EFFECTS OF ATRIAL NATRIURETIC PEPTIDE AND PREGNANCY ON THE CENTRAL AND PERIPHERAL REGULATION OF THE CARDIOVASCULAR FUNCTIONS

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by

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KESAN ATRIAL NATRIURETIK PEPTIDA DAN KEHAMILAN KE ATAS PENGAWALATURAN PUSAT DAN PERIFERI FUNGSI-FUNGSI KARDIOVASKULAR

oleh

NURUL HASNIDA BINTI MOHAMMAD YUSOFF

Tesis yang diserahkan untuk
memenuhi keperluan bagi
Ijazah Sarjana Sains

September 2010

Dedicated to my beloved parents,

Mohammad Yusoff Hassan

&

Zubaidah Md. Zin

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LIST OF ABBRIVIATIONS

ACh	acetylcholine
ANOVA	analysis of variance
ANG II	angiotensin II
ABP	arterial blood pressure
ACSF	artificial cerebrospinal fluid
ANP	atrial natriuretic peptide
ANS	autonomic nervous system
AVP	arginine vasopressin
BP	blood pressure
cAMP	cyclic 3,5-adenosine monophosphate
cGMP	cyclic 3,5-guanosine monophosphate
CNS	central nervous system
CVLM	caudal ventrolateral medulla

DLH	D,L-homocysteic acid
et al.	and others
g	gram
GABA	gamma amino butyric acid
g/kg	gram per kilogram
HEX	hexamethonium bromide
HR	heart rate
5-HT	serotonin
IML	intermediolateral
i.p.	intraperitoneal
i.t.	intrathecal
i.v.	intravenous
L-NAME	nitro-L-arginine methyl ester
MAP	mean arterial pressure
µg	microgram
µl	microliter
mmHg	millimeter mercury
min	minute
n	number of animals
ng	nanogram
nl	nanoliter
nNOS	neuronal nitric oxide synthase
NO	nitric oxide
NPR	natriuretic peptide receptor
NTS	nucleus tractus solitarius
PE	phenylephrine
PVN	paraventricular nucleus
RAS	renin-angiotensin system
RSNA	renal sympathetic nerve activity
RVLM	rostral ventrolateral medulla
RVMM	rostral ventromedial medulla
SEM	standard error mean
SNP	sodium nitroprusside
SNS	sympathetic nervous system

SPN	sympathetic preganglionic neuron
VE	volume expansion
vs	versus

LIST OF SYMBOLS

α	alpha
β	beta
ʎ	gamma
°C	degree celcius
%	percentage
<	less than
>	more than
Δ	change
±	plus minus

KESAN ATRIAL NATRIURETIK PEPTIDA DAN KEHAMILAN KE ATAS PENGAWALATURAN PUSAT DAN PERIFERI FUNGSI-FUNGSI KARDIOVASKULAR

ABSTRAK

Kajian ini tertumpu pada dua topik yang berkaitan dengan pengembangan isipadu (VE); iaitu mengkaji kesan atrial natriuretik peptida (ANP) dan perubahan disebabkan kehamilan, ke atas pengawalaturan fungsi-fungsi kardiovaskular. ANP dirembes dari atria kardiak sebagai respon terhadap keadaan hipervolumia. Walaupun peranannya semasa VE sudah jelas, mekanisma pusat di sebalik kesan sistemik ANP kurang diselidik. Oleh itu, kajian ini bertujuan untuk mengkaji kesan sistemik dan pusat ANP, beserta penglibatan laluan vasopresin supra spina terhadap kesan selepas pemberian ANP secara intra venus. Hasil penemuan kami menunjukkan bahawa pemberian ANP secara sistemik pada dos rendah dan tinggi ke atas tikus jantan spesis Sprague-Dawley yang telah dibius dengan uretana menghasilkan kesan perencatan simpatetik renal yang signifikan, vasodilatasi dan bradikardia, di mana, kesan-kesan ini bergantung kepada efikasi laluan vasopresin supra spina. Pemberian ANP secara intra karotid melalui arteri karotid internal pula tidak menghasilkan sebarang kesan signifikan terhadap pembolehubah kardiovaskular yang direkod, mencadangkan bahawa kesan sistemik ANP tidak melibatkan tindakan ANP pada organ-organ sirkumventrikular. Pemberian ANP secara terus pada nukleus paraventrikular (PVN) menyebabkan kesan perencatan simpatetik renal yang berpanjangan, beserta kesan vasodilatasi dan bradikardia, menunjukkan tindakan ANP secara pusat. Pemerhatian ini, mencadangkan bahawa

tindakan spesifik di PVN kemungkinan terlibat dalam menghasilkan kesan sistemik oleh ANP. Sementara itu, bahagian kedua kajian, bertujuan mengkaji beberapa aspek kehamilan, suatu situasi di mana isipadu darah bertambah secara mendadak. Pengaruhan VE akut menggunakan infusi salina isotonik dalam tikus betina menyebabkan sedikit kenaikan pada purata tekanan arteri (MAP) dan kadar denyutan jantung (HR), dan menurunkan aktiviti saraf simpatetik renal (RSNA). Walaubagaimanapun, refleks takikardia dan perencatan simpatetik renal ini berkurang dalam tikus bunting, menunjukkan bahawa mekanisma refleks dalam mengekalkan keseimbangan cecair tubuh berubah pada fasa akhir kehamilan. Berikutan rangsangan dan penyahrangsangan baroreseptor arteri masing-masing menggunakan fenilefrina dan sodium nitroprusida, kehamilan menunjukkan indeks sensitiviti barorefleks yang sama, sama ada melalui pemberian agonis secara perlahan atau pantas. Kajian mencadangkan bahawa perubahan kronik dalam fungsi barorefleks semasa kehamilan tidak mengubah sensitiviti barorefleks arteri bagi HR dan RSNA terhadap perubahan MAP yang sementara. Dalam siri kajian berlainan, peranan sistem saraf simpatetik (SNS) dan nitrik oksida (NO) dalam pengawalan MAP basal semasa kehamilan diselidik, beserta respon vaskular terhadap beberapa jenis agen vasopressor dan agen vasodepressor ke atas tikus ternyahsaraf. Kajian mendapati peranan SNS terhadap MAP basal berkurang dalam tikus bunting, mencadangkan pengurangan tona simpatetik-kawalan-pusat ke atas sistem vaskular semasa fasa akhir kehamilan. Juga, kehamilan tidak mengubah peranan NO dalam mengekalkan tona vaskular basal, tetapi, pemerhatian kami menunjukkan bahawa interaksi antara SNS dan sistem NO semasa kehamilan telah berubah. Pada peringkat periferal, kehamilan menyebabkan perubahan yang tidak seragam dari segi respon

vaskular terhadap agen vasopressor dan vasodepressor yang diselidik pada seluruh sistem haiwan tanpa sebarang pengaruh kesan pusat dan refleks.

EFFECTS OF ATRIAL NATRIURETIC PEPTIDE AND PREGNANCY ON THE CENTRAL AND PERIPHERAL REGULATION OF THE CARDIOVASCULAR FUNCTIONS

ABSTRACT

The present study focuses on two topics that are related to volume expansion (VE); investigating the effect of atrial natriuretic peptide (ANP) and pregnancy-induced changes, in the regulation of the cardiovascular functions. ANP is released from the cardiac atria in response to hypervolemic state. Even though its role during VE is well established, the central mechanism underlying its systemic effect is less studied. Therefore, this study sets out to examine the systemic and central effect of ANP, as well as the involvement of supraspinal vasopressin pathways in mediating the effect produced by intravenous ANP. Our findings show that the systemic administration of low and high doses of ANP in urethane-anaesthetized male Sprague-Dawley rats produce a significant renal sympathoinhibition, vasodilation and bradycardia, and these effects are dependent on the efficacy of a well established supraspinal vasopressin pathway. Intracarotid administration of ANP via internal carotid artery does not produce any significant change in the recorded cardiovascular variables, suggesting that the systemic effect of ANP is independent of its action on the circumventricular organs. Direct administration of ANP into the paraventricular nucleus (PVN) causes a long lasting renal sympathoinhibition as well as vasodilation and bradycardia, demonstrating the central action of ANP. This observation, suggests that specific actions in the PVN might possibly mediate the expression of systemic effect by ANP. Meanwhile, the second part of the study, aims to investigate several

aspects of pregnancy; a state where the blood volume is greatly expanded. Induction of acute VE using isotonic saline infusion in female rats raises slightly the mean arterial pressure (MAP) and heart rate (HR), and reduces the renal sympathetic nerve activity (RSNA). However, these reflex tachycardia and renal sympathoinhibition are attenuated in pregnant rats, indicating that the reflex mechanisms in maintaining fluid balance are altered during late-term pregnancy. Following activation and deactivation of arterial baroreceptors using phenylephrine and sodium nitroprusside, respectively, pregnancy exhibits similar baroreflex sensitivity index, both by slow and rapid administration of agonists. It is proposed that chronic resetting of arterial baroreceptors, which has been reported during pregnancy, does not alter the arterial baroreflex sensitivity of HR and RSNA following transient changes in MAP. In different series of experiments, the roles of sympathetic nervous system (SNS) and nitric oxide (NO) in maintaining basal MAP during pregnancy are examined, as well as the vascular responsiveness to several vasopressor and vasodepressor agents in pithed rat preparation. It has been demonstrated that the role of SNS on basal MAP is reduced in pregnant rats, suggesting diminished centrally-regulated sympathetic tone to the vasculature during late-term pregnancy. Also, pregnancy does not alter the role of NO in maintaining basal vascular tone, however, our observation shows that the interaction between the SNS and NO system in pregnancy is somehow altered. At the peripheral level, pregnancy causes non-uniform changes in the vascular responsiveness to selective vasopressor and vasodepressor agents in a whole animal system without the influence of central and reflex effects.

CHAPTER 1

GENERAL INTRODUCTION

CHAPTER ONE

GENERAL INTRODUCTION

1.1 Nervous system

The nervous system can be divided into the central nervous system and the peripheral nervous system. A brief description of these two components of the nervous system is discussed below.

1.1.1 Central nervous system (CNS)

The CNS is basically composed of the brain and the spinal cord. Anatomically, the brain can be subdivided into four divisions, which are cerebrum, diencephalon, cerebellum and brainstem. The latter division, namely brainstem, consists of the midbrain, pons and medulla oblongata (Yusof, 1999).

The spinal cord lies within the bony vertebral column. As seen in transverse sections, the central butterfly shaped area, namely the grey matter, is composed of interneurones, the cell bodies and dendrites of efferent neurones, the entering fibres of afferent neurones, and glial cells. The grey appearance of this core is due to the nerve fibres that lack myelin. On the other hand, groups of myelinated axons of interneurones make up an area called the white matter surrounding the grey matter of the spinal cord. These groups of axons run longitudinally through the spinal cord, either ascending, to relay information between different levels of the brain and the spinal cord, or descending, to transmit signals from the brain to the spinal cord. The groups of afferent fibres enter the spinal cord via the dorsal roots; meanwhile, axons

of efferent fibres leave the spinal cord via the ventral roots. Both dorsal and ventral roots from the same level merge to form spinal nerves, one on each side of the spinal cord (Yusof, 1999).

1.1.2 Peripheral nervous system (PNS)

Originating from the central parts, the PNS is made up of nerve fibres which extend from the brain or the spinal cord to the body's muscles, glands and sense organs. This system is responsible for transmitting signals between the CNS and the effectors in all other parts of the body. Anatomically, the PNS consists of 43 pairs of nerves: 12 pairs of cranial nerves and 31 pairs of spinal nerves. All 31 pairs of spinal nerves are then structured into four levels, namely cervical, thoracic, lumbar and sacral. A single nerve contains nerve fibres that are either the axons of afferent neurones or efferent neurones, or both. Based on the types of effectors being innervated, the efferent division can be subdivided into a somatic nervous system and autonomic nervous system (ANS). The neurones of the somatic division control voluntary movements, therefore innervate skeletal muscles. On the other hand, the autonomic neurones, which control involuntary responses, innervate all tissues other than skeletal muscles such as smooth and cardiac muscles, glands and neurones in the gastrointestinal tract (Yusof, 1999).

The importance of the ANS in regulating body functions has been appreciated for many years. It is composed of two major divisions, which are sympathetic and parasympathetic components. Many organs are innervated by both divisions, whereby normally each controls antagonistic function to the other. For instance, in the heart, excitation of the sympathetic fibres causes tachycardia, whereas,

stimulation of the parasympathetic fibres produces bradycardic effect. At each side of the spinal cord, a two-neuron chain exists in a series between the CNS and the effector organs. The first-order neurones, called sympathetic preganglionic neurones (SPNs), pass between the CNS and ganglia, whereas the second-order neurones, namely sympathetic postganglionic neurones, connect the ganglia with the effector organs (Loewy, 1990a; Yusof, 1999; Guyenet, 2006). The sympathetic postganglionic neurones have direct influence on the target organs and their activity is controlled by the SPNs.

With regard to sympathetic innervations, the motor outflow arises predominantly from the intermediolateral (IML) cell column of the thoracic and upper lumbar spinal cord. This outflow originates from the SPNs that are segmentally organized in the spinal cord (Loewy, 1990a). This was evident by neuroanatomical retrograde tracing methods using fluorescent labels or horseradish peroxidase, whereby the target organ-specified preganglionic neurones are located in specific spinal cord segments (Strack *et al*., 1988; Pyner and Coote, 1994). The innervations of two important organs that will be discussed in this present study, mainly the heart and kidney, originate from the upper and lower thoracic innervations, respectively. More specific, sympathetic innervations to the heart originate from T1-T4, whereas sympathetic innervations to the systemic arterioles, including renal arteries, originate predominantly in the thoraco-lumbar cord at T5-L2 level (Kawabe *et al*., 2009; Fig. 1.1). This well organized structure of the spinal cord has made the CNS capable of generating highly differentiated patterns of sympathetic outflow to different target organs in response to various stimuli (Appel and Elde, 1988; Deering and Coote, 2000; Sved *et al*., 2001). Differentiated patterns of regional sympathetic outflow are

probably expressed through activation of specific subgroups of neurones in the medulla committed to specific groups of SPNs which innervate appropriate target organs.

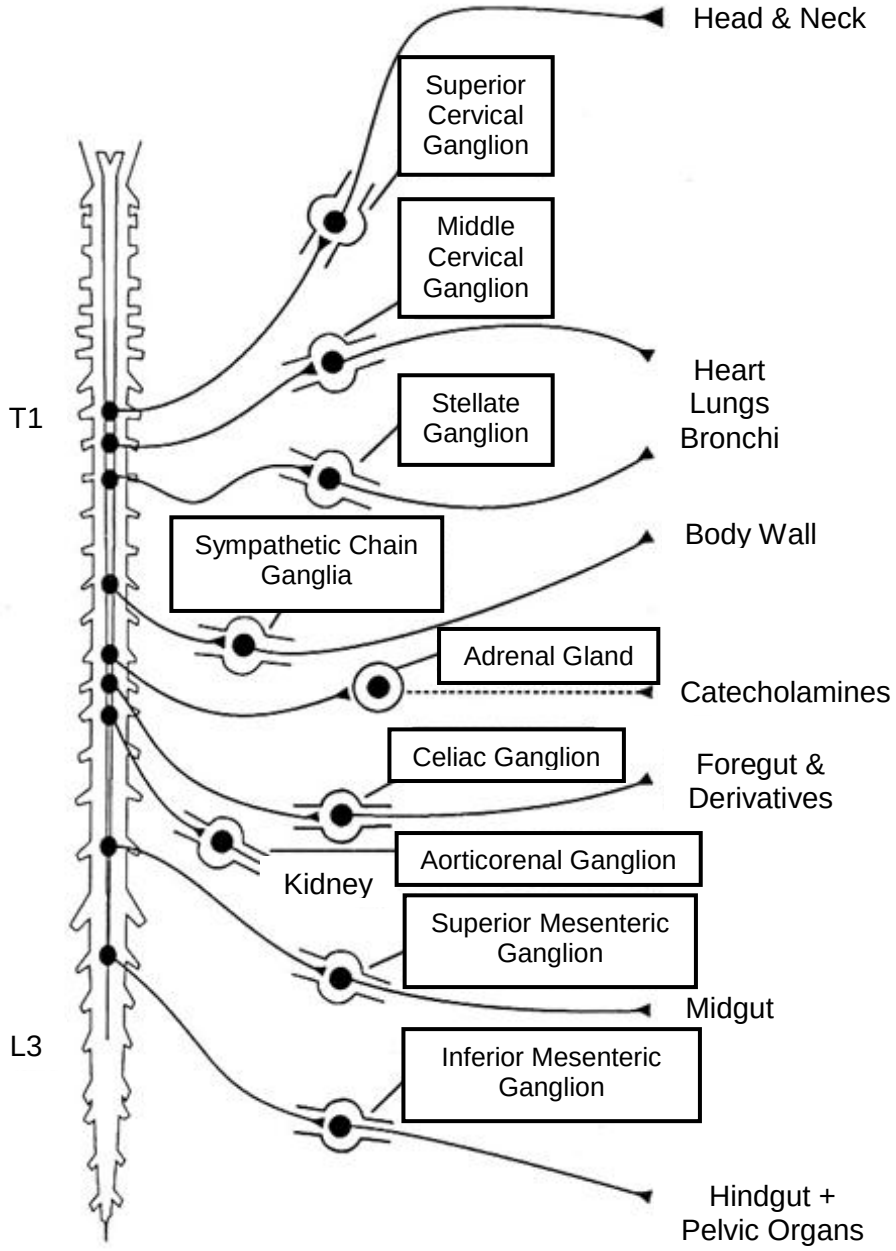

Figure 1.1. Schematic illustration of the sympathetic nervous system (Adapted from Loewy, 1990a)

1.2 Central and peripheral regulation of cardiovascular system

Vascular tissues, except for the capillaries and venules, receive sympathetic innervations of the ANS. These sympathetic fibres cause vasoconstriction, thereby also known as sympathetic vasoconstrictor fibres. The sympathetic nervous system maintains and greatly influences blood pressure (BP) by regulating the rate and force of contraction of the heart, and the calibre of the blood vessels. Therefore, any abnormality in sympathetic function will result in various types of cardiovascular diseases, such as hypertension, neurogenic cardiac arrhythmias and ischemic stroke (Yusof, 1999).

1.2.1 Arterial pressure (AP)

AP is a function of cardiac output and total peripheral resistance, two variables that are controlled by the ANS. Cardiac output, in turn, is dependent on three variables, which are end-diastolic volume, myocardial contractility and heart rate (HR). The heart receives both sympathetic and parasympathetic innervations of nervous system. Impulses in the sympathetic nerves increases the HR (chronotropic effect) and the force of cardiac contraction (ionotropic effect), whereas impulses in the parasympathetic or vagal nerves decrease the HR (Loewy, 1990a; Yusof, 1999).

Total peripheral resistance is determined primarily by the contractile status of the microcirculation, in particular, the arterioles and small arteries (Lucca *et al*., 2000). In general, there are a number of factors that affect the calibre of the arterioles, such as local regulatory mechanism (osmolality, pH, oxygen and carbon dioxide tension), substances secreted by the endothelium (prostaglandins, thromboxanes, nitric oxide; NO, and endothelins), circulating hormones (kinins, vasoactive intestinal peptide,

atrial natriuretic peptide, vasopressin, noradrenaline, adrenaline and angiotensin II) and sympathetic innervations. In normal condition, the ANS and the endothelium works together to maintain the vascular tone, by exerting tonic balance between the release of vasodilators from the endothelium and vasoconstrictors from the sympathetic nerve terminals (Harris and Matthews, 2004). The sympathetic nervous system produces tonus activity on basal AP by releasing norepinephrine from sympathetic nerve endings (Zimmerman, 1962).

1.2.2 Concept of a vasomotor centre

The ideas on how the brain regulates the cardiovascular system starting from studies over the last 200 years, which have given shape to our current concept and better understanding of the cardiovascular mechanisms and their neural control. The basic concept began in 1870 when Dittmar transected a region in the ventral medulla of rabbits, and found that BP fell profoundly, while pressure responses elicited by stimulation of the peripheral nerves were attenuated (Yusof, 1999). In 1873, he concluded that the vasomotor centre lies in the ventrolateral reticular formation near the region of the superior olivary and facila nuclei (Yusof, 1999). These early findings provide a clue to the roles of ventral medulla in the maintenance of AP, thus initiating a better move towards implicating the importance of the medulla region in the regulation of vasomotor tone.

1.2.2.1 Rostral ventrolateral medulla

Several neuroanatomical retrograde tracing studies have identified that within the medulla, there are reticulospinal neurones projecting to the sympathetic columns in the spinal cord (Amendt *et al*., 1978; Dampney *et al*., 1982; Caverson *et al*., 1983;

Reis *et al*., 1984; Ross *et al*., 1984). This region, first identified in the rat by Reis and colleagues, became known as the rostral ventrolateral medulla (RVLM; Ross *et al*., 1981) and was found to have direct monosynaptic connections with SPN (Zagon and Smith, 1993).

The RVLM-spinal projecting sympathoexcitatory neurones play a crucial role in the tonic and phasic regulation of the sympathetic vasomotor activity and AP. This idea was provided by an extracellular recording study, whereby these neurones were found to be tonically active with the firing rates of 5-40 spikes/s (see review Coote, 2006). In addition, the RVLM has been shown to provide major tonic excitatory drive to the sympathetic neurones that maintain AP (Dampney *et al*., 2003). Alteration in the activity of the RVLM-spinally projecting neurones seems to have profound effects on the sympathetic vasomotor tone (Dampney *et al*., 2003). Electrical or chemical stimulation of the RVLM neurones markedly increases sympathetic vasomotor activity, AP, and monosynaptically excited SPN (Dampney *et al*., 1982; Caverson *et al*., 1983; Morrison *et al*., 1988; Deuchars *et al*., 1995). This synaptic regulation of the RVLM sympathoexcitatory neurones is mediated by several neurotransmitters, such as glutamate and gamma-aminobutyric acid (GABA) (Stauss, 2002; Mayorov and Head, 2003; Horiuchi *et al*., 2004).

However, by virtue of development and application of a wide range of new experimental techniques, many traditionally held beliefs about the concept of a vasomotor centre have changed. Studies over the last 30 years or so have shown that the RVLM neurones are not alone in directly controlling the sympathetic vasomotor outflow. The tonic activity exerted by the RVLM neurones is thought to result from

sympathoexcitatory and sympathoinhibitory input from other brain regions to the RVLM (Ito and Sved, 1997; Schreihofer and Guyenet, 2002; Dampney *et al*., 2003; Yusof, 2007). In fact, the use of anaesthetized or decerebrate animals in many studies seems to depress or even abolish the contribution of supramedullary-spinal projections on the cardiovascular responses. Other groups of neurones, particularly in the hypothalamus, have also been shown to have direct projections to the SPNs, thus revealing that the supramedullary regions are capable of influencing sympathetic outflows to cardiovascular tissues independent of the RVLM (Coote, 2006).

In 1989, a method of transneuronal retrograde labelling by means of pseudorabies viruses was introduced to identify CNS cell groups that project their axons directly to the IML of the spinal cord (Strack *et al*., 1989). This study, together with the results from various workers (Jansen *et al*., 1995a), revealed five specific cell groups that innervate the SPNs in the spinal cord, mainly confined to several discrete regions within the brainstem and the hypothalamus. Besides the RVLM, there were 4 other regions demonstrated to have direct innervations to the SPNs, which were rostral ventromedial medulla (RVMM), caudal raphe nuclei, A5 noradrenergic cell group in the pons and the paraventricular nucleus (PVN) in the hypothalamus (Fig. 1.2) , and most importantly, seem to have influence on cardiovascular regulation. These neurones, which project directly to SPNs in the spinal cord, are called presympathetic neurones. These important findings bring a big impact on the current concept of vasomotor control, whereby it is now believed that the sympathetic vasomotor activity partly depends on signals originating from the supramedullary regions. In this particular study, only one of the supramedullary regions will be explained in great detail, which is the PVN.

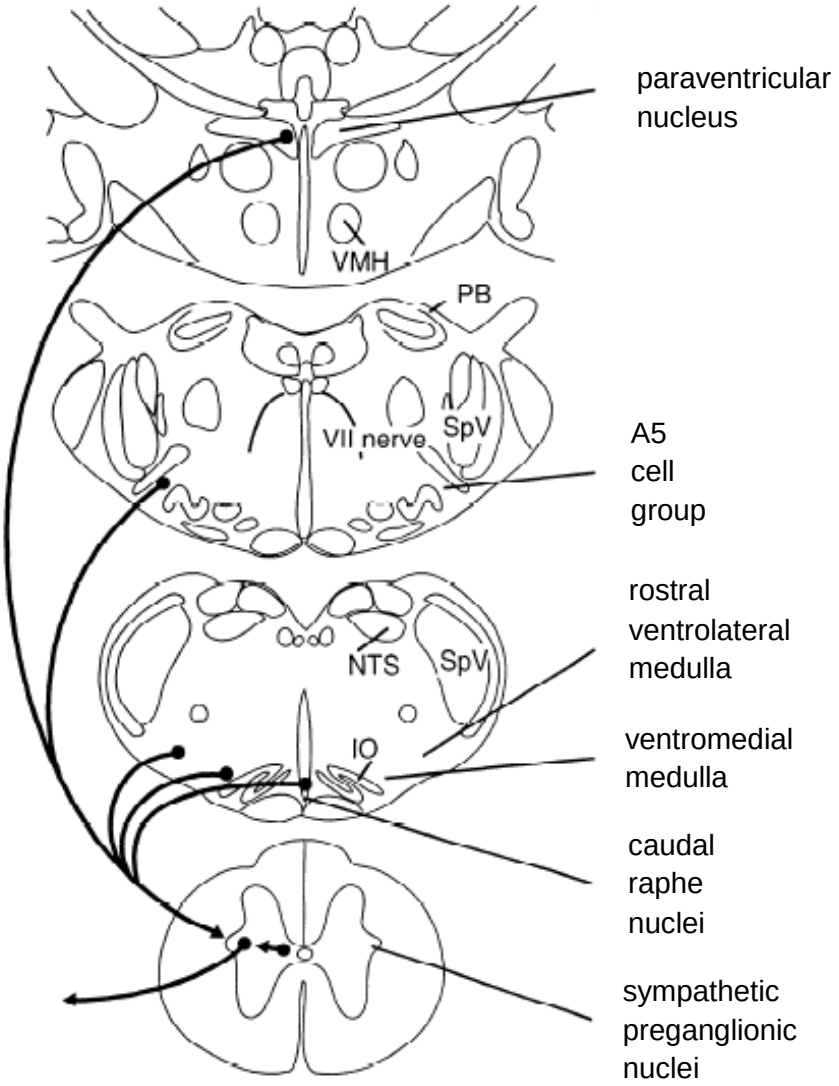

Figure 1.2. Brainstem and hypothalamic inputs to the intermediolateral column of the spinal cord. VII nerve, facial nerve; IO, inferior olive; NTS, nucleus tractus solitarius; PB, parabrachial nucleus; SpV, spinal trigeminal nucleus; VMH, ventromedial nucleus of hypothalamus (Adapted from Dampney, 1994).

1.2.2.2 Paraventricular nucleus

The PVN of the hypothalamus is located adjacent to the third ventricle in the forebrain. It is one of the five brain regions that directly innervate the SPNs in the spinal cord, thus capable of regulating sympathetic nerve outflows. There are three classes of PVN neurones; PVN neurones that directly project to the RVLM, PVN neurones that directly project to the spinal cord, and PVN neurones that have collateral projections to both the RVLM and spinal cord (Strack *et al*., 1989; Shafton *et al*., 1998; Pyner and Coote, 1997, 2000). The PVN-spinal neurones are mainly sympathoexcitatory, wherein the excitatory effect on renal sympathetic neurones is mediated in part by release of vasopressin and in part by glutamate (Yang and Coote, 2007).

With the advent of more sophisticated neuroanatomic tracing and immunohistochemical techniques, the functional cyto- and chemo-architecture of PVN have been explored, particularly in rats. Results from previous studies have driven to the subclassification of PVN neurones into two groups namely magnocellular and parvocellular neurones (Haselton *et al*., 1994; Shafton *et al*., 1998). Large neurones of the magnocellular subdivision of PVN play important roles in neuroendocrine functions, where they synthesize arginine vasopressin and oxytocin. On the other hand, the parvocellular neurones, which contain smaller cells, are involved in the regulation of autonomic functions by virtue of their projections to autonomic nuclei of the brain stem and spinal cord (Haselton *et al*., 1994; Shafton *et al*., 1998).

PVN has been implicated in various body functions, including regulation of food intake, responses to stress, modulating metabolic rate, thermoregulation, and regulation of the cardiovascular function (Schlenker, 2005). With regard to cardiovascular regulation, PVN influences the sympathetic and parasympathetic nervous systems as well as neuroendocrine functions (Kenney *et al*., 2003a, b; Eduardo, 2005; Schlenker, 2005). Apart from its role in normal condition, there is evidence implicating PVN in cardiovascular diseases. For example, increased activity in PVN is associated with the sympathoexcitation observed in congestive heart failure, and ablation of PVN seems to attenuate the progression of increased BP in spontaneously hypertensive rats (Akine *et al*., 2003).

Although PVN plays an important role in various body functions, the major focus of this study is on volume regulation. To date, the importance of PVN in volume regulation has been the subject of numerous articles and reviews (Lovick and Coote 1988a, b; Lovick *et al*., 1993; Haselton *et al*., 1994; Deng and Kaufman, 1995; Badoer, 1997; Coote, 2006). This parallels with its projections with other autonomic nuclei that are also involved in central processing of volume signals, including dorsomedial nucleus of the hypothalamus (DMH), nucleus tractus solitaries (NTS), subfornical organ (SFO) and C1/A1 groups of catecholaminergic neurones of the ventrolateral medulla. The sympathetic nerve discharge bursting pattern of the PVN neurones is mediated by different chemically-coded pathways to the spinal cord (Yang *et al*., 2002; Kenney *et al*., 2003b).

1.3 Role of neurotransmitters and neuromodulators in central cardiovascular regulation

The localization of various neuropeptides in the areas of brain that are known to play pivotal role in cardiovascular regulation has been achieved by the availability of newly advanced and sophisticated immunocytochemical and biochemical techniques. In mammalian CNS, glutamate appears to be the major fast excitatory neurotransmitter; meanwhile, GABA is known to be the principal neurotransmitter mediating fast inhibitory synaptic currents input (Gordon and Sved, 2002). Other examples of neurotransmitters or neuromodulators that are present in the brain are dynorphin, enkephalin, oxytocin, nitric oxide, 5-hydroxytryptamine, angiotensin II, vasopressin, endothelins, catecholamines, acetylcholine and glycine (Kenney *et al*., 2003b; Li *et al*., 2003; Yang and Coote, 2003; Ng *et al*., 2004).

The spinal cord also contains various types of neurotransmitters or neuromodulators that play important roles in the regulation of neuronal functions. The SPNs change their activity pattern, firing rate or membrane potential in response to a variety of neurotransmitters, including amino acids, monoamines and neuropeptides (Cammack and Logan, 1996; Deuchars *et al*., 1997). With regard to the PVN-spinal neurones, studies have shown that these neurones express either arginine vasopressin (25-40%) or oxytocin (20-30%) and, to a lesser extent, enkephalin or dopamine, which are now convincing to act as neurotransmitters in PVN-spinal sympathetic pathways (Coote, 2006). These important findings were achieved by means of intrathecal application of selective receptor antagonists for each of the neurotransmitter employed (Yang *et al*., 2002). By virtue of its great influence on the effect of PVN–spinal neurones on

renal sympathetic activity (Yang and Coote, 2007), vasopressin has been chosen as the topic of discussion in our present study.

1.3.1 Vasopressin and cardiovascular regulation

Arginine vasopressin (AVP), a peptide hormone, is synthesized in magnocellular neurones of the supraoptic and the PVN of the hypothalamus. These neurones send their axons into the posterior lobe of the pituitary gland, from which AVP is released into nearby capillaries and distributed throughout the body. The peripheral actions of AVP are mediated by two subtypes of membrane-bound receptors. The V_{1a} receptor mediates the vasoconstrictor actions of AVP via phosphatidyl inositol biphosphate hydrolysis and a rise in cytosolic Ca^{2+}. Meanwhile, the V_2 receptor mediates the antidiuretic effect of AVP on renal collecting ducts via adenylate cyclase (Berecek, 1992; Brunton *et al*., 2008).

The importance of AVP in cardiovascular regulation has been demonstrated in a number of studies (Porter and Brody, 1986; Berecek, 1992; Malpas and Coote, 1994; Yang *et al*., 2002; Yang and Coote, 2006; 2007; Brunton *et al*., 2008). There are evidences which indicate that AVP innervations of the spinal sympathetic neurones most probably arise from the PVN (Porter and Brody, 1986; Coote, 2004). From PVN, these AVP-containing neurones terminate in the dorsal horn and IML. Following PVN stimulation, the level of spinal AVP in the spinal fluid is increased (Malpas and Coote, 1994). Moreover, application of AVP to the spinal cord results in an increase in the firing rate of SPNs, an increase in BP and vasoconstriction in the renal vascular beds (Malpas and Coote, 1994). Selective blockade of spinal AVP receptors antagonizes excitatory effects on the RSNA following PVN stimulation

(Yang *et al*., 2002). Collectively, these evidences demonstrate the roles of AVP in mediating the descending pathways from the PVN to the IML of the spinal cord. Therefore, investigation at the spinal level, by some means, probably is a good approach for completing neural pathways subserving any cardiovascular mechanism being studied.

1.4 Central and peripheral regulation of reflex cardiovascular system

The preceding paragraphs are about the nervous control of cardiovascular system. Another important issue that also receives detailed attention will be on how these body systems are constantly maintained, for both short- and long-term period. The reflex arc, which forms a complete model for cardiovascular regulation, consists of sensory afferents, an efferent component and a central component that links the afferent input with the efferent output. In brief, the regulation of reflex cardiovascular system involves activation of several groups of afferent receptors, and their afferent input to the CNS modifies the discharge of the autonomic outflows to produce appropriate physiological responses. The variables that are being regulated include ABP, cardiac output, circulating blood volume, and arterial blood gas tensions (Spyer, 1990).

Two types of receptors responsible for the reflex control of circulation are chemoreceptors and mechanoreceptors. The arterial chemoreceptors, located within the carotid bodies and aortic arch, have a chemosensitive function, in which they monitor the blood gas composition of the arterial blood. Meanwhile, the second group of receptors, known as machanoreceptors, can be further subdivided into two categories, namely arterial baroreceptors and cardiac receptors. Arterial

baroreceptors play essential role in monitoring BP whereas cardiac receptors have a great influence on body volume (Spyer, 1990). All these afferent signals are known to terminate in the nucleus tractus solitarius (NTS), an area that is important in cardiovascular regulation. Further details concerning both arterial baroreceptor reflex and cardiac receptor reflex, will be discussed at length later.

1.4.1 Nucleus tractus solitarius (NTS)

The NTS, an ovoid shaped nucleus, has been generally accepted as the major visceral sensory relay cell group in the brain. It receives signals from peripheral baroreceptor, chemoreceptor and cardiopulmonary afferents (Stauss, 2002; Kawabe *et al*., 2009). Anatomically, NTS is composed of several subnuclei, which can be classified on the basis of their position relative to the solitary tract. The incoming primary visceral afferent fibres that project to the NTS are organized in two ways, one for reflex adjustments of the end organ and the others for integrative functions involving more complex changes that affect multiple systems. The first involves an organ-specific projection pattern to individual NTS subnuclei, whereas the second relates to overlapping afferents projecting to a common NTS region, which is the commissural NTS. The reflex pathways seem to be more straightforward since it involves relatively simple neuronal circuits, probably only to specific lower brain stem nuclei. On the other hand, various inputs sent to the commissural NTS will be transmitted to and integrated by a variety of brain stem and forebrain nuclei, thus, commanding specific autonomic motor and neuroendocrine responses (Loewy, 1990b). Having direct projections to other parts of the brain that are known to be involved in cardiovascular control makes NTS a key nucleus for cardiovascular regulation. These include medullary and supramedullary regions such as caudal ventrolateral medulla

(CVLM), RVLM and hypothalamic PVN. Integration of these brain areas is important to elicit such reflex responses due to particular stimulus that will affect the body functions.

1.4.2 Arterial baroreceptor reflex

A growing body of evidence has shown that arterial baroreceptor input has a dominant influence on normal nervous control of BP. In fact, the organization and control of the baroreceptor reflex is a particular reflex mechanism for which the most detailed information is available in the literature (Shafton *et al*., 1999). This reflex seems to be important for stabilization of AP in the face of disturbances of circulatory homeostasis (Stauss, 2002). Mechanoreceptors that are located in the main systemic arteries, or to be more specific, in the aortic arch and carotid sinuses, are known as arterial baroreceptors. These high-pressure receptors are responsible for transmitting BP signals to the CNS via the aortic nerve, a branch of the vagus, for those with endings in the aortic arch. Meanwhile, for the receptors in the carotid sinus, the information is relayed through the sinus nerve, a branch of the glossopharyngeal nerve (Spyer, 1990). These arterial baroreceptor afferents have direct projections to three NTS areas: the dorsolateral NTS, the medial NTS and the commissural nucleus.

A combination of a variety of experimental approaches, including the electrophysiological, pharmacological, neuroanatomical tracing and immunohistochemical studies, has resulted in a model of essential pathways subserving the baroreceptor reflex (Fig. 1.3). In brief, the arterial baroreceptors are activated or stimulated by the increased vascular stretch that accompanies a rise in

AP. Once NTS receives signals from the arterial baroreceptor afferents, the signals are transmitted to the CVLM via its excitatory neuronal projections, mainly involving glutamate neurotransmitters. Since CVLM is rich with GABAergic neurones some of which project directly to the RVLM, therefore, excitation of these neurones will inhibits the spinal-RVLM sympathoexcitatory neurones. Eventually, inhibition of the excitatory input to the SPNs in the spinal cord results in an inhibition of the sympathetic nerve activities to the organs and vascular beds involved in cardiovascular control (Urbanski and Sapru, 1988; Aicher *et al*., 1995; Shafton *et al*., 1999; Schreihofer and Guyenet, 2002; Moreira *et al*., 2005; Yusof, 2007; Kawabe *et al*., 2009). In short, increased frequency of baroreceptor discharge will leads to reflex adjustments that will buffer the rise in AP, including an increase in vagal cardioinhibitory neurone discharges and a decrease in sympathetic preganglionic and postganglionic neurone discharges to the heart and peripheral blood vessels. Consequently, baroreceptor activation results in bradycardia, decreased cardiac contractility, and decreased peripheral vascular resistance. The fall in cardiac output and vascular resistance will then contributes to a decrease in AP. Conversely, a decrease in AP will results in appropriate changes in autonomic activity to increase AP back to the normal level.

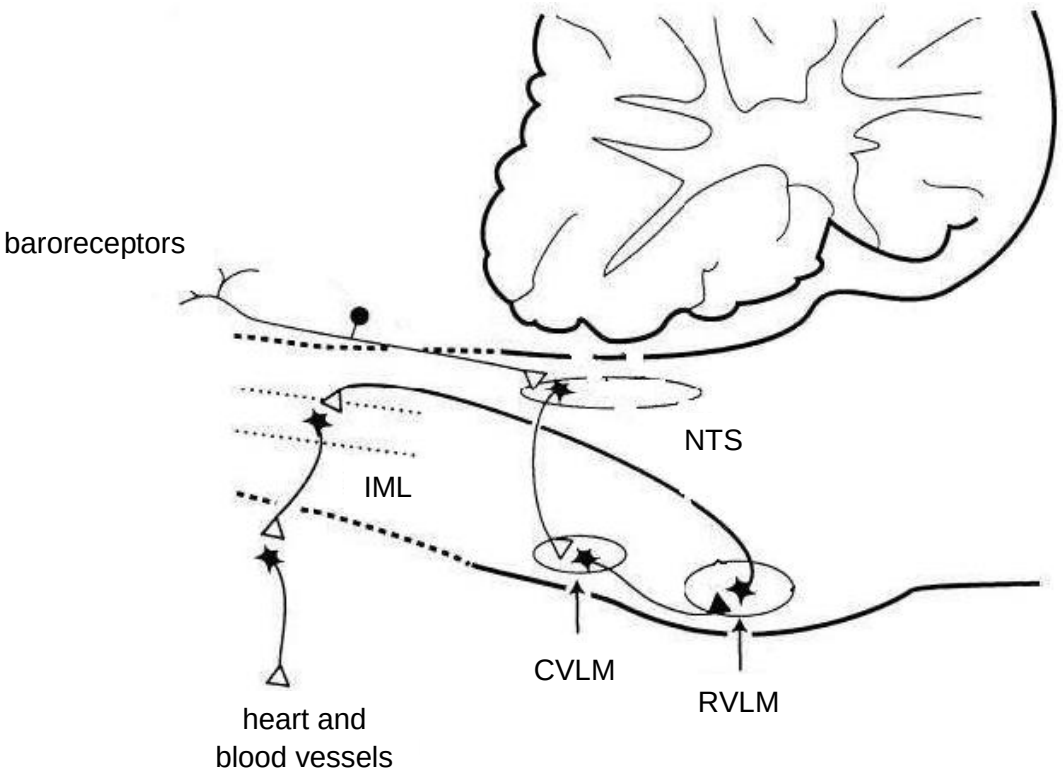

Figure 1.3. Pathways within the lower brain stem and spinal cord that subserve the baroreceptor reflex control of the sympathetic outflow to the heart and blood vessels. The open triangle indicates excitatory synaptic inputs and the filled triangles, inhibitory synaptic inputs. CVLM, caudal ventrolateral medulla: IML, intermediolateral cell column in the spinal cord; NTS, nucleus tractus solitarius (Modified from Dampney *et al*., 2001)

On reviewing the literature, there are two parameters that are often used to estimate the baroreflex function, namely the operating point of the reflex at which the reflex responds most effectively to changes in AP, and the sensitivity of the reflex or the magnitude of the reflex response per unit of ABP deviation from the operating point. In addition to these two parameters, recently, a baroreflex effectiveness index has been used as it provides information on how active the baroreflex is in BP, HR or RSNA regulation (Stauss, 2002).

In several physiological or pathological conditions, resetting of arterial baroreceptors can occur, especially when there is a prolonged and sustained exposure to hypotensive or hypertensive states. This resetting, or sometimes known as adaptation of arterial baroreceptors, involves a shift in the operating set point of the receptor to levels that are closer to the new prevailing pressure. This resetting may or may not change the sensitivity of the arterial baroreflex responses. Examples of situations where chronic resetting of arterial baroreflex functions come into play are during pregnancy and hypertension (Brooks *et al*., 2002).

1.4.3 Cardiac receptor reflex

It has been well accepted that an optimum blood volume is vital for normal performance of all types of body functions; therefore, it is tightly regulated via detection of several plasma volume-related signals. The circulating levels of angiotensin, aldosterone and osmolality are examples of signals for long-term regulation of blood volume. Apart from that, the most important signals that directly reflect changes in vascular volume are the cardiac receptors (Badoer *et al*., 1998; Colombari *et al*., 2000; Potts *et al*., 2000; Coote, 2004). They have been reported to be sensitive enough to give a precise and rapid indication of the fullness of the thoracic circulation (Coote, 2004). These receptors are found to be localized in either the great veins close to their entry to the heart or within the walls of the atria, the atrial apendange, or the walls of the ventricles (Spyer, 1990). Of all the cardiac receptors, atrial receptors are the one that becomes active with each heartbeat and minimal information from epicardial and ventricular responses reach the CNS (Korner, 1971). These atrial receptors are also known as low-pressure receptors. They provide information on the circulating blood volume to the brain based on the

magnitude of the venous return and force of atrial contraction via the vagus nerve (Spyer, 1990; Coote, 2004).

The important components of cardiac or volume reflex include the afferent limb (including receptors and afferent fibers), the central neural processing of afferent input and the efferent limb (RSNA and the release and /or action of humoral factors) (Patel, 1997). Like arterial baroreceptor afferents, cardiac afferents have been shown to terminate in the NTS. From NTS, it seems to connect to other autonomic nuclei which play important roles in blood volume regulation, thereby generating appropriate actions to restore normal blood volume. The most popular examples of situations where these mechanisms are clearly brought into play are haemorrhage and volume expansion (VE). However, in the present study, only reflex regulation of VE will be discussed in great detail.

1.4.3.1 Volume expansion

Blood volume is maintained homeostatically within its physiological level by the intrinsic, neural and humoral regulatory mechanisms. Of these regulatory mechanisms, neural regulation is characterised by its promptness in effect. The best known neural regulation of volume loading, namely Bainbridge reflex, refers to a tachycardic response to a rapid rise in blood volume (Kappagoda *et al*., 1975; Kaufman *et al*., 1981; Morooka *et al*., 2002; Coote, 2004). Simultaneously, a reflex inhibition of RSNA has been reported to compensate the increased in central blood volume (Potts *et al*., 2000; Coote, 2004, 2006), by altering the vascular resistance (Fiol *et al*., 1998) and affect both water and sodium excretion (Kaufman and Deng, 1993). In addition, several studies have demonstrated that an acute increase in blood

volume induces responses such as renal vasodilation (Lovick *et al*., 1993; Colombari and Cravo, 1999; Pedrino *et al*., 2005), reductions in AVP, renin and aldosterone (Badoer *et al*., 1998), release of atrial natriuretic peptide and oxytocin (Godino *et al*., 2005), natriuresis and diuresis (Badoer *et al*., 1998). Collectively, these responses help to restore blood volume to a normal level.

During volume loading, volume receptors will respond to atrial and ventricular filling and eventually transmit the signals to the first synapse in NTS via the vagus nerve. From the NTS, the signal pass first to the CVLM and then to the RVLM, where the integrated afferent signals would be transmitted to the SPNs to produce appropriate responses. Besides medullary regions, the involvement of supramedullary regions such as PVN and other autonomic nuclei in the volume reflex regulation has been shown previously (Potts *et al.*, 2000). This is due to the fact that both NTS and RVLM neurones do project to other parts of the brain regions. Studies using the expression of immediate-early gene c-*fos*, a marker of neuronal activation, have identified regions in the medulla and hypothalamus that are activated by acute VE, including, area postrema, the caudal, intermediate and rostral parts of the ventrolateral medulla, supraoptic nucleus, PVN, arcuate nucleus, suprachiasmatic nucleus and median preoptic nucleus (Potts *et al*., 2000). This and other neuroanatomical findings collectively indicate that the activation and coordination of the volume reflex responses resulted from the integration of CNS (Badoer *et al*., 1997; Randolph *et al*., 1998; Shafton *et al*., 1999; Potts *et al*., 2000; Pyner *et al*., 2002; Godino *et al*., 2005). Finally, the output produced from the central integration is reflected by the sympathetic nerve discharge innervating different target organs.

The present knowledge concerning the involvement of PVN in controlling cardiac reflex pathway is summarized in Figure 1.4. On reviewing the literature, evidence strongly favours the idea that inhibition of RSNA following atrial receptor stimulation is mediated by GABA interneurones in the PVN. These neurones regulate the RSNA by virtue of innervating the PVN-spinally projecting AVP neurones that synapse with renal SPNs (Yang *et al*., 2002; Yang and Coote, 2003, 2004). Meanwhile, the reflex tachycardia responses seem to be attributed to activation of PVN-spinally projecting oxytocin neurones that innervate cardiac SPNs in the spinal cord. This latter response is mediated by the PVN interneurones-containing glutamate.

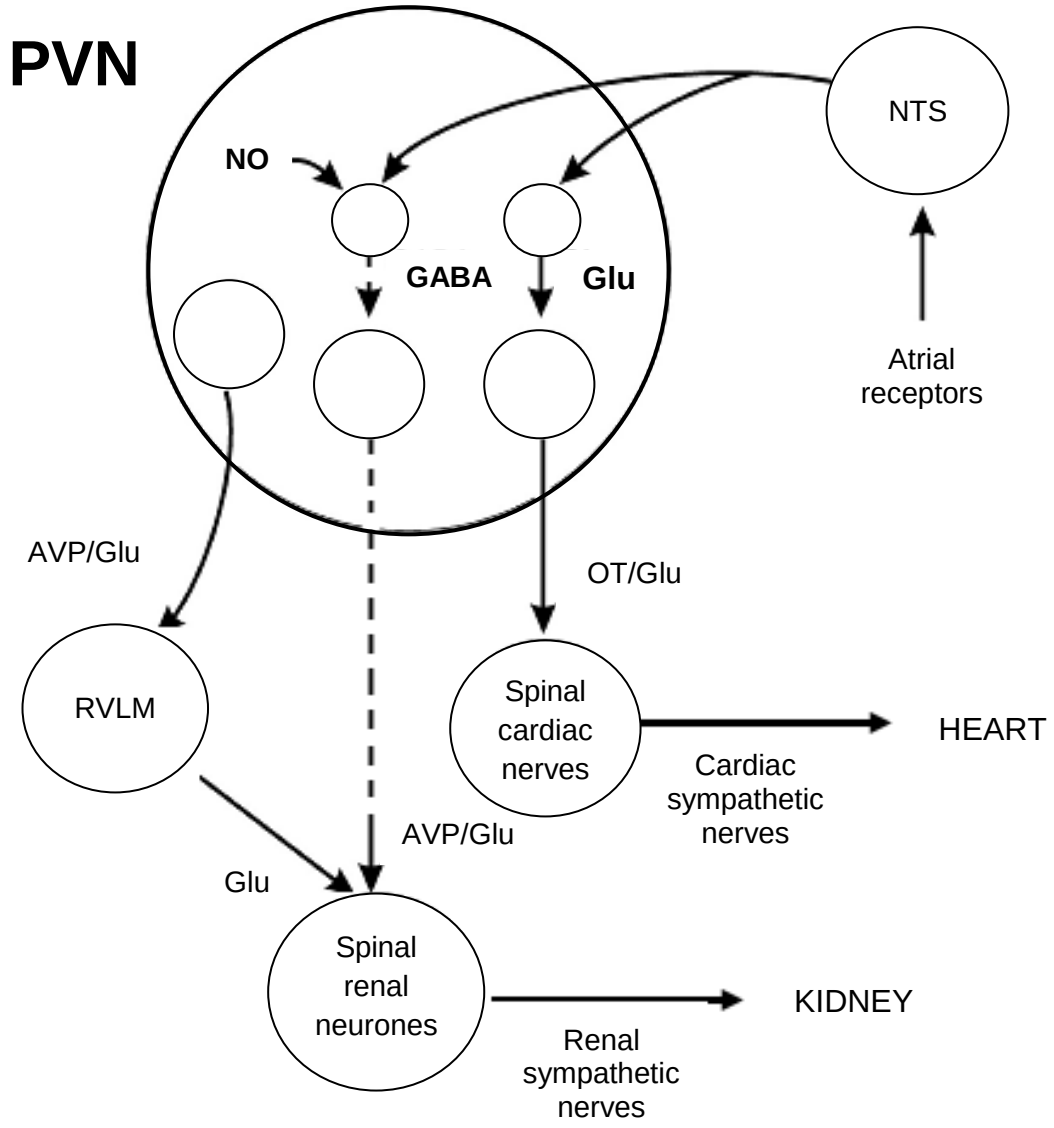

Figure 1.4. Central nervous pathways controlling renal sympathetic neurone activity and the likely reflex pathway activated by atrial receptor stimulation which leads to inhibition of renal sympathetic activity and excitation of cardiac sympathetic activity. Abbreviations: AVP, arginine vasopressin; Glu, glutamate; NO, nitric oxide; NTS, nucleus tractus solitarius; OT, oxytocin; PVN, paraventricular nucleus; and RVLM, rostral ventrolateral medulla. Full arrow, excitatory pathway; dashed arrow, inhibitory pathway (Modified from Coote, 2004)

Examples of normal situations where chronic blood VE come into play are during exercise training and pregnancy (Randolph *et al*., 1998). Several pathological states such as congestive heart failure, cirrhosis, renal failure and nephritic syndrome, result from this volume overload (Shafton *et al*., 1999).

Since VE is thought to be an important issue in clinical practice, the two main areas that will be investigated in the present study are also related to VE. These include the study of ANP, a hormone which is released by cardiac atria in response to VE. The systemic and central effects of ANP are explored, together with the central neurones participating in the systemic effect of ANP. Meanwhile, the second part of the study will be focusing on pregnancy, a situation where blood volume is greatly expanded. This includes the study of reflex regulation of cardiovascular variables, such as MAP, HR and RSNA, in response to cardiac and arterial baroreceptor stimulation, as well as the study of peripheral system in this volume-expanded model. Taken together, the present study aims to investigate the effects of ANP and pregnancy, in relation to the regulation of the cardiovascular functions.