been found in CG neurons, (Carrier, 1995). I_{KCa} consists of two types of K^+ channel currents. One current is I_{BK} (large-conductance calcium-activated K^+ channel current, which has a large conductance that is both calcium and voltage-dependent. The other current is I_{SK} (small-conductance calcium-activated K^+ channel), which has a small conductance that is calcium dependent but voltage insensitive. I_{Kv} repolarizes the membrane following an action potential. Other than repolarization, I_{KCa} and I_A also hyperpolarize the membrane potential, which

creates a time interval between subsequent APs. Increased time interval is what decreases frequency (Hille, 2001). Calcium-activated K^+ channel is closely regulated by Ca^{2+} through voltage-gated Ca^{2+} channels (Akita & Kuba, 2000). During an AP, Ca^{2+} flows into neurons via open voltage-gated Ca^{2+} channels. This Ca^{2+} influx triggers a further release of Ca^{2+} from intracellular depots. Elevated intracellular Ca^{2+} activates calcium-activated K^+ channels, which repolarize and/or hyperpolarize the membrane to inactivate voltage-gated Ca^{2+} channels and prevent further calcium influx. *I_M is a* muscarinic agonist-sensitive persistent outward K^+ current, which decreases neuronal excitability. Blockage of I_M prevents angiotensin Ⅱ induced depolarization in CG neurons. Overall, K^+ and Ca^{2+}channels play a very important role in CG neuronal firing, which modulates SNS activity and BP.

K^+ channels and neuronal firing properties

K^+ channel activation leads to outward K^+ current, which dampens neuronal excitability. K^+ channel activity is involved in AP initiation, AP duration and the period after AP firing. Each neuron exhibits its specific firing pattern. I_A is found to maintain repetitive firing in *Drosophila* neurons (Ping *et al.*, 2011). The relationship between specific K^+ channel current and neuronal firing has been studied in sympathetic ganglionic neurons. In SCG, both I_{Kv} and I_{KCa} are significantly smaller in phasic

neurons than in tonic neurons (Luther & Birren, 2009). Expression of Kv2 encoded delayed rectifier K^+ channel increases the percentage of tonic neurons in SCG (Malin & Nerbonne, 2002). The amplitude of I_M in phasic neurons is significantly larger than that in tonic neurons from sympathetic ganglionic neurons (Cassell *et al.*, 1986;Luther & Birren, 2009;Cassell *et al.*, 1986). M-type K^+ channel agonist, bethanechol, makes phasic neurons fire tonicly (Cassell *et al.*, 1986). Compared to tonic neurons, phasic neurons exhibit smaller amplitude of I_A in guinea-pig sympathetic ganglia (Cassell *et al.*, 1986), but similar amplitude of I_A in rat sympathetic ganglia (Wang & McKinnon, 1995). Genetic elimination of I_A shifts phasic neurons to an adapting firing pattern in SCG neurons (Malin & Nerbonne, 2000). Adapting firing pattern is defined as firing repetitively under low amplitude current injection, but stopping firing when the amplitude of injected current is increased (Malin & Nerbonne, 2000). Overall, specific neuronal firing patterns are not likely to be caused by only a single specific K^+ channel. A variety of K^+ channels may be correlated to neuronal firing pattern changes.

The proportions of phasic and tonic neurons is different in sympathetic ganglia from control rats compared to ganglia from spontaneously hypertensive rats (Jubelin & Kannan, 1990;Yarowsky & Weinreich, 1985). The proportion of phasic and tonic neurons has not been examined in sympathetic ganglia from DOCA-salt hypertensive rats. K^+ channel currents have only been sparsely researched in sympathetic ganglia of hypertensive animals. I_A is reported to be elevated in sympathetic neurons from SHR

rats (Robertson & Schofield, 1999). More K^+ channel currents may also be modified to alter the neuronal firing in sympathetic ganglia from hypertensive rats. I_{Kv} I_{KCa} I_M and I_A and their relation to neuronal firing were explored in this study in CG neurons from DOCA-salt hypertensive rats and control rats.

Reactive Oxygen Species (ROS) and hypertension

Introduction

Reactive oxygen species (ROS) are highly reactive, chemically unstable derivatives of O_2 metabolism, and they include superoxide anion ($O_2^{\bullet-}$), hydrogen peroxide (H_2O_2) and hydroxyl radical (OH•). ROS molecules are generated in all aerobic cells including neurons. $O_2^{\bullet-}$ is generated by nicotinamide adenine dinucleotide phosphate (NADPH) oxidase, xanthine oxidase, NO synthase, mitochondrial respiration and Ca^{2+} (dam-Vizi & Starkov, 2010). H_2O_2 is formed from $O_2^{\bullet-}$ by superoxide dismutase (SOD) (Halliwell, 1992). On contact with ferrous-containing low- molecular-weight proteins or with free cytoplasmic Fe^{2+}, H_2O_2 is a source of OH•, which is one of the most potent of oxidants (Stadtman & Levine, 2000). ROS level is maintained by balance between the formation of ROS and antioxidant defense system, which includes a number of antioxidant enzymes. Catalase, glutathione peroxidase and SOD are three main antioxidant enzymes. As mentioned before, SOD decrease $O_2^{\bullet-}$ level by converting $O_2^{\bullet-}$ to H_2O_2, but it cannot scavenger H_2O_2 which plays an important role in

hypertension (Thakali *et al.*, 2006). Catalase and glutathione peroxidase lower ROS level by changing H_2O_2 to water and molecular oxygen. Overproduction of ROS occurs in the pathogenesis of many neuronal diseases such as Parkinson's disease (Barkats *et al.*, 2006), Alzheimer's dementia (Behl *et al.*, 1994) and multiple sclerosis (Ferretti *et al.*, 2006). Pathological substances such as β-Amyloid peptide in Alzheimer's disease increase central ROS production (Abramov *et al.*, 2004;Sheehan *et al.*, 1997).

Elevated ROS in hypertension

Hypertension is characterized with elevated ROS level. $O_2^{\bullet-}$ and H_2O_2 are elevated in tissues and blood of DOCA-salt hypertensive rats (Somers *et al.*, 2000), spontaneous hypertensive rats (Kerr *et al.*, 1999) and essential hypertensive patients (Sagar *et al.*, 1992). Prolonged antioxidant administration alleviates $O_2^{\bullet-}$ accumulation and decreases systolic BP in DOCA-salt hypertensive rats (Beswick *et al.*, 2001). The pathologies associated with vascular functional and structural changes are related to elevated ROS in hypertension (Briones & Touyz, 2010). Long-term increased H_2O_2 is critical to the development of vascular smooth muscle hypertrophy (Zafari *et al.*, 1998). H_2O_2 causes the aorta to contract more in DOCA-salt hypertensive rats compared to control normotensive rats (Thakali *et al.*, 2006). In addition to elevated ROS in the cardiovascular system, increased levels of ROS are also found in the sympathetic nervous system and are related to BP regulation. Inhibition of ROS generation in cardiovascular centers of the brain prevent epoxide hydrolase induced BP elevation (Sellers *et al.*, 2005). Epoxide hydrolase converts epoxyeicosatrienoic acids to their corresponding diol, and epoxyeicosatrienoic acid is an endogenous antihypertensive

molecule. Epoxide hydrolase is up-regulated in cardiovascular centers of spontaneous hypertensive rats (Sellers *et al.*, 2005). In addition to the cardiovascular center in the brain, $O_2^{\bullet-}$ levels are increased in CG from DOCA-salt hypertensive rats due to the activation of NADPH oxidase (Dai *et al.*, 2006) as previously discussed. However the physiological or pathophysiological effects of ROS on sympathetic ganglia in hypertension are unknown.

Oxidation function of ROS on regulation of ion channels

As free radicals, the main function of ROS is oxidation. All macromolecules including nucleic acids, proteins and lipids are susceptible to ROS oxidation. Excessive ROS are destined to damage cells. For example the ROS released from phagocyte are used to destroy virus infected cells. However, moderate level of ROS is well accepted to be second messenger in many cellular processes. It is not clear how ROS mediated oxidation regulates cellular activities. Selective and reversible oxidation of key residues in protein is most likely the underline mechanism, and it can involve hydroxylation of aromatic groups and aliphatic amino acid side chains, nitration of aromatic amino acid residues, nitrosylation of sulfhydryl groups, sulfoxidation of methionine residues, chlorination of aromatic groups and primary amino groups, and the conversion of some amino acid residues to carbonyl derivatives (Stadtman & Levine, 2000). Amino acid residues in ion channel proteins are highly susceptible to oxidation by ROS (Stadtman & Levine, 2000). Oxidation of K^+ channels in the brain is associated with aging and neurodegeneration (Sesti *et al.*, 2010). In addition to direct oxidation of ion channels, ROS can also oxidize cell signaling molecules, which regulate ion channel functions. In the mitogen-activated protein kinase (MAPK) cascade, ROS activate apoptosis signal-

regulated kinase 1, cGMP-dependent protein kinase, and protein kinase A as well as inhibits MAPK phosphatase (Ray *et al.*, 2012). In phosphoinositide-3 kinase (PI3Ks) pathway, the phosphatase and tensin homology phosphatase is subject to ROS oxidation (Kwon *et al.*, 2004). A-type K^+ channel is down-regulated by activation of protein kinase A in MAPK cascade (Hoffman & Johnston, 1998). Delay rectifier K^+ channel and Ca^{2+} channel are modulated by inhibitors of PI3Ks (Lu *et al.*, 2012). Therefore the elevated ROS in disease status such as hypertension may modulate functions of ion channels in CG neurons by oxidizing ion channels proteins and/or enzymes in the signaling pathways.

ROS and neuronal firing properties in hypertension

Neuronal firing properties are potential targets for ROS in hypertension even though the underlying mechanisms are unclear. Epoxide hydrolase is involved in the central control of BP, and the increased neuronal firing rate that is induced by an inhibitor of epoxide hydrolase is completely abolished by inhibition of ROS generation in cardiovascular central neurons (Sellers *et al.*, 2005). The effects of ROS on the firing properties of sympathetic ganglionic neurons remain unknown; however, some research shows that ROS change neuronal activity by modifying ion channels. The interstitial cells of Cajal are intestinal cells in the gastrointestinal tract that act as pacemakers for the basal electrical rhythm of intestinal peristalsis. The pacemaker activity of interstitial cells of Cajal is modulated by H_2O_2 (Choi *et al.*, 2008). H_2O_2 also enhances Ca^{2+} channels expressed in *Xenopus*oocytes(Li *et al.*, 1998). The ROS scavenger TEMPOL decreases Na^+ channel currents in dorsal root ganglion neurons (Wang *et al.*, 2011).

K^+ channel currents are also targets for ROS. H_2O_2 modulates voltage-gated K^+ currents in cultured hippocampal neurons (Muller & Bittner, 2002). It also changes K(ATP) channel openings, which alters neuronal excitability in midbrain dopamine neurons (Avshalumov *et al.*, 2005). All these findings indicate that H_2O_2 may change the neuronal firing properties of sympathetic ganglionic neurons. In this study, I explore the effects of H_2O_2 on firing properties of dissociated CG neurons as a means to evaluate the possible impact of elevated ROS in sympathetic ganglia from hypertensive rats.

6. Summary:

Increased SNS activity is an important factor for hypertension pathogenesis. However, the roles of SNS in hypertension are not fully understood. Sympathetic ganglia receive inputs from sympathetic preganglionic nerves and send signals to target organs through sympathetic postganglionic nerves. It has been shown that both preganglionic nerve activity and postganglionic nerve firing rate are elevated in hypertension. However, the functional role of sympathetic ganglia is unclear. The celiac ganglion (CG) is the largest prevertebral sympathetic ganglion that controls the splanchnic circulation, which contributes to 30% of total blood volume. This study focused on comparing the electrophysiological properties of CG neurons from hypertensive rats to those from matched normotensive rats. DOCA-salt hypertensive rat was selected as an appropriate animal model to explore the roles of the sympathetic ganglia in hypertension.

In response to stimuli from preganglionic nerves, sympathetic postganglionic neurons in sympathetic ganglia generate specific firing patterns of APs, which are propagated to postganglionic nerve terminals that control neurotransmitter release to target organs. The neuronal firing properties of postganglionic neurons are explored *in vitro* by injecting a sustained suprathreshold depolarizing current. Using this technique, the distribution of different firing patterns has been found to be different in sympathetic ganglia from some hypertensive rats. The firing patterns are formed by precisely regulated openings and closings of different ion channels. The purpose of this study is to explore the neuronal firing properties of sympathetic postganglionic neurons including their firing patterns and firing related ion channel currents, specifically K^+ and Ca^{2+} currents, in CG neurons from DOCA-salt hypertensive rats and their matched normotensive rat.

Moderate level of ROS is involved in the regulations of cellular activities. Elevated ROS have been found in several tissues in hypertension, and ROS are elevated in sympathetic ganglia from DOCA-salt hypertensive rats. However, little is known about the function of ROS on sympathetic ganglia in hypertension. Neuronal firing and ion channels have been shown to be modulated by ROS in other types of neurons. This study plans to investigate the association between neuronal firing properties and H_2O_2, one type of ROS in CG neurons, which would aid in the elucidation of the pathological role of increased ROS in hypertension.

Sympathetic ganglion is not just a relay center in SNS. Its modification controls the final output of SNS, and it is shown to be modulated in hypertension. This study is valuable because it will help to clarify the changes and roles of sympathetic ganglionic

neurons in hypertension, and it is very helpful to further understand and cure hypertension.

BIBLIOGRAPHY

BIBLIOGRAPHY

Abramov AY, Canevari L, & Duchen MR (2004). Beta-amyloid peptides induce mitochondrial dysfunction and oxidative stress in astrocytes and death of neurons through activation of NADPH oxidase. *J Neurosci* **24**, 565-575.

Aileru AA, De Albuquerque A, Hamlyn JM, Manunta P, Shah JR, Hamilton MJ, & Weinreich D (2001). Synaptic plasticity in sympathetic ganglia from acquired and inherited forms of ouabain-dependent hypertension. *Am J Physiol Regul Integr Comp Physiol* **281**, R635-R644.

Akita T & Kuba K (2000). Functional triads consisting of ryanodine receptors, Ca(2+) channels, and Ca(2+)-activated K(+) channels in bullfrog sympathetic neurons. Plastic modulation of action potential. *J Gen Physiol* **116**, 697-720.

Anderson EA, Sinkey CA, Lawton WJ, & Mark AL (1989). Elevated sympathetic nerve activity in borderline hypertensive humans. Evidence from direct intraneural recordings. *Hypertension* **14**, 177-183.

Appel LJ, Frohlich ED, Hall JE, Pearson TA, Sacco RL, Seals DR, Sacks FM, Smith SC, Jr., Vafiadis DK, & Van Horn LV (2011). The importance of population-wide sodium reduction as a means to prevent cardiovascular disease and stroke: a call to action from the American Heart Association. *Circulation* **123**, 1138-1143.

Avshalumov MV, Chen BT, Koos T, Tepper JM, & Rice ME (2005). Endogenous hydrogen peroxide regulates the excitability of midbrain dopamine neurons via ATP-sensitive potassium channels. *J Neurosci* **25**, 4222-4231.

Badyal DK & Dadhich AP (2003). Animal models of hypertension and effect of drugs. *Indian J of Pharmacol* **35**, 349-362.

Barkats M, Horellou P, Colin P, Millecamps S, Faucon-Biguet N, & Mallet J (2006). 1-methyl-4-phenylpyridinium neurotoxicity is attenuated by adenoviral gene transfer of human Cu/Zn superoxide dismutase. *J Neurosci Res* **83**, 233-242.

Beevers G, Lip GY, & O'Brien E (2001). ABC of hypertension: The pathophysiology of hypertension. *Br Med J* **322**, 912-916.

Behl C, Davis JB, Lesley R, & Schubert D (1994). Hydrogen peroxide mediates amyloid beta protein toxicity. *CELL* **77**, 817-827.

Bell C, Seals DR, Monroe MB, Day DS, Shapiro LF, Johnson DG, & Jones PP (2001). Tonic sympathetic support of metabolic rate is attenuated with age, sedentary lifestyle, and female sex in healthy adults. *J Clin Endocrinol Metab* **86**, 4440-4444.

Beswick RA, Zhang H, Marable D, Catravas JD, Hill WD, & Webb RC (2001). Long-term antioxidant administration attenuates mineralocorticoid hypertension and renal inflammatory response. *Hypertension* **37**, 781-786.

Briones AM & Touyz RM (2010). Oxidative stress and hypertension: current concepts. *Curr Hypertens Rep* **12**, 135-142.

Cao X, Dai X, Parker LM, & Kreulen DL (2007). Differential regulation of NADPH oxidase in sympathetic and sensory ganglia in deoxycorticosterone acetate salt hypertension. *Hypertension* **50**, 663-671.

Carretero OA & Oparil S (2000). Essential hypertension. Part I: definition and etiology. *Circulation* **101**, 329-335.

Carrier GO (1995). Whole-cell and perforated patch recordings of four distinct K+ currents in acutely dispersed coeliac-superior mesenteric ganglia neurons of adult rats. *Brain Res* **701**, 1-12.

Cassell JF, Clark AL, & McLachlan EM (1986). Characteristics of phasic and tonic sympathetic ganglion cells of the guinea-pig. *J Physiol (Lond)* **372**, 457-483.

Chobanian AV, Bakris GL, Black HR, Cushman WC, Green LA, Izzo JL, Jr., Jones DW, Materson BJ, Oparil S, Wright JT, Jr., & Roccella EJ (2003). Seventh report of the Joint National Committee on Prevention, Detection, Evaluation, and Treatment of High Blood Pressure. *Hypertension* **42**, 1206-1252.

Choi S, Yeum CH, Kim YD, Park CG, Kim MY, Park JS, Jeong HS, Kim BJ, So I, Kim KW, & Jun JY (2008). Receptor Tyrosine and MAP Kinase are Involved in Effects of H(2)O(2) on Interstitial Cells of Cajal in Murine Intestine. *J Cell Mol Med*.

Chou SH, Kao EL, Lin CC, Chuang HY, & Huang MF (2005). Sympathetic hypertensive

syndrome: a possible surgically curable type of hypertension. *Hypertens Res* **28**, 409-414.

Crowcroft PJ & Szurszewski JH (1971). A study of the inferior mesenteric and pelvic ganglia of guinea pigs with intracellular electrodes. *J Physiol (Lond)* **219**, 421-441.

Dai X, Cao X, & Kreulen DL (2006). Superoxide anion is elevated in sympathetic neurons in DOCA-salt hypertension via activation of NADPH oxidase. *Am J Physiol Heart Circ Physiol* **290**, H1019-H1026.

Dai X, Fink GD, & Kreulen DL. Elevation of superoxide anions in sympathetic neurons in DOCA-salt hypertension via activation of NADPH oxidase. FASEB J . 2004a. Ref Type: Abstract

Dai X, Galligan JJ, Watts SW, Fink GD, & Kreulen DL (2004b). Increased O2*- production and upregulation of ETB receptors by sympathetic neurons in DOCA-salt hypertensive rats. *Hypertension* **43**, 1048-1054.

dam-Vizi V & Starkov AA (2010). Calcium and mitochondrial reactive oxygen species generation: how to read the facts. *J Alzheimers Dis* **20 Suppl 2**, S413-S426.

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

Dornas WC & Silva ME (2011). Animal models for the study of arterial hypertension. *J Biosci* **36**, 731-737.

Dovinova I, Gardlik R, Palffy R, Kristek F, Cacanyiova S, Vantova Z, & Paulikova H (2009). Modulation of antioxidative response in the therapy of hypertension and other cardiovascular diseases. *Neuro Endocrinol Lett* **30 Suppl 1**, 32-35.

Ferretti G, Bacchetti T, DiLudovico F, Viti B, Angeleri VA, Danni M, & Provinciali L (2006). Intracellular oxidative activity and respiratory burst of leukocytes isolated from multiple sclerosis patients. *Neurochem Int* **48**, 87-92.

Ferrier C, Jennings GL, Eisenhofer G, Lambert G, Cox HS, Kalff V, Kelly M, & Esler MD (1993). Evidence for increased noradrenaline release from subcortical brain regions in essential hypertension. *J Hypertens* **11**, 1217-1227.

Fink GD (2009). Sympathetic Activity, Vascular Capacitance, and Long-Term Regulation of Arterial Pressure. *Hypertension* **53**, 307-312.

Fink GD, Johnson RJ, & Galligan JJ (2000). Mechanisms of increased venous smooth muscle tone in desoxycorticosterone acetate-salt hypertension. *Hypertension* **35**, 464-469.

Fisher JP & Paton JF (2011). The sympathetic nervous system and blood pressure in humans: implications for hypertension. *J Hum Hypertens*.

Friedman SM & Tanaka M (1987). Increased sodium permeability and transport as primary events in the hypertensive response to deoxycorticosterone acetate (DOCA) in the rat. *J Hypertens* **5**, 341-345.

Geerling JC, Engeland WC, Kawata M, & Loewy AD (2006). Aldosterone target neurons in the nucleus tractus solitarius drive sodium appetite. *J Neurosci* **26**, 411-417.

Gibbins IL (1995). Chemical neuroanatomy of sympathetic ganglia. In *Autonomic Ganglia*, ed. McLachlan EM, pp. 73-121. Harwood Academic Publishers, Luxembourg.

Gray MA, Rylander K, Harrison NA, Wallin BG, & Critchley HD (2009). Following one's heart: cardiac rhythms gate central initiation of sympathetic reflexes. *J Neurosci* **29**, 1817-1825.

Guyenet PG (2006). The sympathetic control of blood pressure. *Nat Rev Neurosci* **7**, 335-346.

Guyton AC (1991). Blood pressure control--special role of the kidneys and body fluids. *Science* **252**, 1813-1816.

Halliwell B (1992). Reactive oxygen species and the central nervous system. *J Neurochem* **59**, 1609-1623.

Han Y, Fan ZD, Yuan N, Xie GQ, Gao J, De W, Gao XY, & Zhu GQ (2011). Superoxide anions in the paraventricular nucleus mediate the enhanced cardiac sympathetic afferent reflex and sympathetic activity in renovascular hypertensive rats. *J Appl Physiol* **110**, 646-652.

Hille B (2001). Potassium Channels and Chloride Channels. In *Ion Channels of Excitable Membranes* pp. 131-168. Sinauer Associates, Massachusetts.

Hoffman DA & Johnston D (1998). Downregulation of transient K+ channels in dendrites of hippocampal CA1 pyramidal neurons by activation of PKA and PKC. *J Neurosci* **18**, 3521-3528.

Iriuchijima J, Mizogami S, & Sokabe H (1975). Sympathetic nervous activity in renal and DOC hypertensive rats. *Jpn Heart J* **16**, 36-43.

Jacob F, Clark LA, Guzman PA, & Osborn JW (2005). Role of renal nerves in development of hypertension in DOCA-salt model in rats: a telemetric approach. *Am J Physiol Heart Circ Physiol* **289**, H1519-H1529.

Joyner MJ, Charkoudian N, & Wallin BG (2008). A sympathetic view of the sympathetic nervous system and human blood pressure regulation. *Exp Physiol* **93**, 715-724.

Jubelin BC & Kannan MS (1990). Neurons from neonatal hypertensive rats exhibit abnormal membrane properties in vitro. *Am J Physiol* **259**, C389-C396.

Kandlikar SS & Fink GD (2011). Splanchnic sympathetic nerves in the development of mild DOCA-salt hypertension. *Am J Physiol Heart Circ Physiol* **301**, H1965-H1973.

Kerr S, Brosnan MJ, McIntyre M, Reid JL, Dominiczak AF, & Hamilton CA (1999). Superoxide anion production is increased in a model of genetic hypertension: role of the endothelium. *Hypertension* **33**, 1353-1358.

King AJ, Osborn JW, & Fink GD (2007). Splanchnic circulation is a critical neural target in angiotensin II salt hypertension in rats. *Hypertension* **50**, 547-556.

Koepke JP, Jones S, & DiBona GF (1987). Alpha 2-adrenoceptors in amygdala control renal sympathetic nerve activity and renal function in conscious spontaneously hypertensive rats. *Brain Res* **404**, 80-88.

Koga Y, Hirooka Y, Araki S, Nozoe M, Kishi T, & Sunagawa K (2008). High salt intake enhances blood pressure increase during development of hypertension via oxidative stress in rostral ventrolateral medulla of spontaneously hypertensive rats. *Hypertens Res* **31**, 2075-2083.

Kreulen DL (2005). Neurobiology of Autonomic Ganglia. In *Peripheral Neuropathy*, eds. Peter J.Dyck & P.K.Thomas, pp. 233-248. Elsevier Saunders, Philadelphia.

Kreulen DL & Szurszewski JH (1979). Nerve pathways in celiac plexus of the guinea pig. *Am J Physiol* **237**, E90-E97.

Kubo T, Fukumori R, Kobayashi M, & Yamaguchi H (1996). Enhanced cholinergic activity in the medulla oblongata of DOCA-salt hypertensive and renal hypertensive rats. *Hypertens Res* **19**, 213-219.

Kwon J, Lee SR, Yang KS, Ahn Y, Kim YJ, Stadtman ER, & Rhee SG (2004). Reversible oxidation and inactivation of the tumor suppressor PTEN in cells stimulated with peptide growth factors. *Proc Natl Acad Sci U S A* **101**, 16419-16424.

Lee TM, Chen CC, & Chang NC (2012). Cardiac sympathetic hyperinnervation in deoxycorticosterone acetate-salt hypertensive rats. *Clin Sci (Lond)* **123**, 445-457.

Li A, Segui J, Heinemann SH, & Hoshi T (1998). Oxidation regulates cloned neuronal voltage-dependent Ca2+ channels expressed in Xenopus oocytes. *J Neurosci* **18**, 6740-6747.

Li L, Watts SW, Banes AK, Galligan JJ, Fink GD, & Chen AF (2003). NADPH Oxidase-Derived Superoxide Augments Endothelin-1-Induced Venoconstriction in Mineralocorticoid Hypertension. *Hypertension*.

Li M, Dai X, Watts SW, Kreulen DL, & Fink GD (2008). Increased superoxide levels in ganglia and sympathoexcitation are involved in sarafotoxin 6c-induced hypertension. *Am J Physiol Regul Integr Comp Physiol* **295**, R1546-R1554.

Li M, Galligan J, Wang D, & Fink G (2010). The effects of celiac ganglionectomy on sympathetic innervation to the splanchnic organs in the rat. *Auton Neurosci* **154**, 66-73.

Lu Z, Wu CY, Jiang YP, Ballou LM, Clausen C, Cohen IS, & Lin RZ (2012). Suppression of phosphoinositide 3-kinase signaling and alteration of multiple ion currents in drug-induced long QT syndrome. *Sci Transl Med* **4**, 131ra50.

Lundin S, Ricksten SE, & Thoren P (1984). Interaction between "mental stress" and baroreceptor reflexes concerning effects on heart rate, mean arterial pressure

and renal sympathetic activity in conscious spontaneously hypertensive rats. *Acta Physiol Scand* **120**, 273-281.

Luther JA & Birren SJ (2009). p75 and TrkA signaling regulates sympathetic neuronal firing patterns via differential modulation of voltage-gated currents. *J Neurosci* **29**, 5411-5424.

Macefield VG, Elam M, & Wallin BG (2002). Firing properties of single postganglionic sympathetic neurones recorded in awake human subjects. *Auton Neurosci* **95**, 146-159.

Madden CJ & Sved AF (2003). Cardiovascular regulation after destruction of the C1 cell group of the rostral ventrolateral medulla in rats. *Am J Physiol Heart Circ Physiol* **285**, H2734-H2748.

Magee JC & Schofield GG (1992). Neurotransmission through sympathetic ganglia of spontaneously hypertensive rats. *Hypertension* **20**, 367-373.

Magee JC & Schofield GG (1994). Alterations of synaptic transmission in sympathetic ganglia of spontaneously hypertensive rats. *Am J Physiol* **267**, R1397-R1407.

Malin SA & Nerbonne JM (2000). Elimination of the fast transient in superior cervical ganglion neurons with expression of KV4.2W362F: molecular dissection of IA. *J Neurosci* **20**, 5191-5199.

Malin SA & Nerbonne JM (2002). Delayed rectifier K+ currents, IK, are encoded by Kv2 alpha-subunits and regulate tonic firing in mammalian sympathetic neurons. *J Neurosci* **22**, 10094-10105.

Malpas SC (2010). Sympathetic nervous system overactivity and its role in the development of cardiovascular disease. *Physiol Rev* **90**, 513-557.

Mano T, Iwase S, & Toma S (2006). Microneurography as a tool in clinical neurophysiology to investigate peripheral neural traffic in humans. *Clin Neurophysiol* **117**, 2357-2384.

Marsh SJ & Brown DA (1991). Potassium currents contributing to action potential repolarization in dissociated cultured rat superior cervical sympathetic neurones. *Neurosci Lett* **133**, 298-302.

Matsumura K, Abe I, Tsuchihashi T, Tominaga M, Kobayashi K, & Fujishima M (1991). Central effect of endothelin on neurohormonal responses in conscious rabbits. *Hypertension* **17**, 1192-1196.

McAllen RM & Malpas SC (1997). Sympathetic burst activity: characteristics and significance. *Clin Exp Pharmacol Physiol* **24**, 791-799.

McLachlan EM (2003). Transmission of signals through sympathetic ganglia--modulation, integration or simply distribution? *Acta Physiol Scand* **177**, 227-235.

McLachlan EM & Meckler RL (1989). Characteristics of synaptic input to three classes of sympathetic neurone in the coeliac ganglion of the guinea-pig. *J Physiol* **415**, 109-129.

Mishra RC, Tripathy S, Gandhi JD, Balsevich J, Akhtar J, Desai KM, & Gopalakrishnan V (2010). Decreases in splanchnic vascular resistance contribute to hypotensive effects of L-serine in hypertensive rats. *Am J Physiol Heart Circ Physiol* **298**, H1789-H1796.

Mortensen LH, Pawloski CM, Kanagy NL, & Fink GD (1990). Chronic hypertension produced by infusion of endothelin in rats. *Hypertension* **15**, 729-733.

Muller W & Bittner K (2002). Differential oxidative modulation of voltage-dependent K+ currents in rat hippocampal neurons. *J Neurophysiol* **87**, 2990-2995.

Murphy SM, Matthew SE, Rodgers HF, Lituri DT, & Gibbins IL (1998). Synaptic organisation of neuropeptide-containing preganglionic boutons in lumbar sympathetic ganglia of guinea pigs. *J Comp Neurol* **398**, 551-567.

Navar LG (2005). The role of the kidneys in hypertension. *J Clin Hypertens (Greenwich)* **7**, 542-549.

O'Donaughy TL & Brooks VL (2006). Deoxycorticosterone acetate-salt rats: hypertension and sympathoexcitation driven by increased NaCl levels. *Hypertension* **47**, 680-685.

O'Donaughy TL, Qi Y, & Brooks VL (2006). Central action of increased osmolality to support blood pressure in deoxycorticosterone acetate-salt rats. *Hypertension* **48**, 658-663.

Olson N, DeJongh B, Hough A, & Parra D (2012). Plasma renin activity-guided strategy for the management of hypertension. *Pharmacotherapy* **32**, 446-455.

Ping Y, Waro G, Licursi A, Smith S, Vo-Ba DA, & Tsunoda S (2011). Shal/K(v)4 channels are required for maintaining excitability during repetitive firing and normal locomotion in Drosophila. *PLoS One* **6**, e16043.

Pinto YM, Paul M, & Ganten D (1998). Lessons from rat models of hypertension: from Goldblatt to genetic engineering. *Cardiovasc Res* **39**, 77-88.

Ray PD, Huang BW, & Tsuji Y (2012). Reactive oxygen species (ROS) homeostasis and redox regulation in cellular signaling. *Cell Signal* **24**, 981-990.

Rettig R, Schmitt B, Pelzl B, & Speck T (1993). The kidney and primary hypertension: contributions from renal transplantation studies in animals and humans. *J Hypertens* **11**, 883-891.

Robertson WP & Schofield GG (1999). Primary and adaptive changes of A-type K+ currents in sympathetic neurons from hypertensive rats. *Am J Physiol* **276**, R1758-R1765.

Rodrigo R, Gonzalez J, & Paoletto F (2011). The role of oxidative stress in the pathophysiology of hypertension. *Hypertens Res* **34**, 431-440.

Sagar S, Kallo IJ, Kaul N, Ganguly NK, & Sharma BK (1992). Oxygen free radicals in essential hypertension. *Mol Cell Biochem* **111**, 103-108.

Schiffrin EL (2001). Role of endothelin-1 in hypertension and vascular disease. *Am J Hypertens* **14**, 83S-89S.

Schlaich MP, Lambert E, Kaye DM, Krozowski Z, Campbell DJ, Lambert G, Hastings J, Aggarwal A, & Esler MD (2004). Sympathetic augmentation in hypertension: role of nerve firing, norepinephrine reuptake, and Angiotensin neuromodulation. *Hypertension* **43**, 169-175.

Sellers KW, Sun C, ez-Freire C, Waki H, Morisseau C, Falck JR, Hammock BD, Paton JF, & Raizada MK (2005). Novel mechanism of brain soluble epoxide hydrolase-mediated blood pressure regulation in the spontaneously hypertensive rat. *FASEB J* **19**, 626-628.

Sesti F, Liu S, & Cai SQ (2010). Oxidation of potassium channels by ROS: a general mechanism of aging and neurodegeneration? *Trends Cell Biol* **20**, 45-51.

Sheehan JP, Swerdlow RH, Miller SW, Davis RE, Parks JK, Parker WD, & Tuttle JB (1997). Calcium homeostasis and reactive oxygen species production in cells transformed by mitochondria from individuals with sporadic Alzheimer's disease. *J Neurosci* **17**, 4612-4622.

Shonis CA & Waldrop TG (1993). Augmented neuronal activity in the hypothalamus of spontaneously hypertensive rats. *Brain Res Bull* **30**, 45-52.

Somers MJ, Mavromatis K, Galis ZS, & Harrison DG (2000). Vascular superoxide production and vasomotor function in hypertension induced by deoxycorticosterone acetate-salt. *Circulation* **101**, 1722-1728.

Stadtman ER & Levine RL (2000). Protein oxidation. *Ann N Y Acad Sci* **899**, 191-208.

Strazzullo P, D'Elia L, Kandala NB, & Cappuccio FP (2009). Salt intake, stroke, and cardiovascular disease: meta-analysis of prospective studies. *Br Med J* **339**, b4567.

Sved AF, Ito S, & Sved JC (2003). Brainstem mechanisms of hypertension: role of the rostral ventrolateral medulla. *Curr Hypertens Rep* **5**, 262-268.

Szurszewski JH (1981). Physiology of mammalian prevertebral ganglia. *Annu Rev Physiol* **43**, 53-68.

Thakali K, Davenport L, Fink GD, & Watts SW (2006). Pleiotropic effects of hydrogen peroxide in arteries and veins from normotensive and hypertensive rats. *Hypertension* **47**, 482-487.

Toal CB & Leenen FH (1987). Dietary sodium restriction, blood pressure and sympathetic activity in spontaneously hypertensive rats. *J Hypertens* **5**, 107-113.

Wang H, Chen AF, Watts SW, Galligan JJ, & Fink GD (2005a). Endothelin in the splanchnic vascular bed of DOCA-salt hypertensive rats. *Am J Physiol Heart Circ Physiol* **288**, H729-H736.

Wang HJ, Li YL, Zhang LB, Zucker IH, Gao L, Zimmerman MC, & Wang W (2011). Endogenous reactive oxygen species modulates voltage-gated sodium channels in dorsal root ganglia of rats. *J Appl Physiol* **110**, 1439-1447.

Wang HS & McKinnon D (1995). Potassium currents in rat prevertebral and paravertebral sympathetic neurones: control of firing properties. *J Physiol (Lond)* **485**, 319-335.

Wang JG, Staessen JA, Franklin SS, Fagard R, & Gueyffier F (2005b). Systolic and diastolic blood pressure lowering as determinants of cardiovascular outcome. *Hypertension* **45**, 907-913.

Watson R.E. & Dipette D.J. (2008). Experimental Models of Hypertension. In *Hypertension Primer* pp. 181-183. Lippincott Williams &Wilkins, Philadelphia.

Xu H, Fink GD, & Galligan JJ (2004). Tempol lowers blood pressure and sympathetic nerve activity but not vascular O2- in DOCA-salt rats. *Hypertension* **43**, 329-334.

Yarowsky P & Weinreich D (1985). Loss of accommodation in sympathetic neurons from spontaneously hypertensive rats. *Hypertension* **7**, 268-276.

Zafari AM, Ushio-Fukai M, Akers M, Yin Q, Shah A, Harrison DG, Taylor WR, & Griendling KK (1998). Role of NADH/NADPH oxidase-derived H2O2 in angiotensin II-induced vascular hypertrophy. *Hypertension* **32**, 488-495.

CHAPTER 2

NEURONAL MEMBRANE FIRING PROPERTIES OF DISSOCIATED CELIAC GANGLION NEURONS IN DOCA-SALT HYPERTENSIVE RATS

Abstract:

The activity of both sympathetic neurons in the central nervous system and peripheral sympathetic nerves that innervate the heart and blood vessels is elevated in hypertension. However, it is unclear about the roles of sympathetic ganglia, which integrate central sympathetic signals and send their signals to cardiovascular end organs via sympathetic nerve. The celiac ganglion (CG) plays an important function in regulating blood pressure by the controlling splanchnic circulation containing 30% of total blood volume. The purpose of this study is to find if the electrophysiological properties of CG neurons are modulated in DOCA-salt hypertensive rats. Using whole-cell patch clamp, dissociated CG neurons from hypertensive and matched normotensive rats were divided into three groups based on their action potential (AP) firing patterns to a sustained suprathreshold depolarizing current (100pA; 5s). Phasic neurons fired several APs and adapted quickly; adaptive neurons fired several APs and adapted slowly; tonic neurons fired continuously. When compared to CG neurons from normotensive rats, the distribution of these three groups was significantly different in neurons from hypertensive rats. There were more phasic neurons, fewer adaptive neurons and tonic neurons in hypertensive rats. Moreover, the firing frequencies of tonic neurons were significantly lower in hypertensive rats compared to normotensive rats. Furthermore, the electrophysiological properties of a single AP were compared between normotensive and hypertensive rats. , the AP repolarization rate and the area of afterhyperpolarization were significantly lower in hypertensive rats. When the CG neurons from normotensive rats were treated with specific K^+ channel blockers, the neuronal firing pattern and firing frequencies were changed in a way mimicking the

changes shown in hypertensive rats. Under treatment of tetraethylammonium (TEA), a delay rectifier K^+ channel blocker, the firing frequencies of phasic, adaptive and tonic neurons were all significantly attenuated. When Paxilline, a big-conductance Ca^{2+}-activated K^+ channel blocker, was used, both tonic and adaptive neurons were converted to phasic neurons. After application of 4-Aminopyridine (4-AP), A-type K^+ channel blocker, some tonic neurons were converted to adaptive or phasic neurons. This study demonstrates that the CG neuronal firing patterns and firing frequencies are modulated in DOCA-salt hypertensive rats, which may be contributed by some changes of K^+ channel currents including delay rectifier K^+ current, big-conductance Ca^{2+}-activated K^+ current and A-type K^+ current.

Introduction

Hypertension as a multifactorial disease is closely related to increased activity of sympathetic nervous system (Beevers *et al.*, 2001). The DOCA-salt hypertensive animal model is an appropriate model for exploring the mechanisms of human essential hypertension. It is characterized by excessive salt intake, increased retention of salt and water, and increased sympathetic nerve activity (Watson R.E. & Dipette D.J., 2008). The CG is the largest prevertebral sympathetic ganglia controlling splanchnic circulation which contains 30% of the total blood volume.

Sympathetic postganglionic neurons in sympathetic ganglia, relay integrate and modify the signals from sympathetic preganglionic neurons. Then they send the

electrical signals to target organs through sympathetic nerves (Gibbins, 1995). The sympathetic nerve activity is enhanced in hypertension (Schlaich *et al.*, 2004). The elevated nerve activity could be caused by increased central sympathetic drive and/or by enhanced neurotransmission in the sympathetic ganglia. The neuronal membrane firing properties of sympathetic postganglionic neurons play an important role in controlling the neurotransmission in sympathetic ganglia and neurotransmitter release from postganglionic nerve terminals. Therefore, the membrane firing properties of CG neurons may be an important factor contributing to elevated sympathetic activity in hypertension.

The membrane firing properties of CG neurons could be studied by injecting a sustained suprathreshold depolarizing current *in vitro*. According to previous finding, CG neurons are divided into phasic (rapidly adapting) neurons and tonic (slowly adapting) neurons in response to the depolarizing current, (Wang & McKinnon, 1995). Cultured neurons from superior cervical ganglia, the paravertebral sympathetic ganglia, show different distribution of neuronal firing patterns in spontaneous hypertensive and neonatal hypertensive rats (Jubelin & Kannan, 1990;Yarowsky & Weinreich, 1985). However the neuronal firing patterns of CG neurons have not been explored in hypertension. In this study, the neuronal firing properties in response to a sustained suprathreshold depolarizing current were tested in CG neurons from DOCA-hypertensive rats and matched normotensive rats.

Materials and Methods

Animals

The animals and number used in the experiments were conformed to “National Institutes of Health Guide for the Care and Use of Laboratory Animals”. The study was approved by the Animal Use and Care Committee of Michigan State University. Adult male Sprague Dawley rats (250-300g; Charles River Laboratories Inc., Portage, MI) underwent uninephrectomy and subcutaneous implantation of deoxycorticosterone acetate (DOCA; 200 mg kg^{-1}) under isoflurane anesthesia. The rats were then given drinking water containing 1% NaCl and 0.2% KCl (herein, the hypertensive group is referred to as HT group). Normotensive controls were also uninephrectomized; however, these control rats did not receive an implant of DOCA and were maintained on normal drinking water (herein, the control group is referred to as NT group). Blood pressure was monitored by tail cuff pletheysmography and the animals were euthanized using an intraperitoneal injection of pentobarbital (80 mg kg^{-1}). Animals were housed two per cage with access to food and water *ad libitum* while being kept on a 12:12 h light-dark cycle in a room with regulated temperature (22-24°C). The systolic arterial pressures for the HT and NT rats were 202±4 mm Hg and 133±1 mm Hg, respectively. The body weight for the HT rats (344.8±9.2 g) was significantly lower than that for NT rats (419.4±6.8 g). In the experiments examining the effects of K^+ channel blockers, normal 3-5 week old Sprague Dawley rats were used and housed as described above.

Tissue collection

The instruments and abdominal area of the euthanized rat were disinfected with 70% alcohol. The abdomen was opened and the back of abdominal cavity was exposed. The CG surrounds the root of the celiac artery appearing as a large irregularly shaped semitransparent mass. Surrounding fatty tissues were torn away with fine

forceps, and the CG ganglion was dissected out with micro-dissecting scissors under the microscope. The ganglion was further partially cut 5-6 times and placed immediately in a culture tube containing 2ml Hank's Balanced Salt Solution (HBSS) (137 NaCl, 5.4 KCl, 4.2 $NaHCO_3$, 1.3 $CaCl_2$, 1.0 $MgCl_2$, 0.44 KH_2PO_4, 0.25 Na_2HPO_4, and 10 HEPES in mM). All the protocols and procedures were reviewed and approved by the Animal Care and Use Committee of the Michigan State University.

***Cell culture*:**

The harvested CG ganglion was dissociated enzymatically with 4ml HBSS containing 6.7U/ml papain for 10 min at 37°C. The papain solution was activated by 1mg/ml dithioerythritol for 15mins before the enzymatically dissociation. After papain application, the ganglion was dissociated with 4ml HBSS containing 0.375% type-1 collagenase and 0.25% trypsin inhibitor for 10 min at 37°C. Half of enzyme solution was replaced with HBSS, and the tissue fragments were triturated ten times at room temperature with fire-polished Pasteur pipettes, which were made with gradually decreasing tip diameter. The ganglion was returned to the enzymatic solution for 2-5 min at 37°C and then triturated another 30 times. The number of neurons in one drop of solution was tested during trituration. The dissociation was stopped when the number of neurons was approximately 10 in one drop of solution. After dispersal, the suspension was centrifuged with 5 ml plating medium (Dulbecco's modified Eagle Medium supplemented with 10% fetal bovine serum and 100U/ml penicillin-streptomycin) at 3000rpm for 4 min. Supernatant was remove, and another 5ml plating medium was added and then centrifuged at 3000rpm for 3 min. The pellet was resuspended in approximately 1 ml plating medium and plated at a density of approximately 1000

cells/cm^{2}on glass bottom culture dishes coated overnight with poly-D-lysine.

The neurons were maintained in cell culture incubator at 37°C in an atmosphere of 5% CO_2/95% O_2 and 90% humidity. After 2 hours, 1.5 ml feeding medium (plating medium supplemented with 100ng/ml 2.5S nerve growth factor) was added to each dish. Dispersed neurons were always roughly spherical in geometry (30-50 μm in diameter) and devoid of dendrites and round or oval within 24 hours.

Whole-cell patch clamp

The culture dish with neurons was mounted on the stage of an inverted microscope (Olympus IX50) and then superfused with extracelluar solution at room temperature (20-23℃). All experiments were carried out within 24 h after cell dispersal. The neurons were perfused at 1.5ml/min by extracellular solution (120 NaCl, 4.7 KCl, 2.5 $CaCl_2$, 1.2 $MgCl_2$, 1.2 $NaHPO_4$, 25 $NaHCO_3$, and 11 Glucose in mM) which was equilibrated with a 95% O_2-5%CO_2 mixture. Whole-cell patch-clamp recordings were made using CV-4 headstage and Axopatch 1D amplifier digitized by Digidata 1322A (Axon Instruments, Inc., Foster City, CA, USA). Command-voltage protocols and data acquisition were performed with pClamp software including Clampex and Clampfit (Molecular Devices, Version 9.2).

The neurons were patched by a recording electrode filled with intracellular solution (150 K Acetate, 3 $MgCl_2$, 40 HEPES, 10 ATP, and 2.5 GTP in mM). Patch electrodes (O.D. 1.5mm, I.D. 1.12 mm; World Precision Instruments, Inc., FL, USA) with open-tip resistances of 3-9 MΩ were made by micropipette puller model P-87 (Sutter Instruments, Inc., Novato, CA, USA) and filled with intracellular solution. A seal resistance of 1.5-2.0 GΩ was created by suction. After sealing, the pipette capacitance

was compensated for by the circuit of the amplifier. A holding potential of -70mv was applied and the cell membrane was disrupted by further quick suction. Access resistance (R_a) was monitored at regular intervals. The resting membrane potential (RMP) lower than -45mv, were accepted for data acquisition.

Data acquisition

Neurons were ruptured under -70mv holding potential in voltage clamp mode. The cell capacitance and input resistance (R_{in}) were measured automatically by Clampex's membrane test function under a 20mv (20ms) depolarizing pulse from -70mv holding potential. Then neurons were carefully switched to current clamp mode without damaging the cells. Neuronal firing was induced by injecting current steps from -20pA to 100pA for 5s with a sampling rate of 10 kHz under current clamp mode. Steps were separated by a 35s delay. A single AP was induced by injecting a short duration current from 0pA to 2000pA for 2ms. The peak amplitude (the amplitude from RMP to highest point of AP), mid-width (the time duration between depolarizing to half peak amplitude and repolarizing to half peak amplitude), repolarization rate (the decline rate from AP peak point to lowest point of AP), AHP peak amplitude (the amplitude from RMP to lowest point of AHP), AHP mid-width (the time duration between hyperpolarizing to half peak amplitude of AHP and depolarizing to half peak amplitude of AHP) , and AHP area (the area under RMP and enclosed by AHP) were measured using Clampfit software.

Statistical analysis:

All values were presented as means ± SEM and n refers to the number of neurons. Less than 4 neurons were recorded per animal. Statistical significance between means was determined using two-tailed unpaired t-test. The neuronal firing

pattern changes were analyzed by χ^2 test. The statistical significance was set for $P<0.05$.

Results

Electrical membrane firing properties of dissociated CG neurons from NT and HT rats

The fundamental electrical membrane properties were compared in dissociated CG neurons. The values of cell capacitance and R_{in} were not significantly different (unpaired t-test, p>0.05) between these neurons from HT rats and those from NT rats (Table 2-1). RMP was in the range of -45 to -65 mv both in NT and HT rats. The RMP for each neuron remained stable (<5mv difference) during the experiment. The RMP was not significantly different (unpaired t-test, p>0.05) between neurons from HT rats and those from NT rats (Table 2-1). These data indicate that the passive electrical membrane properties of CG neurons were not modified in hypertension. However, the rheobasic current (I_r), estimated by the minimum current inducing APs in 20pA, 40pA, 60pA, 80pA, 100pA current injection, was significantly higher (unpaired t-test, p<0.05) in neurons from HT rats compared to those from NT rats (Table 2-1).

Different neuronal firing patterns in CG neurons from HT rats

In response to long-duration depolarizing current steps (20pA, 40pA, 60pA, 80pA, 100pA, 5s for each at 35s interval), CG neurons fired action potentials (APs). The neurons were divided to three groups by their different firing patterns: phasic, adaptive and tonic neurons. ***Phasic*** neurons fired less than 4 APs and adapted quickly; ***adaptive***

neurons fired several APs, adapted slowly and then stop firing even at the highest current injection; ***tonic*** neurons fired continuously in the highest level (100pA) of depolarizing current step (Figure. 2-1, A). Some phasic neurons did not fire in response to 100pA current injection, but fired APs when more than 100pA current step was injected. The distribution of these three groups was significantly different (χ^2 test, $p<0.05$, NT: n=101; HT: n=97) in CG neurons from HT compared to those from NT rats. Among neurons from HT rats there was higher percentage of phasic neurons (44% for HT and 29% for NT), a lower percentage of adaptive neurons (8% for HT and 18% for NT) and a lower percentage of tonic neurons (48% for HT and 53% for NT) compared to NT (Figure. 2-1, C). There were significantly more percentage of phasic neurons not firing in response to 100pA current injection in HT rats compared to NT rats (x^2 test, $p<0.05$, NT: n=29; HT: n=43) (Figure. 2-1, D). This finding indicates the CG neurons in HT rats were more accommodating to sustained depolarizing current injection compared to those in NT rats.

The neuronal firing frequency in the different types of CG neurons in NT and HT rats

The firing frequency was analyzed in response to 100pA depolarizing current step. The neuronal firing frequency was not constant during sustained depolarizing current injection. The instantaneous firing frequency (fi) was tested as the reciprocal of the time duration between the previous AP peak and the next AP peak. It declined gradually and stopped firing in phasic and adaptive neurons or reduced to a stable plateau in tonic neurons (Figure. 2-1, B). To address to the CG neuronal firing frequency, the time from initiation of current injection to the first AP peak (t_1), the time

from the first AP peak to the second AP peak (t_2) and the time from the penultimate AP peak and the last AP peak (t_3) in the plateau region were measured. The first instantaneous firing frequency (f_{1st}) is equal to 1/ t_2.The last instantaneous firing frequency (f_{last}) is equal to 1/ t_3. t_3 and f_{last} were only obtained from tonic neurons because only tonic neurons have plateau regions. f_{1st} was measured in phasic neurons that showed at least 2 APs during current injection. t_1 was measured in phasic neurons which showed at least 1 AP in response to current injection. In all three types of neurons, the firing frequency was lower in CG neurons from HT rats. In phasic neurons, t_1 and f_{1st} were not significantly different between NT and HT rats (Figure. 2-2, A). In adaptive neurons, the t_1 was significantly lower in HT rats compared to NT rats (t-test, $p<0.05$, NT: n=17; HT: n=7). However there was no significant difference in f_{1st} between NT and HT rats (t-test, $p>0.05$, NT: n=17; HT: n=7) (Figure. 2-2, B). In tonic neurons, t_1 and the first instantaneous firing frequency (1/ t_2) were not significantly different between NT and HT rats (t-test, $p>0.05$, NT: n=30; HT: n=29) (Figure. 2-2 C). The last instantaneous firing frequency (1/ t_3) was significantly lower in HT rats compared to NT rats (t-test, $p>0.05$, NT: n=30; HT: n=29) (Figure. 2-2 C). The action potential firing frequency for the entire 5 s of depolarization increased with increased current injection in tonic neurons. The action potential firing frequency for the entire 5 s was measured as number of AP fired during the current injection. The firing frequency at 100pA current

injection was significantly lower in HT rats compared to NT rats. (two-way ANOVA followed by Bonferroni posttest, $p<0.01$) (Figure. 2-2, C). These data indicate adaptive and tonic neurons tend to fire less frequently in HT rats.

AP properties of CG neurons from HT and NT rats

To explore the reasons which may contribute to neuronal firing changes in hypertension, the first AP of neuronal firing in response to 100pA, 5ms depolarizing current injection was measured as AP amplitude, duration and repolarizing rate. The AP peak amplitude and duration were not significantly different between NT and HT rats. However, the repolarization rate was significantly lower in HT (t-test, $p<0.05$, NT: n=89; HT: n=76) (Table 2-2). Because the afterhyperpolarization (AHP) was covered by the current injection depolarization, a quick and strong depolarizing current injection (2000pA, 2ms) was applied to generate a single AP. The AHP peak amplitude, mid-width and area were measured. The AHP mid-width was significantly higher in HT (t-test, $p<0.05$, NT: n=26; HT: n=19). However the AHP area was significantly lower in HT (t-test, $p<0.05$, NT: n=26; HT: n=19). The peak amplitude was not different between NT and HT rats (Table 2-3). The changes in AP repolarization and AHP may be due to modifications of the K^+ currents in HT.

The effects of K^+ channel blockers on neuronal firing in NT rats

The changes in single AP firing properties indicated K^+ currents could be modified in HT, which may be related to the changes in neuronal firing in HT. Potassium channel blockers were used in NT rats to further investigate and dissect the relative contribution of each potassium channel group to the alterations in AP properties of HT

neurons.

Tetraethylammonium (TEA) was used to block delayed rectifier K^+ currents (Hille, 2001). The outward potassium currents were generated through series of voltage steps from -140mv to +60mv in 10mV increments at a membrane potential of -70mV in the presence of $3x10^{-7}$M Na channel blocker, tetrodotoxin (TTX). TEA decreased the outward potassium currents in dose-dependent manner (Figure. 2-3). Its EC_{50}, calculated as 23.4mM, was used to test the effects of TEA on neuronal firing. Under current clamp mode, the firing frequency was decreased by TEA (23.4mM) in tonic neurons (n=4). However the firing pattern was not changed in these tonic neurons (Figure. 2-4, E). The firing frequency was also decreased in phasic (n=4) and adaptive (n=4) neurons (Figure. 2-4, A-D). However the firing pattern changes are variable in phasic and adaptive neurons. The firing pattern was not changed in two phasic neurons, but was changed to tonic neurons in the other two phasic neurons (Figure. 2-4, A & B). The firing pattern was changed to phasic neurons in two adaptive neurons, but was changed to tonic neurons in the other adaptive neurons (Figure. 2-4, C& D).

Paxilline is a blocker for big-conductance Ca^{2+}-activated K^+ channel (BK). The outward potassium current under +50mv voltage step was decreased by paxilline in dose-dependent manner in concentration below 10 μM. At 10 μM, the effect of paxilline was reached to its plateau by reducing 25% of the outward potassium current (Figure. 2-5). Paxilline converted adaptive and some tonic neurons to phasic neurons (Figure. 2-6, B & C) and decreased the number of AP in phasic neurons (Figure. 2-6, A). However the firing pattern was not changed by paxilline in other tonic neurons (data not shown). The A-type K^+ channel blocker 4-AP (5mM) was tested on tonic neurons. 4-AP decreased

firing frequency in some tonic neurons (n=2) (Figure. 2-7, A), converted some tonic neurons to adaptive neurons (n=2) (Figure. 2-7, B) and converted some tonic neurons to phasic neurons (n=2) (Figure. 2-7, C). Adaptive neurons remained adaptive neurons after 4-AP treatment (data not shown).

Discussion

In this study, the neuronal firing in response to a sustained depolarizing current injection was compared between dissociated CG neurons from NT rats and those from HT rats. It has been shown that the acutely dissociated CG neurons retained electrical properties similar to intact ganglia (Carrier & Ikeda, 1992). Dissociated CG neurons are conveniently isolated from the influences of adjacent neurons and the CNS. In HT rats, the main findings are there were more phasic neurons and less adaptive and tonic neurons in CG neurons; the firing frequency was lower in tonic neurons from HT rats. These results may indicate that the neurons from HT rats were more accommodating to sustained suprathreshold depolarizing current injection. To explore the underlying mechanisms, single AP parameters were measured. The repolarization and AHP of the AP were modified in HT rats, which lead us to test the effects of K^+ channel blockers on neuronal firing. The main findings are TEA decreased neuronal firing frequency, paxilline changed neuronal firing patterns by converting tonic and adaptive neurons to phasic neurons and 4-AP converted some tonic neurons to adaptive/phasic neurons. The effects of TEA on firing frequency and paxilline and 4-AP on firing pattern mimic the neuronal firing changes from neurons in NT rats to neurons in HT rats. These data indicate that the more accomodating neuronal firing pattern and decreased firing

frequency in HT rats may be contributed by decreased certain K^+ currents.

Most studies classified CG neurons as phasic or tonic neurons based on their response to a sustained depolarizing current (Wang & McKinnon, 1995). In my study, I classified another group of neurons in addition to phasic and tonic neuron using a 5s sustained depolarizing current. These “adaptive neurons” stop firing in the middle of sustained depolarizing current injection. They were more accommodating than tonic neurons, but less accommodating than phasic neurons. This detailed classification might give us more information on neuronal firing properties in hypertension.

Specific ion channel blockers are available for different kinds of ion channels. Na^+ current I is irreversibly blocked by tetrodotoxin (TTX), a potent neurotoxin. Its binding sit is located at the pore opening of the voltage-gated Na^+ channel. K^+ channels compose of several subgroups. Specific blocker is developed for each subgroup. In this study, three K^+ channel blockers including TEA (tetraethylammonium), paxilline and 4-AP (4-Aminopiridine), were used to explore the effects of decreased I_{Kv}, I_{BK}, and I_A on neuronal firing in CG neurons. TEA applied externally blocked the delay rectifier K^+ channel in concentration-dependent manner (Bokvist *et al.*, 1990). 4-AP is relatively selective blocker for A-type K^+ channel. Some research work compares TEA with 4-AP on blocking I_{Kv} and I_A. TEA not 4-AP, is found to block I_{Kv} (Thompson, 1977), whereas I_A is blocked by 4-AP, but much less sensitive to TEA (Sonner & Stern, 2007;Thompson, 1977). Paxilline is a potent neurotoxin and selective to large-conductance calcium-activated K^+ channel (BK) (Li & Cheung, 1999). Previous studies demonstrate more

tonic neurons in superior cervical ganglia from the spontaneous hypertensive animal model, which are opposite to my finding. However they prove that neuronal firing pattern is not an unchanged electrical property of sympathetic neurons, and it could be modulated in hypertension. The different firing properties of CG neurons from DOCA-salt hypertensive rats may be not related to genetic modulation. To generate DOCA-salt hypertensive animal model, DOCA, high concentration salt and uninephrectomy were applied. The changed firing properties are probably related to DOCA, high salt or high BP.

It has been found that the firing frequency of sympathetic preganglionic nerve, which sending nerve stimulus to celiac ganglion is higher in this model compared to control rats (Iriuchijima *et al.*, 1975). In this study, CG neurons from HT rats exhibited more accommodating properties with more phasic and less adaptive and tonic neurons. The increased activity of central sympathetic drives could be dampened by these changes in celiac ganglia. On the other hand, the postganglionic nerves exhibit burst firing patterns. The burst might contribute to neurotransmitter release. More accommodating of the CG neurons may generate more burst firing and then increase neurotransmitter release. This study cannot make it clear if the enhanced accommodation in CG neurons in HT will enhance or dampen sympathetic activity. Further studies could be done in intact celiac ganglia in vivo to explore the effects of changed neuronal accommodation properties on postganglionic nerve firing in hypertension.

APPENDIX

	R_{in} (MΩ)	cell capacitance (pF)	RMP (mv)	I_r (pA)
NT	750.7±73.7	98.3±3.5	-55.1±0.91	49±4
HT	616.1±41.1	100.4±4.9	-53.7±1.0	63±6*

Table 2-1. Electrical membrane properties of dissociated CG neurons from NT and HT rats

The cell capacitance and input resistance (R_{in}) were measured using a 20mv, lasting 20 ms, depolarizing pulse from -70mv holding potential in voltage clamp mode. R_{in}, cell capacitance and RMP (resting membrane potential) were not significantly different between dissociated CG neurons from HT rats and those from NT rats (unpaired t-test, $p>0.05$, NT: n=98; HT: n=94). The rheobasic current (I_r) was significantly higher in neurons from HT rats compared to NT rats (unpaired t-test, $p<0.05$, NT: n=81; HT: n=79). n refers to the number of neurons.

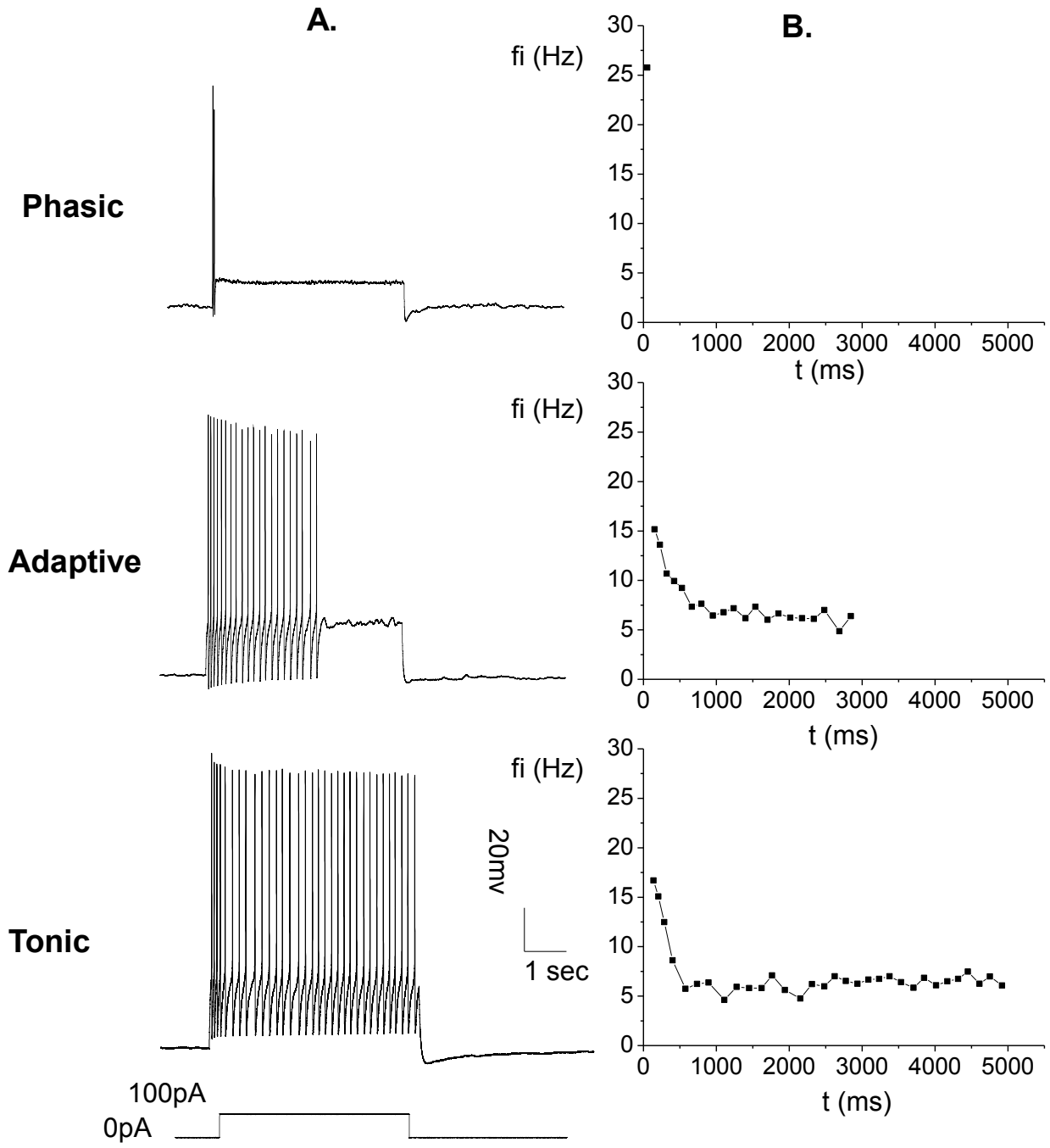
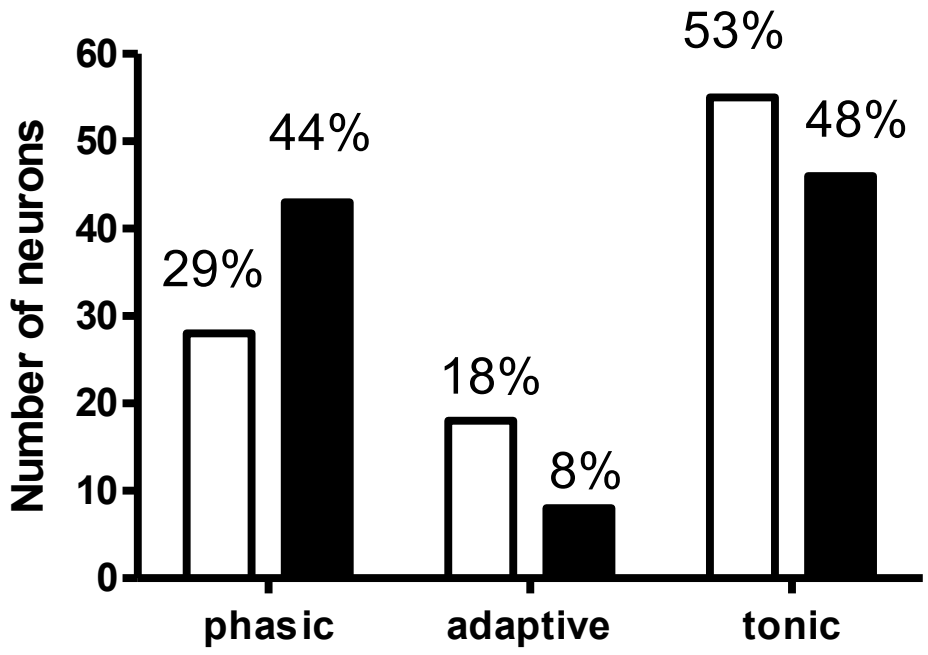


Figure 2-1. Neuronal firing patterns of CG neurons from NT and HT rats

Figure 2-1 (cont'd)

C.



D.

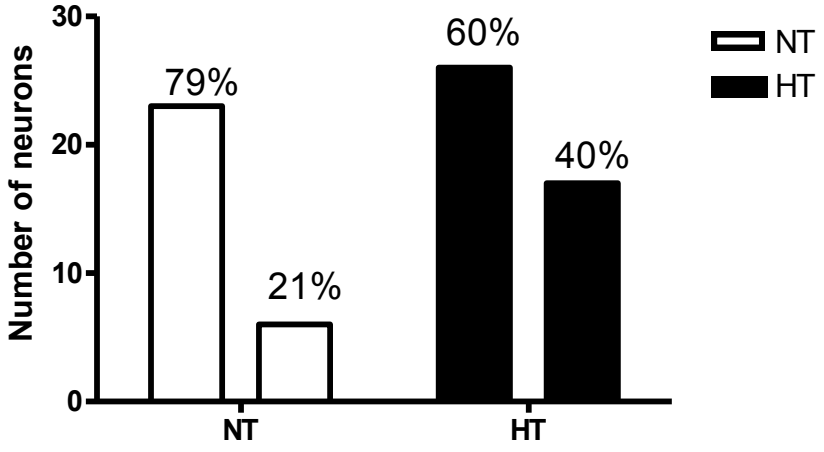


Figure 2-1 (cont'd)

Characteristic firing patterns of dissociated CG neurons from NT and HT rats in response to a sustained 100pA depolarizing current were shown (A). The instantaneous firing frequency (fi) was tested as the reciprocal of the time duration between the previous AP peak and the next AP peak. It declined gradually and stopped firing in phasic and adaptive neurons or reduced to a stable plateau in tonic neurons (B). The distribution of firing patterns were different between CG neurons from NT and those from HT rats (χ^2 test, $p<0.05$, NT: n=101; HT: n=97) (C). The percentage of phasic, adaptive, and tonic neurons out of the total number of neurons in NT (open column) and HT (closed column) rats were shown on the top of corresponding columns. In response to 100pA current injection step, some phasic neurons didn't generate AP, but they fired in response to more current injection. There were more number of phasic neurons not firing in response to 100pA current injection in HT rats compared to NT rats (X^2 test, $p<0.05$, NT: n=29; HT: n=43) (D).

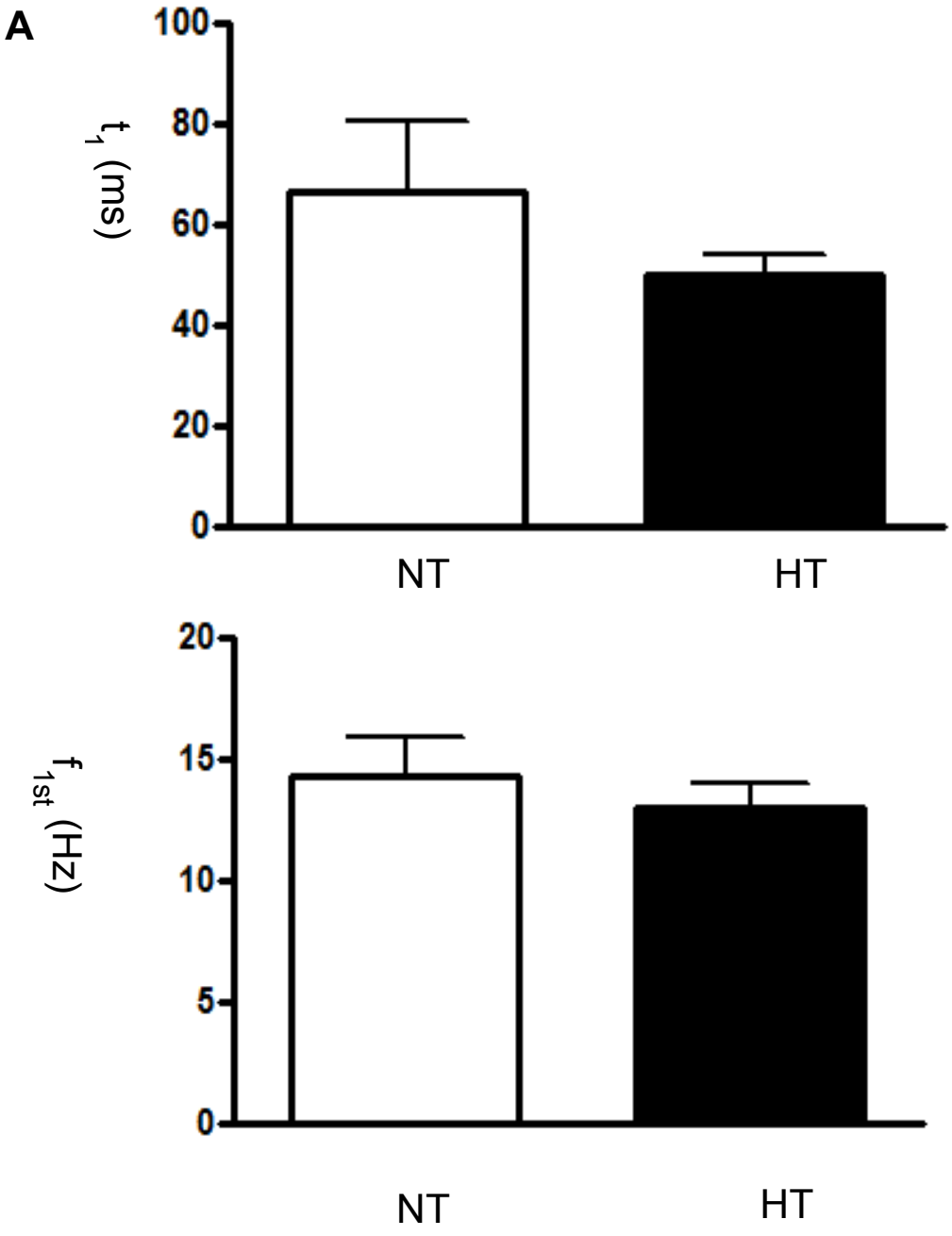


Figure 2-2. The characteristics of firing frequency change in response to a sustained current injection in CG neurons

Figure 2-2 (cont'd)

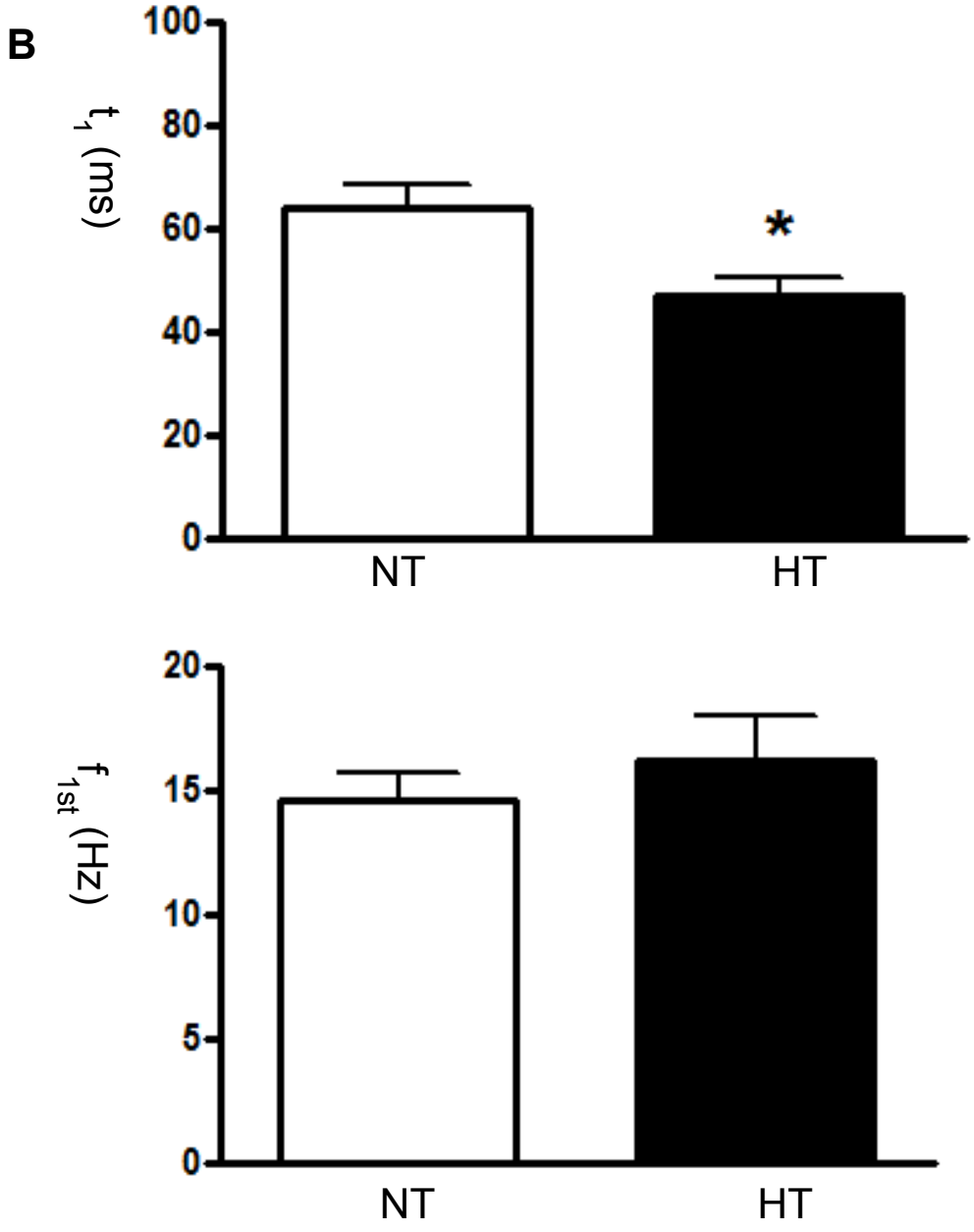


Figure 2-2 (cont'd)

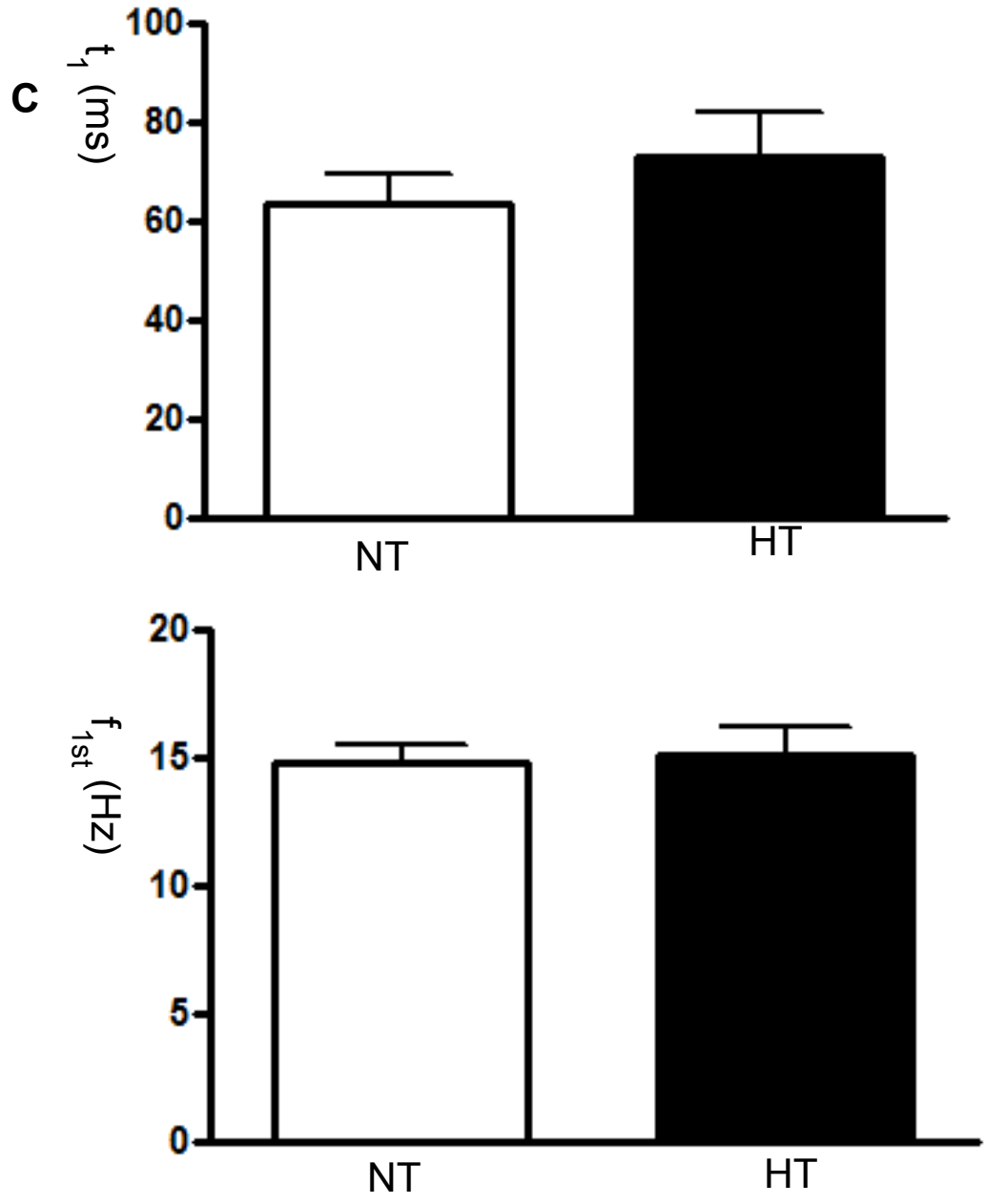


Figure 2-2 (cont'd)

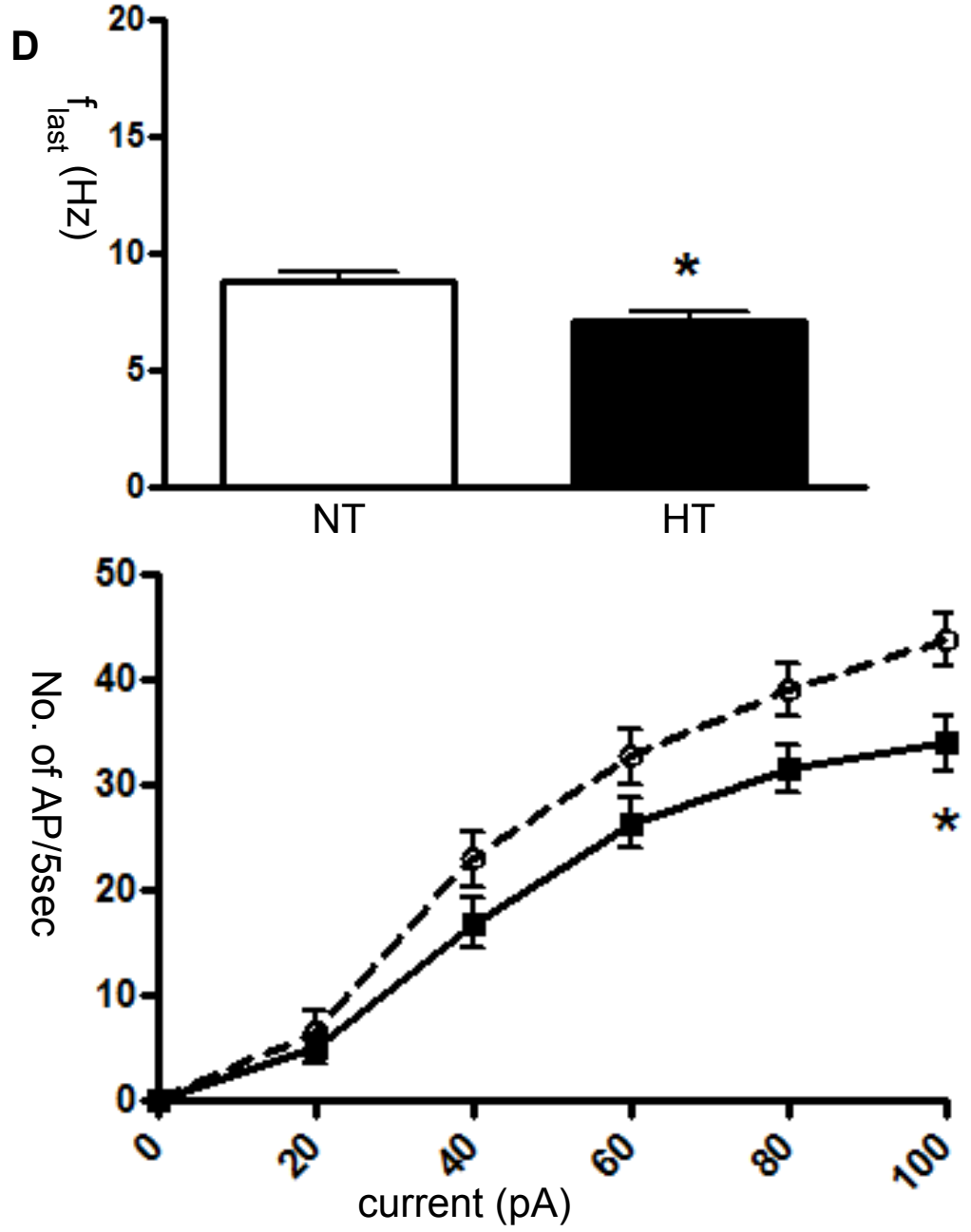


Figure 2-2 (cont'd)

t_1 , t_2 , t_3 were measured to describe CG neuronal firing. t_1 is the time from current injection to the first AP peak, t_2 is the time from the first AP peak to the second AP peak and t_3 is the time from the penultimate AP peak and the last AP peak in the plateau region. (A) In phasic neurons, t_1 was not significantly different between neurons from NT rats and those from HT rats (t-test, $p>0.05$, NT: n=23; HT: n=26). f_{1st} (the first Instantaneous firing frequency) which equals $1/t_2$, was not significantly different between NT and HT (t-test, $p>0.05$, NT: n=16; HT: n=20). (B) In adaptive neurons, t_1 was significantly lower in neurons from NT rats compared to those from HT rats (t-test, $p<0.05$, NT: n=17; HT: n=7). f_{1st} was not significantly different between NT and HT rats(t-test, $p<0.05$, NT: n=17; HT: n=7).(C) In tonic neurons, t_1 and were not significantly different between NT and HT rats (t-test, $p>0.05$). (D) In tonic neurons, f_{last} (the last instantaneous firing frequency) which equals to $1/ t_3$ was significantly lower in HT rats compared to NT rats (t-test, $p<0.05$). The firing frequency for the entire 5 s of depolarization was significantly lower in HT rats compared to NT rats (two-way ANOVA followed by Bonferroni posttest, $p<0.01$ at 100pA current injection).

	Peak amplitude (mv)	Mid-width (ms)	Repolarizing rate (mv/ms)
NT	113.6±1.7	2.5±0.1	41.6±2.2
HT	109.8±2.4	2.6±0.1	35.4±1.8 *

Table 2-2. Single AP properties in response to sustained current injection of CG neurons in NT and HT rats .

The first AP of firing in response to 100pA depolarizing current injection was measured. The AP peak amplitude and duration were not significantly different between NT and HT rats. However, the repolarizing rate was significantly lower in HT (t-test, *$p<0.05$, NT: n=89; HT: n=76).

	AHP peak amplitude (mv)	AHP Mid-width (ms)	AHP area (mv•ms)
NT	13.87±1.30	55.83±6.92	1097±150
HT	14.04±1.46	77.53±14.66 *	882±71 *

Table 2-3. Afterhyperpolarizaion of single AP of CG neurons in NT and HT rats

The AHP peak amplitude and area were measured in single APs generated by a quick and strong depolarizing current injection (2000pA, 2ms) in NT and HT rats (NT: n=26; HT: n=19). The peak amplitude of AHP was not different between NT and HT rats (unpaired t-test, $p>0.05$). The AHP area was significantly lower in HT (unpaired t-test, *$p<0.05$), whereas the AHP mid-width was significantly higher in HT (unpaired t-test, *$p<0.05$).

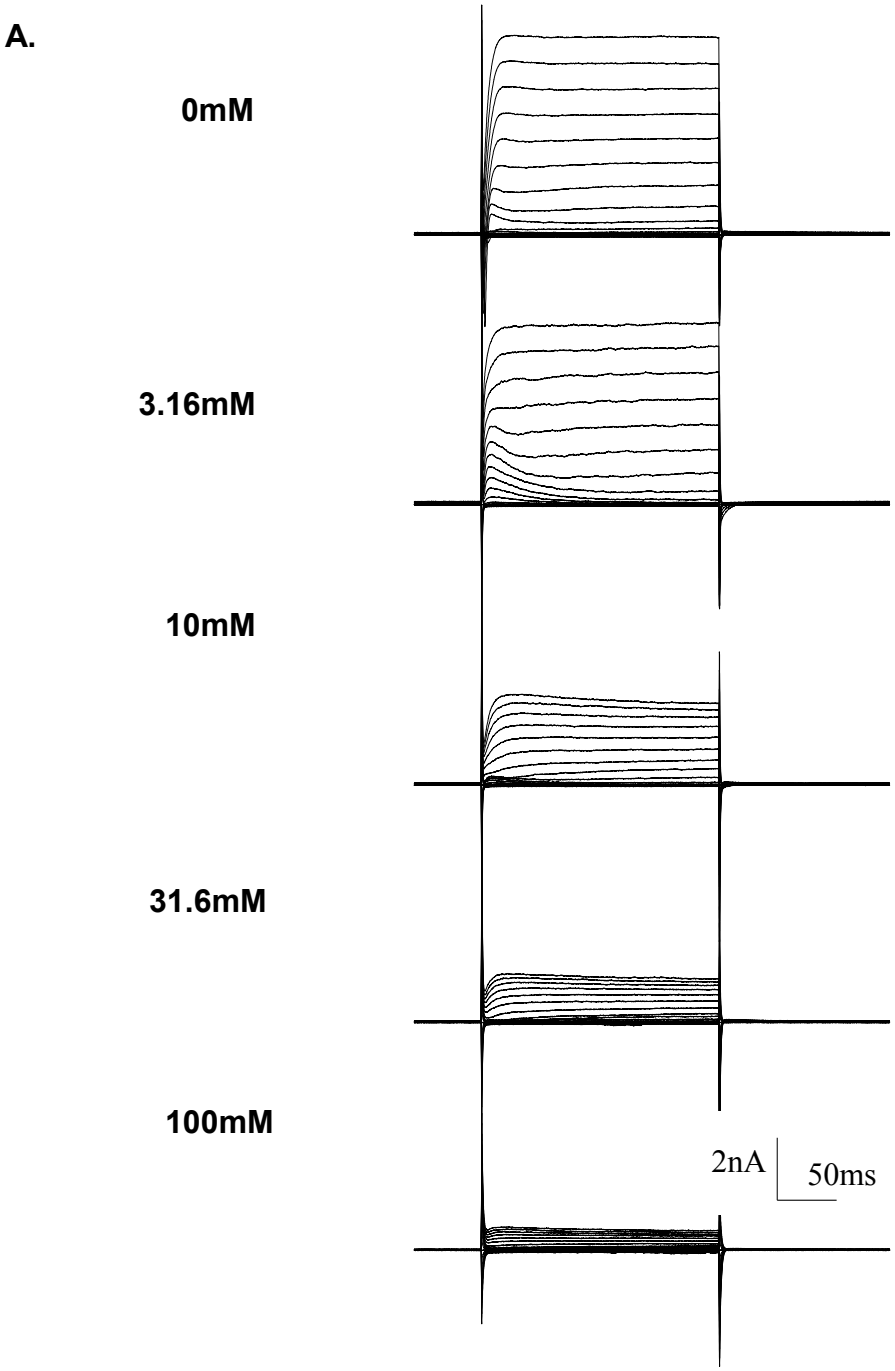


Figure 2-3. The effect of TEA on outward K^+ currents

Figure 2-3 (cont'd)

B.

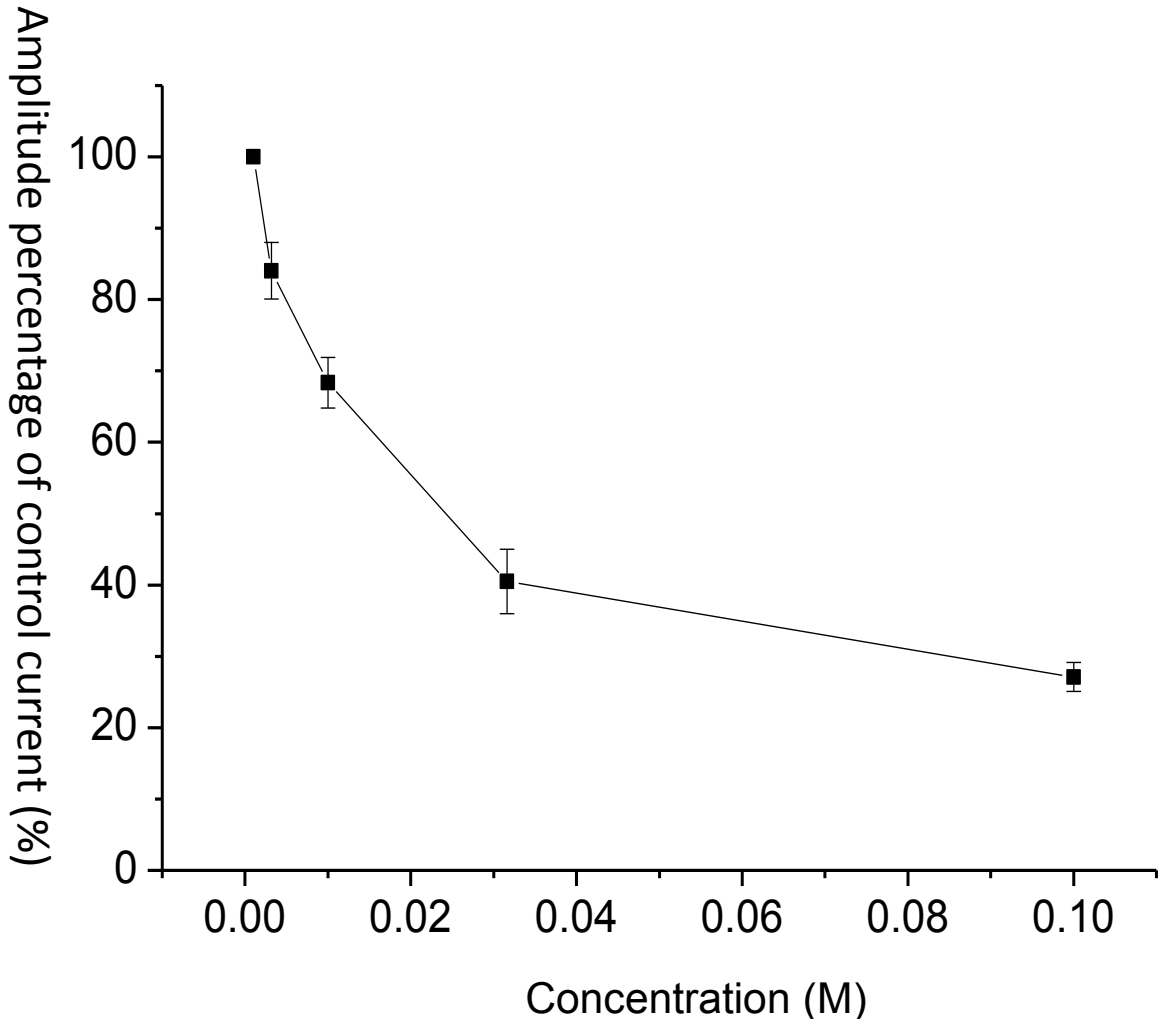


Figure 2-3 (cont'd)

Tetraethylammonium (TEA) was used to block delayed rectifier K^+ currents. The outward potassium currents were generated through series of voltage steps from -140mv to +60mv in 10mV increments at a membrane potential of -70mV in the presence of $3x10^{-7}$M Na channel blocker, tetrodotoxin (TTX). TEA decreased sustained outward potassium currents in dose-dependent manner (A). Dose-response curve was generated (n=8) (B) The EC_{50} was calculated 23.4mM.

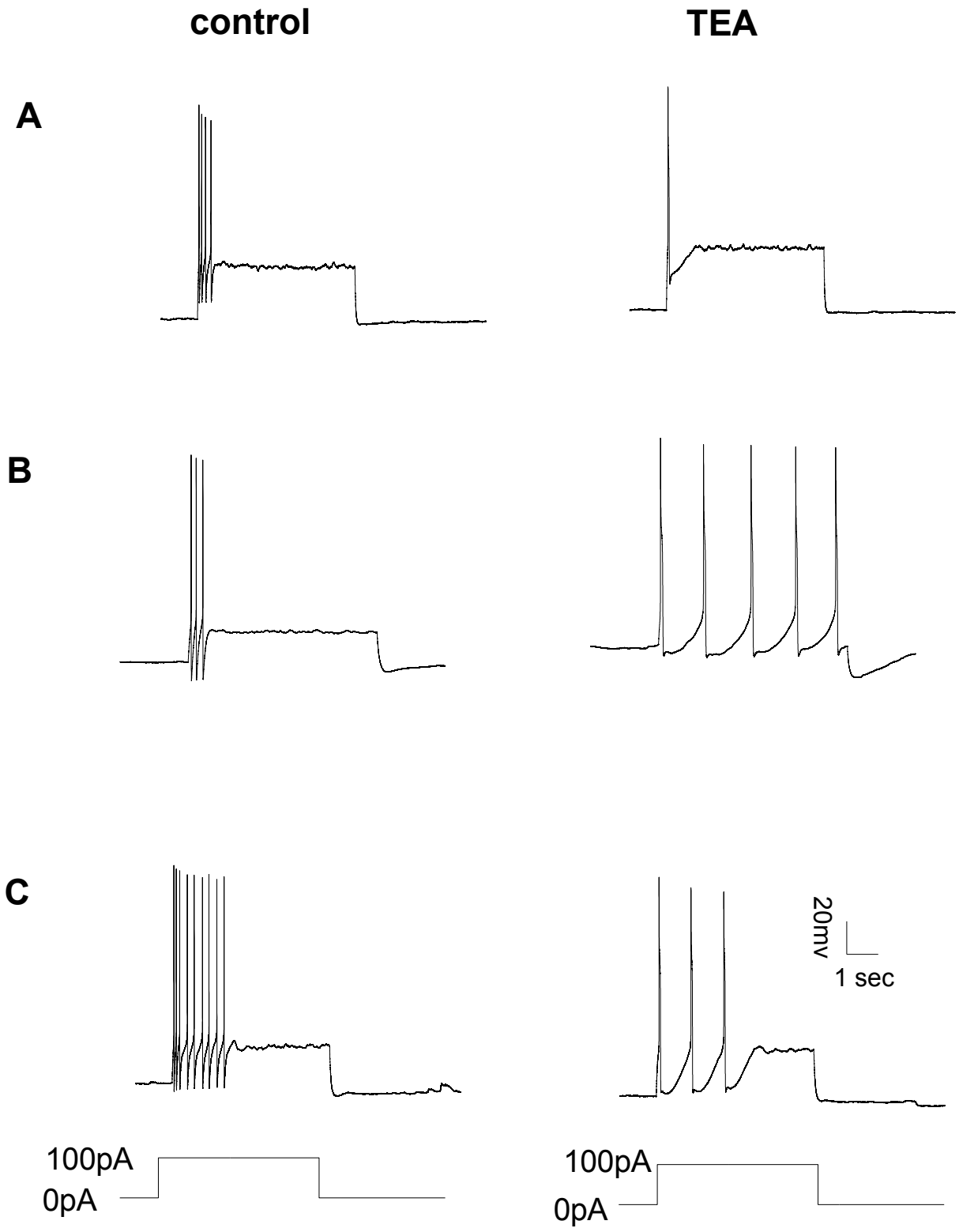


Figure 2-4. Effect of TEA on neuronal firing of dissociated CG neurons

Figure 2-4 (cont'd)

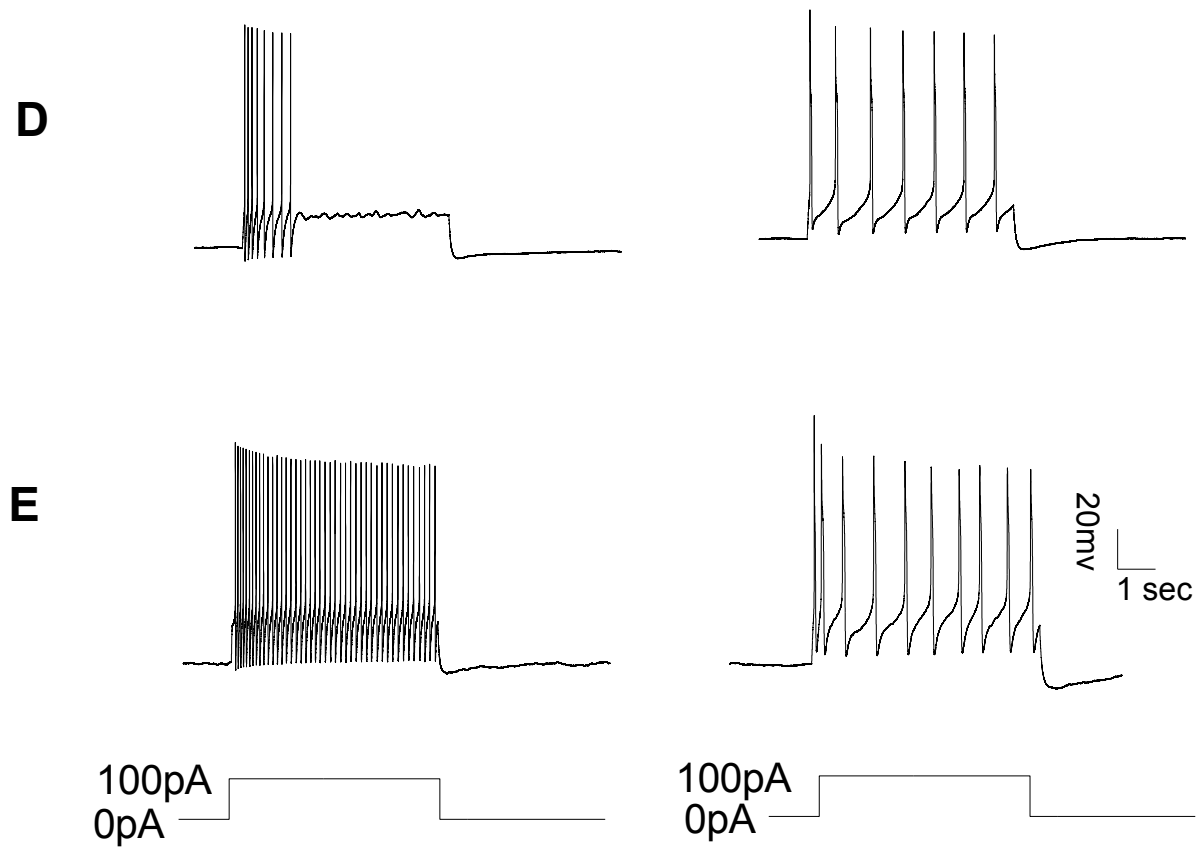


Figure 2-4 (cont'd)

Under current clamp mode, the firing frequency was decreased by TEA (23.4mM) in tonic neurons (n=4). However the firing pattern was not changed in these tonic neurons (E). The firing frequency was also decreased in phasic (n=4) and adaptive (n=4) neurons (A-D). However the firing pattern changes are variable in phasic and adaptive neurons. The firing pattern was not changed in two phasic neurons, but was changed to tonic neurons in the other two phasic neurons (A, B). The firing pattern was changed to phasic neurons in two adaptive neurons, but was changed to tonic neurons in the other adaptive neurons (C, D).

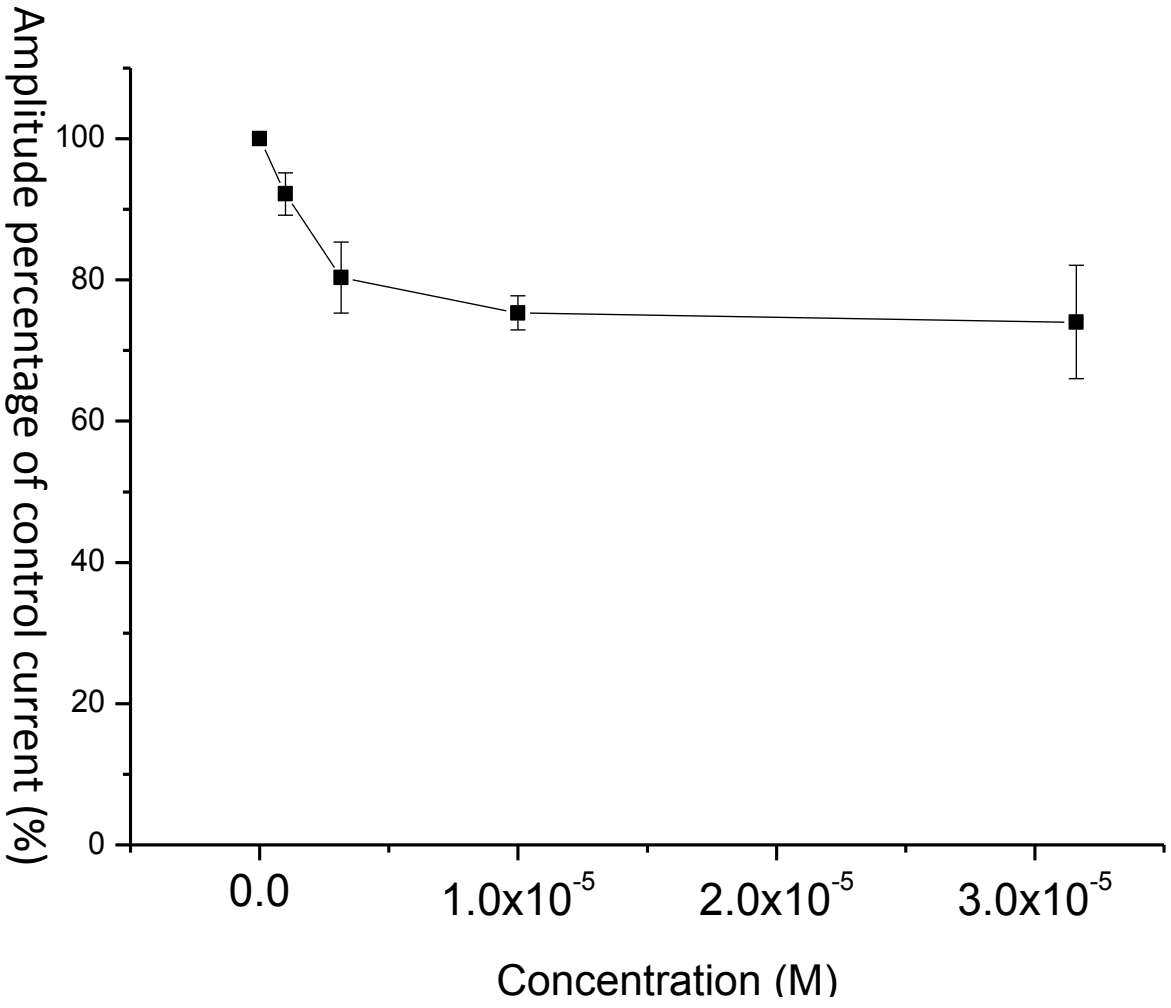


Figure 2-5. Effect of paxilline on outward K^+ currents

The outward potassium currents were generated through series of voltage steps from -120mv to +50mv in 10mV increments at a membrane potential of -70mV. The outward potassium current under +50mv voltage step was decreased by paxilline in dose-dependent manner in concentration below 10 μ M. At 10 μ M, the effect of paxilline was reached to its plateau by reducing 25% of the outward potassium current.

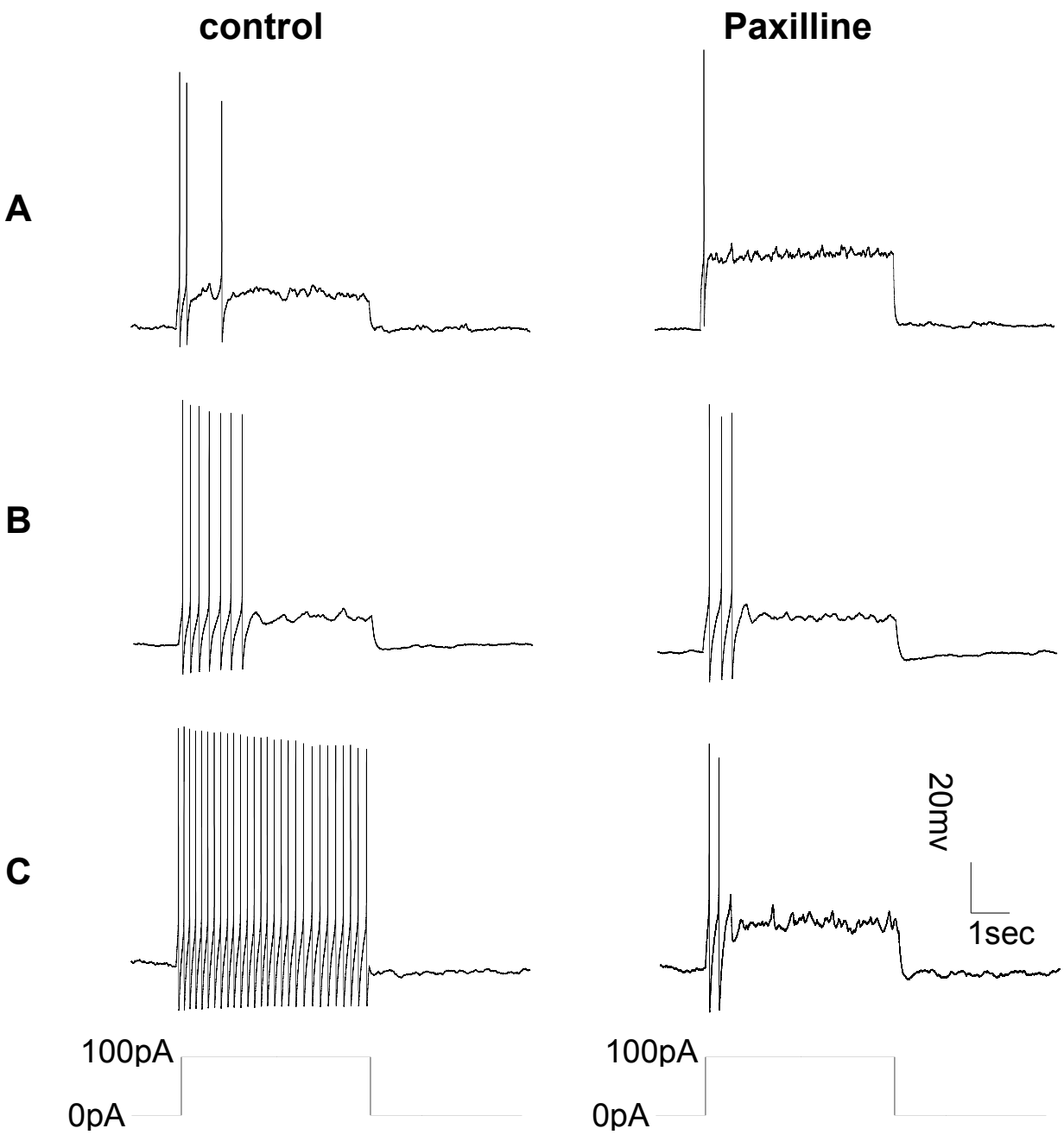


Figure 2-6. Effect of paxilline on neuronal firing of dissociated CG neurons

Paxilline (10 μM) converted adaptive and tonic neurons to phasic neurons (B,C) and decreased the number of AP phasic neurons (A) in response to a 100pA sustained depolarizing current for 5 s.

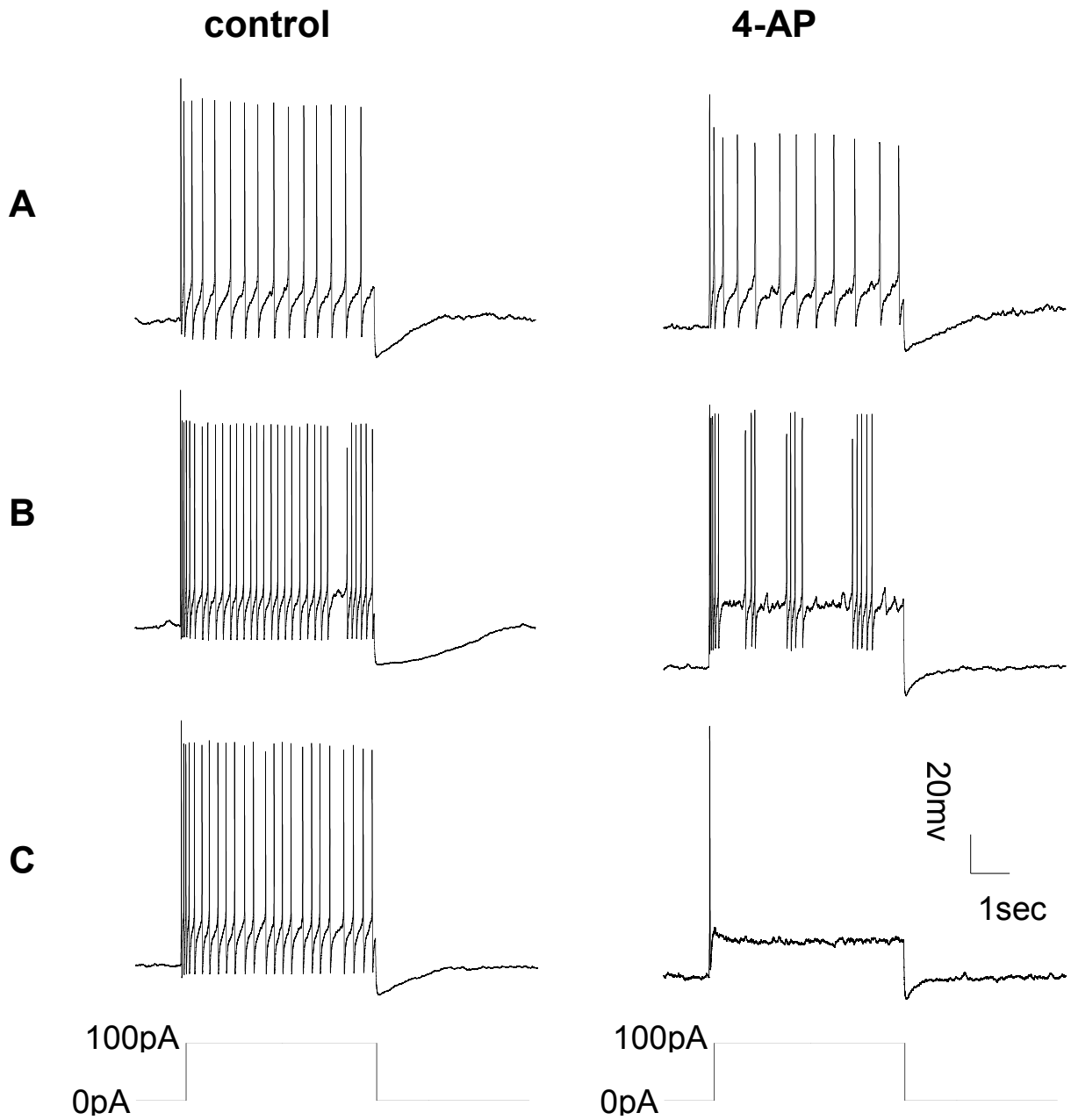


Figure 2-7. Effect of 4-AP on neuronal firing of dissociated CG neurons

In response to a sustained 100pA current injection for 5s, 4-AP (5mM) decreased firing frequency in some tonic neurons (A), converted some tonic neurons to adaptive neurons (B) and converted some tonic neurons to phasic neurons (C).

BIBLIOGRAPHY

BIBLIOGRAPHY

Beevers G, Lip GY, & O'Brien E (2001). ABC of hypertension: The pathophysiology of hypertension. *Br Med J* **322**, 912-916.

Bokvist K, Rorsman P, & Smith PA (1990). Effects of external tetraethylammonium ions and quinine on delayed rectifying K+ channels in mouse pancreatic beta-cells. *J Physiol* **423**, 311-325.

Carrier GO & Ikeda SR (1992). TTX-sensitive Na+ channels and Ca2+ channels of the L- and N-type underlie the inward current in acutely dispersed coeliac-mesenteric ganglia neurons of adult rats. *Pflugers Arch* **421**, 7-16.

Gibbins IL (1995). Chemical neuroanatomy of sympathetic ganglia. In *Autonomic Ganglia*, ed. McLachlan EM, pp. 73-121. Harwood Academic Publishers, Luxembourg.

Hille B (2001). Potassium Channels and Chloride Channels. In *Ion Channels of Excitable Membranes* pp. 131-168. Sinauer Associates, Massachusetts.

Iriuchijima J, Mizogami S, & Sokabe H (1975). Sympathetic nervous activity in renal and DOC hypertensive rats. *Jpn Heart J* **16**, 36-43.

Jubelin BC & Kannan MS (1990). Neurons from neonatal hypertensive rats exhibit abnormal membrane properties in vitro. *Am J Physiol* **259**, C389-C396.

Li G & Cheung DW (1999). Effects of paxilline on K+ channels in rat mesenteric arterial cells. *Eur J Pharmacol* **372**, 103-107.

Schlaich MP, Lambert E, Kaye DM, Krozowski Z, Campbell DJ, Lambert G, Hastings J, Aggarwal A, & Esler MD (2004). Sympathetic augmentation in hypertension: role of nerve firing, norepinephrine reuptake, and Angiotensin neuromodulation. *Hypertension* **43**, 169-175.

Sonner PM & Stern JE (2007). Functional role of A-type potassium currents in rat presympathetic PVN neurones. *J Physiol* **582**, 1219-1238.

Thompson SH (1977). Three pharmacologically distinct potassium channels in molluscan neurones. *J Physiol (Lond)* **265**, 465-488.

Wang HS & McKinnon D (1995). Potassium currents in rat prevertebral and paravertebral sympathetic neurones: control of firing properties. *J Physiol (Lond)* **485**, 319-335.

Watson R.E. & Dipette D.J. (2008). Experimental Models of Hypertension. In *Hypertension Primer* pp. 181-183. Lippincott Williams &Wilkins, Philadelphia.

Yarowsky P & Weinreich D (1985). Loss of accommodation in sympathetic neurons from spontaneously hypertensive rats. *Hypertension* **7**, 268-276.

CHAPTER 3

CALCIUM AND POTASSIUM CHANNEL CURRENTS OF DISSOCIATED CELIAC GANGLION NEURONS IN DOCA-SALT HYPERTENSIVE RATS

Abstract

In Chapter 2, it was shown that neuronal firing properties were different between celiac ganglion (CG) neurons from hypertensive (HT) rats and those from normotensive (NT) rats. The underlying ionic mechanisms have not yet been elucidated. The results in chapter two also show that the neuronal firing patterns and frequencies were changed by K^+ channel blockers in NT rats. The goal of this study is to analyze, with the voltage clamp technique, the behavior of ion channels in sufficient detail to account for the changed neuronal firing in HT. The K^+ currents concomitantly with neuronal firing and Ca^{2+} current in dissociated CG neurons from HT and NT rats were tested. The sustained outward K^+ current (I_{so}) was expressed in all neurons and the amplitude of I_{so} was significantly lower in HT rats compared to NT rats. The big-conductance Ca^{2+}-activated K^+ channel blocker, paxilline, was used as tool to separate delayed rectifier K^+ current (I_{Kv}) and big-conductance Ca^{2+}-activated K^+ current (I_{BK}) within I_{so}. The amplitudes of both I_{Kv} and I_{BK} were significantly smaller in HT rats than those in NT rats. The A-type K^+ current (I_A) was expressed in 70% of neurons from NT rats and 65% of neurons from HT rats. The peak amplitude of I_A was statistically attenuated in HT rats than that in NT rats. M-type K^+ current (I_m) was shown in 70% of neurons from NT rats and 75% of neurons from HT rats. The amplitude of I_m was not significantly different between NT and HT rats. There was no strong correlation between the amplitude of a specific K^+ current and neuronal firing pattern. The whole Ca^{2+} channel current (I_{Ca}) was also tested in neurons from NT and HT rats. There was no significant difference in the amplitude of

I_{Ca} between NT and HT rats. In summary, I_{KV}, I_{BK} and I_A were decreased, while I_m and I_{Ca} were not modulated in CG neurons from HT rats. All the changes in K^+ channel currents may be related to altered neuronal firing in HT rats and other neuronal cell activities such as Ca^{2+} influx, neurotransmitters release in hypertension.

Introduction

This study in this chapter used the same hypertension animal model as the one used in chapter 2. In Chapter 2, I found that the neuronal firing pattern distribution of dissociated CG neurons from DOCA-salt hypertensive (HT) rats was significantly different than those from normotensive control (NT) rats. There were more highly accommodating phasic neurons and fewer slightly accommodating adaptive and not accommodating tonic neurons in HT rats than in NT rats. Furthermore, the neuronal firing frequency was lower in tonic neurons from HT rats. These differences found between HT and NT rats were shown similar to those changes after and before the treatment of K^+ channel blockers, TEA, paxilline, and 4-AP in NT rats.

Voltage-dependent ion-channel currents in a neuron are involved in determining its action potential (AP) discharge and firing pattern (Hille, 2001a). The depolarizing phase of the AP results from activation of voltage-dependent Na^+ and Ca^{2+} channel currents, while the time course of repolarization is determined by a combination of Na^+, Ca^{2+} channel current inactivation and K^+ channel currents

activation. In CG neurons, the inward current is contributed by TTX-sensitive Na^+ channels and high voltage-activated Ca^{2+} channels (Carrier & Ikeda, 1992); the outward current is composed of A-type K^+ current (I_A), delayed rectifier K^+ current (I_{Kv}), calcium-activated K^+ currents (I_{BK}), and M-type K^+ current (I_m) (Vanner *et al.*, 1993). I_{BK} include big-conductance calcium-activated K^+ current (I_{BK}) and small-conductance calcium-activated K^+ currents (I_{SK}) (Hille B 7717). With the exception of Na^+ currents and I_{SK}, all other currents were evaluated in this study.

From previous study, I_A was found to be elevated in sympathetic ganglionic neurons from genetic hypertensive rats (Robertson & Schofield, 1999). Other types of ion channel currents have not been studied in sympathetic ganglia from hypertensive rats. However K^+ channel currents in vascular smooth muscle cells have been extensively tested in hypertensive animal models. In DOCA-salt hypertensive rats, I_{Kv} is smaller in HT rats compared to control rats in the smooth muscle cells from the small artery (Martens & Gelband, 1996). The changes of I_{BK} in smooth muscle cells are varied in different hypertensive animal models. It is decreased in N^{ω}-nitro-L-arginine induced hypertension and pulmonary hypertension (Bratz *et al.*, 2005;Muraki *et al.*, 2001). However, it is increased in genetic hypertensive rats and angiotensin Ⅱ and aldosterone-salt hypertensive animal model (Cox *et al.*, 2003;Liu *et al.*, 1995;Liu *et al.*, 1997). In addition to the changes in K currents, intracellular calcium is increased in smooth muscle cells from DOCA-salt hypertensive rats (Aguas & Nickerson, 1983). These findings of vascular smooth muscle cells in hypertension give the possibility that the levels of K^+ and Ca^{2+}

channel currents may also be changed in CG neurons from HT rats. Their modulations might contribute to altered membrane firing activities in dissociated CG neurons from HT rats found in Chapter 2.

Materials and Methods

Animals

The number of animals used in the experiments, as well as the animals themselves, was conformed to the National Institutes of Health Guide for the Care and Use of Laboratory Animals. The study was approved by the Animal Use and Care Committee of Michigan State University. DOCA-salt hypertensive rats and normotensive control rats were prepared (details seen in Chapter 2).

Tissue collection

CG was dissected out from the animals (details seen in Chapter 2).

Cell culture

The harvested celiac ganglia were dissociated enzymatically and plated in glass bottom culture dishes for electrophysiological studies (details seen in Chapter 2).

Whole-cell patch clamp

The dissociated CG neurons were tested under whole-cell patch clamp technique (details seen in Chapter 2). Access resistance (R_a) was monitored at regular intervals. The ruptured neurons with R_a higher than 20MΩ, were not accepted for data acquisition. The series resistance and cell capacitance were electronically compensated. The experiments were performed at room temperature

(20-23℃). When measuring outward K^+ currents, the neurons were kept in voltage clamp mode after membrane rupture. K^+ currents were induced in neurons held at a membrane potential of -70mv through a series of voltage steps from -140mv to +60mv (from -120mv to +50mv in some cases) in 10mv increments for 200 ms per step in the presence of $3x10^{-7}$M TTX.

Solutions for measuring K^+ currents: The extracellular solution contained 120 NaCl, 4.7 KCl, 2.5 $CaCl_2$, 1.2 $MgCl_2$, 1.2 $NaHPO_4$, 25 $NaHCO_3$, and 11 Glucose in mM which was equilibrated with a 95% O_2-5%CO_2 mixture. Using a drug administration capillary tube, which was positioned 500-800 μM from the patched neurons $3x10^{-7}$M TTX was applied by gravity. The intracellular solution contained 150 K Acetate, 3 $MgCl_2$, 40 HEPES, 10 ATP, and 2.5 GTP in mM. In the experiments to separate sustained K^+ currents, 10 μM paxilline in TTX-containing extracellular solution was applied through the drug administration capillary tube.

Solutions for measuring Ca^{2+} currents: The extracellular solution included 97 NaCl, 4.7 CsCl, 5.0 $CaCl_2$, 1.2 $MgCl_2$, 1.2 NaH_2PO_4, 25 $NaHCO_3$, 11 Glucose and 20 TEA in mM equilibrated with a 95% O_2-5%CO_2 mixture. The intracellular solution included 160 CsCl Acetate, 2 $MgCl_2$, 10 HEPES, 10 EGTA, 10 ATP, and 2.5 GTP in mM. $3x10^{-7}$M TTX was also applied the same way as measuring K^+ currents.

Data acquisition

The cell capacitance and cell input resistance (R_{in}) were measured automatically by software Clampex described in Chapter 2. Before TTX application, neuronal firing was recorded in current-clamp mode by injecting current step from -

0pA to 100pA for 5 s. Afterwards it was switched back to voltage-clamp mode to test different K^+ currents under specific protocol with TTX applied. 10uM paxilline was applied for separating I_{KV} and I_{BK}. Neuronal firing was not recorded before Ca^{2+} current measurement because extracellular and intracellular solutions for measuring Ca^{2+} current is inappropriate for testing neuronal firing. The amplitudes of K^+ currents were measured by Clampfit software.

Statistical analysis:

All values were presented as mean ± SEM and n refers to the number of neurons. Less than 4 neurons were recorded per animal. Statistical significance between means was determined using two-tailed unpaired t-test or two-way ANOVA. The statistical significance was set for $P<0.05$.

Results

The characteristic outward K^+ currents studied were described as an initial transient outward I_A, followed by a I_{SO} under voltage steps (from -140mv to 60mv at 10mv step, 200ms) (Figure. 8A). The neurons were held at -70mv in voltage-clamp mode. The neuronal firing was also recorded in the same neuron under a constant depolarizing current (100pA, 5sec) injection in current-clamp mode. In Chapter 2, the CG neurons were divided to phasic, adaptive or tonic neurons according to different neuronal firing patterns induced by 100pA current injection. The same classification in neuronal firing pattern was used in this study.

I_{SO} of CG neurons in HT and NT rats

I_{SO} was detected in all the neurons. It was activated at about -30mv. The relationship between the amplitudes of I_{SO} and voltage steps was generated as current-voltage curve (I-V curve). I-V curves of phasic, adaptive and tonic neurons overlapped in NT and HT rats (Figure. 3-1, B, C). However, in the range of 20-60 mV the amplitude of I_{SO} was significantly lower in neurons from HT rats by 24-26% compared to NT rats (NT n=32, HT n=40, two-way ANOVA, $p<0.05$) (Figure. 3-1, D). In order to resolve the component currents of I_{SO}, a selective BK channel blocker, paxilline was used to separate these two K^+ currents in I_{SO}. According to paxilline's dose-response curve shown in chapter 2, 10μM was used in this study because it is the lowest concentration required for the maximum effect. The amplitudes of I_{SO} before and after paxilline application were recorded under voltage steps (from -120mv to 50mv, at 10mv step 200ms) (Figure 3-2, A). The amplitudes of I_{SO} were decreased by paxilline both in NT and HT rats with the percentages of 15 and 11 respectively. I_{SO} before paxilline application, which indicates the summation of I_{KV} and I_{BK}, was significantly lower in CG neurons from HT rats compared to those from NT rats (NT n=18, HT n=18, two-way ANOVA, $p<0.05$) (Figure. 3-2, B). This result was consistent with previous data (Figure.3-1, C). I_{SO} after paxilline application, which indicates only I_{KV}, was also significantly lower at 20-60 mV depolarizations in CG neurons from HT rats compared to NT rats (NT n=18, HT n=18, two-way ANOVA, $p<0.05$) (Figure. 3-2, C). The current obtained by subtracting I_{SO} after paxilline application from I_{SO} before paxilline application, which indicates I_{BK}, was significantly smaller at 20-60 mV depolarizations in HT rats compared to NT rats (Figure. 3-2, D).

All these results demonstrated both I_{KV} and I_{BK} in I_{so} were decreased in CG neurons from HT rats.

I_A of CG neurons in HT and NT rats

I_A was expressed in 70% of neurons from NT rats and 65% of neurons from HT rats. It rapidly activated and moderately rapidly inactivated shown in the beginning of the outward K^+ current. I_A was separated from total outward current by subtracting current generated by voltage step (from -40mv to 30mv) from current by the step (from-70mv to 30mv) in NT and HT rats(Figure. 3-3, A). The peak amplitude of I_A from all neurons expressing I_A was statistically smaller in HT rats than N NT rats (NT n=16; HT n=13, *p<0.05 t-test) (Figure. 3-3, D). I_A was exhibited in all types of neuronal firing patterns including phasic, adaptive and tonic neurons. Furthermore the amplitude of I_A was not correlated to neuronal firing patterns in NT rats (n=22, p>0.05) (Figure. 10B) or HT rats (n=20, p>0.05) (Figure. 3-3, C).

M-type K^+ current of CG neurons in HT and NT rats

M-type K^+ current (I_m) was shown in 70% of neurons from NT rats and 75% of neurons from HT rats. The M-type potassium channel current (I_m) was tested as a slowly decaying outward current from the step (-30mv to -70mv, held at -70mv) (Figure. 3-4, A). Similar to I_A, I_m was shown in all types of neuronal firing patterns. The amplitude of I_m was not correlated with neuronal firing patterns in NT rats (n=24, p>0.05) (Figure. 3-4, B), or HT rats (n=20, p<0.05) (Figure. 3-4, C). The amplitude of I_m from all neurons expressing I_m was not significantly different between NT and HT rats (Figure. 3-4, D).

Voltage-gated Ca^{2+} current of CG neurons from HT and NT rats

The high-voltage activated calcium channel current (I_{Ca}) was generated under voltage steps (from -70mv to 60mv, at 10mv step 200ms) with Na^+ channel blocker TTX and K+ channel blocker TEA (Figure. 3-5, A). The amplitudes of ICa plateau was plotted with voltage steps as I-V curves in NT and HT rats. The current activated at about -20mv and reached its maximum amplitude close to -10mv. The mean reversal potential estimated by interpolation of linear portion of the I-V curves was around 45mv. The amplitude of ICa was not significantly different in CG neurons from HT and NT rats (n=17, 2-way ANOVA $p>0.05$) (Figure. 3-5, B).

Discussion

In this study, K^+ and Ca^{2+} currents were examined in dissociated CG neurons from NT and HT rats. The amplitudes I_{Kv}, I_{BK} and I_A were significantly lower in CG neurons from HT rats compared to NT rats. However, I_m and I_{Ca} were not statistically different between NT and HT rats. There was no prominent correlation between neuronal firing patterns and the amplitude of a specific K^+ current. These results indicated that attenuated K^+ currents could work together not alone to lead to neuronal firing pattern changes found in CG neurons from HT rats.

This study showed I_{Kv}, I_{BK} and I_{Ca} were expressed in all CG neurons. I_A, and I_m were expressed in about 70% of neurons associated with all types of neuronal firing patterns. However another study showed that the M-current was only expressed in all phasic neurons (Wang 1995)

The decreased I_{KV} may contribute to decreased neuronal firing frequency in HT. It was shown in Chapter 2 that delayed rectifier K^+ channel (Kv) blocker, Tetraethylammonium (TEA) decreased the firing frequency of tonic neurons in NT rats. Previous studies also showed that TEA increases the AP duration and decreases neuronal firing frequency (Yoshida *et al.*, 2007). Fewer tonic neurons were shown in cultured sympathetic neurons expressing dominant negative Kv channel subunits (Malin & Nerbonne, 2002), which indicates the decreased I_{KV} may also lead to changed firing pattern with fewer tonic CG neurons in HT rats.

The effect of I_{BK} on neuronal firing can be explained in two ways. In one way decreased I_{BK} may decrease AP frequency and lead to more accommodating firing pattern. Since BK channel activation is involved in AP repolarization, the attenuated I_{BK} would elongate AP duration and increase calcium influx resulting in more SK channels opening. SK channels contribute to small and long-lasting hyperpolarizing currents (Faber & Sah, 2003) and dampen the neuronal firing (Greffrath *et al.*, 2004;Wolfart *et al.*, 2001). Also elongated AP and attenuated afterhyperpolarization keep more Na^+ channels in an inactivated state, which resulting fewer Na^+ channels available for activation in next AP (Lovell *et al.*, 2004).

Another way of explaining the effect of BK channels on HT neurons is centered on its effect on AHP which prevents to generate another AP. Decreased I_{BK} would attenuate fast AHP and lead to increased AP frequencies. In chapter 2, I found BK channel blocker, paxilline, converted tonic neurons to phasic neurons. This result indicates the effect of I_{BK} on neuronal firing may be mainly caused by increased SK

channel opening and Na^+ channel inactivation. The SK channel blocker (Greffrath *et al.*, 2004;Wolfart *et al.*, 2001) increases the firing frequency of tonic and phasic neurons sympathetic ganglionic neurons (Wang & McKinnon, 1995). Unfortunately, in my study I didn't address the SK current, which is much smaller than I_{BK}, consistent with the immeasurable change in K^+ current by SK channel blocker, apamin (Data not shown). Local minor changes in intracellular Ca^{2+} levels can alter the gating of Ca^{2+} activated K^+ channels. Ca^{2+} channels will decrease action potential frequencies via increased repolarization through BK channels and increased afterhyperpolarization through BK and SK channels(Vanner *et al.*, 1993). Although the whole voltage-gated calcium current was not found to be different in HT rats in this study, it would be beneficial to explore further the different subtypes of voltage-gated Ca^{2+} channel (L-, N-, P/Q, R ,T type) in hypertension.

As for I_A, decreased I_A could increase firing frequency by attenuating a damping effect on the developing depolarization between two adjacent APs (Hille, 2001b). This effect is proved by A-type K^+ channel blocker, 4-Aminopyridine (4-AP) in paraventricular nucleus neurons (Sonner & Stern, 2007). However, I_A was also related with AP duration other than I_{KV}, I_{BK} (Louise ES, 2003, Nerbonne, 1989 4749 /id). Decreased I_A would cause increased AP duration and more Ca^{2+} influx which activates more SK channels leading to decreased neuronal firing. Overexpression of A-type K^+ channels in neurons from superior cervical ganglion, a paravertebral sympathetic ganglion, increases the percentage of tonic neurons(Malin & Nerbonne, 2001). In Chapter 2, 4-AP converted tonic neurons to adaptive or phasic neurons in

CG neurons.

In summary, attenuated I_{KV} I_{BK} and I_A in CG neurons found in this study may all contribute to the altered neuronal firing pattern seen in hypertension. From the functions of different K^+ currents, phasic-type neurons are predicted to exhibit Ca^{2+}-activated K^+ currents or M-currents, whereas tonic type neurons express a A-type K^+ current (Hille 1992). However my study showed there is no relation between a specific neuronal firing pattern and the amplitude of a specific K^+ current, and the specific K^+ current coexists with all types of neuronal firing patterns. This indicates the specific firing pattern is contributed by a combination of several K^+ currents.

Other than neuronal firing, K^+ and Ca^{2+} channels are also an important determinant of cellular activities including neural signal transduction, transmitter release and gene transcription (Hille, 2001b). Decreased K^+ currents may contribute to increased neurotransmitter release from sympathetic nerve terminal in hypertension.

APPENDIX

A.

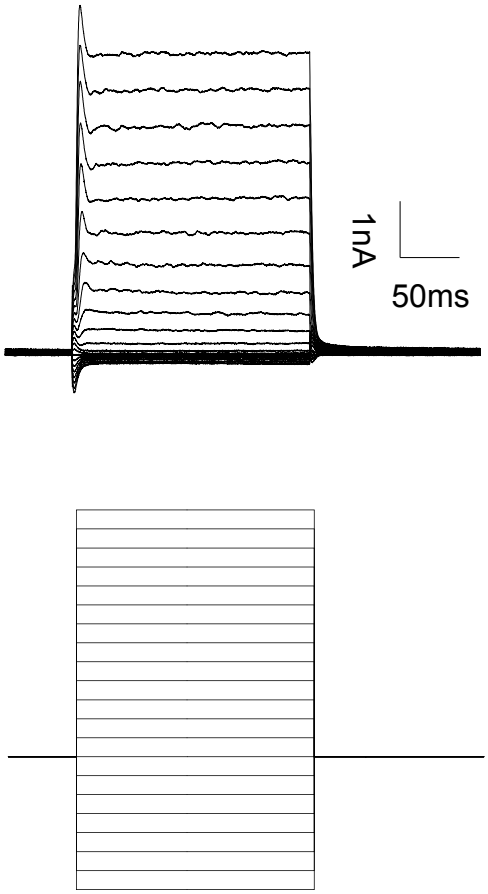
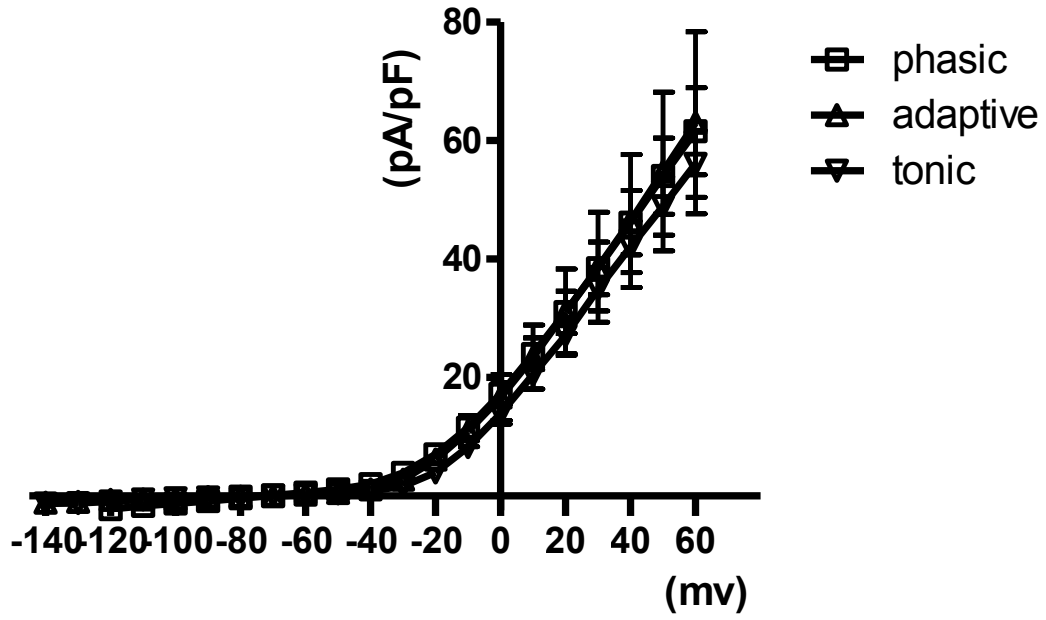


Figure 3-1. Sustained outward K^+ currents of CG neurons in HT and NT rats

Figure 3-1 (cont'd)

B.



C.

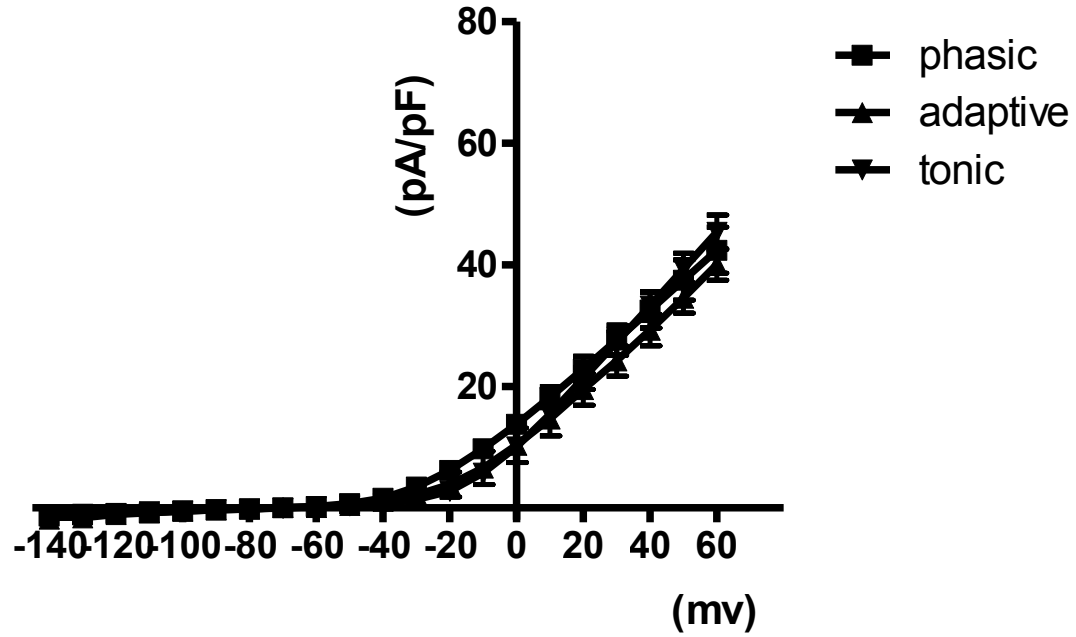
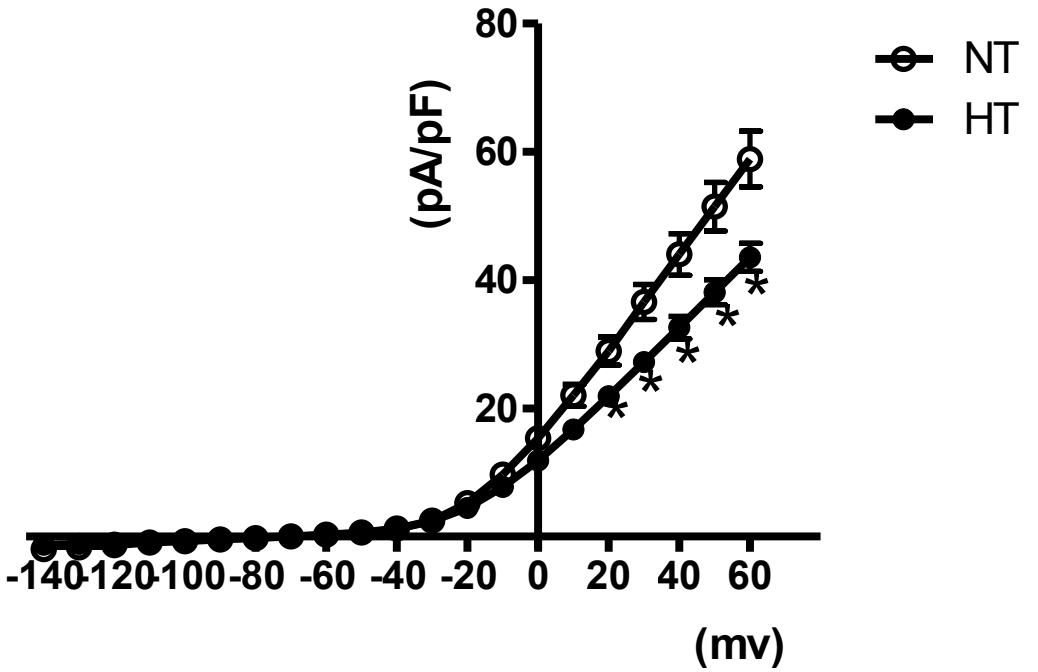


Figure 3-1 (cont'd)

D.



The sustained outward K^+ currents (I_{so}) were induced by voltage steps (from -140mv to 60mv, at 10mv step 200ms). A sample trace and protocol is shown in (A). The amplitude of I_{so} was not significantly different among phasic, adaptive and tonic neurons both in NT (phasic n=10, adaptive n=5, tonic n=11) and HT rats (phasic n=19, adaptive n=3, tonic n=18) ($p>0.05$) (B, C). However the amplitude of I_{so} was significantly decreased in HT rats compared to NT rats (NT n=32, HT n=40 * $p<0.05$) (D). Data were analyzed by two-way ANOVA followed by Bonferroni post-test.

A.

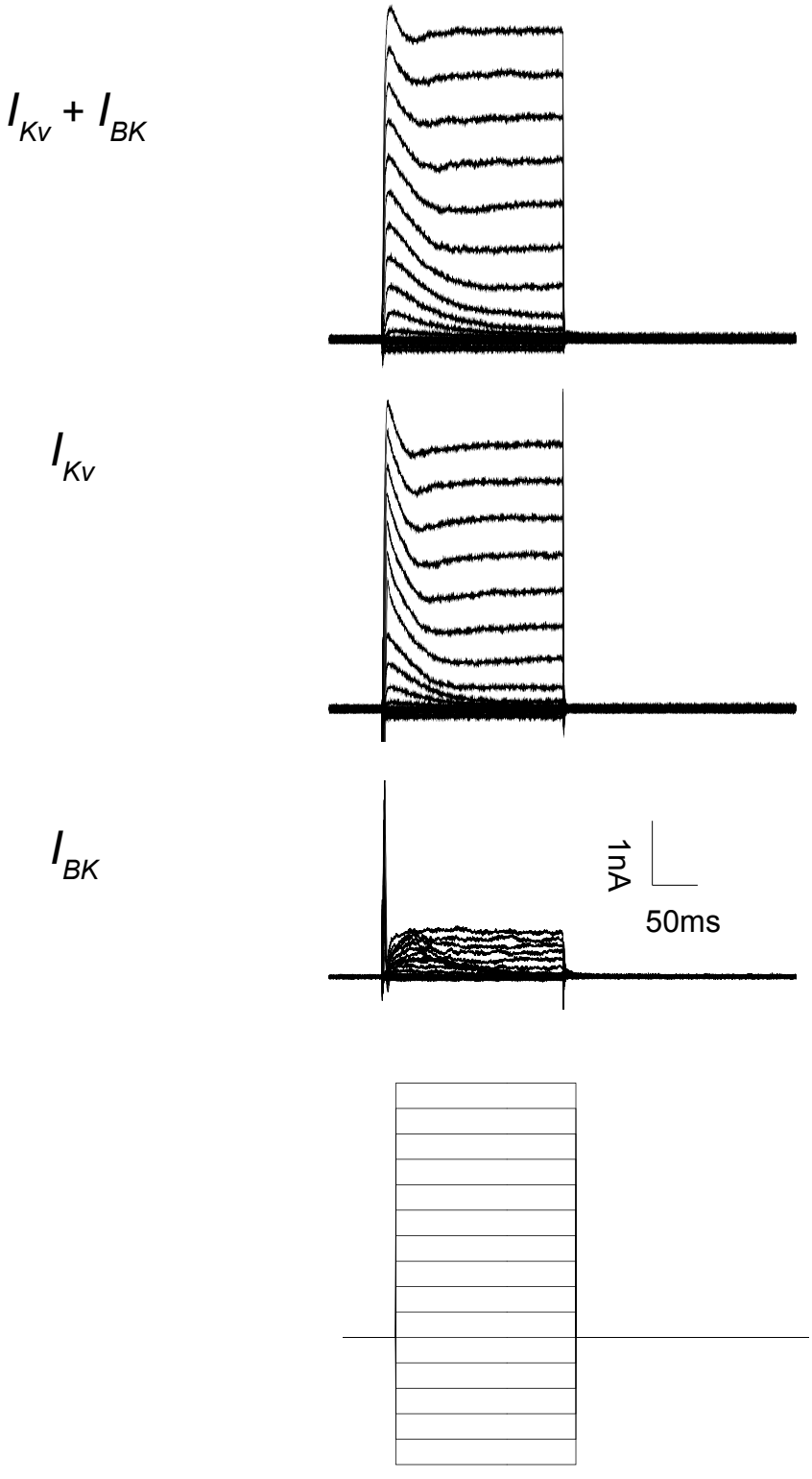
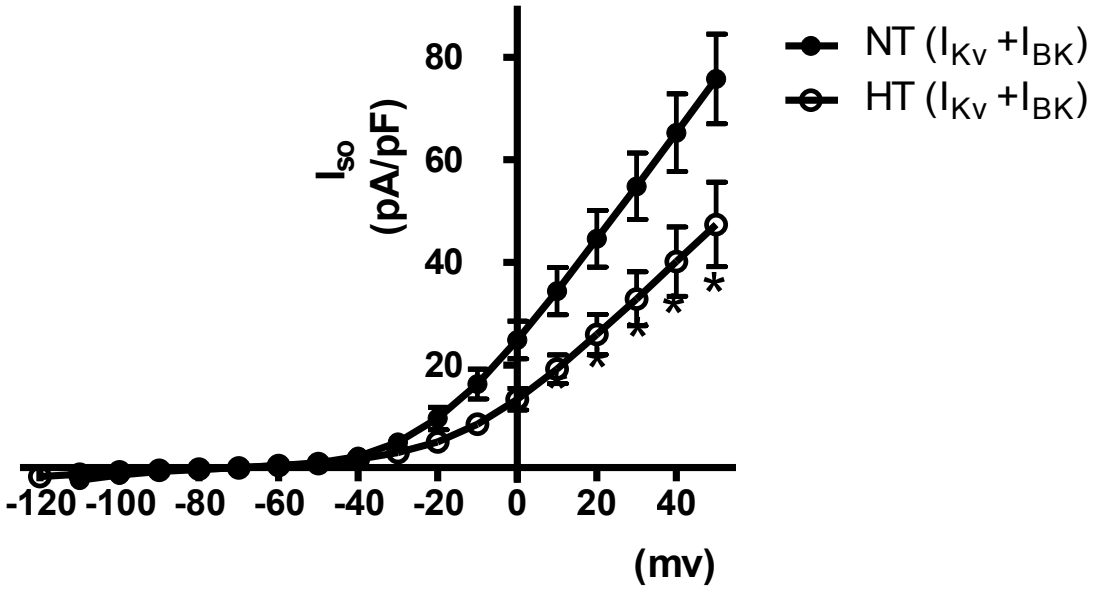


Figure 3-2. *I_{Kv} and I_{BK}* of CG neurons in HT and NT rats

Figure 3-2 (cont'd)

B.



C.

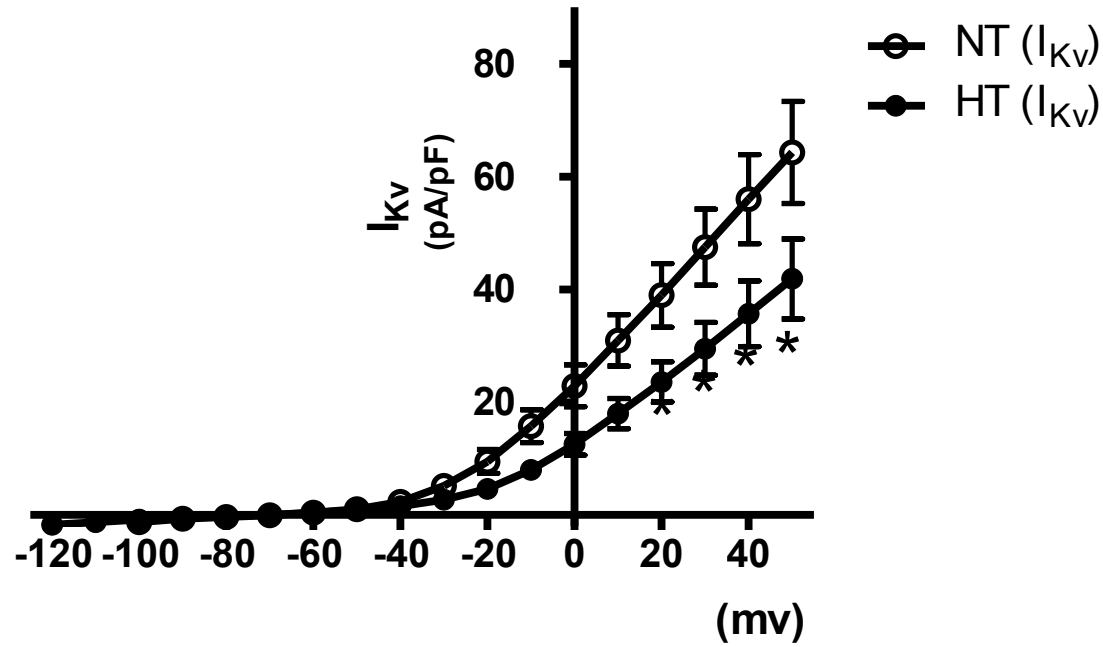
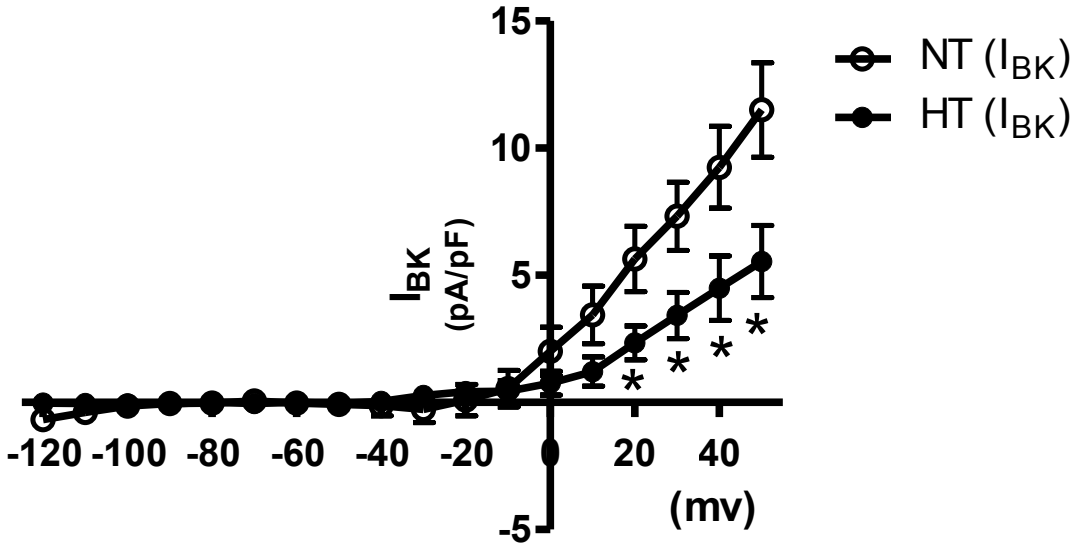


Figure 3-2 (cont'd)

D.



10 μM paxilline was used to separate I_{KV} and I_{BK} in I_{so} under voltage steps (from -140mv to 60mv, at 10mv step 200ms). The characteristic current traces were shown in (A). I_{so} before paxilline application equals to I_{KV}+ I_{BK}. I_{so} after paxilline application was I_{KV}. The current obtained by subtracting I_{so} after paxilline application from I_{so} before paxilline application is the I_{BK}. All of them were significantly smaller in HT rats compared to NT rats (n=18, * $p<0.05$, two-way ANOVA followed by Bonferroni post-test) (B, C, D).

A.

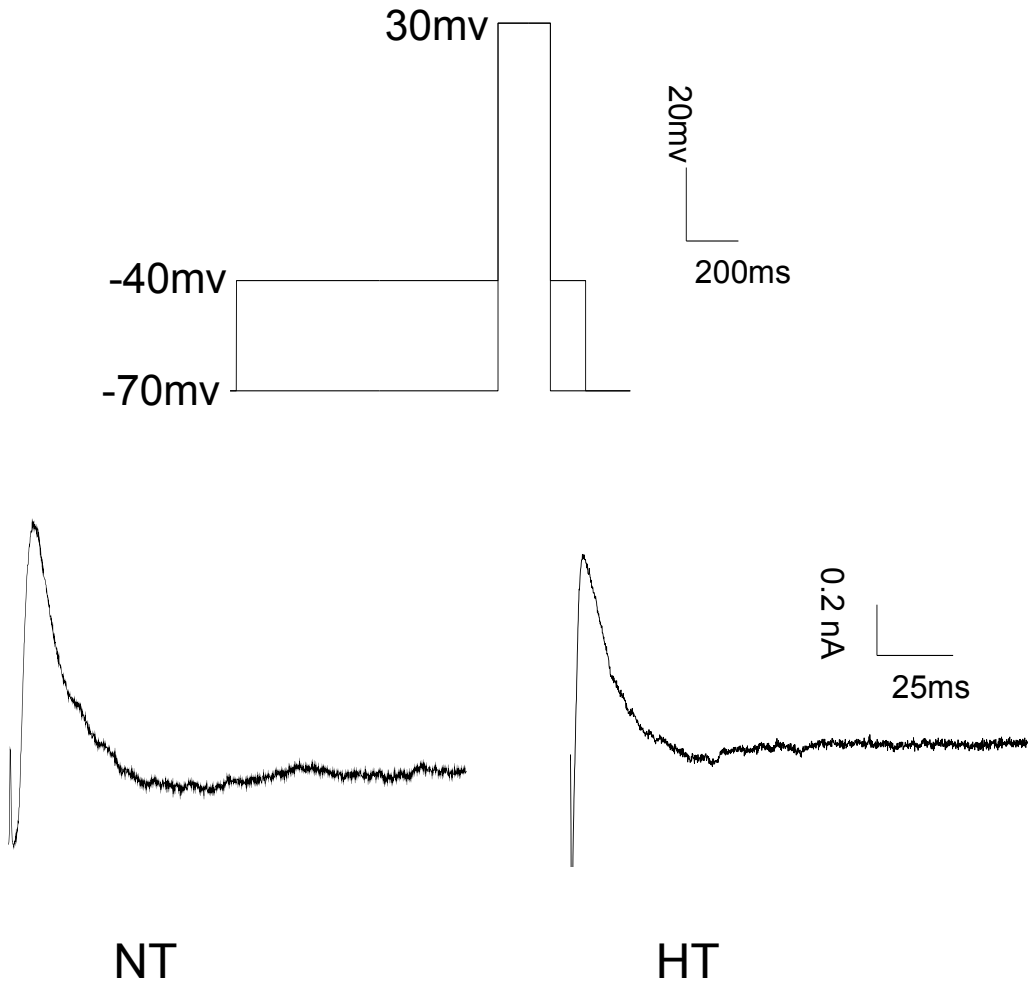
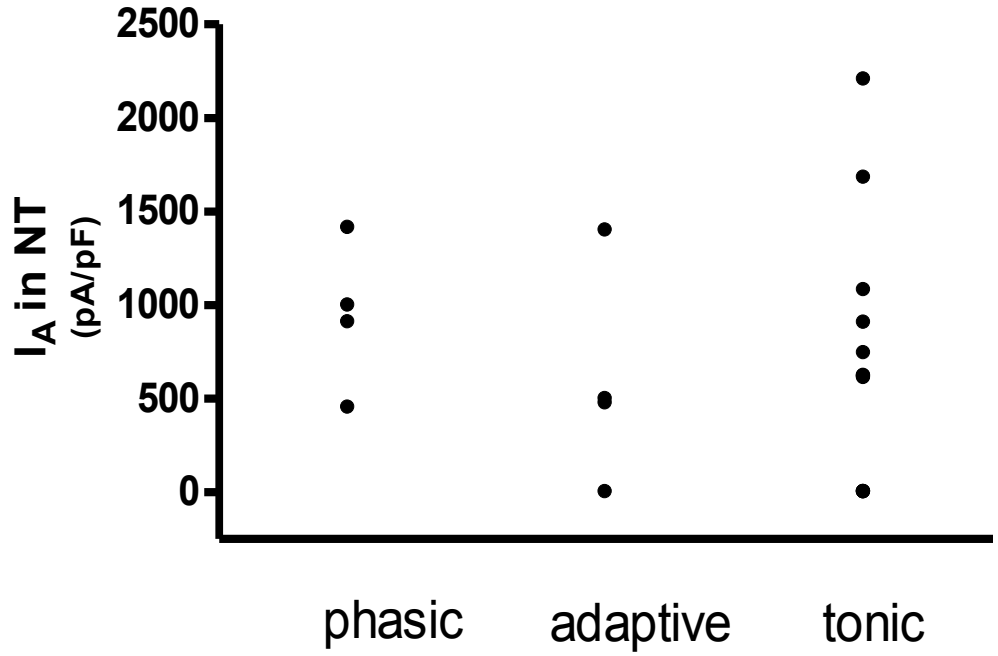


Figure 3-3. I_A of CG neurons in HT and NT rats

Figure 3-3 (cont'd)

B.



C.

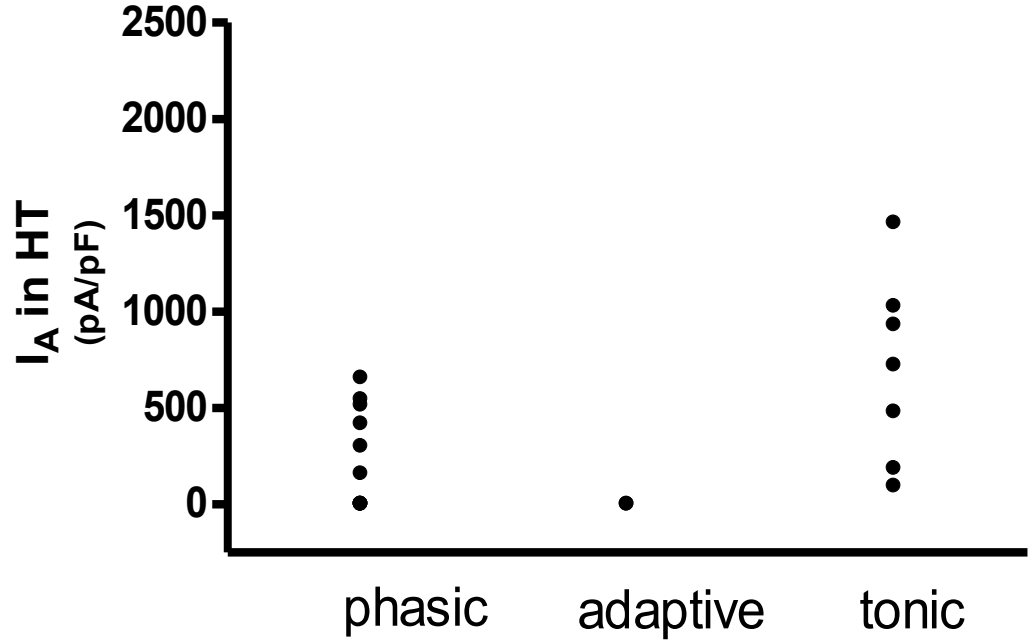
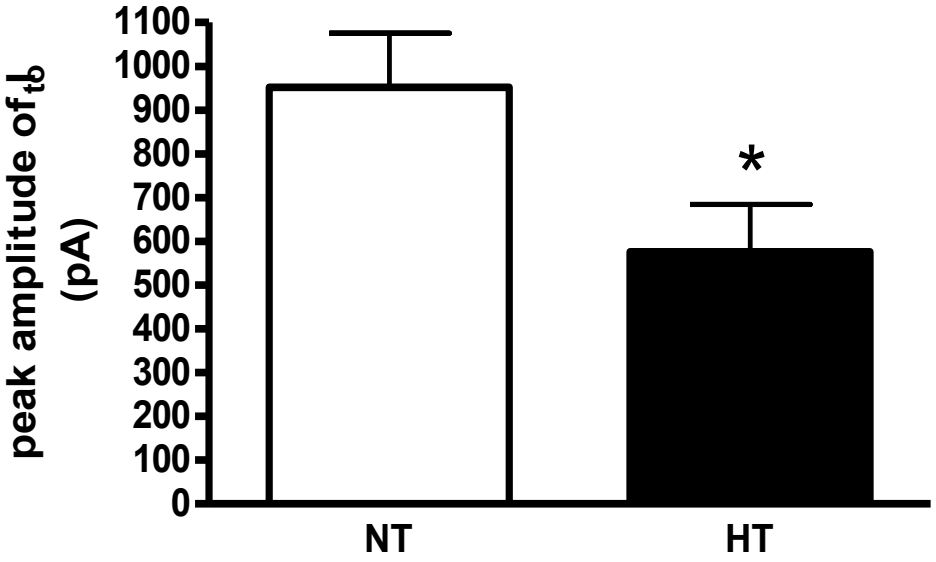


Figure 3-3 (cont'd)

D.



I_A was measured as the current subtraction of voltage step (from -40mv to 30mv) from the step (from-70mv to 30mv) in NT and HT rats (A). The peak amplitude of I_{to} was not correlated to neuronal firing patterns in NT rats (n=22, p>0.05) or HT rats (B, C), The peak amplitude of I_A from all neurons expressing I_A was statistically lower in HT rats than N NT rats (NT n=16; HT n=13, p<0.05 t-test) (D).

A.

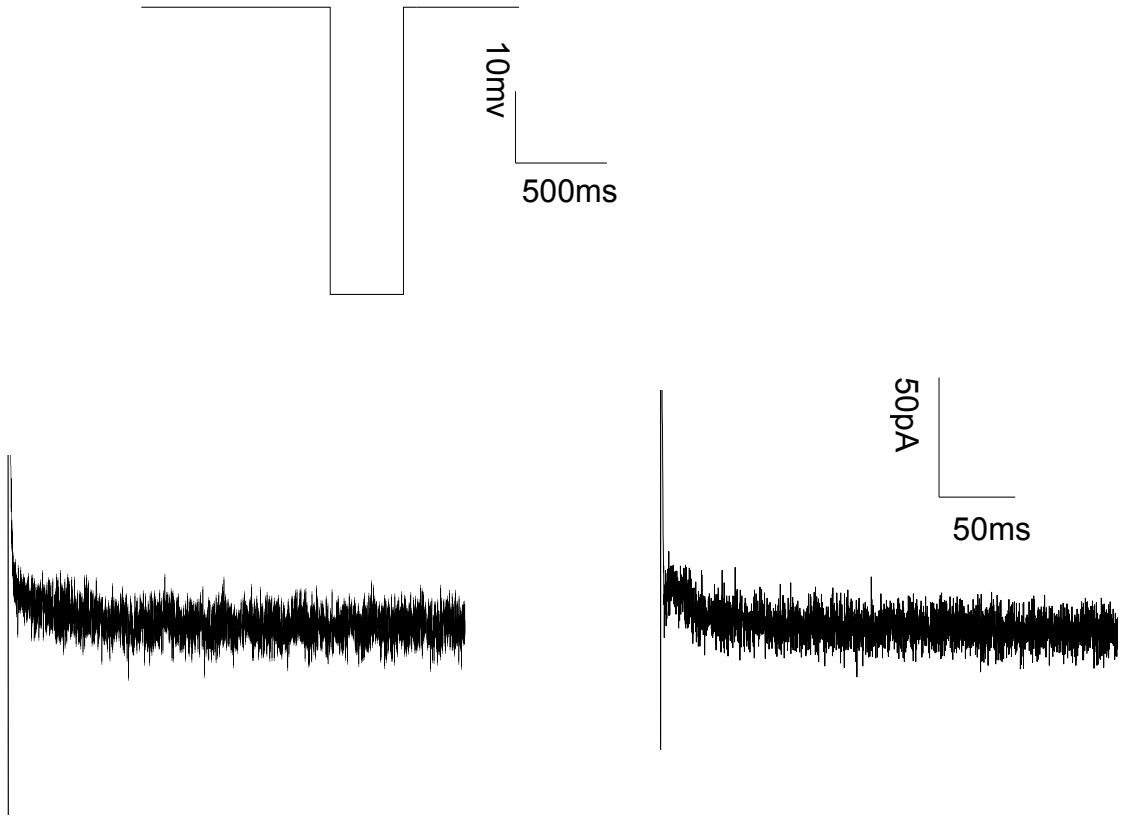
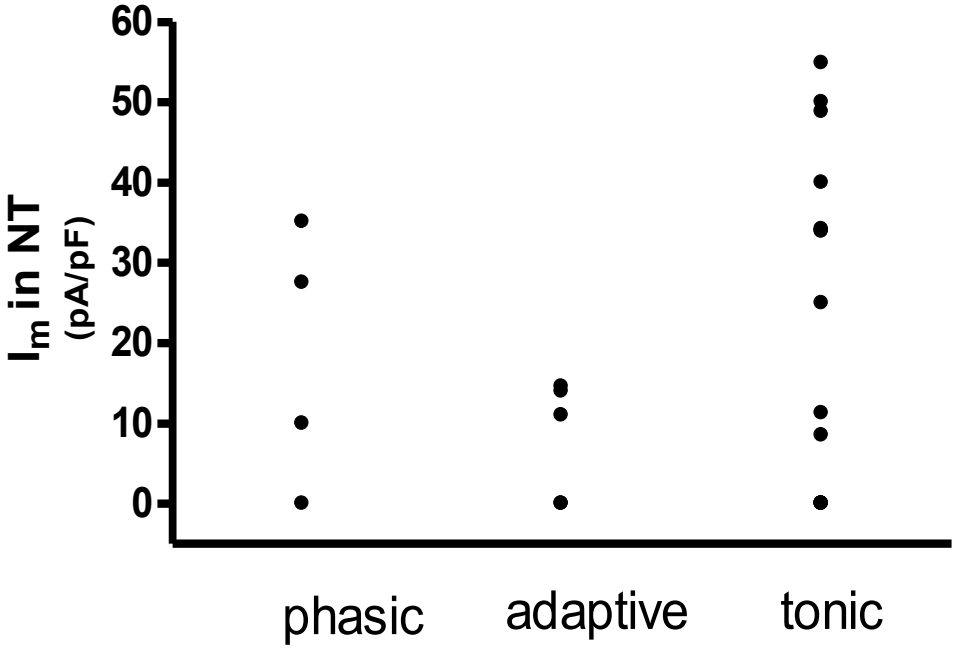


Figure 3-4. I_m of CG neurons in HT and NT rats

Figure 3-4 (cont'd)

B.



C.

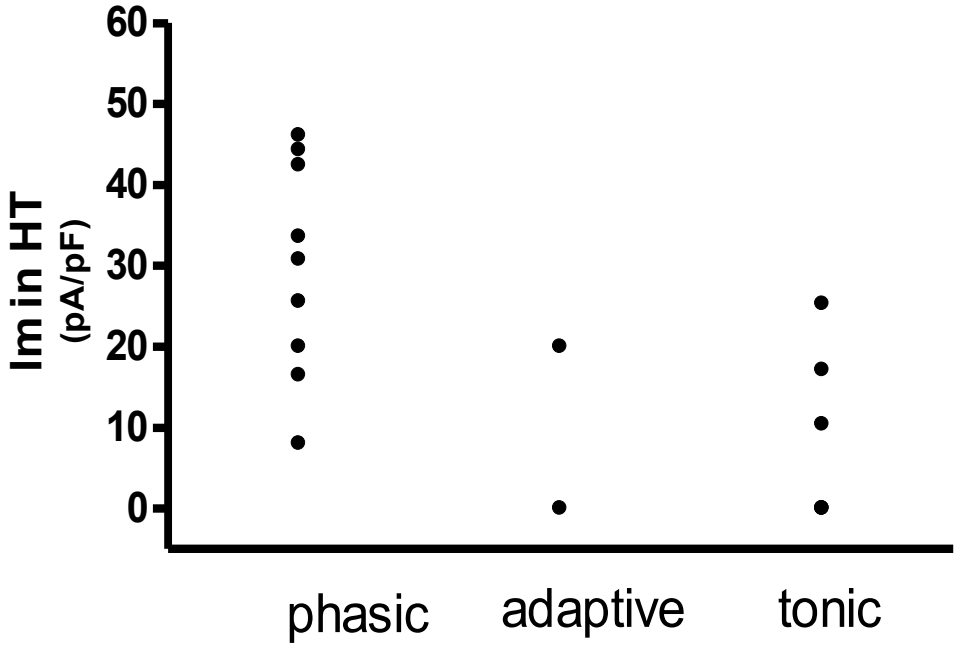
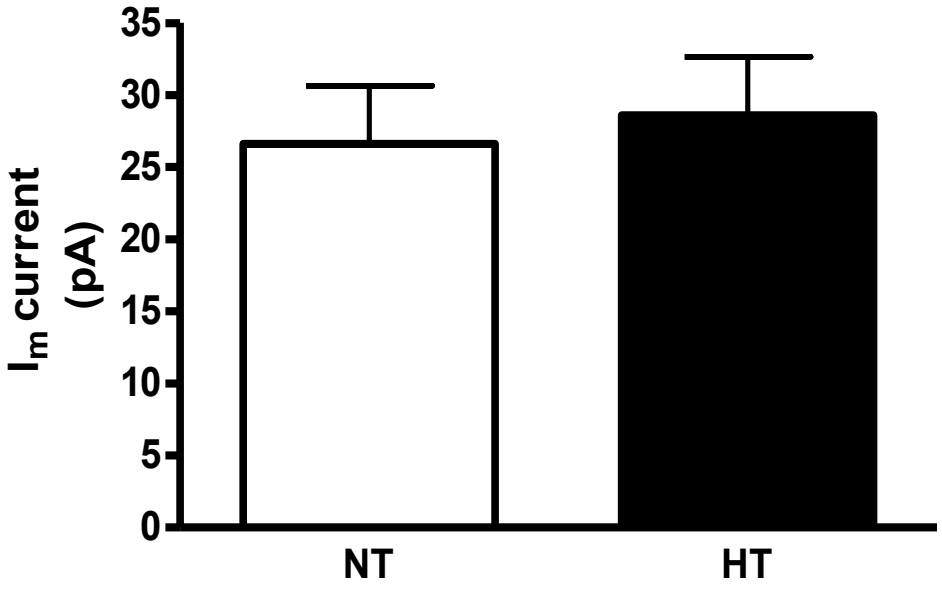


Figure 3-4 (cont'd)

D.



The M-type potassium channel current (I_m) was tested as a slowly decaying outward current from the step (-30mv to -70mv, held at -70mv). The characteristic traces and running protocol were shown in (A). The amplitude of I_m was not correlated with neuronal firing pattern in NT rats (n=24, p>0.05) (B), or in HT rats (n=20, p>0.05) (C). The peak amplitude of I_m was also not significantly different between NT and HT rats (D).

A.

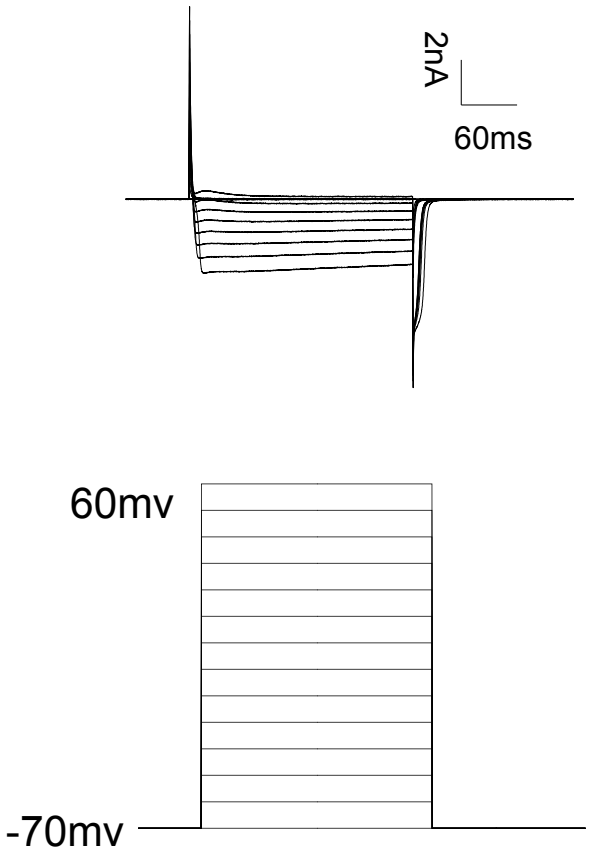
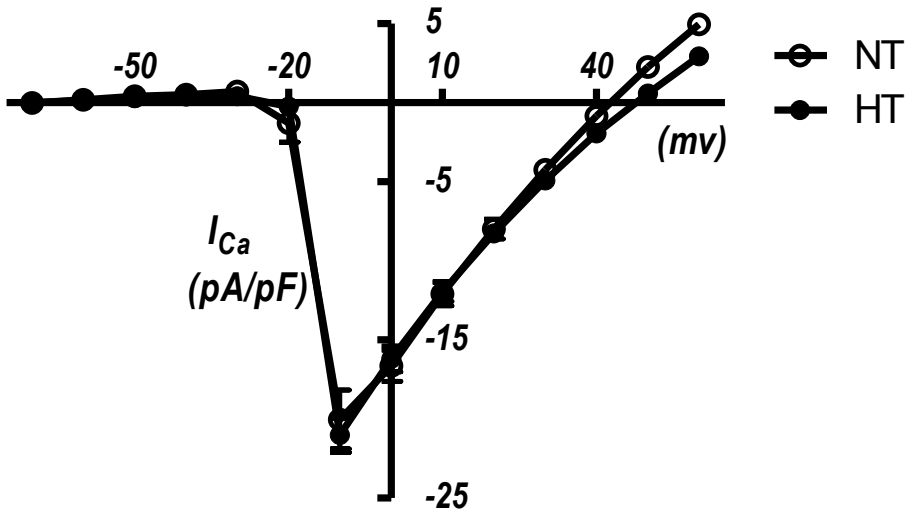


Figure 3-5. I_{Ca} of CG neurons in HT and NT rats

Figure 3-5(cont'd)

B.



The calcium channel current (I_{Ca}) was tested under voltage steps (from -140mv to 60mv, at 10mv step 200ms). The characteristic traces and running protocol were shown in (A). The amplitude of I_{Ca} was not significantly different between NT and HT rats (n=17, 2-way ANOVA, $p>0.05$) (B).

BIBLIOGRAPHY

BIBLIOGRAPHY

Aguas AP & Nickerson PA (1983). Increased Ca2+ in the sarcoplasm of aortic smooth muscle cells from rats made hypertensive with DOC. A quantitative ultrastructural and cytochemical study. *J Submicrosc Cytol* **15**, 425-431.

Bratz IN, Swafford AN, Jr., Kanagy NL, & Dick GM (2005). Reduced functional expression of K(+) channels in vascular smooth muscle cells from rats made hypertensive with N{omega}-nitro-L-arginine. *Am J Physiol Heart Circ Physiol* **289**, H1284-H1290.

Carrier GO & Ikeda SR (1992). TTX-sensitive Na+ channels and Ca2+ channels of the L- and N-type underlie the inward current in acutely dispersed coeliac-mesenteric ganglia neurons of adult rats. *Pflugers Arch* **421**, 7-16.

Cox RH, Lozinskaya I, & Dietz NJ (2003). Calcium exerts a larger regulatory effect on potassium+ channels in small mesenteric artery mycoytes from spontaneously hypertensive rats compared to Wistar-Kyoto rats. *Am J Hypertens* **16**, 21-27.

Faber ES & Sah P (2003). Calcium-activated potassium channels: multiple contributions to neuronal function. *Neuroscientist* **9**, 181-194.

Greffrath W, Magerl W, Disque-Kaiser U, Martin E, Reuss S, & Boehmer G (2004). Contribution of Ca2+-activated K+ channels to hyperpolarizing after-potentials and discharge pattern in rat supraoptic neurones. *J Neuroendocrinol* **16**, 577-588.

Hille B (2001a). Introduction. In *Ion Channels and Excitable Membranes* pp. 1-21. Sinauer Associates, Sunderland.

Hille B (2001b). Potassium Channels and Chloride Channels. In *Ion Channels of Excitable Membranes* pp. 131-168. Sinauer Associates, Massachusetts.

Liu Y, Jones AW, & Sturek M (1995). Ca(2+)-dependent K+ current in arterial smooth muscle cells from aldosterone-salt hypertensive rats. *Am J Physiol* **269**, H1246-H1257.

Liu Y, Pleyte K, Knaus HG, & Rusch NJ (1997). Increased expression of Ca2+-sensitive K+ channels in aorta of hypertensive rats. *Hypertension* **30**, 1403-1409.

Lovell PV, King JT, & McCobb DP (2004). Acute modulation of adrenal chromaffin cell BK channel gating and cell excitability by glucocorticoids. *J Neurophysiol* **91**, 561-570.

Malin SA & Nerbonne JM (2001). Molecular heterogeneity of the voltage-gated fast transient outward K+ current, I(Af), in mammalian neurons. *J Neurosci* **21**, 8004-8014.

Malin SA & Nerbonne JM (2002). Delayed rectifier K+ currents, IK, are encoded by Kv2 alpha-subunits and regulate tonic firing in mammalian sympathetic neurons. *J Neurosci* **22**, 10094-10105.

Martens JR & Gelband CH (1996). Alterations in rat interlobar artery membrane potential and K+ channels in genetic and nongenetic hypertension. *Circ Res* **79**, 295-301.

Muraki S, Tohse N, Seki S, Nagashima M, Yamada Y, Abe T, & Yabu H (2001). Decrease in the Ca2+-activated K+ current of pulmonary arterial smooth muscle in pulmonary hypertension rats. *Naunyn Schmiedebergs Arch Pharmacol* **364**, 183-192.

Robertson WP & Schofield GG (1999). Primary and adaptive changes of A-type K+ currents in sympathetic neurons from hypertensive rats. *Am J Physiol* **276**, R1758-R1765.

Sonner PM & Stern JE (2007). Functional role of A-type potassium currents in rat presympathetic PVN neurones. *J Physiol* **582**, 1219-1238.

Vanner S, Evans RJ, Matsumoto SG, & Surprenant A (1993). Potassium currents and their modulation by muscarine and substance P in neuronal cultures from adult guinea pig celiac ganglia. *J Neurophysiol* **69**, 1632-1644.

Wang HS & McKinnon D (1995). Potassium currents in rat prevertebral and paravertebral sympathetic neurones: control of firing properties. *J Physiol (Lond)* **485**, 319-335.

Wolfart J, Neuhoff H, Franz O, & Roeper J (2001). Differential expression of the small-conductance, calcium-activated potassium channel SK3 is critical for pacemaker control in dopaminergic midbrain neurons. *J Neurosci* **21**, 3443-3456.

Yoshida S, Takahashi M, Kadoi J, Kitagawa J, Saiki C, Takeda M, & Matsumoto S (2007). The functional difference between transient and sustained K(+) currents on the action potentials in tetrodotoxin-resistant adult rat trigeminal ganglion neurons. *Brain Res* **1152**, 64-74.

CHAPTER 4

EFFECTS OF HYDROGEN PEROXIDE ON NEURONAL FIRING AND POTASSIUM CHANNEL CURRENTS OF DISSOCIATED RAT CELIAC GANGLION NEURONS

Abstract:

Elevated reactive oxygen species (ROS) have been found in celiac ganglia from DOCA-salt hypertensive rats. In chapters 2 and 3, altered neuronal firing patterns associated with reduced K^+ currents were detected in dissociated CG neurons from hypertensive rats. In this study, one type of ROS, H_2O_2 was used in CG neurons to explore if the changes in neuronal firing properties are related to elevated ROS and whether the changes by H_2O_2 mimic differences in neuronal firing properties found in hypertensive rats. Using whole-cell patch clamp, dissociated CG neurons from normal SD rats were divided into three groups based on their action potential (AP) firing patterns in response to a sustained suprathreshold depolarizing current step (100pA; 5s). Phasic neurons fired several APs and adapted quickly; adaptive neurons fired several APs and adapted slowly; tonic neurons fired continuously. Neurons were exposed to 3mM H_2O_2 for 1 min. After treatment, the phasic neurons remained phasic but fired fewer APs and the adaptive neurons became phasic neurons. 21% of tonic neurons were changed to phasic neurons, 14% were changed to adaptive neurons, and 65% tonic remained tonic but with lower firing frequency. Further effects on K^+ currents were explored by applying 3mM H_2O_2 for 1min. The amplitude of the sustained outward potassium current was increased at potentials positive to -20mv in Ca^{2+}-free extracellular solution but not in regular extracellular solution with Ca^{2+}. The peak amplitude and mid-width of A-type K^+ current were decreased by H_2O_2. The

inactivation curve of A-type K^+ current was shifted to left by H_2O_2. The effects of H_2O_2 on K^+ currents were partially blocked by catalase (100 kU/ml) applied intracellularly. These findings demonstrate that H_2O_2 acts intracellularly in CG neurons to reduce neuronal firing frequency and alter firing patterns. The changes in firing properties by H_2O_2 may be associated with changes by H_2O_2 in sustained outward K^+ currents and decreased A-type K^+ current.

Introduction:

ROS are reactive derivatives of oxygen metabolism and include superoxide anion ($O_2^{\bullet -}$), hydrogen peroxide (H_2O_2) and hydroxyl radical (OH•). This study targeted H_2O_2 as a potential candidate for neuronal firing properties changes in CG neurons from hypertensive rats. One reason is that H_2O_2 is a potent and stable molecule. It is also a source of OH• in the presence of ferrous-containing low-molecular-weight proteins or free cytoplasmic Fe^{2+}. OH• is one of the most potent oxidants that oxidizes proteins and lipids and breaks apart DNA strands (Stadtman & Levine, 2000). The other reason is that H_2O_2 is lipid soluble, and it can diffuse out into the cytoplasm (Halliwell, 1992). H_2O_2 applied extracellularly would diffuse into the neurons and function intracellularly. The production of H_2O_2 is checked by enzymes such as catalase and glutathione peroxidase that convert H_2O_2 to water and molecular oxygen.

ROS are produced during normal metabolism and might be associated with physiological or pathological functions, even though an excessive amount of ROS can result in tissue damage (Halliwell, 1992). Elevated ROS is found in many neuronal diseases such as Parkinson's disease (Barkats *et al.*, 2006), Alzheimer's dementia (Behl *et al.*, 1994) and multiple sclerosis (Ferretti *et al.*, 2006). It also has been found that ROS is associated with hypertension. ROS are elevated in blood and tissues in hypertension (Somers *et al.*, 2000).Prolonged antioxidant administration prevents superoxide accumulation and attenuate systolic BP elevation in DOCA-salt hypertensive rats (Beswick *et al.*, 2001).Interestingly, elevated ROS are also shown in celiac ganglia from hypertensive rats (Dai *et al.*, 2006). However it is unknown about the pathological role of elevated ROS in sympathetic ganglia for hypertension

Different CG neuronal firing pattern exist in response to a sustained depolarizing current. The firing pattern is generated by activation and inactivation of a series of ion channels. Membrane ion channels are the possible targets of ROS (Kourie, 1998). It has been demonstrated that H_2O_2 can modulate ICC pacemaker activity and this occurs by the activation of K_{ATP} channels (Choi *et al.*, 2008). Voltage-gated K^+ currents are also altered by H_2O_2 in cultured hippocampal neurons (Muller & Bittner, 2002). In chapter 2 and 3, It was found that the neuronal firing patterns and K^+ currents were modulated in CG neurons from DOCA-salt hypertensive rats. It is not known if the elevated the ROS level in sympathetic ganglia wound contribute to the neuronal firing property changes of CG neurons in this hypertensive animal model. In this study, I explored the effects of H_2O_2 on neuronal firing properties of dissociated CG neurons,

which would illustrate if there is a relationship between ROS and neuronal firing properties in sympathetic ganglion neurons.

Materials and Methods:

Animals

The animals and number used in the experiments were conformed to "National Institutes of Health Guide for the Care and Use of Laboratory Animals". 3-4 week male Sprague Dawley (SD) rats (100-150g) were housed and used in accordance with guidelines established by the Animal Use and Care Committee of Michigan State University.

Tissue collection

CG was removed from the animals (details seen in Chapter 2).

Cell culture

The harvested celiac ganglia were dissociated enzymatically and plated in glass bottom culture dishes for electrophysiological studies (details seen in Chapter 2).

Whole-cell patch clamp

The dissociated CG neurons were tested under whole-cell patch clamp (details seen in Chapter 2). Access resistance (R_a) was monitored at regular intervals. The ruptured neurons with R_a higher than 20MΩ were not accepted for data acquisition in voltage clamp mode. The neurons with resting membrane potential more depolarized than -45mv were not included in current clamp mode.

The extracellular solution contained 120 NaCl, 4.7 KCl, 2.5 $CaCl_2$, 1.2 $MgCl_2$, 1.2 $NaHPO_4$, 25 $NaHCO_3$, and 11 Glucose in mM which was equilibrated with a 95% O_2-

5%CO_2 mixture. The intracellular solution contained 150 K Acetate, 3 $MgCl_2$, 40 HEPES, 10 ATP, and 2.5 GTP in mM. In no Ca^{2+} extracellular solution, $CaCl_2$ was replaced by NaCl. 3mM H_2O_2 was applied through drug administration capillary tube by gravity which was positioned 500-800 µM from the patched neurons. The perfusion system was still running at the rate of 20-30 drops/min during H_2O_2 application. $3x10^{-7}$M tetrodotoxin (TTX) was applied to neurons during the experiments for measuring the K^+ currents through drug administration capillary tube. Some recording were made with intracellular solution containing catalase (100kU/ml). Catalase did not alter the pH of the intracellular solution at the concentrations used.

Statistical analysis:

All values were presented as mean ± SEM and n refers to the number of neurons Less than 4 neurons were recorded per animal. Statistical significance between means was determined using two-tailed paired t-test for two independent variables or one-way ANOVA for more than two independent variables. The neuronal firing pattern change was analyzed by χ^2-test. The statistical significance was set for $P<0.05$.

Results:

Effects of H_2O_2 on firing patterns of dissociated CG neurons

Using whole-cell patch clamp, dissociated rat CG neurons (n=54) were tested for neuronal firing in current clamp mode by injecting a sustained suprathreshold

depolarizing current (100pA; 5 s). The neurons were divided into three groups, phasic, adaptive and tonic neurons based on their AP firing pattern (Figure 4-1, A). The same classification standard was used as in Chapter 2. Some phasic neurons did not fire after 100pA current injection, but did fire after injection of a larger current (100 to 300pA). In these 54 neurons, 13% of them were phasic neurons, 9% were adapting neurons and 78% were tonic neurons. After 1min H_2O_2 (3mM) application, the neurons were tested again for neuronal firing. The phasic neurons (n=7) remained phasic (Figure 4-1, B). All adapting neurons (n=4) changed to phasic (Figure 4-1, C). As for tonic neurons, 21% of them (n=9) changed to phasic neurons, 14% (n=6) changed to adapting neurons, and 65% (n=27) remained tonic (Figure 4-1, D). In summary there were 39% phasic, 11% adapting and 50% tonic neurons after H_2O_2 application, which was statistically different (χ^2-test, $p<0.05$) from the firing pattern distribution before H_2O_2 application (Figure 4-1, E). These data indicate that H_2O_2 increased the accommodating capacity of CG neurons to sustained depolarizing current injection. The effects of H_2O_2 were reversible in 50% patched neurons by 4min wash with extracellular solution. The effects of H_2O_2 on neuronal firing pattern were not blocked by applying 100kU/ml catalase intracellularly. The changes by H_2O_2 in neuronal firing pattern with catalase were similar to those without catalase shown before. In the presence of intracellular catalase, one adaptive neuron changed to phasic neuron, 25% of tonic neurons (n=2) changed to phasic neurons, 12% of tonic (n=1) changed to adaptive neuron and 62% of tonic (n=5) remained tonic after H_2O_2 treatment.

Effects of H_2O_2 on neuronal firing frequency

The neuronal firing frequency was not constant when depolarizing current injection applied. It declined gradually and stopped firing in phasic and adapting neurons or went to a stable plateau in tonic neurons. To examine CG neuronal firing frequency, the time to first AP (t_1), the time from the first AP to the second AP (t_2) and the time from the penultimate AP and the last AP (t_3) in the plateau region were measured before and after 1 min 3mM H_2O_2 treatment (Figure 4-2, A). The t_3 value was not tested in phasic and adapting neurons because they did not have a stable plateau. t_2 and t_1 were measured when they occurred. t_2 was measured when there were more than 2 APs and t_1 when there was an AP in 100pA current injection. 3mM H_2O_2 significantly increased t_1 (t-test, $p<0.05$, n=52) in all measurable neurons including phasic, adapting and tonic neurons (Figure 4-2, B). The first firing frequency ($1/ t_2$) was significantly decreased by H_2O_2 in all accessible neurons (t-test, $p<0.05$, n=44) (Figure 4-2, C). The last firing frequency ($1/ t_3$) was only tested in tonic neurons that remained tonic after H_2O_2 treatment. It was significantly decreased by H_2O_2 (t-test, $p<0.05$, n=27) (Figure 4-2, D). The phasic, adaptive and tonic neurons were tested for the action potential firing frequency during the entire 5 s of depolarization (f_{5s}) as number of APs during current injecting. The f_{5s} was statistically decreased by H_2O_2 in all three types of neurons (Figure 4-2, E). In summary, these data showed that H_2O_2 delayed the time

to generate the first AP and decreased firing frequencies under a sustained depolarizing current injection.

Effects of H_2O_2 on electrical membrane parameters of a single AP

Neuronal firing is composed of a series of APs. Altered AP shape may reflect altered neuronal firing pattern and firing frequency. AP electrical membrane properties were measured before and after H_2O_2 treatment. The capacitance of these dissociated neurons was 53±10pF, which is not changed by H_2O_2 treatment. The input resistance (R_{in}) was measured by applying a constant 20pA hyperpolarizing current for 5s. R_{in} was lowered by H_2O_2 from 1039±68MΩ to 821±69 MΩ (n=42, paired t-test $p<0.05$). The resting membrane potential (RMP) was hyperpolarized by H_2O_2 from -56.0±1.4mv to -60.1±2.2mv (n=42, paired t-test $p<0.05$). The peak amplitude and the mid-width of the first AP of neuronal firing were measured under the sustained 100pA depolarizing current injection. The peak amplitude was not changed by H_2O_2. However the mid-width was statistically increased by H_2O_2 from 2.8 ±0.2ms to 3.2±0.2ms (n=50, paired t-test $p<0.05$) (Table 4-1).

To characterize the AP in more detail, a short depolarizing current injection (400pA, 2ms) was applied to the neurons to induce just one AP. It was confirmed that the effects of H_2O_2 on RMP and AP peak amplitude under this short current injection were consistent with those found under 5 s depolarizing current injection. The RMP was

hyperpolarized significantly from -49.8±2.7mv to -52.7±2.9mv (n=13, paired t-test $p<0.05$). The amplitude of AP was not changed by H_2O_2 application. However, H_2O_2 did not alter the mid-width of the single AP despite being significantly increased in the first AP under 5-s depolarizing current injection. Furthermore it has been found that the repolarizing rate of the single AP was significantly decreased by H_2O_2 from 29.4.0±6.7mv/ms to 25.8±5.5mv/ms (n=13, paired t-test $p<0.05$); the amplitude of afterhyperpolarization (AHP) was not changed by H_2O_2, but the area of AHP was significantly decreased from 1400±193.8mv•ms to 996±192.2mv•ms (n=13, paired t-test $p<0.05$) (Table 4-2). In summary, H_2O_2 treatment lead to hyperpolarized RMP, decreased R_{in}, slower AP repolarization and attenuated AHP in dissociated CG neurons.

Effects of H_2O_2 on sustained outward potassium currents

Previous findings in electrical membrane firing properties indicate that the voltage-gated K^+ channels might be modulated by H_2O_2. To explore the mechanisms of changed neuronal firing by H_2O_2, the voltage-gated K^+ currents were tested before and after H_2O_2 treatment. Current-voltage (I-V) relationships were generated in voltage-clamped neurons held at a membrane potential of -70mV and then stepped in 10mV increments from -120 to +50 mV. Each Voltage step was 0.2s duration. 0.19s intervals were allowed between steps. Furthermore, a single-step depolarization protocol, stepping from -70mv to +50mv for 0.2 s was also applied to neurons before and after

H_2O_2 treatment. The amplitudes of K^+ currents were normalized to cell capacitance to obtain the current densities. The outward K^+ currents included a transient outward K^+ current (I_A) and a sustained outward K^+ current (I_{so}) (Figure. 4-3, A). I_{so} was composed of a delayed rectifier potassium current (I_{KV}) and a big-conductance calcium-activated potassium channel (BK) current (I_{BK}). 3mM H_2O_2 applied for 1 min, did not significantly change the amplitude of I_{so} under protocol for I-V relationships (n=17, two-way ANOVA $p>0.05$) (Figure 4-3, B). However, it significantly increased the amplitude of I_{so} under single-step depolarization protocol (n=5, paired t-test $p<0.05$) (Figure 4-3, C). There would be no Ca^{2+} influx in single step depolarization from -70mv to +50mv because the equilibrium potential for Ca^{2+} current is around +45 (for details see Chapter 3). Therefore, the BK channel current would be less activated under single-step depolarization compared to the last step of the series depolarization steps. The I-V relationships of I_{so} were further tested in Ca^{2+}-free extracellular solution. The amplitudes of I_{so} were significantly enhanced 5 to 6 pA/pF at the voltage steps positive to -20mv in the Ca^{2+}-free extracellular solution (n=7, two-way ANOVA $p<0.05$) (Figure 4-3, D). This effect was blocked by addition of the H_2O_2 catalyzing enzyme, catalase. When 100kU/ml catalase was applied in the intracellular solution, I_{so} was not significantly changed by H_2O_2 in Ca^{2+}-free extracellular solution (Figure 4-3, E).

Effects of H_2O_2 on A-type potassium current

In the beginning of the outward K^+ currents generated by depolarization steps, there was transient outward A-type potassium current (I_A) exhibiting quick inactivation. Two depolarization steps (from -40mv to 30mv and from-70mv to 30mv) were used to separate I_A from I_{so} in the whole outward K^+ current. I_A was obtained by subtracting current under -40mv to 30mv step from one under -70mv to 30mv step. It was compared between before and after H_2O_2 application (Figure 4-4, A). The H_2O_2 significantly decreased the peak amplitude of I_A by 50%-60% and the mid-width from 7.3±1.ms to 5.8±0.6ms (n=9, paired t-test $p<0.05$) (Figure 4-4, B).

The activation and inactivation of I_A were further studied before and after H_2O_2 treatment. Voltage-dependent I_A activation was generated using two protocols. One protocol is to depolarize neurons from -70mv to +50mv in 10mv. Extra -20mv depolarization for 0.5 s was applied right before depolarization from -70mv to +50mv in the other protocol. I_A was achieved by subtracting currents from these two protocols (Figure 4-5, A). I_A inactivated was studied by depolarizing neurons to +50mv proceeded by prepulse, lasting 0.5s, from -120mv to -20mv (Figure 4-5, B). The neurons were held at a membrane potential of -70mV. I_A activation curves and inactivation curves were compared before and after H_2O_2 treatment using the conductance-voltage plot. The conductance was calculated by peak current during the test pulse divided by maximum peak current. H_2O_2 shifted voltage dependence of steady –state inactivation to the left but did not affect the voltage dependence of

activation (Figure 4-5, C). When catalase (100kU/ml) was applied intracellularly, the effects H_2O_2 on I_A were partially checked. Peak amplitude was not significantly changed by H_2O_2, but the mid-width remained decreased by H_2O_2 in the presence of catalase (Figure 4-6, A). With catalase, H_2O_2 still decreased the mid-width of I_A from 7.0±1.6ms to 6.1±1.6ms (n=8, paired t-test $p<0.05$). There were no shifts of either voltage dependence activation curve or voltage dependence steady-state inactivation curve by H_2O_2 with intracellular catalase application (Figure 4-6, B).

The effects of H_2O_2 on K^+ currents were not reversible after a 4 min wash with control solution.

Discussion:

The major findings of this study are that, in dissociated CG neurons 1) H_2O_2 converted neuronal firing pattern to more accommodating type from tonic to phasic or adaptive neurons and from adaptive to phasic neurons; 2) it decreased neuronal firing frequency in all three types of neurons; 3) it enhanced I_{so} in no Ca^{2+} extracellular solution and inhibited I_A in regular extracellular solution. 4) The effects of H_2O_2 on CG neuronal firing and K^+ current were rarely reversible by simple wash. The effects were partially eliminated by adding catalase in the intracellular solution. Taken together,

these findings suggest that H_2O_2 exert different effects on the various K^+ currents which may contribute the effects of H_2O_2 on neuronal firing.

In the previous study, firing properties changes were seen in dissociated CG neurons from normotensive and DOCA-salt hypertensive rats. The changes in neuronal firing pattern from NT rats to HT rats are similar to those before and after H_2O_2 treatment. There were more phasic neurons and less adaptive and tonic neurons in HT rats; the firing frequency was lower in tonic neurons from HT rats. It is shown that superoxide level is increased in sympathetic ganglia from this hypertensive animal model (Dai *et al.*, 2004). Elevated superoxide would lead to increased H_2O_2, which may contribute CG neuronal firing pattern change in hypertension.

The effect of H_2O_2 on voltage-dependent K^+ currents was examined in this study. There is evidence indicating that chemical oxidation and reduction may modulate the activity of ion channels (Gulbis, 2002;Ruppersberg *et al.*, 1991). I have shown that the sustained potassium channel currents (I_{so}) were increased by H_2O_2 in calcium free extracellular solution but unchanged in normal calcium extracellular solution. I_{so} include mainly I_{KV} and I_{BK}. Since BK channel are activated by calcium, the increased effect by H_2O_2 is probably through delay rectifier potassium channel currents. The unchanged effect in normal calcium solution, which is also shown in colonic smooth muscle cells (Prasad & Goyal, 2004), may be contributed to decreased BK_{Ca} channel currents which cover the increased K_v current. The increased AP duration by H_2O_2 may also

contributed by decreased BK_{Ca} channel current. Decreased peak amplitude of afterhyperpolarization (AHP) is also consistent with decreased BK_{Ca} channel current. It also shown that the neuronal firing was more phasic when BK_{Ca} channel was blocked in Chapter 2.

It has been show that I_A was reduced by H_2O_2 in rat CA1 pyramidal neurons (Angelova & Muller, 2006). The I_A contributes to the RMP by the so-called window current. Because of its fast activation, it is primarily responsible for repolarization, the AP duration, and repetitive firing (Connor & Stevens, 1971a;Connor & Stevens, 1971b). It would increase AP duration, which was consistent with the finding in the membrane electrical properties of single AP. The role of A-type K^+ channel on neuronal firing was discussed in Chapter 3. Via action potential duration, I_A channels would also strongly affect presynaptic Ca^{2+} influx and transmitter release (Pongs, 1999).

A large range of H_2O_2 concentrations from several μM to 10mM (Prasad & Goyal, 2004) are used in the research work. It has been estimated that local H_2O_2 concentration can be higher than 1-2mM (Bychkov *et al.*, 1999). 3mM H_2O_2 applied for 1 min give me the consistent effect and also the effect can be partially washable. I used high concentration due to my drug administration system. My drug perfusion tube is 600-800μm away from patched cell. The extracellular solution perfusion system is still on during the drug perfusion to ensure the neuron gets enough O_2. The actual H_2O_2

concentration working on the neurons will be much less than 3mM. It has been shown that the effect of H_2O_2 is hardly washed away, partly because it is related to covalent chemical modification (Prasad & Goyal, 2004). The effects of H_2O_2 on neuronal firing pattern are partially washed away, whereas the changes on K^+ currents by H_2O_2 are not washed away in this study. It should be mentioned in this study that I focused only on short-term effects of H_2O_2, which occurred at the first 1 min after application of H_2O_2 to the bath solution. Further research could be done to elucidate the long-term effects of H_2O_2.

In conclusion, H_2O_2 converted neurons to more accommodating neuronal firing pattern and decreased firing frequency in response to sustained suprathreshold depolarizing currents, which could be related to its inhibitory effect on BK channels, and A-type K^+ channel. Interestingly all these effects by H_2O_2 are similar to the changes found in CG neurons from HT rats. Therefore, the changes in neuronal firing and K^+ currents are possibly related to elevated ROS in hypertension.

APPENDIX

A.

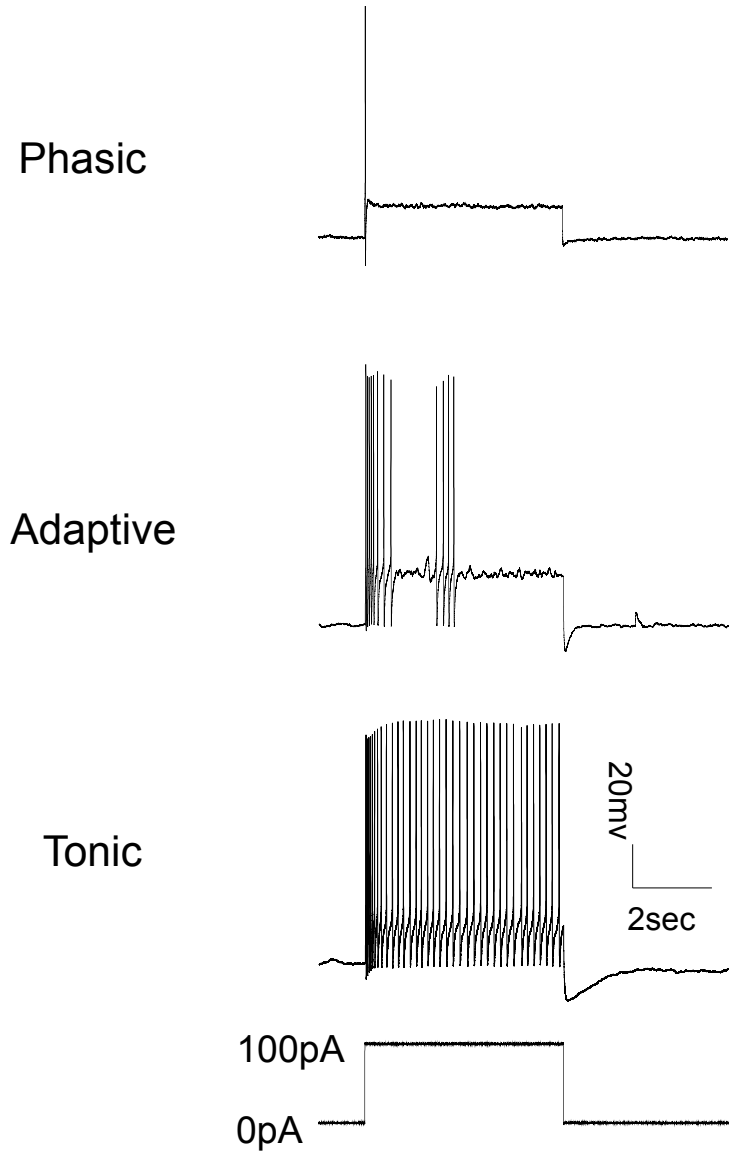


Figure 4-1: Effects of H_2O_2 on firing patterns of dissociated CG neurons.

Figure 4-1(cont'd)

B.

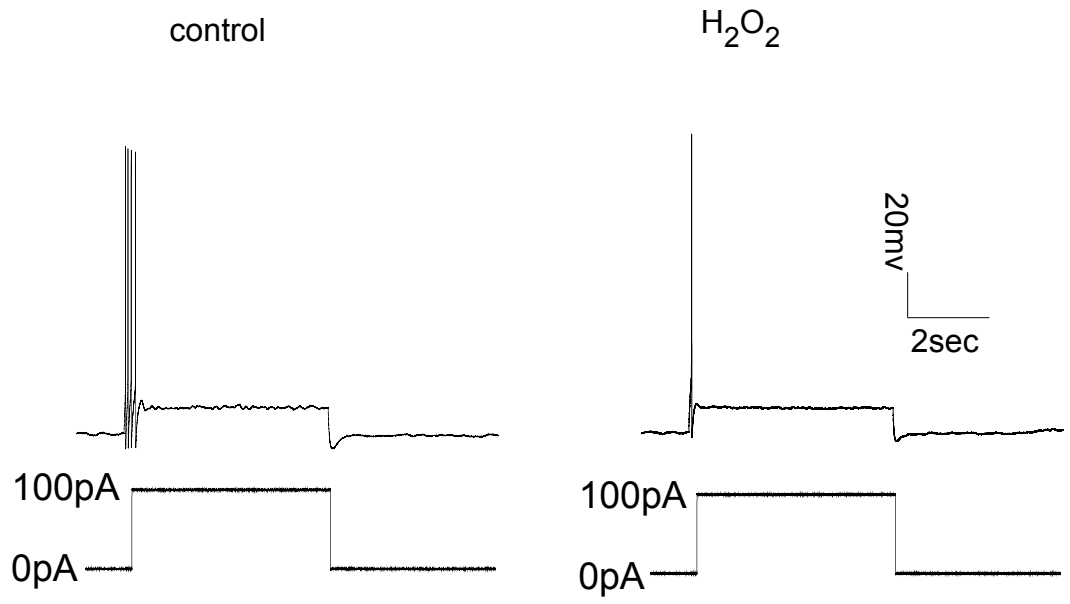


Figure 4-1(cont'd)

C.

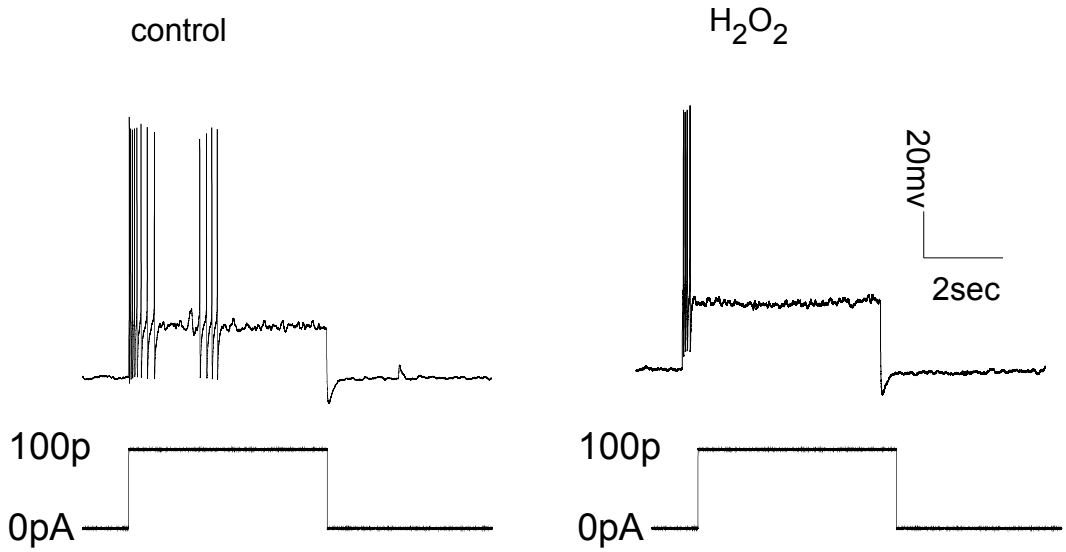


Figure 4-1(cont'd)

D.

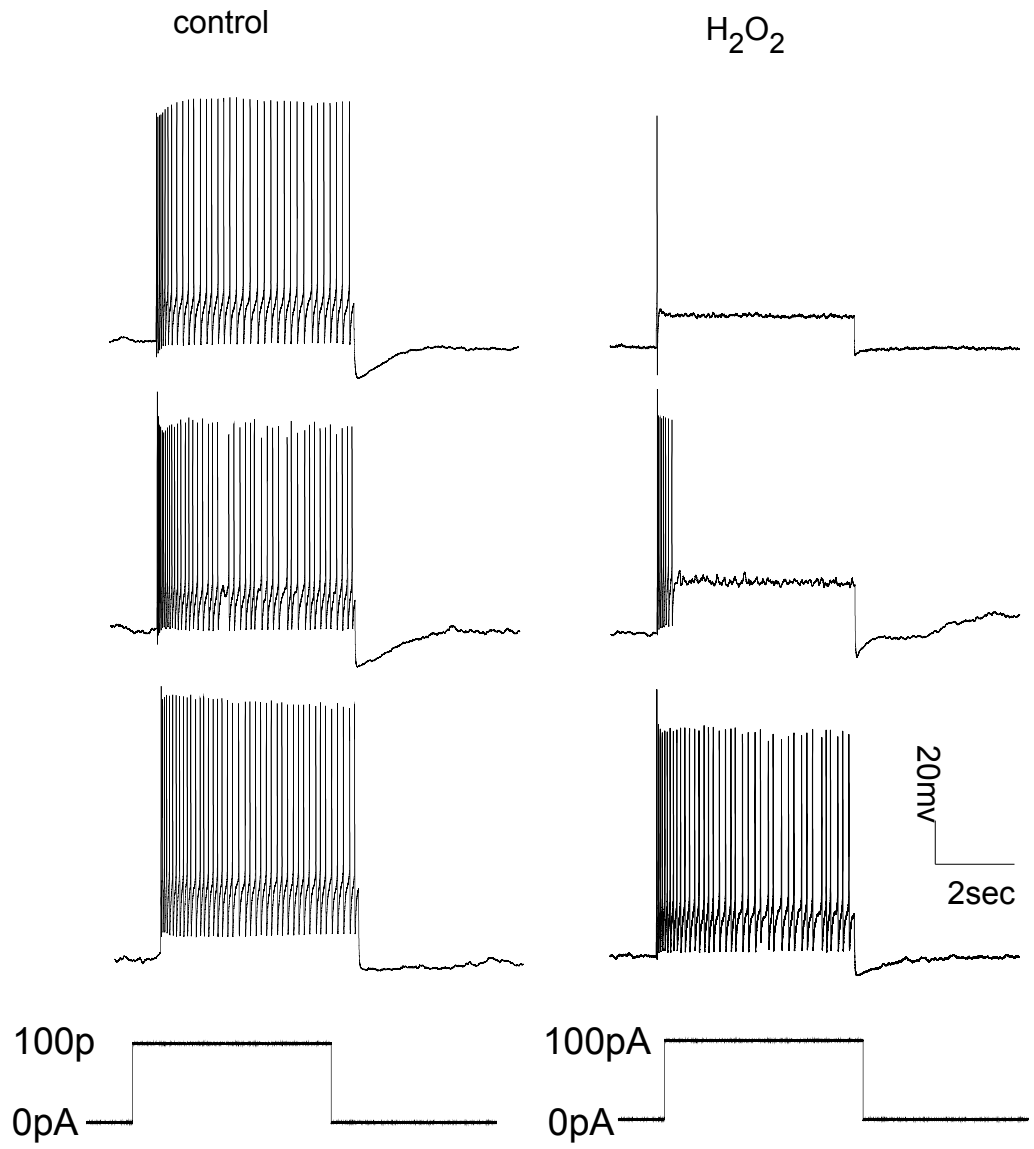


Figure 4-1(cont'd)

E.

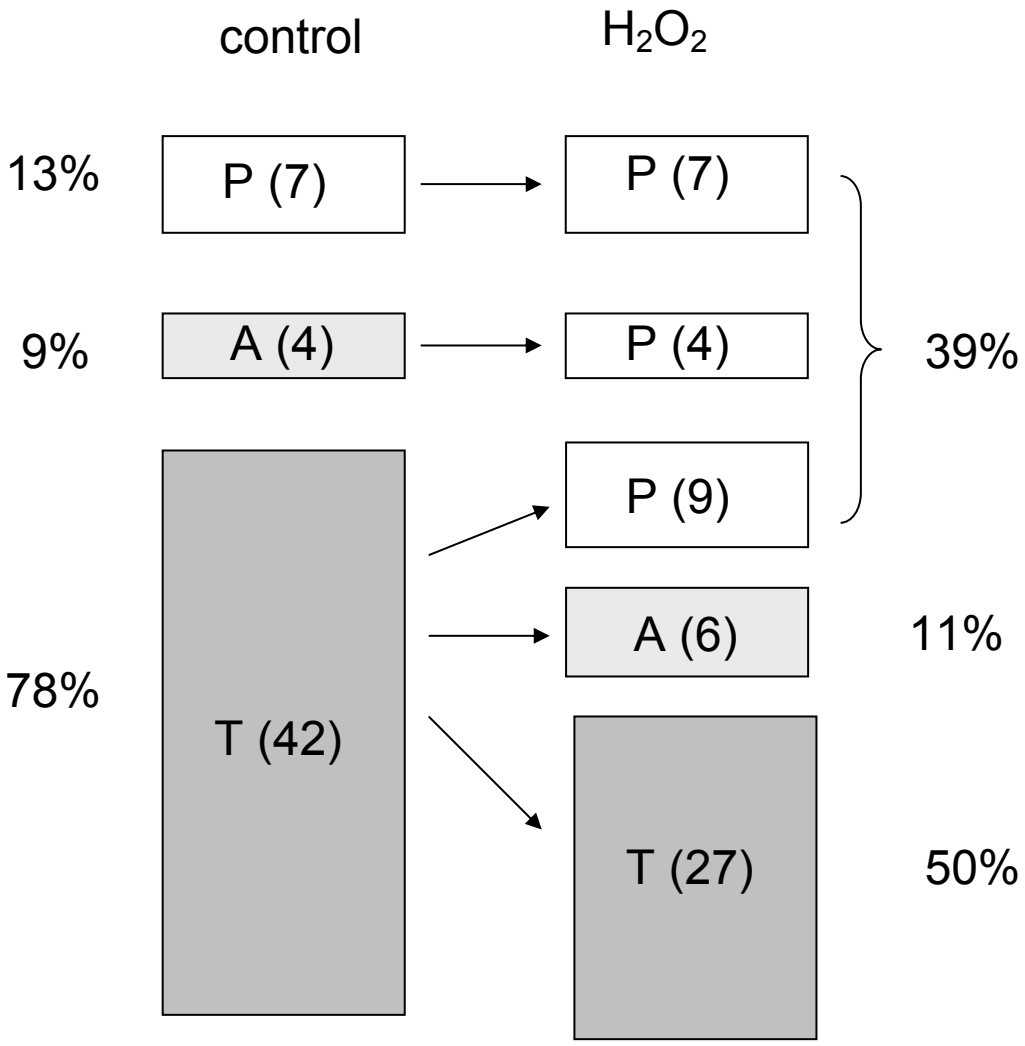


Figure 4-1(cont'd)

Three categorical firing patterns including phasic, adapting, or tonic were found in dissociated CG neurons in response to sustained suprathreshold 100pA depolarizing current injection in current clamp mode. Firing patterns from three representative cells (A). H_2O_2 (3mM) was applied on 54 neurons for 1 min. The neuronal firing pattern was tested before and after H_2O_2 application. Phasic neurons (n=7) remained phasic after H_2O_2 treatment (B). A representative cell is shown. Adapting neurons (n=4) changed to be phasic by H_2O_2(C). A representative cell is shown. Out of 42 tonic neurons, nine changed to phasic, six changed to adapting, and the remaining twenty-seven remained tonic (D). Representative cells are shown. The schematic summary diagram was shown (E) "P" "A" and "T" represents phasic, adaptive and tonic neurons, respectively. The firing pattern was statistically different between before and after H_2O_2 treatment (χ_2-test, $p<0.05$). The percentages of specific neuronal firing pattern before and after H_2O_2 treatment were shown adjacent to the representative schematic boxes.

A.

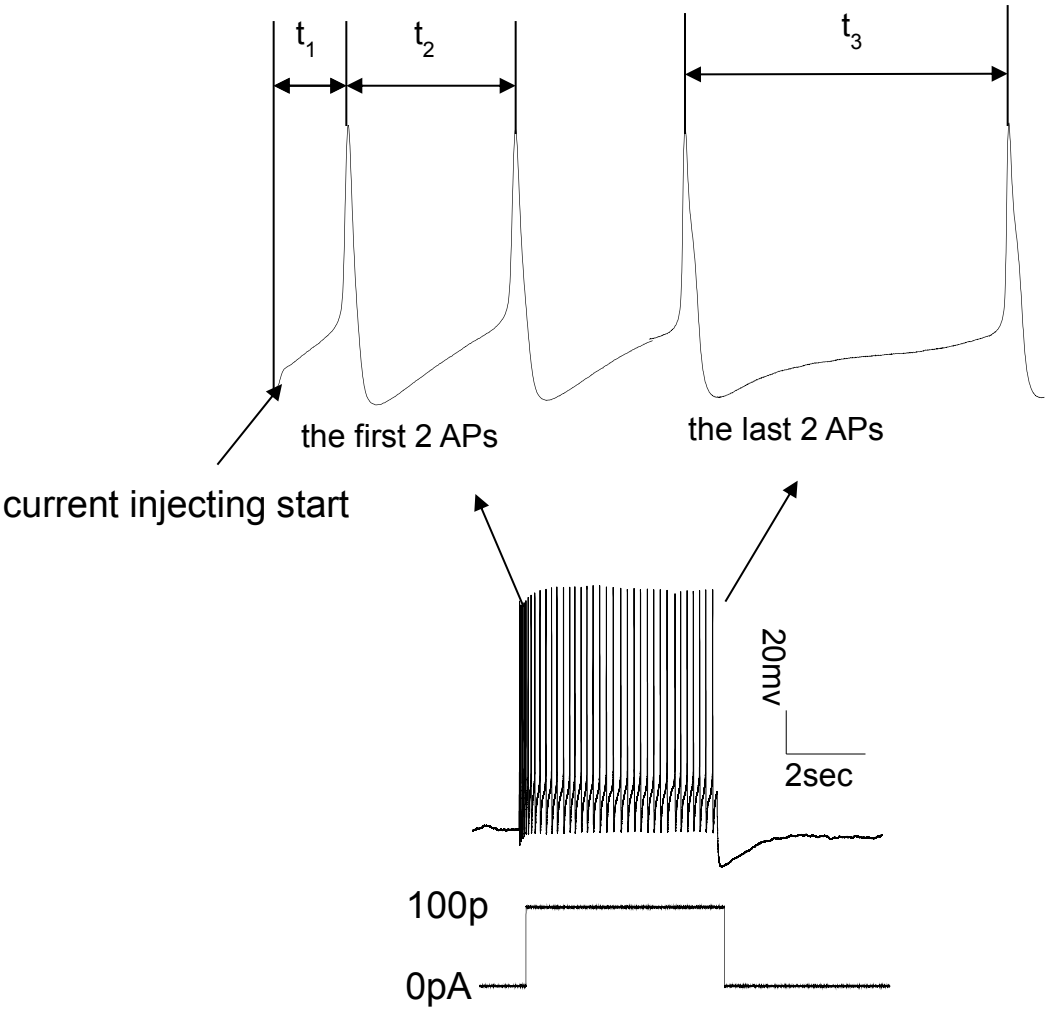
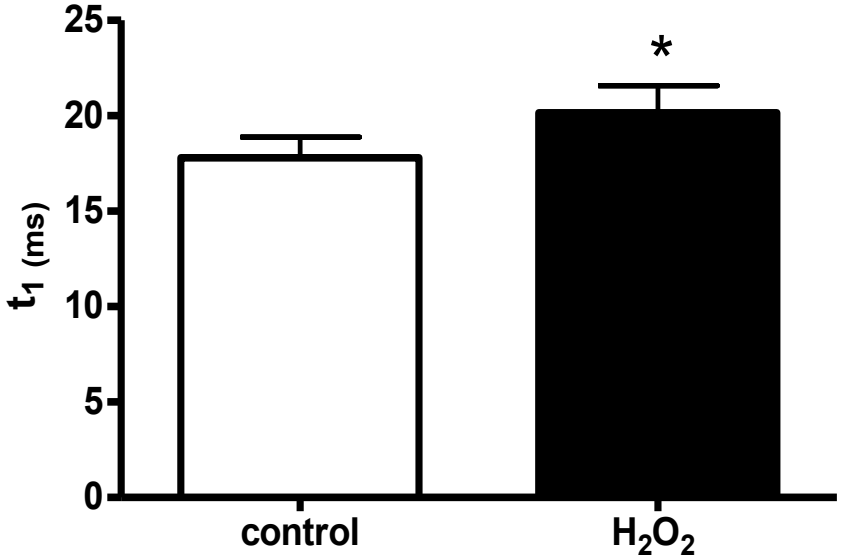


Figure 4-2: Effects of H_2O_2 on firing frequency in dissociated CG neurons.

Figure 4-2(cont'd)

B.



C.

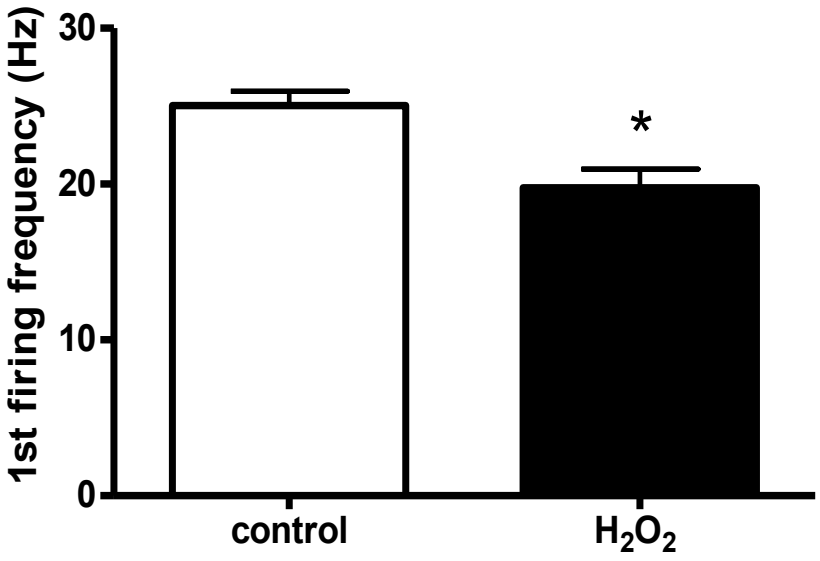
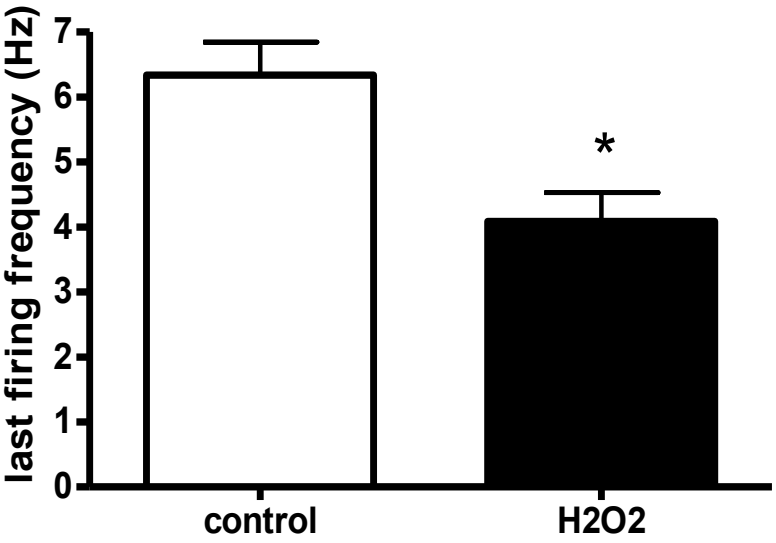


Figure 4-2(cont'd)

D.



E.

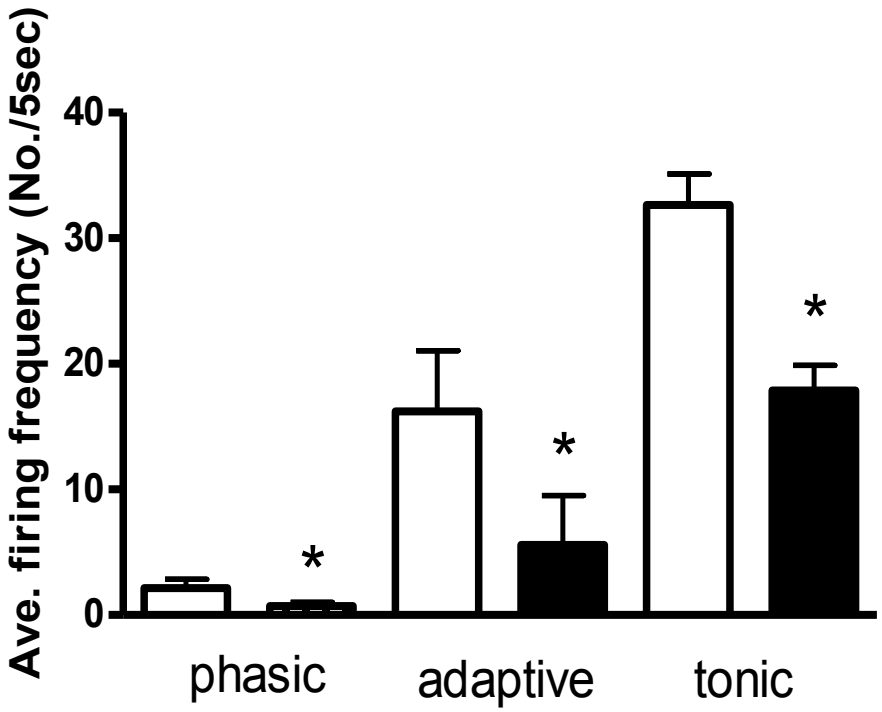


Figure 4-2(cont'd)

CG neuronal firing frequency declined gradually and stopped firing in phasic and adapting neurons or went to a stable plateau in tonic neurons. The time to first AP (t_1), the time from the first AP to the second AP (t_2) and the time from the penultimate AP and the last AP (t_3) in plateau region were measured before and after 1-min 3mM H_2O_2 treatment. A schematic graph was shown to illustrate how to measure t_1, t_2 *and* t_3 (A). H_2O_2 significantly increased t_1 *(*n=52, paired t-test, $p<0.05$) in measurable neurons (B). H_2O_2 significantly decreased the first firing frequency *(*n=44, paired t-test, $p<0.05$) (C) and significantly decreased the last firing frequency (1/ t_3) *(*n=27, paired t-test, $p<0.05$) (D). H_2O_2 also significantly decreased the action potential firing frequency during the entire 5 s of depolarization (number of APs during 5-s current injection) (n=7 in phasic group, n=5 in adapting group, n=41 in tonic group; paired t-test, $p<0.05$) (E)

	RMP (-mv)	R_{in} (MΩ)	peak amplitude (mv)	mid-width (ms)
control	56.0±1.4	1039±68	121.7±2.6	2.8±0.2
H_2O_2	60.1±2.2 *	821±69*	120.0±3.0	3.2±0.2*

Table 4-1. The effects of H_2O_2 on electrical parameters of CG neurons in response to a sustained depolarizing current injection

The electrical parameters of dissociated CG neurons in response to a sustained 100pA depolarizing current were measured in before and after H_2O_2 treatment. R_{in} was lowered by H_2O_2 from 1039±68MΩ to 821±69 MΩ (n=42, paired t-test $p<0.05$). The resting membrane potential (RMP) was hyperpolarized by H_2O_2 from -56.0±1.4mv to -60.1±2.2mv (n=42, paired t-test $p<0.05$). The peak amplitude of the first AP was not changed by H_2O_2. However the mid-width of the first AP was statistically increased by H_2O_2 from 2.8 ±0.2ms to 3.2±0.2ms (n=50, paired t-test $p<0.05$).

	RMP (-mv)	peak amplitude (mv)	mid-width (ms)
control	49.8±2.7	117.4±4.0	2.7±0.3
H_2O_2	52.7 ±2.9 *	110.2±5.5	2.8±0.3

	Repolarizing rate (mv/ms)	AHP amplitude (mv)	AHP area (mv•ms)
control	29.4±6.7	13.3±1.8	1400±193.8
H_2O_2	25.8±5.5*	13.4±2.4	996±192.2*

Table 4-2. The effects of H_2O_2 on membrane electrical parameters of a single AP

Membrane electrical parameters was measured in response to a short depolarizing current injection (400pA, 2ms). The RMP was hyperpolarized significantly from -49.8±2.7mv to -52.7±2.9mv (n=13, paired t-test $p<0.05$). The amplitude of AP was not changed by H_2O_2 application. However, H_2O_2 did not alter the mid-width of the single AP. Furthermore it has been found that the repolarizing rate of the single AP was significantly decreased by H_2O_2 from 29.4.0±6.7mv/ms to 25.8±5.5mv/ms (n=13, paired t-test $p<0.05$); the amplitude of afterhyperpolarization (AHP) was not changed by H_2O_2, but the area of AHP was significantly decreased from 1400±193.8mv•ms to 996±192.2mv•ms (n=13, paired t-test $p<0.05$)

A.

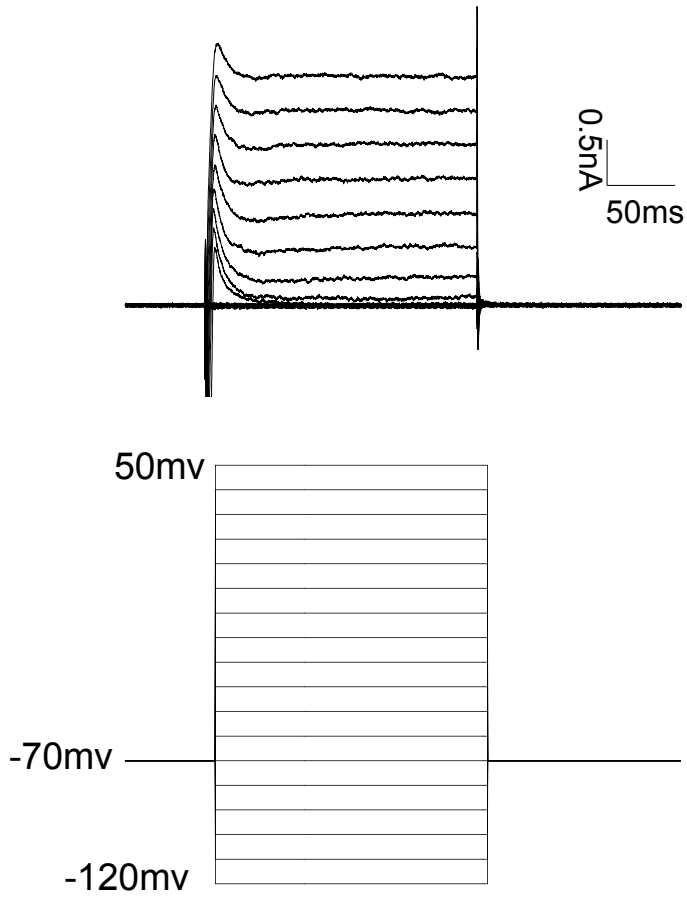


Figure 4-3. Effects of H_2O_2 on sustained outward K^+ currents in dissociated CG neurons.

Figure 4-3(cont'd)

B.

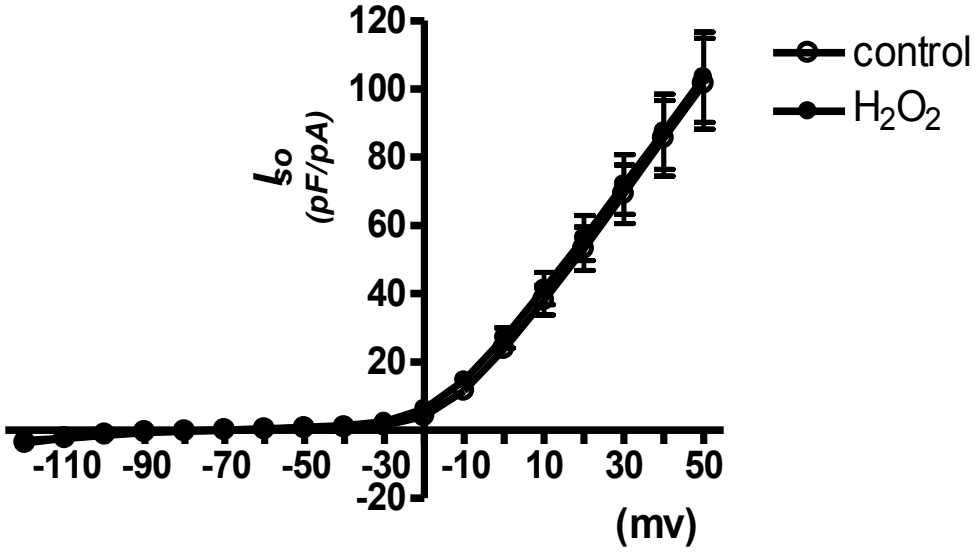


Figure 4-3(cont'd)

C.

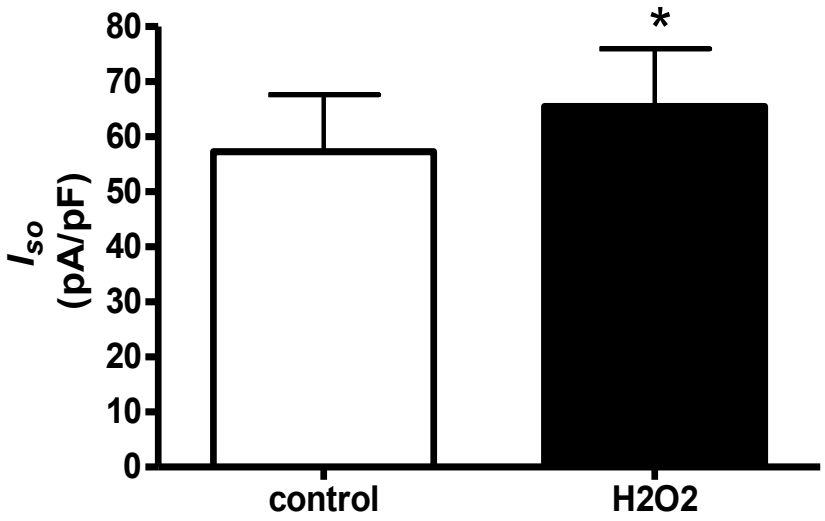


Figure 4-3(cont'd)

D

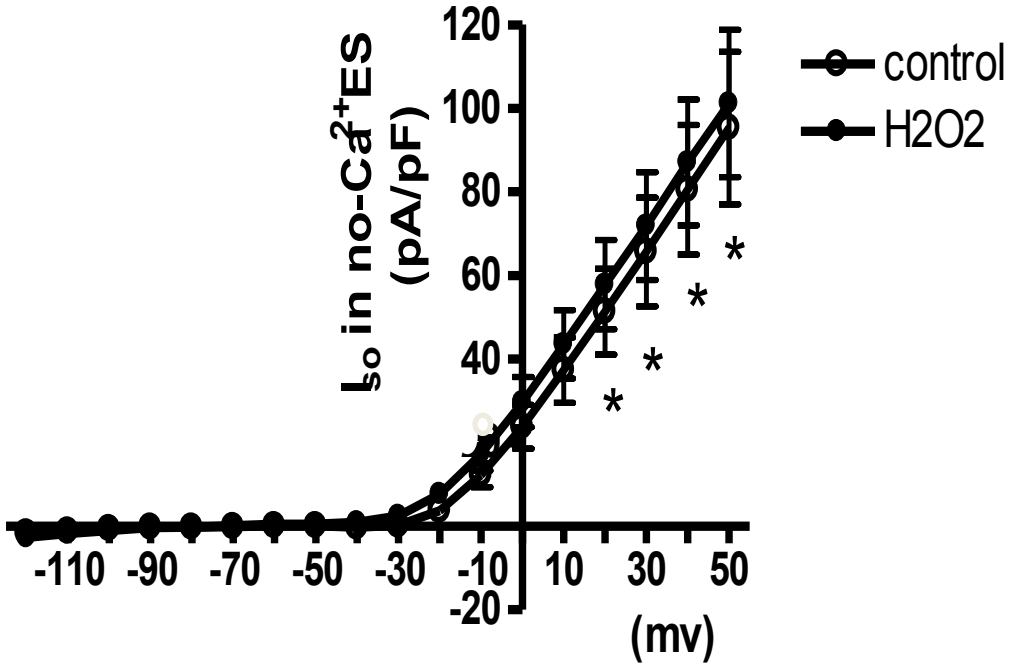


Figure 4-3(cont'd)

E.

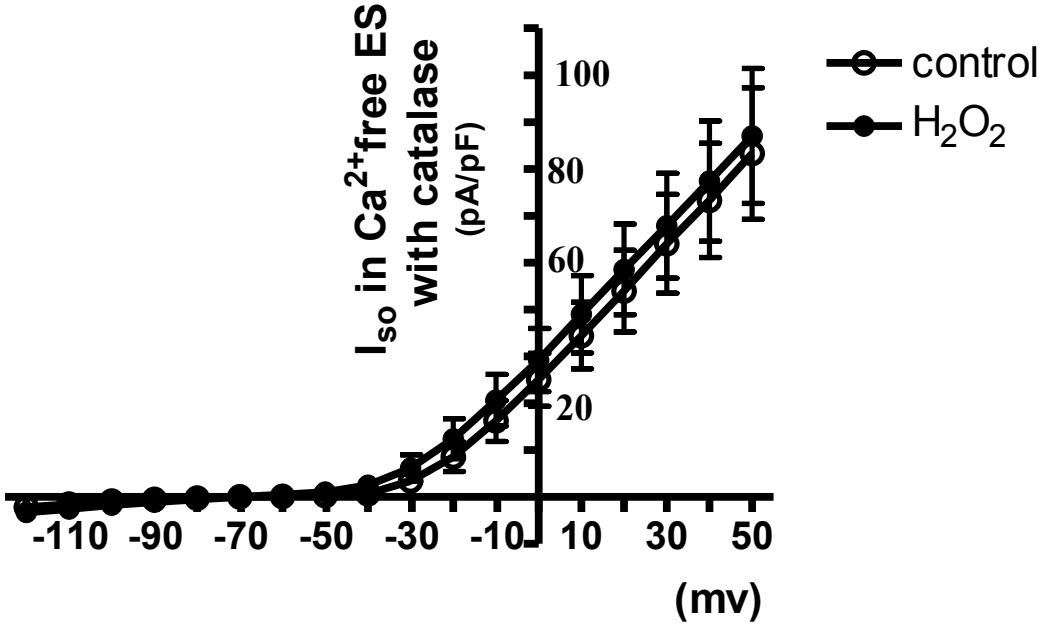
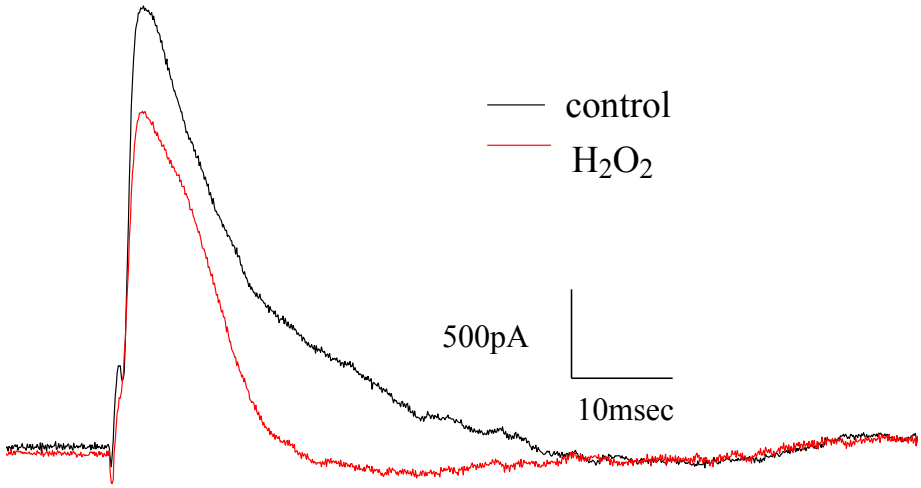


Figure 4-3(cont'd)

The CG neurons were held at -70mv in voltage clamp mode. Outward K^+ currents were generated by an I-V relationship protocol (from -120 to +50 mV at 10mv step, 0.2s) (A). H_2O_2 (3mM) applied for 1 min did not change the amplitude of I_{so} under I-V relationship protocol (n=17, two-way ANOVA $p>0.05$) (B), but significantly increased the amplitude of I_{so} under one-step depolarization protocol from -70mv to +50mv, 0.2s) (n=5, paired t-test $p<0.05$) (C). In the Ca^{2+}-free extracellular solution, H_2O_2 significantly enhanced I_{so} by 5 to 6 pA/pF at the voltage steps positive to -20mv (n=7, two-way ANOVA $p<0.05$) (D), However H_2O_2 did not change the amplitude of I_{so} in the Ca^{2+}-free extracellular solution when 100kU/ml catalase applied intracellularly (n=5, two-way ANOVA $p>0.05$) (E).

A.



B.

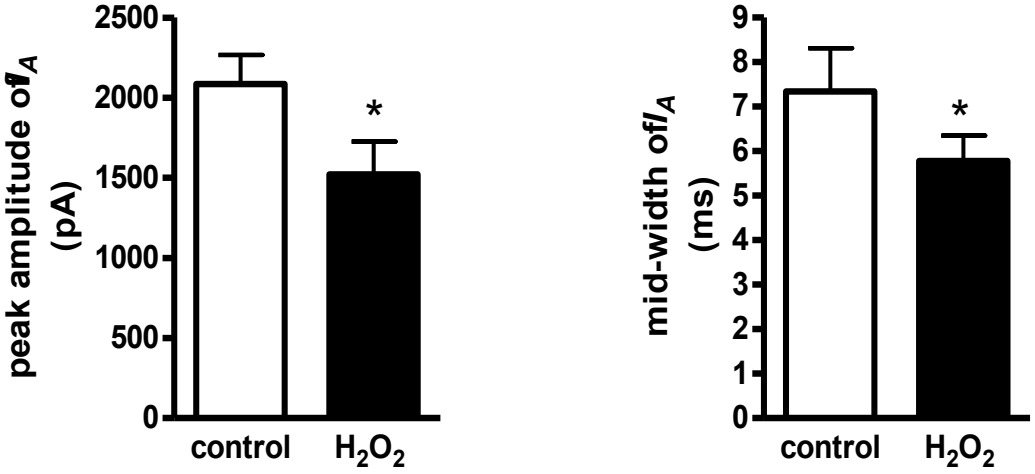
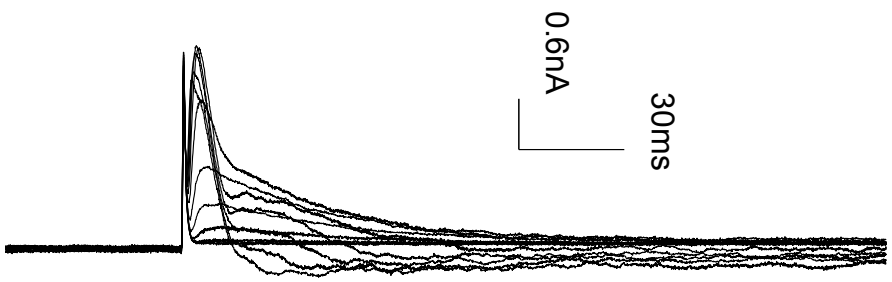


Figure 4-4. The effects of H_2O_2 on transient outward K^+ current

Figure 4-4(cont'd)

I_A obtained by subtracting current under -40mv to 30mv step from one under -70mv to 30mv step was compared between before and after H_2O_2 application. The subtracting traces were shown before and after H_2O_2 treatment (A). The dark line represents the control; the red line presents the H_2O_2 treatment. Both the peak amplitude and mid-width of were I_A significantly decreased by H_2O_2 (t-test, $p<0.05$, $n=9$) (B). (For interpretation of the references to color in this figure, the reader is referred to the electronic version of this dissertation)

A.



B.

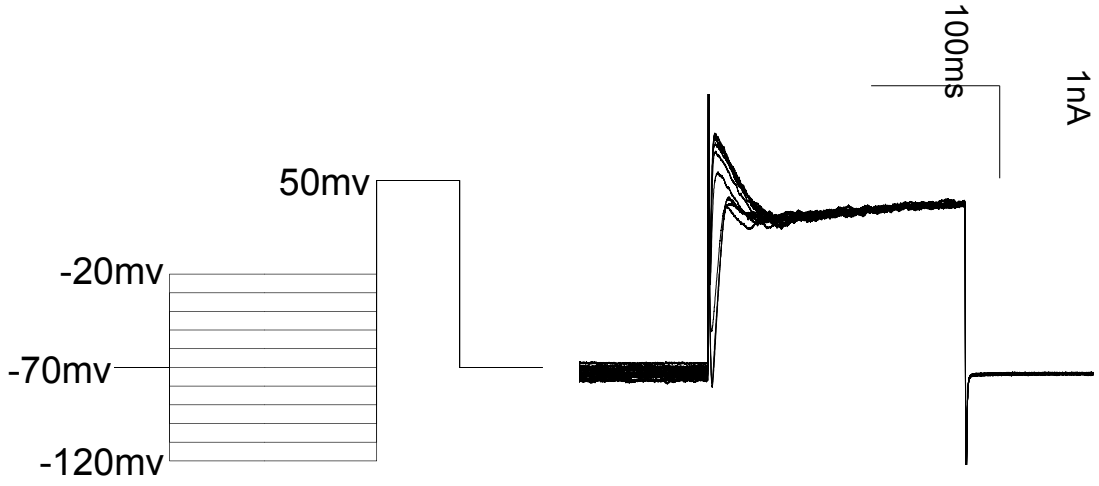


Figure 4-5. The effects of H_2O_2 on activation and inactivation transient outward K^+ current

Figure 4-5(cont'd)

C.

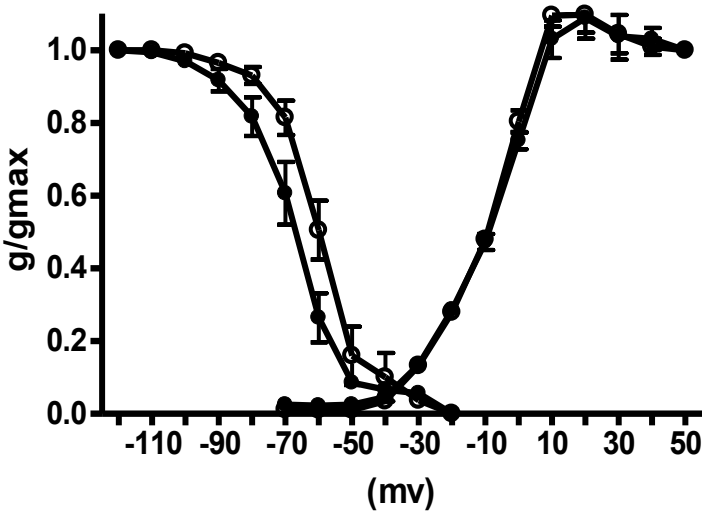
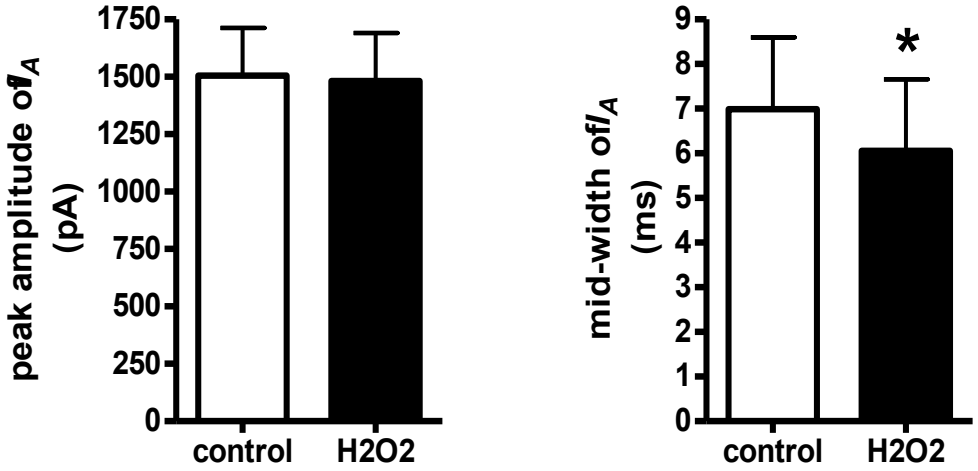


Figure 4-5(cont'd)

Voltage-dependent I_A activation was generated using two protocols. One protocol is to depolarize neurons from -70mv to +50mv in 10mv. Extra -20mv depolarization for 0.5 s was applied right before depolarization from -70mv to +50mv in the other protocol. I_A was achieved by subtracting currents from these two protocols (A). I_A inactivated was studied by depolarizing neurons to +50mv proceeded by a prepulse, lasting 0.5s, from -120mv to -20mv (B). I_A activation curves and inactivation curves were compared before and after H_2O_2 treatment using the conductance-voltage plot. H_2O_2 shifted voltage dependence of steady –state inactivation to the left but did not affect the voltage dependence of activation (C)

A.



B.

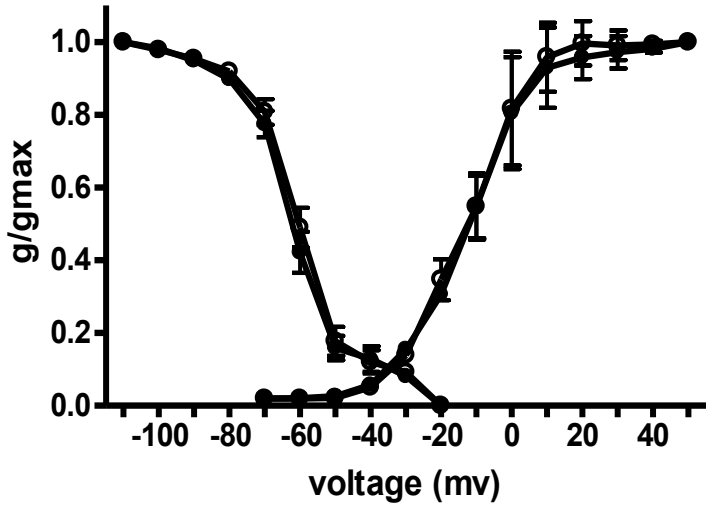


Figure 4-6. The effects of H_2O_2 on activation and inactivation transient outward K^+ current in presence of intracellular catalase

Figure 4-6(cont'd)

In presence of intracellular catalase (100kU/ml), I_A obtained by subtracting current under step from -40mv to 30mv from one under step from -70mv to 30mv was compared between before and after H_2O_2 application. Peak amplitude was not significantly changed by H_2O_2 (t-test, $p>0.05$, $n=8$), but the mid-width was significantly decreased by H_2O_2 (t-test, $p<0.05$, $n=8$) (A). There were no shifts of both voltage dependence activation curve and voltage dependence steady-state inactivation curve by H_2O_2 with intracellular catalase application (B).

BIBLIOGRAPHY

BIBLIOGRAPHY

Angelova P & Muller W (2006). Oxidative modulation of the transient potassium current IA by intracellular arachidonic acid in rat CA1 pyramidal neurons. *Eur J Neurosci* **23**, 2375-2384.

Barkats M, Horellou P, Colin P, Millecamps S, Faucon-Biguet N, & Mallet J (2006). 1-methyl-4-phenylpyridinium neurotoxicity is attenuated by adenoviral gene transfer of human Cu/Zn superoxide dismutase. *J Neurosci Res* **83**, 233-242.

Behl C, Davis JB, Lesley R, & Schubert D (1994). Hydrogen peroxide mediates amyloid beta protein toxicity. *CELL* **77**, 817-827.

Beswick RA, Zhang H, Marable D, Catravas JD, Hill WD, & Webb RC (2001). Long-term antioxidant administration attenuates mineralocorticoid hypertension and renal inflammatory response. *Hypertension* **37**, 781-786.

Bychkov R, Pieper K, Ried C, Milosheva M, Bychkov E, Luft FC, & Haller H (1999). Hydrogen peroxide, potassium currents, and membrane potential in human endothelial cells. *Circulation* **99**, 1719-1725.

Choi S, Yeum CH, Kim YD, Park CG, Kim MY, Park JS, Jeong HS, Kim BJ, So I, Kim KW, & Jun JY (2008). Receptor Tyrosine and MAP Kinase are Involved in Effects of H(2)O(2) on Interstitial Cells of Cajal in Murine Intestine. *J Cell Mol Med*.

Connor JA & Stevens CF (1971a). Prediction of repetitive firing behaviour from voltage clamp data on an isolated neurone soma. *J Physiol (Lond)* **213**, 31-53.

Connor JA & Stevens CF (1971b). Voltage clamp studies of a transient outward membrane current in gastropod neural somata. *J Physiol (Lond)* **213**, 21-30.

Dai X, Cao X, & Kreulen DL (2006). Superoxide anion is elevated in sympathetic neurons in DOCA-salt hypertension via activation of NADPH oxidase. *Am J Physiol Heart Circ Physiol* **290**, H1019-H1026.

Dai X, Fink GD, & Kreulen DL. Elevation of superoxide anions in sympathetic neurons in DOCA-salt hypertension via activation of NADPH oxidase. FASEB J . 2004.
Ref Type: Abstract

Ferretti G, Bacchetti T, DiLudovico F, Viti B, Angeleri VA, Danni M, & Provinciali L (2006). Intracellular oxidative activity and respiratory burst of leukocytes isolated from multiple sclerosis patients. *Neurochem Int* **48**, 87-92.

Gulbis JM (2002). The beta subunit of Kv1 channels: voltage-gated enzyme or safety switch? *Novartis Found Symp* **245**, 127-141.

Halliwell B (1992). Reactive oxygen species and the central nervous system. *J Neurochem* **59**, 1609-1623.

Kourie JI (1998). Interaction of reactive oxygen species with ion transport mechanisms. *Am J Physiol* **275**, C1-24.

Muller W & Bittner K (2002). Differential oxidative modulation of voltage-dependent K+ currents in rat hippocampal neurons. *J Neurophysiol* **87**, 2990-2995.

Pongs O (1999). Voltage-gated potassium channels: from hyperexcitability to excitement. *FEBS Lett* **452**, 31-35.

Prasad M & Goyal RK (2004). Differential modulation of voltage-dependent K+ currents in colonic smooth muscle by oxidants. *Am J Physiol Cell Physiol* **286**, C671-C682.

Ruppersberg JP, Stocker M, Pongs O, Heinemann SH, Frank R, & Koenen M (1991). Regulation of fast inactivation of cloned mammalian IK(A) channels by cysteine oxidation. *Nature* **352**, 711-714.

Somers MJ, Mavromatis K, Galis ZS, & Harrison DG (2000). Vascular superoxide production and vasomotor function in hypertension induced by deoxycorticosterone acetate-salt. *Circulation* **101**, 1722-1728.

Stadtman ER & Levine RL (2000). Protein oxidation. *Ann N Y Acad Sci* **899**, 191-208.

CHAPTER 5

DISCUSSION AND CONCLUSION

Two major electrophysiological differences between dissociated celiac ganglion (CG) neurons from DOCA-salt hypertensive rats and normotensive control rats were identified in the present study. 1) The distribution of neuronal firing patterns among neurons from hypertensive rats differed significantly from the firing pattern distribution among neurons from normotensive rats. Specifically, hypertensive rats had more phasic neurons, fewer adaptive neurons and tonic neurons than normotensive rats. The firing frequencies of the tonic neurons from the hypertensive rats were also significantly lower than the firing frequencies of the tonic neurons from the normotensive rats. 2) The delayed rectifier K^+ current (I_{KV}), the BK K^+ current (I_{BK}) and the A-type K^+ current (I_A) were significantly attenuated in CG neurons from hypertensive rats compared with these currents in normotensive rat neurons. However, there were no significant differences in either the M-type K^+ currents or the whole-cell Ca^{2+} currents in CG neurons from the two groups.

The relationships between these two main differences were further investigated by applying specific K^+ channel blockers to normal CG neurons. Using an appropriate channel blocker to reduce either the I_{KV}, the I_{BK} or the I_A changed the neuronal firing patterns and/or firing frequencies in such a way that neurons from normal rats resembled neurons that had been obtained from hypertensive rats. To further explore other possible factors that might contribute to the observed differences between hypertensive and normal rats, normal CG neurons were exposed to a reactive oxygen species (ROS), H_2O_2. Following H_2O_2 treatment, more phasic neurons were observed, and there appeared to be fewer adaptive neurons and tonic neurons, similar to what

was observed in neurons from hypertensive rats. Moreover, exposure to H_2O_2 resulted in lower neuronal firing frequencies, attenuated I_{BK} and I_A and an increased I_{KV} current. These data indicate that elevated ROS levels in the celiac ganglia (CGs) that had been isolated from DOCA-salt hypertensive rats might partially account for the observed electrophysiological changes in the CG neurons from the hypertensive rats. The significance of these findings regarding hypertension is discussed in this chapter.

The sympathetic nervous system in hypertension

Increased sympathetic activity in hypertension

Elevated sympathetic nervous system (SNS) activity is widely accepted as an important contributor to both the development and maintenance of hypertension (Bohr & Dominiczak, 1991). Some research findings have shown that the enhanced sympathetic activity occurs during the early stages of hypertension; muscle sympathetic activity is frequently elevated in patients with borderline hypertension, which is defined as a diastolic pressure that occasionally rises above 90 mmHg (Anderson *et al.*, 1989). In addition, normotensive adults with familial predispositions toward developing hypertension generally exhibit higher levels of total body norepinephrine spillover when compared with adults who have no family history of hypertension (Ferrier *et al.*, 1993). Elevated plasma levels of norepinephrine have also been reported in young hypertensive patients; similar elevations have not been reported in older hypertensive patients (Esler *et al.*, 1986). However, long-term elevations in sympathetic activity accompany hypertension as the disease progresses; the observed enhanced sympathetic activity does not disappear once hypertension has been established.

Instead, it becomes a typical pathological change that occurs in hypertension (Schlaich *et al.*, 2004). Blocking sympathetic activity surgically lowers the observed BP elevation in hypertensive patients (Chou *et al.*, 2005). Both renal nerves and the CNS are proposed to be the key contributors to hypertension maintenance (Malpas, 2010). CG neurons receive and integrate synaptic input from preganglionic neurons in the spinal cord and distribute impulses to various abdominal organs. However, the specific role that the CG plays in hypertension is not entirely clear. In the present study, DOCA-salt hypertensive rats, which have increased sympathetic activity, were selected for use in our exploration of the electrophysiological changes that occur in the CGs of hypertensive rats. In the present study, the CGs were dissected from the rats immediately after the rats had undergone 4 weeks of DOCA and salt treatment. During the 4-week treatment period, the BPs of the rats gradually increased and reached plateaus; thus, it is likely that the observed changes in the CGs from these rats are related to the maintenance of hypertension, not the initiation of it.

The sympathetic nervous system in DOCA-salt hypertensive rats

Although previous studies have established that DOCA-salt hypertensive rats have elevated sympathetic activity levels (de Champlain, 1990), the various components of the SNS, including the cardiovascular centers in the brain stem, the sympathetic preganglionic neurons in the spinal cord, the sympathetic ganglia and the sympathetic nerves have not been fully examined in this animal model. The existing studies of the SNS in DOCA-salt hypertensive rats are summarized as follows. Sympathetic neurons in the CNS have been shown to be hyperactive in hypertensive rats (Gomez Sanchez, 1991;Kubo *et al.*, 1996); the observed hyperactivity may result

from activation of mineralocorticoid receptors in CNS neurons (McManus *et al.*, 2009). Moreover, the splanchnic nerve (a preganglionic nerve that sends signals to the CG) maintains its elevated activity levels in hypertensive rats (Iriuchijima *et al.*, 1975), and that the overall level of sympathetic activity, which is measured in terms of the circulating catecholamine level, is enhanced these animals (de Champlain, 1990). However, the values of one index of splanchnic sympathetic activity, nonhepatic splanchnic NE spillover, does not significantly differ between normotensive and hypertensive rats. Interestingly, surgical removal of the CG diminishes the degree to which BP increases during DOCA treatment (Kandlikar & Fink, 2011). The present study explores the ways in which CG neurons from normotensive and hypertensive rats differ.

Predicting the possible roles of altered firing properties in CG neurons from hypertensive rats

The CG could contribute directly to hypertension, or it could have a completely opposite function, namely, playing a compensatory role. CG neurons may have one of the following possible roles: 1) relaying the enhanced sympathetic activity in the CNS to target organs in the absence of changes to their own excitation properties; 2) further increasing SNS activity by becoming more sensitive to preganglionic stimulation; or 3) dampening enhanced sympathetic activity signals from the CNS by becoming less excitable.

Instead of being activated by preganglionic nerve stimulation, the CG neurons in the present *in vitro* study were stimulated via a sustained current injection. Although a sustained current injection clearly differs from the currents generated by the release of neurotransmitters at preganglionic nerve terminals, this type of stimulation provides

valuable insight into the electrophysiological properties of these neurons by providing a means of isolating the different factors that contribute to those firing properties. The postganglionic neurons used in the present study have been dissociated from other related neurons and satellite cells, which allows their properties to be studied without interference from the related cells. The results showed that the firing properties of CG neurons appear to have changed in ganglia from HT animals and that this might be a response to chronically increased firing of preganglionic nerves. I predict that these changes in the firing properties of the CG neurons in these rats might contribute to the enhancement of SNS activity in hypertensive rats. Previous research on neuronal firing patterns and signal transmission in sympathetic ganglia will be summarized in the following paragraphs to explain this prediction.

Neuronal firing pattern is a changeable firing property

The specific neuronal firing patterns that occur in response to sustained suprathreshold current injection have been thought to be permanent firing characteristics of neurons that have specific functions. These patterns have been considered electrophysiological markers of various groups of neurons, such as vasoconstriction neurons or visceral neurons (Cassell *et al.*, 1986). Thus, the phasic neurons in the CG were predicted to be vascular neurons because nerves that innervate blood vessels tend to fire in a bursting manner (Schlaich *et al.*, 2004). However, neurons in the inferior mesenteric ganglion (IMG) that project to mesenteric blood vessels fire tonically when subjected to sustained depolarizing current (Browning *et al.*, 1999). More research studies indicate that the firing patterns of neurons in sympathetic ganglia are changeable. The firing patterns of these neurons can be altered

by exposure to physiological substances and in different disease states. For example, exposure to substance P causes phasic neurons in the CG to become adaptive neurons (Vanner *et al.*, 1993). and treatment with nerve growth factor shifts the firing patterns of neurons in the superior cervical ganglion (SCG) from tonic to phasic (Luther & Birren, 2009). Both my study and research performed by others indicate that the firing patterns of sympathetic ganglionic neurons may be modified in hypertensive rats (Jubelin & Kannan, 1990;Yarowsky & Weinreich, 1985).

Neurotransmission in sympathetic ganglia

Assuming that neuronal firing patterns are modifiable, the next question is how the observed changes in neuronal firing patterns contribute to elevated sympathetic activity in hypertension. Let us first consider the anatomical and physiological characteristics of the CG. Nerve fiber impulses that originate from several preganglionic neurons converge on a single postganglionic neuron in the CG (Janig & McLachlan, 1992b;Kreulen & Szurszewski, 1979). Upon receiving stimulation from preganglionic nerve cells, the CG neurons generate EPSCs (excitatory postsynaptic currents), which include both fast, excitatory preganglionic acetylcholine (Ach) inputs and other weak inputs. The number of Ach quanta released from a preganglionic nerve terminal is usually sufficient to depolarize the postsynaptic neuron and generate a subsequent AP (Bratton *et al.*, 2010;Janig & McLachlan, 1992a); weak, subthreshold excitatory postsynaptic potentials depolarize membrane and increase the probability of generating APs(Peters & Kreulen, 1986). Neurons in the CG can receive suprathreshold Ach stimulations from each of several preganglionic nerve terminals that are summed spatially, and/or high-frequency presynaptic activity from a central sympathetic signal

may produce several overlapping EPSCs that are summed temporally. The resulting spatial and/or temporal summations of EPSCs can then be graded in a fashion similar to the *in vitro* current injections used in the present study. Activity in the sympathetic nervous system is precisely regulated in accordance with the physiological needs of the body; the level of sympathetic activity is low when the body is at rest and high when it performs physical or mental activities. As the activity level of the sympathetic nervous system increases, the CG neurons are more prone to generating spatial and/or temporal summations of EPSCs; these have been mimicked by the *in vitro* current injections used in the present study. The firing frequency of a sympathetic postganglionic nerve is approximately 0.2 Hz. In contrast, preganglionic nerves fire much more frequently; their firing rate is approximately 5 Hz (Su *et al.*, 2010). This difference in firing frequency is most likely accounted for by the responses of ganglionic neurons to the different strengths and frequencies of the synaptic inputs.

Predicting the significance of alterations in the firing properties of CG neurons for hypertension

Many postganglionic nerve fibers that follow the same sympathetic pathway are bundled together to form a sympathetic nerve. Thus, the sympathetic nerve fibers from tonic, adaptive and phasic neurons may be combined in a single sympathetic nerve, and the firing patterns generated by these postganglionic neurons then propagate along the postganglionic nerve fibers of a sympathetic nerve. Combined firing of several postganglionic nerve fibers within a given sympathetic nerve can be described as sympathetic bursts that occur in the background of high-frequency firing spikes, and these so-called sympathetic bursts may be explained by the arrangement of specific

postganglionic nerve fibers. Firing along the neuronal fibers of phasic and adaptive neurons are more likely to contribute to a bursting firing pattern than fibers originating from tonic neurons. Although firing along the neuronal fibers that originate from tonic neurons may be involved in high-frequency background firing, the frequency of sympathetic nerve firing is determined on the basis of the frequency of burst firing (Schlaich *et al.*, 2004). Thus, phasic and adaptive neurons would play a key role in determining the firing frequencies of sympathetic nerves. In the present study, more phasic neurons were found in dissociated CG neurons from hypertensive rats than in dissociated CG neurons from normal rats. On the basis of the assumption that the phasic and adaptive neurons are the primary contributors to burst firing patterns in sympathetic nerves, the presence of additional phasic and/or adaptive neurons implies that more bursts will occur during sympathetic nerve firing. These additional bursts result in increases in the firing frequencies of sympathetic nerves, which are subsequently identified as elevated sympathetic activity. Therefore, I predict that alterations in the firing properties of CG neurons would enhance an already elevated central sympathetic central and thereby contribute to the elevated sympathetic activity observed in DOCA-salt hypertensive rats.

My prediction is based on a reasonable assumption sympathetic nerve activity is determined by the percentages of phasic and adaptive neurons in the sympathetic ganglia. The burst firing pattern has been shown to provide meaningful information to dopamine neurons in the central nervous system (Jeong *et al.*, 2012). In CG neurons, the release of neurotransmitters is likely related to burst firing patterns, not high-frequency nerve firing. If the high-frequency nerve firing caused the release of

neurotransmitters, the supply of neurotransmitters in any given nerve terminal would be depleted very quickly. Compared with continuous nerve stimulation, using a bursting pattern when stimulating sympathetic nerves has been shown to cause enhanced vasoconstriction (Hardebo, 1992), which suggests that the burst firing pattern is an effective means of inducing the release of neurotransmitters. Therefore, I predict that the presence of additional phasic and adaptive neurons in the CGs of DOCA-salt hypertensive rats would lead to in an increase in the frequency of sympathetic burst firing that would then result in increased sympathetic neurotransmitter release and the subsequent enhancement of vasoconstriction.

Changes in K^+ currents and neurotransmitter release in hypertension

In addition to their contributions to neuronal firing, voltage-gated K^+ channel currents are also involved in regulating the release of neurotransmitters. Reductions in K^+ currents could increase the AP duration for a given neuron by decreasing its repolarization rate. This increase in AP duration would then allow greater Ca^{2+} influx, which in turn triggers the release of additional neurotransmitters. The BK channel is involved in the prostanoid TP receptor-mediated inhibition of norepinephrine (NE) release in sympathetic nerve terminals (Nakamura & Yokotani, 2010). Delayed rectifier K^+ channel activity contributes to the inhibition of neurotransmitter release in the urinary bladder and skeletal muscles (Szulczyk & Szulczyk, 2003;Brooke *et al.*, 2004). In the present study, attenuated I_{KV}, I_{BK} and I_A were found in CG neurons from DOCA-salt hypertensive rats. These changes in current flow could result in increased Ca^{2+} influx,

subsequent increases in the release of neurotransmitters, and ultimately in elevated levels of sympathetic activity.

In summary, changes in neuronal firing properties in CG neurons from hypertensive rats would contribute to increased sympathetic activity by two ways. One is to increase sympathetic nerve burst firing frequency. The other is to enhance neurotransmitters release from sympathetic nerve terminals (Figure 5-1).

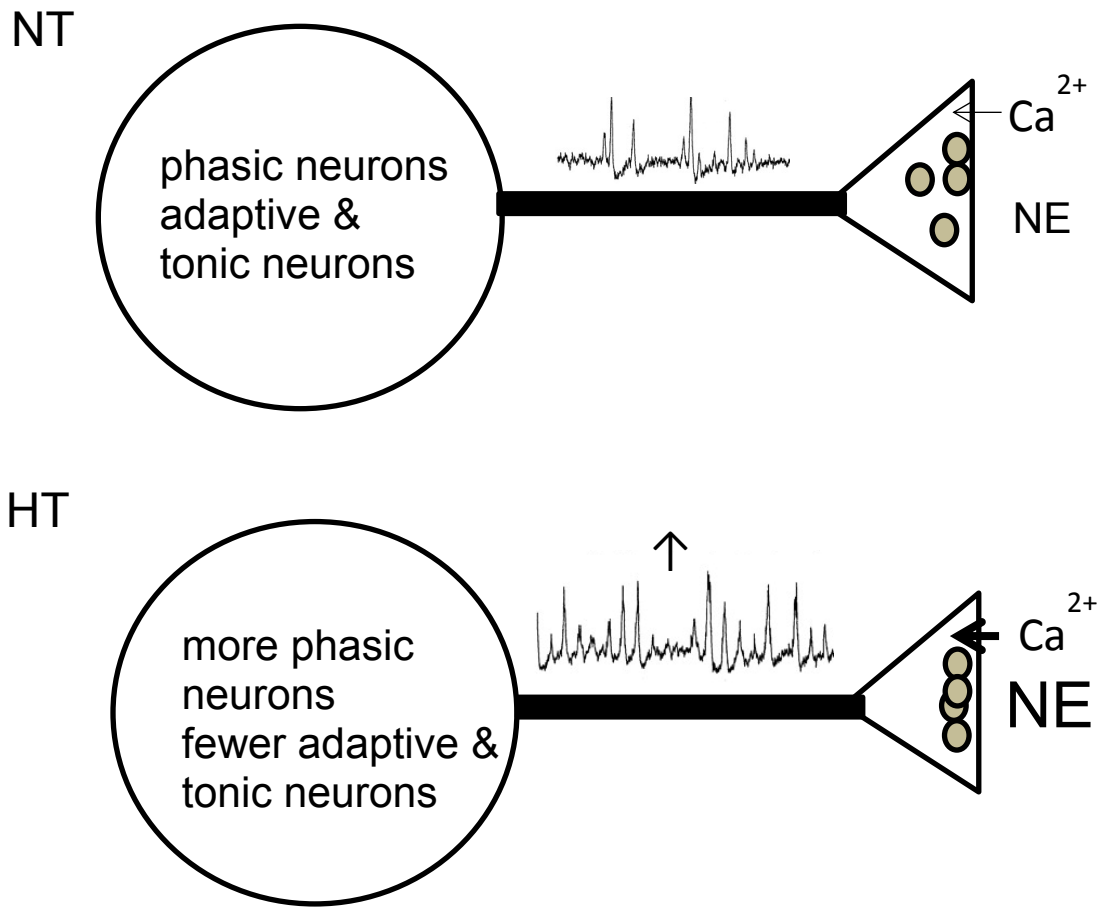


Figure 5-1. Prediction of the role of altered firing properties of CG neurons in HT. The traces of nerve activity are from Schlaich group's recording of nerve activity in normotensive and hypertensive patients (Schlaich *et al.*, 2004).

Changes in the neuronal firing properties of SCG neurons in other animal models of hypertension

The firing properties of sympathetic postganglionic neurons have been examined in SCG neurons from both SHRs (Jubelin & Kannan, 1990;Yarowsky & Weinreich, 1985). Two main firing patterns, tonic and phasic, that appear to occur in response to sustained suprathreshold current injections have been identified. More tonic neurons and fewer phasic neurons were found in the populations of SCG neurons that were isolated from both of the aforementioned hypertensive models, which shows that these hypertensive animal models have altered distributions of neuronal firing patterns. However, the alterations in the firing patterns of SCG neurons that were identified in the aforementioned studies are opposite to the findings from my study. The present study considered dissociated CG neurons from DOCA-salt hypertensive rats and found evidence that there were more neurons with phasic firing patterns and fewer with tonic firing patterns in response to sustained suprathreshold current injection. This inconsistency may be related to inherent differences in the types of hypertensive models used, namely differences between genetic and non-genetic models. The observed discrepancy might also be caused by differences between paravertebral and prevertebral sympathetic ganglia.

My study indicates that the observed changes in the distribution of neuronal firing patterns could be related to the altered voltage-gated K^+ currents and elevated ROS levels that occur in the CG of DOCA-salt hypertensive rats. The ROS levels in SCG from SHR animals have not been determined, but in contrast to my finding, the densities of A-type K^+ currents (I_A) are known to be significantly elevated in the SCG of SHR rats

(Robertson, 1999). The amplitude of the I_A was attenuated in CG neurons from DOCA-salt hypertensive rats, and it was also decreased following H_2O_2 exposure in CG neurons from normal rats. Therefore, the elevated ROS concentration in CG neurons might reduce the I_A and thereby contribute to the altered neuronal firing pattern distribution observed in DOCA-salt hypertensive rats. Similarly, the firing pattern changes observed in SCG neurons from SHR might be related to an elevated I_A.

Neuronal firing properties and ROS

K^+ currents and neuronal firing

K^+ currents generated by the activation of corresponding ion channels are the key elements in the formation of various neuronal firing patterns, and they determine neuronal firing frequencies. Each type of K^+ channel is composed of several subcomponents that are either expressed by different genes or via different splices of a single gene. There is more diversity among K^+ channels than among any other type of channel, and thus, it can be concluded that K^+ channels help neurons encode trains of APs and facilitate rhythmic electrical activity. K^+ channels can be divided into three categories: voltage-gated K^+ channels, ligand-gated K^+ channels and ungated K^+ channels. Voltage-gated K^+ channel currents have been extensively studied in vascular smooth muscle cells derived from hypertensive model animals (see chapter 3 for a

detailed discussion). However, voltage-gated K^+ channel currents in the cells of the sympathetic ganglia in hypertensive rats have not been studied. The present study examined the voltage-gated K^+ channel currents in CG neurons from hypertensive and normotensive rats. Carrier identified the I_{Kv}, I_{KCa}, I_A and I_M in CG neurons isolated from normal SD rats, and these currents are consistent with the currents considered in my findings (Carrier, 1995).

The present study found that the amplitudes of the I_{Kv}, I_{BK} and I_A in the CG neurons from the DOCA-salt hypertensive rats were smaller than the amplitudes of those currents in the corresponding neurons from control rats. It also indicates that the altered neuronal firing patterns that are associated with hypertension result from the combined contributions of changes in several K^+ currents and that they cannot be accounted for by modulation of a single K^+ current. Decreased K^+ currents contribute to increased AP duration, decreased repolarization rate and smaller afterhyperpolarization found in CG neurons from hypertensive rats. Elangated AP duration would lead to increased Ca^{2+} influx, which enhances I_{SK} and dampens neuronal firing. Therefore, the changed distribution in neuronal firing patterns and decreased neuronal firing frequency in hypertension could be related to increased AP duration and elevated I_{SK} , which caused by decreased I_{Kv}, I_{BK} and I_A (see chapter 3 for a more detailed discussion). Some research studies further prove that the down-regualtions in these currents lead to the differences in firing properties I explored (see 4.3 in chapter 1 for a more detailed

discussion). My results by using blockers specific to these channels also indicates decreased I_{KV}, I_{BK} and I_A are the contributors for CG neuronal firing changes in hypertension (see chapter 2 for a more detailed description).

ROS and neuronal firing in the SNS

ROS, such as superoxide anions ($O_2^{\bullet-}$), hydrogen peroxide (H_2O_2) and hydroxyl radicals (OH•), are active chemicals that are involved in the pathogenesis of hypertension. ROS are widely accepted as being both important intracellular signaling molecules and molecules that cause tissue damage. $O_2^{\bullet-}$, H_2O_2 and OH• are generally thought to have similar effects on various cellular activities. In the present study, H_2O_2 was applied to CG neurons to explore the way in which ROS exposure affects neuronal firing properties. However, the underlying mechanism(s) that mediate the effects of H_2O_2 on neuronal firing remain unclear. One possible mechanism may be the ROS-induced oxidation of K^+ channels because the amino acid residues in several K^+ channel proteins are highly susceptible to ROS-mediated oxidation (Stadtman & Levine, 2000). Protein oxidation can lead to hydroxylation of aromatic groups and aliphatic amino acid side chains, nitration of aromatic amino acid residues, nitrosylation of sulfhydryl groups, sulfoxidation of methionine residues, chlorination of aromatic groups and primary amino groups, and the conversion of some amino acid residues to carbonyl derivatives (Stadtman & Levine, 2000). Interestingly, a recent study found that the oxidation of sulfur-containing amino acid residues is reversible; this result may provide a possible mechanism for the regulation of sulfur-containing proteins, such as the BK

channel protein (Yi *et al.*, 2010). Moreover, the K_V, BK and A-type K^+ channels may become oxidized as a result of the elevated ROS concentrations in CG neurons, which in turn may result in diminished K^+ currents in neurons from hypertensive rats.

Studies of the effects that ROS have on neuronal firing in the central SNS have shown that ROS are involved in the central neural control of BP. Intracerebroventricular infusions of Tempol (a SOD mimetic that lowers ROS concentrations) result in decreased blood pressure and sympathetic nerve activity (Campese *et al.*, 2004). In addition, inhibiting NADPH oxidase activity in central sympathetic neurons prevents an angiotensin II-induced increase in neuronal firing (Sun *et al.*, 2005). Angiotensin II is one of the major circulating factors that affects the sympathetic cardiovascular center in hypertension (Zimmerman & Davisson, 2004). Taken together, these results suggest that the elevated ROS concentrations that occur in conjunction with hypertension may increase neuronal firing in central sympathetic neurons and thereby contribute to the observed elevations in sympathetic activity levels.

Research regarding the peripheral sympathetic nervous system has shown that ROS concentrations are elevated in sympathetic ganglia neurons from DOCA-salt hypertensive (Dai *et al.*, 2006). However, it is unclear about the physiological roles of ROS in these neurons. Other studies indicate that both neuronal firing and K^+ channel behavior may be regulated by ROS (see detailed discussion in Chapter 4).The present study found that ROS exposure resulted in the modulation of neuronal firing properties in dissociated CG neurons. H_2O_2 converts adaptive and some tonic neurons to phasic neurons. It also decrease the firing frequency. These changes can be contributed by its

inhibitory effects on I_{BK} and I_A as I discussed before. However, it also enhances I_{Kv} , which may not be related to its effects on neuronal firings. The decreased AP repolarization rate and smaller AHP by H_2O_2 treatment are consistent with decreased I_{BK} and I_A. Compared to the neuronal firing property differences I found between hypertensive and normotensive rats, the changes by H_2O_2 mimic altered neuronal firing properties in hypertensive rats except increased I_{Kv}.

H_2O_2 in sympathetic ganglia and hypertension

In the present study, ROS-mediated effects on neuronal firing were tested using H_2O_2, which is a product of the dismutation of $O_2^{\cdot -}$ by superoxide dismutase (SOD). In hypertension, changes in the concentration of $O_2^{\cdot -}$ would lead to subsequent alterations in H_2O_2 concentrations. The DHE (dihydroethidium) staining technique is a popular method of measuring cellular $O_2^{\cdot -}$ levels in scientific research, but this technique is not strictly specific to $O^{2\bullet -}$. The compounds used in DHE staining also react with H_2O_2, so the observed elevations in the $O_2^{\bullet -}$ concentrations in cells from hypertensive organisms that have been found using DHE staining techniques may also indicate increased H_2O_2 levels in these cells. H_2O_2 is more stable than $O_2^{\bullet -}$, which is a short-lived ROS, and thus, it may be a more prominent contributor to chronic diseases such as hypertension.

H_2O_2 can be generated both outside and inside of CG neurons. First, the satellite

cells that surround CG neurons may be responsible for the extracellular production of H_2O_2. The activity level of NADPH oxidase is elevated in CG cells from DOCA-salt hypertensive rats (Cao *et al.*, 2007), which results in the accumulation of $O_2^{\bullet-}$ in the extracellular space (Barbacanne *et al.*, 2000). The extracellular $O_2^{\bullet-}$ generated by NADPH oxidase in satellite cells could then be converted into the lipid-soluble compound H_2O_2, which is subsequently able to freely enter the neurons via diffusion through their cell membranes (Halliwell, 1992). DHE staining of IMG samples showed that satellite cells exhibit higher levels of $O_2^{\bullet-}$ in comparison to the $O_2^{\bullet-}$ observed in neurons (Dai *et al.*, 2004a). $O_2^{\bullet-}$ can also be converted into H_2O_2 within the CG neurons. In the present study, the firing properties of dissociated CG neurons were tested both before and after receiving extracellular applications of H_2O_2. Because H_2O_2 is lipid soluble, the extracellular application of H_2O_2 would mimic both its extracelluar and intracelluar effects on neurons. The extracellular H_2O_2 can be converted from $O_2^{\bullet-}$ that had been released from the satellite cells. The intracellular H_2O_2 can be generated from observed elevated intracellular ROS concentrations that occur in hypertension.

In summary, the present study illustrates the way in which elevated ROS concentrations may attenuate the I_{BK} and I_A, thereby leading to alterations in the distributions of various neuronal firing patterns in CG neurons from hypertensive rats.

Factors that may contribute to the observed changes in neuronal firing properties

and elevated ROS levels in CGs from DOCA-salt hypertensive rats

The present study found evidence of altered firing properties in CG neurons from DOCA-salt hypertensive rats that may in part be caused by elevated ROS levels in the CG of these animals. In addition, some factors related to the development of the DOCA-salt hypertensive animal model may also contribute to the findings of this study.

Increased central sympathetic drive

The splanchnic nerve, a preganglionic nerve to the CG, exhibits increased activity in DOCA-salt hypertensive rats (Iriuchijima *et al.*, 1975). This enhancement of preganglionic activity may contribute to the observed changes in voltage-gated K^+ currents in CG neurons from this animal model. Moreover, increases in I_A have been observed in SCG neurons when the SCG was decentralized by severing its preganglionic nerves (Szulczyk & Szulczyk, 2003). From this finding, we can infer that I_A would be diminished in conditions in which preganglionic nerves exhibit enhanced control of the SCG, as they do in HT. This deduction is consistent with the finding of decreased I_A in the present study.

In hypertensive rats, the increase in the central sympathetic drive leads to increased Ach release from preganglionic nerve terminals, which then results in the enhanced activation of nicotinic Ach receptors in postganglionic neurons. The nicotinic Ach receptor is a ligand-gated ion channel that is permeable to Na^+ , K^+ and Ca^{2+}; therefore, enhancing the activation of the nicotinic Ach receptor will result in a higher level of Ca^{2+} influx. Because ROS can be generated by mitochondria via a process

induced by intracellular Ca^{2+} (dam-Vizi & Starkov, 2010), it can be concluded that the aforementioned Ca^{2+} influx is able to increase ROS concentrations in DOCA-salt hypertensive rats (Xu *et al.*, 2011). The elevated ROS concentrations could then modulate the K^+ currents in CG neurons, as shown in the present study. Elevated intracellular Ca^{2+} concentrations could also modulate the activity and/or expression of K^+ channels by modifying the expression of various genes. Ca^{2+} influxes stimulate both c-fos transcription and the phosphorylation of c-AMP response element binding protein (CREB) in sympathetic ganglionic neurons (Zhao *et al.*, 2007), and CREB phosphorylation is involved in the regulation of various K^+ channels (Tong *et al.*, 2010). Therefore, the increased Ach release that results from a central sympathetic drive may increase the intracellular Ca^{2+} concentrations in CG neurons from hypertensive rats, and the increased intracellular Ca^{2+} concentrations could then contribute to the observed K^+ current changes via the enhancement of ROS production and/or changes in the levels of expression of genes that encode K^+ channels. Moreover, the attenuated Kv, BK and A-type K^+ currents found in this study would also lead to increased Ca^{2+} influxes by increasing the durations of APs and thereby forming a positive feedback loop that continues to enhance Ca^{2+} influx. On the other hand Ca^{2+} influx activates BK channels which repolarizes the membrane and prevent further Ca^{2+} influx. This negative feedback for Ca^{2+} influx may help to maintain Ca^{2+}level below a lethal level in

hypertension. Even though increased Ca^{2+} influx would enhance I_{BK}, I_{BK} was found to be attenuated in CG neurons from hypertensive rats, which could be resulted from the effects of elevated ROS level.

Upregulation of the ET-B receptor

In addition to increased central input, the expression of an endothelin-1 (ET-1) receptor, the endothelin-B (ETB) receptor, is upregulated in CGs from DOCA-salt hypertensive rats (Dai *et al.*, 2004b). There are two types of endothelin receptors: endothelin-A receptor and endothelin-B receptor. Endothelin-1 is a potent peptide that mediates vasoconstriction, and it is mainly secreted from endothelial cells. The tissue concentrations of ET-1 in both CGs and splanchnic circulation are similar between DOCA-salt hypertensive rats and normotensive control rats (Dai *et al.*, 2004b;Wang *et al.*, 2005). Enhanced ET-1 effects in hypertension would be mediated by increased ETB receptor expression. The elevated levels of this ETB receptor are known to be associated with increased ROS concentrations in the sympathetic ganglia of DOCA-salt hypertensive rats (Dai *et al.*, 2004b). Similarly, sarafotoxin 6c is a selective agonist of endothelin-B receptor, and the ROS levels in the sympathetic ganglia are also elevated in sarafotoxin 6c-induced hypertension (Li *et al.*, 2008). These data indicate that elevated ROS concentrations are associated with enhanced endothelin-B receptor activity in the sympathetic ganglia of hypertensive rats. As I have already mentioned, the present study found that both the neuronal firing patterns and K^+ currents were altered following exposure to one type of ROS, H_2O_2. Therefore, K^+ currents could be modulated by increased ROS levels that result from enhanced ET-1 functioning in the

CG neurons of DOCA-salt hypertensive rats. Some studies have previously indicated that K^+ channel activity can be modulated by ET-1. In the cardiac sympathetic ganglia, for example, the ET-1-induced inhibition of signal transmission is blocked by the application of an SK channel blocker (Tsutsumi *et al.*, 1995). In addition, voltage-gated K^+ channels are modulated by protein kinase C in smooth muscle cells from hypertensive rats (Barman, 2007;Moreno *et al.*, 2007). Both protein kinase C and Rho kinase mediate ET-1 induced vessel constriction (Barman, 2007), so it may be that the upregulation of the ETB receptor in CG neurons from hypertensive rats result in elevated ROS concentrations that then modulate both the neuronal firing properties of these neurons and the behavior and/or expression of their K^+ channels.

Other possible factors

In addition to increased central sympathetic drive and the upregulation of the ETB receptor, previous research has also proposed certain factors that are associated with hypertension and that may or may not contribute to the findings in the present study. First, ROS concentrations are elevated in both high-renin angiotensin II-induced (Rajagopalan *et al.*, 1996) and low-renin DOCA-salt hypertensive animal models (Somers *et al.*, 2000). These findings suggest that increased ROS levels may not be modulated by renin in cases of hypertension. Second, it is not clear whether the ROS elevations in DOCA-salt rats are caused by high levels of salt intake. However, they may be related to DOCA treatment; aldosterone, a mineralocorticoid hormone mimicked by DOCA, is known to be related to increased ROS concentrations (Shimoni *et al.*, 2008). Third, the short-term increase in BP that results from norepinephrine exposure

does not increase ROS production, whereas long-term increases in BP, such as those that occur as a result of DOCA-salt hypertension, are associated with elevated ROS levels. Taken together, these observations suggest that increased levels of ROS could be related to the consistent pathological changes that occur in organisms with hypertension. Fourth, another important change that occurs in DOCA-salt hypertensive model animals is the activation of pro-inflammatory cytokines (Seifi *et al.*, 2010). Extravasation of activated neutrophils at inflammation sites is accompanied by increased levels of ROS production (Vogalis & Harvey, 2003), so it is very likely that inflammation could increase the ROS concentrations in the sympathetic ganglia of hypertensive rats.

Vascular neurons in the CG

Substructure of the CG

The CG controls functions in several visceral organs, including the stomach, intestines, liver, pancreas and spleen. In this regard, the CG neurons are definitely not homogenous. There are vascular neurons in the CG, but there are also other types of neurons, such as those modulate gastrointestinal motility, splenic capsule contraction, lipolysis, etc. The vascular neurons that control splanchnic circulation are key contributors to BP regulation. One limitation of the present study is that the CG neurons that were examined were not specific vascular neurons. Some studies have focused on exploring the substructure of the CG. Morphologically, the CG comprises several ganglionic units that are separated by nerve fibers, capillaries and septa composed of connective tissue (Ribeiro *et al.*, 2002). In total, 12 different functional groups of

sympathetic nerves have been identified within the entire sympathetic nervous system (Janig & McLachlan, 1992a). Nerve fibers that originate from the CG may be included in different functional groups of nerves that control specific functions in specific organs. The ganglionic units may be arranged in a systematic way and may then be related to the specific innervations of the target organs.

Identification of vascular neurons

It is not clear whether a particular ganglionic unit is related to the distribution of vascular neurons. Using retrograde tracing techniques some research groups identified vascular neurons in prevertebral sympathetic ganglia that innervate mesenteric arteries or veins. For example, the Hsieh group reported that the vascular neurons are dispersed along the celiac ganglia in rats and that these neurons do not group into specific units (Hsieh *et al.*, 2000). Their study also showed that 54% of the stained vascular neurons innervate both the mesenteric artery and the mesenteric vein, which contradicts the results in the IMG from our laboratroy that used a different retrograde tracing method. We have demonstrated a more organized distribution of vascular neurons in the IMG: neurons that project to the artery are localized to the central part of the IMG and the neurons that project to the vein are generally localized to the peripheral part of the ganglion (Browning *et al.*, 1999). However, neither of these studies indicates that the vascular neurons are confined to a specific area within the sympathetic ganglia, so it is not reasonable to attempt to identify vascular neurons based on their location within the ganglia. Retrograde labeling is a more reliable means of identifying these neurons that can be used in future studies. However, the technique used by Hsieh's group requires further modification to acquire more accurate information regarding

vascular neurons. In their study, they labeled the blood vessels by applying dye to the surrounding the vessel walls, which could result in the staining of both the nerve terminal of a nerve that innervated the blood vessel and nerve fibers that ran parallel to the vessel before innervating other parts of other organs. We used a procedure that involved removing both the blood vessels and the IMG as well as injecting dye into the blood vessel. This method reliably identifies vascular neurons, but the IMG is such a small ganglion (2-3 mm in length) that following enzymatic dissociation it does not yield enough vascular neurons to conduct patch clamp studies.

Conclusion and perspectives

The present study focused on the CG, which is an important sympathetic ganglion that contributes to BP elevation in DOCA-salt hypertensive rats. More phasic neurons, fewer adaptive neurons and fewer tonic neurons were identified among dissociated CG neurons from hypertensive rats in response to sustained suprathreshold current injections, and the neurons had lower firing frequencies. These changes in the firing patterns of the CG neurons were related to the decreased K^+ currents in the CG neurons from hypertensive rats. Most of the electrophysiological changes that were found in the CG neurons from hypertensive rats could be mimicked by treating CG neurons from normal rats a type of ROS, H_2O_2 (Table 5-1). These findings allow the establishment of a relationship between the elevated ROS levels observed in the CG neurons from DOCA-salt hypertensive rats that have been shown in previous research work and the electrophysiological changes that were illustrated in the present study. Other factors, such as increased central sympathetic drive, upregulated endothelin-B

receptor expression, inflammatory status and even DOCA application and hypertension itself could contribute to the elevated levels of ROS production in DOCA-salt hypertensive rats, which further affects both neuronal firing and K^+ currents in CG neurons. According to these findings, I predict that the observed changes in the firing properties of CG neurons from hypertensive rats would positively contribute to the elevated sympathetic activity levels observed in DOCA-salt hypertensive rats (Figure 5-3). Decreased K^+ currents would also lead to elevations in the levels of neurotransmitter released from sympathetic nerve terminals in hypertension.

The present study provides evidence of the electrophysiological alterations that occur in sympathetic ganglionic neurons in an animal model of hypertension, and these changes could be targets of hypertension treatment protocols. Although some of the underlying mechanisms of these findings have been tested, additional studies are needed to clarify the role(s) played by the sympathetic ganglia in hypertension.

In vivo study of firing changes in CG neurons from hypertensive animals: Electrical probes could be placed in CG neurons in normotensive and hypertensive rats and could then be used to record the neuronal firing patterns that occur while the rats follow normal activity patterns. This type of study would help answer questions about the way in which the CG modulates the central sympathetic drive of an animal and the role CG plays in hypertension.

Possible mechanisms that underlie the hypertension-induced changes in neuronal firing properties: Additional research is needed to further elucidate the mechanisms that underlie the observed changes in the K^+ currents in CG neurons from

hypertensive rats. Molecular biological techniques could be used to determine whether there are changes in the K^+ channels at the gene and/or protein expression level. Moreover, other factors involved in the modulation of ROS production, such as endothelin-1, sympathetic central stimulation and inflammatory factors, could be applied to CG neurons. In addition to H_2O_2, other ROS could be applied to CG neurons to better understand the role(s) that ROS play in the modulation of neuronal firing. Finally, it is worth exploring the underlying mechanisms of the observed ROS-mediated effects on neuronal firing.

Functions of vascular neurons in the sympathetic ganglia of hypertensive rats: Vascular neurons in the CG could be identified by placing a retrograde tracer into splanchnic circulation. The CG with abdominal aorta, celiac artery and superior mesenteric artery could excised out and put into a dish with Kreb's solution. After the vascular openings are sealed with cyanoacrylate glue, a retrograde tracer such as fast blue could be injected into the vessels. After several days of organ culture, CG could be dissociated and vascular neurons would be labeled by the retrograde tacer. This technique would be valuable for predicting the roles of sympathetic ganglia in hypertension by allowing for the exploration of the neuronal firing properties of specific vascular neurons and the mechanisms by which they modulate.

APPENDIX

	HT compared to NT	Changes by H_2O_2
firing patterns	more phasic fewer adaptive & tonic neurons	more phasic fewer adaptive &tonic neurons
firing frequency	lower	decreased
I_{Kv}	lower	increased
I_{BK}	lower	decreased
I_A	lower	decreased

Table 5-1. Summaries of the differences between HT and NT rats and the changes by H_2O_2 in CG neuronal firing properties and K^+ channel current amplitudes of CG neurons

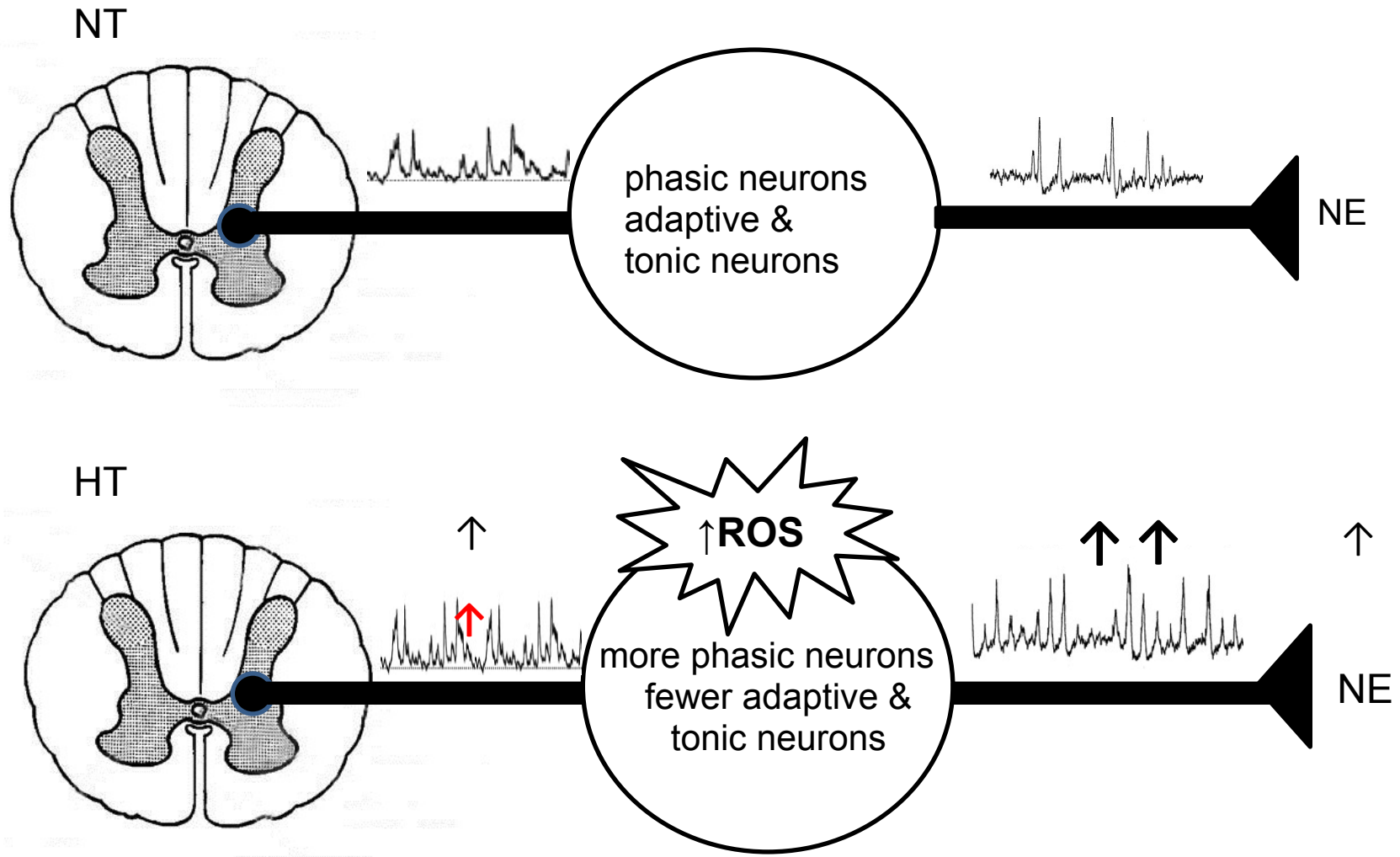


Figure 5-2. A diagram illustrating the possible role of CG neurons in sympathetic neurotransmission in DOCA-salt hypertensive rats

BIBLIOGRAPHY

BIBLIOGRAPHY

Anderson EA, Sinkey CA, Lawton WJ, & Mark AL (1989). Elevated sympathetic nerve activity in borderline hypertensive humans. Evidence from direct intraneural recordings. Hypertension 14, 177-183.

Barbacanne MA, Souchard JP, Darblade B, Iliou JP, Nepveu F, Pipy B, Bayard F, & Arnal JF (2000). Detection of superoxide anion released extracellularly by endothelial cells using cytochrome c reduction, ESR, fluorescence and lucigenin-enhanced chemiluminescence techniques. Free Radic Biol Med 29, 388-396.

Barman SA (2007). Vasoconstrictor effect of endothelin-1 on hypertensive pulmonary arterial smooth muscle involves Rho-kinase and protein kinase C. Am J Physiol Lung Cell Mol Physiol 293, L472-L479.

Bohr DF & Dominiczak AF (1991). Experimental hypertension. Hypertension 17, I39-I44.

Bratton B, Davies P, Janig W, & McAllen R (2010). Ganglionic transmission in a vasomotor pathway studied in vivo. J Physiol 588, 1647-1659.

Brooke RE, Moores TS, Morris NP, Parson SH, & Deuchars J (2004). Kv3 voltage-gated potassium channels regulate neurotransmitter release from mouse motor nerve terminals. Eur J Neurosci 20, 3313-3321.

Browning KN, Zheng ZL, Kreulen DL, & Travagli RA (1999). Two populations of sympathetic neurons project selectively to mesenteric artery or vein. Am J Physiol 276, H1263-H1272.

Campese VM, Ye S, Zhong H, Yanamadala V, Ye Z, & Chiu J (2004). Reactive oxygen species stimulate central and peripheral sympathetic nervous system activity. Am J Physiol Heart Circ Physiol 287, H695-H703.

Cao X, Dai X, Parker LM, & Kreulen DL (2007). Differential regulation of NADPH oxidase in sympathetic and sensory ganglia in deoxycorticosterone acetate salt hypertension. Hypertension 50, 663-671.

Carrier GO (1995). Whole-cell and perforated patch recordings of four distinct K+ currents in acutely dispersed coeliac-superior mesenteric ganglia neurons of adult rats. Brain Res 701, 1-12.

Cassell JF, Clark AL, & McLachlan EM (1986). Characteristics of phasic and tonic sympathetic ganglion cells of the guinea-pig. J Physiol (Lond) 372, 457-483.

Chou SH, Kao EL, Lin CC, Chuang HY, & Huang MF (2005). Sympathetic hypertensive syndrome: a possible surgically curable type of hypertension. Hypertens Res 28, 409-414.

Dai X, Cao X, & Kreulen DL (2006). Superoxide anion is elevated in sympathetic neurons in DOCA-salt hypertension via activation of NADPH oxidase. Am J Physiol Heart Circ Physiol 290, H1019-H1026.

Dai X, Fink GD, & Kreulen DL. Elevation of superoxide anions in sympathetic neurons in DOCA-salt hypertension via activation of NADPH oxidase. FASEB J . 2004a. Ref Type: Abstract

Dai X, Galligan JJ, Watts SW, Fink GD, & Kreulen DL (2004b). Increased O2*- production and upregulation of ETB receptors by sympathetic neurons in DOCA-salt hypertensive rats. Hypertension 43, 1048-1054.

dam-Vizi V & Starkov AA (2010). Calcium and mitochondrial reactive oxygen species generation: how to read the facts. J Alzheimers Dis 20 Suppl 2, S413-S426.

de Champlain J (1990). Pre- and postsynaptic adrenergic dysfunctions in hypertension. J Hypertens Suppl 8, S77-S85.

Esler MD, Jennings G, Biviano B, Lambert G, & Hasking G (1986). Mechanism of elevated plasma noradrenaline in the course of essential hypertension. J Cardiovasc Pharmacol 8 Suppl 5, S39-S43.

Ferrier C, Cox H, & Esler MD (1993). Elevated total body noradrenaline spillover in normotensive members of hypertensive families. Clin Sci (Colch) 84, 225-230.

Gomez Sanchez EP (1991). What is the role of the central nervous system in mineralocorticoid hypertension? Am J Hypertens 4, 374-381.

Halliwell B (1992). Reactive oxygen species and the central nervous system. J Neurochem 59, 1609-1623.

Hardebo JE (1992). Influence of impulse pattern on noradrenaline release from sympathetic nerves in cerebral and some peripheral vessels. Acta Physiol Scand 144, 333-339.

Hsieh NK, Liu JC, & Chen HI (2000). Localization of sympathetic postganglionic neurons innervating mesenteric artery and vein in rats. J Auton Nerv Syst 80, 1-7.

Iriuchijima J, Mizogami S, & Sokabe H (1975). Sympathetic nervous activity in renal and DOC hypertensive rats. Jpn Heart J 16, 36-43.

Janig W & McLachlan EM (1992a). Characteristics of function-specific pathways in the sympathetic nervous system. TINS 15, 475-481.

Janig W & McLachlan EM (1992b). Specialized functional pathways are the building blocks of the autonomic nervous system. J Auton Nerv Sys 41, 3-14.

Jeong J, Shi WX, Hoffman R, Oh J, Gore JC, Bunney BS, & Peterson BS (2012). Bursting as a source of non-linear determinism in the firing patterns of nigral dopamine neurons. Eur J Neurosci.

Jubelin BC & Kannan MS (1990). Neurons from neonatal hypertensive rats exhibit abnormal membrane properties in vitro. Am J Physiol 259, C389-C396.

Kandlikar SS & Fink GD (2011). Splanchnic sympathetic nerves in the development of mild DOCA-salt hypertension. Am J Physiol Heart Circ Physiol 301, H1965-H1973.

Kreulen DL & Szurszewski JH (1979). Reflex pathways in the abdominal prevertebral ganglia: evidence for a colo-colonic inhibitory reflex. J Physiol (Lond) 295, 21-32.

Kubo T, Fukumori R, Kobayashi M, & Yamaguchi H (1996). Enhanced cholinergic activity in the medulla oblongata of DOCA-salt hypertensive and renal hypertensive rats. Hypertens Res 19, 213-219.

Li M, Dai X, Watts SW, Kreulen DL, & Fink GD (2008). Increased superoxide levels in ganglia and sympathoexcitation are involved in sarafotoxin 6c-induced hypertension. Am J Physiol Regul Integr Comp Physiol 295, R1546-R1554.

Luther JA & Birren SJ (2009). p75 and TrkA signaling regulates sympathetic neuronal firing patterns via differential modulation of voltage-gated currents. J Neurosci 29, 5411-5424.

Malpas SC (2010). Sympathetic nervous system overactivity and its role in the development of cardiovascular disease. Physiol Rev 90, 513-557.

McManus F, MacKenzie SM, & Freel EM (2009). Central mineralocorticoid receptors, sympathetic activity, and hypertension. Curr Hypertens Rep 11, 224-230.

Moreno L, Frazziano G, Cogolludo A, Cobeno L, Tamargo J, & Perez-Vizcaino F (2007). Role of protein kinase Czeta and its adaptor protein p62 in voltage-gated potassium channel modulation in pulmonary arteries. Mol Pharmacol 72, 1301-1309.

Nakamura K & Yokotani K (2010). Presynaptic BK type Ca(2+)-activated K(+) channels are involved in prostanoid TP receptor-mediated inhibition of noradrenaline release from the rat gastric sympathetic nerves. Eur J Pharmacol 629, 111-117.

Peters S & Kreulen DL (1986). Fast and slow synaptic potentials produced in a mammalian sympathetic ganglion by colon distension. Proc Natl Acad Sci USA 83, 1941-1944.

Rajagopalan S, Kurz S, Munzel T, Tarpey M, Freeman BA, Griendling KK, & Harrison DG (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.

Ribeiro AA, Elias CF, Liberti EA, Guidi WL, & de Souza RR (2002). Structure and ultrastructure of the celiac-mesenteric ganglion complex in the domestic dog (Canis familiaris). Anat Histol Embryol 31, 344-349.

Robertson WP (1999). Primary and adaptive changes of A-type K+ currents in sympathetic neurons from hypertensive rats.

Schlaich MP, Lambert E, Kaye DM, Krozowski Z, Campbell DJ, Lambert G, Hastings J, Aggarwal A, & Esler MD (2004). Sympathetic augmentation in hypertension: role of nerve firing, norepinephrine reuptake, and Angiotensin neuromodulation. Hypertension 43, 169-175.

Seifi B, Kadkhodaee M, Xu J, & Soleimani M (2010). Pro-inflammatory cytokines of rat vasculature in DOCA-salt treatment. Mol Biol Rep 37, 2111-2115.

Shimoni Y, Chen K, Emmett T, & Kargacin G (2008). Aldosterone and the autocrine modulation of potassium currents and oxidative stress in the diabetic rat heart. Br J Pharmacol 154, 675-687.

Somers MJ, Mavromatis K, Galis ZS, & Harrison DG (2000). Vascular superoxide production and vasomotor function in hypertension induced by deoxycorticosterone acetate-salt. Circulation 101, 1722-1728.

Stadtman ER & Levine RL (2000). Protein oxidation. Ann N Y Acad Sci 899, 191-208.

Su CK, Fan YP, Chen CC, & Chern Y (2010). Supraspinal contribution to splanchnic sympathetic activity in neonatal mouse and rat brainstem-spinal cord in vitro. Auton Neurosci.

Sun C, Sellers KW, Sumners C, & Raizada MK (2005). NAD(P)H oxidase inhibition attenuates neuronal chronotropic actions of angiotensin II. Circ Res 96, 659-666.

Szulczyk B & Szulczyk P (2003). Postdecentralization plasticity of voltage-gated K+ currents in glandular sympathetic neurons in rats. Eur J Neurosci 18, 43-52.

Tong H, Steinert JR, Robinson SW, Chernova T, Read DJ, Oliver DL, & Forsythe ID (2010). Regulation of Kv channel expression and neuronal excitability in rat medial nucleus of the trapezoid body maintained in organotypic culture. J Physiol 588, 1451-1468.

Tsutsumi S, Kushiku K, Kuwahara T, Tokunaga R, & Furukawa T (1995). Ionotropic mechanisms involved in postsynaptic inhibition by the endothelins of ganglionic transmission in dog cardiac sympathetic ganglia. J Cardiovasc Pharmacol 26, 707-713.

Vanner S, Evans RJ, Matsumoto SG, & Surprenant A (1993). Potassium currents and their modulation by muscarine and substance P in neuronal cultures from adult guinea pig celiac ganglia. J Neurophysiol 69, 1632-1644.

Vogalis F & Harvey JR (2003). Altered excitability of intestinal neurons in primary culture caused by acute oxidative stress. J Neurophysiol 89, 3039-3050.

Wang H, Chen AF, Watts SW, Galligan JJ, & Fink GD (2005). Endothelin in the splanchnic vascular bed of DOCA-salt hypertensive rats. Am J Physiol Heart Circ Physiol 288, H729-H736.

Xu B, Chen S, Luo Y, Chen Z, Liu L, Zhou H, Chen W, Shen T, Han X, Chen L, & Huang S (2011). Calcium signaling is involved in cadmium-induced neuronal apoptosis via induction of reactive oxygen species and activation of MAPK/mTOR network. PLoS One 6, e19052.

Yarowsky P & Weinreich D (1985). Loss of accommodation in sympathetic neurons from spontaneously hypertensive rats. Hypertension 7, 268-276.

Yi L, Morgan JT, & Ragsdale SW (2010). Identification of a thiol/disulfide redox switch in the human BK channel that controls its affinity for heme and CO. J Biol Chem 285, 20117-20127.

Zhao R, Liu L, & Rittenhouse AR (2007). Ca2+ influx through both L- and N-type Ca2+ channels increases c-fos expression by electrical stimulation of sympathetic neurons. Eur J Neurosci 25, 1127-1135.

Zimmerman MC & Davisson RL (2004). Redox signaling in central neural regulation of cardiovascular function. Prog Biophys Mol Biol 84, 125-149.