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# Role of the hypothalamic arcuate nucleus in cardiovascular regulation

**Hreday N. Sapru**
Department of Neurological Surgery, UMDNJ-New Jersey Medical School, 185 South Orange Avenue, Newark, NJ 07103, USA

## Abstract

Recently the hypothalamic arcuate nucleus (Arc) has been implicated in cardiovascular regulation. Both pressor and depressor responses can be elicited by the chemical stimulation of the Arc. The direction of cardiovascular responses (increase or decrease) elicited from the Arc depends on the baseline blood pressure. The pressor responses are mediated via increase in sympathetic nerve activity and involve activation of the spinal ionotropic glutamate receptors. Arc-stimulation elicits tachycardic responses which are mediated via inhibition of vagal input and excitation of sympathetic input to the heart. The pathways within the brain mediating the pressor and tachycardic responses elicited from the Arc have not been delineated. The depressor responses to the Arc-stimulation are mediated via the hypothalamic paraventricular nucleus (PVN). Gamma aminobutyric acid type A receptors, neuropeptide Y1 receptors, and opiate receptors in the PVN mediate the depressor responses elicited from the Arc. Some circulating hormones (e.g., leptin and insulin) may reach the Arc via the leaky blood-brain barrier and elicit their cardiovascular effects. Although the Arc is involved in mediating the cardiovascular responses to intravenously injected angiotensin II and angiotensin-(1-12), these effects may not be due to leakage of these peptides across the blood-brain barrier in the Arc; instead, circulating angiotensins may act on neurons in the SFO and mediate cardiovascular actions via the projections of SFO neurons to the Arc. Cardiovascular responses elicited by acupuncture have been reported to be mediated by direct and indirect projections of the Arc to the RVLM.

### Keywords

Angiotensin-(1-12); Angiotensin II; Blood pressure; Leptin; Insulin; Sympathetic nerve activity

## 1. Introduction

The hypothalamus lies below the thalamus on both sides of the third ventricle and extends from the optic chiasm rostrally to the midbrain tegmentum caudally. In the anterio-posterior direction, the hypothalamus can be arbitrarily divided into three regions: anterior, tuberal and posterior regions. It is a complex structure and contains several groups of neurons. The preoptic and suprachiasmatic nuclei are located in the anterior region. The periventricular, paraventricular (PVN), anterior, supraoptic, dorsomedial (DMN), ventromedial (VMN) and



Contact information: H.N. Sapru, Ph.D. Department of Neurological Surgery, MSB H-586, UMDNJ - New Jersey Medical School, 185 South Orange Avenue, Newark, N.J. 07103, USA, Telephone 973-972-5876, FAX 973-972-5986, sapru@umdnj.edu.

arcuate (Arc) nuclei are located in the tuberal region. The posterior nucleus and mammillary body are present in the posterior region. The lateral nucleus extends from the tuberal to the posterior regions. All of these hypothalamic nuclei are located on both sides of the third ventricle (Siegel and Sapru, 2011).

Among other vital functions, the hypothalamus plays a critical role in the regulation of cardiovascular function (Coote, 2004). Extensive literature is available on the role of the paraventricular, dorsomedial, lateral and posterior hypothalamic nuclei in the regulation of cardiovascular function (Coote, 2004). However, information regarding the role of the Arc in cardiovascular regulation and autonomic functions is just beginning to be accumulated.

The main focus of this review is to discuss available literature on the participation of the Arc in cardiovascular regulation. However, other information relevant to the role of Arc in autonomic regulation has also been included. In the beginning, basic neuroanatomy of the Arc, its projections and chemical phenotypes of its neurons are presented. This description is followed by a discussion of different types of cardiovascular responses elicited by the chemical stimulation of the Arc and the pathways mediating these responses. Next, cardiovascular effects of microinjections of leptin, angiotensin II (Ang II), angiotensin-(1-12) (Ang-(1-12) and insulin into the Arc are discussed. The role of the Arc in mediating cardiovascular responses to these circulating hormones is also discussed in the context of leaky blood-brain barrier of the ventromedial part of this nucleus. Finally, the role of Arc in mediating the cardiovascular effects of acupuncture is discussed.

## 2. Basic anatomy of the Arc

The Arc is located in the ventral hypothalamus on both sides of the base of the third ventricle. Ventrally it has a short extension into the median eminence of the tuber cinereum. In the rat, the Arc extends along the base of the 3$^{rd}$ ventricle from 1.72 to 4.36 mm caudal to the bregma (about 2.64 mm length in rostro-caudal direction). In the rostral regions (1.72 to 3.36 caudal to the bregma), the Arc has been divided into dorsal, medial and lateral regions. In the caudal regions (3.48 to 4.36 mm caudal to the bregma), only the medial and lateral regions of the nucleus are prominent (Paxinos and Watson, 2007). The neurons in the Arc are generally polymorphic and small to medium in size. They usually give rise to 2–3 dendrites which do not arborize extensively. In a majority of Arc neurons (72%), the axons arise from the perikarya while in some neurons (28%) they arise from the proximal dendrites. The axons of some Arc neurons synapse locally while other neurons project to the median eminence and other nuclei in the central nervous system (CNS). In the rostral part of the Arc, the axons of some neurons cross under the third ventricle to the contralateral Arc (Carpenter and Sutin, 1983; Bleier and Byne, 1985).

A large percentage of the total tissue volume of the Arc consists of tanycytes which are descendants of radial glial cells. The cell bodies of tanycytes are located either in the lateral walls or floor of the third ventricle and their main processes proceed ventrally and end on blood vessels or pial surface. Tanycytes have been implicated in the transport of hormones from the cerebrospinal fluid to the capillaries of the hypophyseal portal system and from hypothalamic neurons to the cerebrospinal fluid (Peruzzo et al., 2004). This function may involve the process of transcytosis in which materials are taken up by the tanycyte on one end by endocytosis and moved to the opposite side where they are released through the plasma membrane by exocytosis.

## 3. Arc projections

Anatomical tracing techniques have revealed that neurons in the Arc project to the forebrain (the nucleus accumbens, bed nucleus of stria terminalis, lateral septal nucleus and

amygdaloid nucleus), thalamus (the paraventricular and centromedian nuclei), hypothalamus, midbrain (ventrolateral periaqueductal gray; vlPAG), pons (the lateral parabrachial nucleus, dorsal raphe nucleus and locus coeruleus) and medulla (the nucleus raphe magnus and pallidus, nucleus reticularis gigantocellularis and nucleus tractus solitarius) (Li et al., 2006; Li et al., 2009; Sim and Joseph, 1991). Some retrogradely labeled cells were found in the lateral portion of the Arc after microinjections of Fluoro-Gold (FG) or fast blue dye (tracers) into the intermediate gray matter and adjacent lateral funiculus of the spinal cord at T1–T4 level in adult rats suggesting that some Arc cells may project directly to the intermediolateral cell column of the spinal cord (IML) (Cechetto and Saper, 1988; Elias et al., 1998). Electrophysiological experiments and anatomical tracing studies have shown that neurons located in the subfornical organ (SFO) can be activated or inhibited by electrical or chemical stimulation of the Arc and that Arc neurons may directly project to the SFO (Rosas-Arellano et al, 1993, 1995, 1996a, 1996b). Arc neurons also project to the median eminence for endocrine modulation (Van Den Pol et al., 1982).

It is well established that nucleus tractus solitarius (NTS), nucleus ambiguus (nAmb), caudal ventrolateral medullary depressor area (CVLM), rostral ventrolateral medullary pressor area (RVLM), PVN, and IML play an important role in cardiovascular regulation (Coote, 2004; Dampney et al., 2005; Guyenet, 2006; Pilowsky et al., 2009; Sapru, 2002). The projections of the Arc to the PVN have been well characterized while information regarding the Arc projections to other CNS structures is sketchy (Coote, 2004). Li et al. (2006) injected a tracer in the RVLM of cats and observed retrograde labeling in the Arc neurons; twenty two percent of the retrogradely labeled Arc cells contained beta-endorphin. Moreover, Arc neurons could be antidromically activated by electrical stimulation of the RVLM. These results indicated the presence of direct projections from the Arc to the RVLM.

We carried out anatomical tracing studies in adult male Wistar rats to identify the projections of the Arc to some of brain areas known to be involved in cardiovascular regulation. FG (4%; Fluorochrome Inc., Denver, CO, USA) was microinjected (5–10 nl) into the target area (e.g., NTS, CVLM, RVLM or IML) under aseptic conditions. After a survival period of 7–10 days, the brains were perfused, fixed in paraformaldehyde, sections (40 μm) of the diencephalon were cut in a vibratome and examined for retrograde labeling. Following the microinjections of FG in the NTS (Fig. 1A), some retrogradely labeled cells were found in the ipsilateral Arc but the labeling was not robust (Figs. 1B). However, in the same experiment, retrograde labeling of the ipsilateral PVN was prominent (Fig. 1C) indicating that the technique used was reliable. Similar microinjections of FG into the CVLM (Fig. 1D) in another experiment, resulted in retrograde labeling of only few cells in the ipsilateral Arc (Figs. 1E) while the labeling in the ipsilateral PVN was robust (Fig. 1F). In another experiment, microinjections of FG into the RVLM (Fig. 2A) resulted in retrograde labeling of only a few cells in the ipsilateral Arc (Fig. 2B) while retrograde labeling in the ipsilateral PVN was prominent (Fig. 2C). Microinjections of FG into the IML and adjacent lateral funiculus in another experiment (Fig. 2D) resulted in retrograde labeling of only a few cells in the ipsilateral Arc (Fig. 2E) while many cells in the ipsilateral PVN were labeled (Fig. 2F). No cells were labeled in the Arc or PVN after microinjections of the tracers into the nAmb. Our results showing the lack of labeling in the Arc after the microinjections of tracers in the nAmb are in agreement with the observations reported by Ciriello et al. (2003). Based on our anatomical tract tracing studies, it was concluded that the projections from the PVN to the NTS, CVLM, RVLM and IML are robust. On the other hand, projections of the Arc to these areas are sparse. It is possible that the Arc neurons may send collateral axonal projections to neurons regulating cardiovascular function in these brain areas (i.e., NTS, CVLM, RVLM and IML). Such instances of collateral projections have been reported in the literature. For example, hypocretin (orexin) containing neurons in

the perifornical and lateral hypothalamic areas send collateral projections to the NTS and nAmb in the rat (Ciriello et al., 2003).

In other experiments, we microinjected (5–10 nl) an anatomical tract tracer (e.g., FG) into the PVN and retrograde labeling was examined in the Arc; robust bilateral retrograde labeling, with ipsilateral preponderance, was observed in the Arc (Kawabe et al., 2012). This observation suggested that some of the cardiovascular responses elicited from the Arc may be mediated via the PVN (see section 7). Our studies using retrograde tracing combined with immunohistochemistry showed that Arc neurons containing beta-endorphin, glutamic acid decarboxylase 67 (GAD67; a marker for gamma aminobutyric acid [GABA] containing neurons), and neuropeptide Y (NPY) projected to the ipsilateral PVN (Kawabe et al., 2012). Previous reports from other laboratories have also reported the presence of beta-endorphin, GABA and NPY containing neurons in the Arc (Ghamari-Langroudi, 2012; Horvath et al., 1997; Li et al., 1998; Meister, 2007; Morton et al., 2006; Ovesjo et al., 2001; Sanchez-Lasheras et al., 2010; Schwartz et al., 2000).

## 4. Chemical phenotypes of Arc neurons

Most of the information about the chemical nature of neurons in the Arc has been generated from studies on feeding behavior and energy homeostasis (Belgardt et al., 2009; Chronwall, 1985; Sanchez-Lasheras et al., 2010; Schwartz et al., 2000). A group of neurons, co-expressing proopiomelanocortin (POMC) and cocaine and amphetamine-regulated transcript (CART), is located in the ventrolateral part of the Arc (POMC/CART neurons). Adrenocorticotropin (ACTH), beta-endorphin, and alpha-melanocyte stimulating hormone (alpha-MSH) are metabolic products of POMC (Pritchard et al., 2002); these peptides are colocalized in the POMC neurons of the Arc (Ibata et al., 1985; Lantos et al., 1995). POMC/CART neurons in the Arc project to the PVN (Schwartz et al., 2000). Activation of POMC/CART neurons in the Arc results in the release of alpha-MSH in the PVN. Alpha-MSH acts as an agonist at melanocortin receptors ($MC_4$ receptors) in the PVN and increases their activity above the basal level which results in a decrease in feeding and increase in energy expenditure (anorexigenic functions). Another group of neurons containing NPY is located in the ventromedial part of the Arc. About 90% of NPY neurons contain agouti-related peptide (AgRP). NPY/AgRP neurons in the Arc also project to the PVN (Schwartz et al., 2000). AgRP acts as an endogenous competitive antagonist at $MC_4$ receptors in the PVN (Meister, 2007). AgRP also acts as an inverse agonist at $MC_4$ receptors; it binds with these receptors in the PVN and elicits responses opposite to those of the agonist (alpha-MSH). Thus, AgRP decreases the activity of $MC_4$ receptors below the basal level which results in an increase in feeding and decrease in energy expenditure (orexigenic functions).

NPY/AgRP and POMC/CART neurons are regulated by peripheral hormones (e.g., leptin and insulin), as well as nutrients (e.g., glucose, amino acids and fatty acids). Leptin and insulin inhibit NPY/AgRP neurons and stimulate POMC/CART neurons (Dampney 2011; Rahmouni and Morgan, 2007; Sanchez-Lasheras et al., 2010). In the Arc NPY type 1 receptors (NPY1Rs) are present on the POMC/CART neurons but not on the NPY/AgRP neurons (Meister, 2007; Fuxe et al., 1997). Both NPY/AgRP and POMC/CART neurons contain GABA (Ghamari-Langroudi, 2012; Horvath et al., 1997; Ovesjo et al., 2001; Sanchez-Lasheras et al., 2010). Two discrete subpopulations of POMC neurons exist in the ventrolateral Arc: one containing GABA and the other expressing glutamate (Dicken et al., 2012; Hentges et al., 2009).

## 5. Role of Arc glucose sensing neurons in sympathoexcitation

### 5.1. Glucose sensing neurons

Available information about glucose sensing neurons has been reviewed in recent years and salient features of this information can be summarized as follows (Routh 2002; 2010). There are two types of glucose sensing neurons namely glucose excited (GE) and glucose inhibited (GI) neurons. The firing frequency of GE neurons is increased while that of GI neurons is decreased as the concentration of interstitial glucose concentration in the brain increases from 0.1–2.5 mM. Under normal physiological conditions glucose concentration in brain parenchyma ranges between 0.7–2.5 mM. At higher interstitial glucose concentrations, there are other subsets of neurons that increase (HGE) or decrease (HGI) their firing when brain glucose levels are increased from 5–20 mM. The physiological relevance of these neurons (i.e., HGE and HGI neurons) is not clear because the concentration of glucose in the brain parenchyma does not usually exceed 5 mM when the blood-brain barrier is intact. Most of the studies on glucose sensing neurons have been done on ventromedial hypothalamus (VMH) which includes the Arc and VMN.

The presence of GE neurons is concentrated in the ventrolateral border of the VMN (vl-VMN) in the region between the Arc and VMN where the cell population is sparse. All GE neurons contain adenosine triphosphate (ATP)-sensitive potassium channels ($K_{ATP}$ channels) which play a role in glucose sensing in these neurons, although other glucose sensing mechanisms may also be utilized by different subtypes of GE neurons.

GI neurons are present in the Arc as well as VMN. Decreased glucose concentration in the brain parenchyma results in decreased glucose levels in the neurons. The ratio of adenosine monophosphate (AMP) and ATP (AMP/ATP) is increased which results in the activation of alpha-2 subunit of 5′ AMP-activated protein kinase (AMPK). Neuronal nitric oxide synthase (nNOS) is stimulated and nitric oxide (NO) is generated. NO stimulates soluble guanylate cyclase (sGC) and cyclic guanosine monophosphate (cGMP) levels are increased; the GI neurons are depolarized and their firing frequency is increased. Stimulation of AMPK also results in the phosphorylation of cystic fibrosis transmembrane regulator (CFTR) channel causing its closure leading to depolarization of the GI neurons and increase in their firing rate.

The chemical phenotype of GI and GE neurons is not firmly established. It has been suggested that POMC neurons in the Arc are GE neurons but there is no firm evidence to support this contention. It is known that about 40% of NPY neurons in the Arc are GI neurons (Routh, 2002). However, GI neurons are also present in the VMN where NPY neurons are not present.

### 5.2. Role of glucose sensing neurons in counter regulatory response to hypoglycemia

One of the functions of glucose sensing neurons is the initiation of counter-regulatory response to hypoglycemia which involves increase in sympathetic nerve activity (SNA). Decrease in the plasma glucose concentration to hypoglycemic levels (2–3 mM) results in the activation of sympatho-adrenal system. Increase in SNA causes the release of epinephrine, norepinephrine and glucagon. These events cause glycogenolysis and inhibition of the release of insulin from the pancreas. Counter regulatory response is a protective mechanism by which euglycemia is restored and the brain is protected from hypoglycemia. The chemical phenotype of glucose sensing neurons that mediate increase in SNA during counter regulatory response is not known.

## 6. Pressor responses elicited from the Arc

There are very few studies in which the role of the Arc in cardiovascular regulation has been studied systematically (Coote, 2004). Recently we investigated the cardiovascular effects of chemical stimulation of the Arc in urethane-anesthetized, artificially ventilated, adult male Wistar rats (Nakamura et al., 2009). Baseline value for mean arterial pressure (MAP) in urethane-anesthetized rats was 87–94 mmHg. Microinjections (50 nl) of N-methyl-D-aspartic acid (NMDA; 1–10 mM) into the Arc elicited increases in MAP (6–17 mmHg). The maximal increases in MAP were elicited by 10 mM concentration of NMDA. The onset and duration of pressor responses to microinjections of NMDA (10 mM) were 6 sec and 10 min, respectively. The peak effect was observed at 40–45 sec. Repeated microinjections of NMDA (3 microinjections at 20 min intervals) did not elicit tachyphylaxis. Controls for all microinjections consisted of artificial cerebrospinal fluid (aCSF; pH 7.4); microinjections of aCSF alone into the Arc did not elicit significant changes in MAP. The coordinates for the Arc were: 3.6–3.8 mm caudal to the bregma, 0.1–0.2 mm lateral to the midline, and 9.8–10.2 mm deep from the dura. Microinjections (50 nl) of AMPA (non-NMDA receptor agonist; 10 mM) into the Arc also elicited increases in MAP (Nakamura et al., 2009). Although microinjections of L-Glutamate monosodium (L-Glu) into the Arc also elicited cardiovascular responses, the concentrations needed for this effect were relatively high (100 mM). Therefore, NMDA was chosen to stimulate the Arc in our studies (Nakamura et al., 2009).

Compared to the concentrations of NMDA, AMPA or L-Glu needed to stimulate medullary cardiovascular areas, the concentrations of these agonists to stimulate the Arc were higher (Nakamura et al., 2009). Tonic inhibition of Arc neurons by GABA and NO (Meister, 2007; Riediger et al., 2006) may be responsible for the necessity to use higher concentrations of NMDA, AMPA and L-Glu to stimulate the Arc. Moreover, microinjections of excitatory amino acid receptor agonists into the Arc may activate inhibitory projections of the Arc to different brain regions which may attenuate the effects of excitatory projections from the Arc to RVLM and IML. For example, the Arc is known to send excitatory projections to the vlPAG which, in turn, sends inhibitory projections to the RVLM (Li et al., 2006). At baseline MAP levels below normal (e.g., 87–94 mmHg), it appears that microinjections of NMDA into the Arc activated the projections to the RVLM and IML eliciting pressor responses despite possible activation of inhibitory projections emerging from the Arc (Nakamura et al., 2009).

Microinjections of NMDA at different rostro-caudal and medio-lateral levels of the Arc elicited similar pressor responses. The diameter of the diffusion sphere of a 50 nl microinjection has been estimated to be about 500 μm (Nicholson, 1985). Therefore, the microinjections made into the Arc were restricted to this nucleus as confirmed by microinjections of India ink or fluorescent microspheres (Lumafluor Corp., Durham, NC, USA). The pressor responses to Arc stimulation were not altered by microinjections of muscimol into the hypothalamic dorsomedial nucleus (DMN) indicating that NMDA did not spread to the DMN.

Microinjections of NMDA into the Arc elicited an increase in greater splanchnic nerve activity (GSNA) (Nakamura et al., 2009). The barosensitivity of the GSNA was tested by reflex inhibition of GSNA and bradycardia elicited by a bolus intravenous (i.v.) injection of phenylephrine (PE; 10 μg/kg) (Fig. 3A). When the effects of PE subsided (about 10 min), microinjection of NMDA (10 mM) into the Arc elicited an increase in the GSNA (Fig. 3B). Intrathecal injections of NBQX disodium (non-NMDA receptor antagonist; 2 mM) and D-AP7 (NMDA receptor antagonist; 5 mM) at T9–T10 (volume 20 μl and duration 10 sec) blocked the increase in GSNA induced by microinjections of NMDA into the Arc (Fig. 3C).

In other experiments, chemical stimulation of the Arc elicited increases in renal sympathetic nerve activity (RSNA) also (Nakamura et al., 2009).

Thus, when the baseline MAP is below normal levels (e.g., 87–94 mmHg), chemical stimulation of the Arc resulted in an increase in MAP and GSNA. The increases in MAP and GSNA were mediated by the activation of spinal cord ionotropic glutamate receptors (iGLURs).

It has been suggested that the increases in blood pressure (BP) following the chemical stimulation of the Arc is mediated via vasopressinergic neurons in the PVN (Brody et al., 1986). This possibility was excluded in our study because the pressor responses to chemical stimulation of the Arc were abolished by intrathecal injections of iGLUR antagonists at T9–T10. However, the participation of the projections from the Arc to the PVN in mediating the increases in BP and SNA cannot be excluded. Indeed, we have reported that other types of cardiovascular responses (e.g., depressor responses) elicited from the Arc are mediated via the PVN (Kawabe et al., 2012) (see section 7).

As mentioned earlier in this paper (section 4), different chemical phenotypes of neurons are present in the Arc. The cardiovascular functions of all types of Arc neurons have not been studied. However, it is known that POMC/CART neurons are involved in catabolic activity (i.e., decrease in food intake and increase in energy expenditure including increase in SNA). It can, therefore, be speculated that microinjections of NMDA into the Arc may stimulate POMC/CART neurons to elicit increase in SNA and BP.

## 7. Depressor responses elicited from the Arc

The presence of GABA, beta-endorphin and NPY containing neurons in the Arc has been reported previously by several authors (Hentges et al., 2009; Horvath et al., 1997; Ibata et al., 1985; Lantos et al., 1995; Ovesjo et al., 2001). These neurotransmitters/neuromodulators have been reported to exert inhibitory effects on neurons (Albers et al., 1990; Kow et al, 1989; Pennock et al., 2011). Because one of the target areas of the Arc neurons is the PVN (Schwartz et al., 2000) and this nucleus has been reported to play a significant role in regulating cardiovascular function (Badoer, 2001; Coote, 2004; Dampney et al., 2005; Kawabe et al., 2008; Stern, 2004), we hypothesized that decreases in BP and SNA may be elicited by the chemical stimulation of the Arc and these responses may be mediated via the PVN. We carried out experiments in urethane-anesthetized, artificially ventilated, adult male Wistar rats (Kawabe et al., 2012). Baseline value for MAP (98–100 mmHg) in these rats was higher than the rats used to study “pressor responses” described in section 6. Unilateral microinjections (30 nl) of NMDA (10 mM) into the Arc elicited decreases in MAP and GSNA. The decreases in MAP and SNA were reproducible after repeated microinjections of NMDA (at least 3 microinjections into the same site of the Arc at 20 min intervals).

In other experiments, we stimulated the Arc by microinjections of NMDA (10 mM) before and after the blockade of receptors mediating inhibitory responses in the ipsilateral PVN. The coordinates for the microinjections into the Arc were: 4.0–5.4 mm caudal to the bregma, 0.2–0.4 mm lateral to the midline, and 9.7–10.0 mm deep from the dura and the coordinates for microinjections into the PVN were: 1.6–1.9 mm caudal to the bregma, 0.3–0.5 mm lateral to the midline and 7.7–8.0 mm deep from the dura. The volumes of all microinjections into the Arc and PVN were 20 and 30 nl, respectively. The blockade of either GABA type A receptors ($GABA_A$Rs) by gabazine (1 mM), or NPY1Rs by BMS193885 (15 mM), or opiate receptors (OPRs) by naloxone (20 mM) in the ipsilateral PVN significantly attenuated the decreases in MAP and GSNA responses elicited from the Arc. When all three of these receptors (i.e., $GABA_A$Rs, NPY1Rs and OPRs) were blocked simultaneously in the ipsilateral PVN, the decreases in MAP and GSNA elicited by Arc-

stimulation were converted to increases in MAP and GSNA. The concentrations of gabazine, naloxone and BMS193885 microinjected into the PVN did not alter the responses to microinjections of NMDA at the same site suggesting that these antagonists did not exert any deleterious effects at the microinjection site.

It is well established that chemical stimulation or disinhibition of the PVN neurons elicits increases in BP and SNA (Chen et al., 2003; Kannan et al., 1988; Kawabe et al., 2008; Li et al., 2006). Direct projections from the PVN to the RVLM and the IML have been reported (Holstege, 1987; Pyner and Coote, 1999; Pyner and Coote, 2000; Yang and Coote, 1998). RVLM neurons projecting to the IML have been reported to be excited by PVN stimulation (Pyner and Coote, 1999; Pyner and Coote, 2000; Yang and Coote, 1998; Yang et al., 2001). Chemical stimulation of the direct projection from the RVLM to the IML has been reported to elicit increases in BP and RSNA via the release of glutamate (Sundaram and Sapru, 1991) or vasopressin (Yang et al., 2002) in the IML. Therefore, in our study (Kawabe et al., 2012), NPY, GABA and beta-endorphin release following the Arc stimulation may have caused inhibition of PVN neurons and elicited decreases in BP and SNA.

It may be argued that microinjections of NMDA into the Arc may have spread to the VMN and elicited decreases in MAP. This possibility is unlikely because the responses elicited from the Arc persisted even in the region where VMN is not present (e.g., 3.5–4.1 mm caudal to the bregma) (Paxinos and Watson, 2007). Diffusion of NMDA microinjected into the Arc to the DMN was also ruled out because pressor, instead of depressor, responses were elicited from the DMN.

Collectively, our results presented in this section indicated that when the baseline MAP was relatively normal (e.g., 98–100 mmHg), chemical stimulation of the Arc resulted in decreases in MAP and GSNA. The depressor responses were mediated via $GABA_A$Rs, NPY1Rs and OPRs in the ipsilateral PVN. Both NPY/AgRP and POMC neurons in the Arc contain GABA (Ghamari-Langroudi, 2012; Horvath et al., 1997; Ovesjo et al., 2001; Sanchez-Lasheras et al., 2010); these neurons may be the source of GABA release in the PVN following the chemical stimulation of the Arc. Simultaneous blockade of $GABA_A$Rs, NPY1Rs and OPRs in the ipsilateral PVN converted decreases in MAP and GSNA to increases in MAP and GSNA. Furthermore, it was concluded that NMDA stimulated neurons with inhibitory as well as excitatory influence on MAP and GSNA in the Arc. When the receptors mediating actions of inhibitory neurotransmitters in the PVN were blocked simultaneously, excitatory responses were unmasked.

## 8. Effect of baseline BP on cardiovascular responses elicited from the Arc

Our results show that both pressor (section 6) and depressor (section 7) responses can be elicited by the chemical stimulation of the Arc. The diversity in cardiovascular and sympathetic responses elicited from the Arc is not unexpected considering the presence of different chemical phenotypes of neurons in the Arc (section 4). As discussed in section 7, stimulation of the Arc may cause activation of NPY, GABA and beta-endorphin containing neurons in the PVN, causing release of their respective peptides in the PVN, which, in turn, results in the inhibition of PVN neurons regulating cardiovascular function and consequent decreases in BP and SNA (Kawabe et al., 2012). There are reports in the literature describing diverse cardiovascular responses elicited by Arc stimulation. For example, stimulation of POMC containing neurons in the Arc by an alpha-2-adrenergic receptor agonist resulted in the release of beta-endorphin in the NTS causing hypotension and bradycardia (Li et al, 1996). In another report, electrical stimulation of the mid-anterior region of the Arc resulted in a biphasic BP response (depressor/pressor); blockade of the

pressor component of the response by an intravenous injection of V1-receptor antagonist revealed only a depressor response (Mastrianni et al., 1989).

We have noticed that the direction of the cardiovascular responses elicited from the Arc depended on baseline MAP (Kawabe et al., 2012). When the baseline MAP was relatively normal (e.g., 98–100 mmHg), chemical stimulation of the Arc elicited depressor responses (section 7) and when the baseline MAP was below normal (e.g., 87–94 mmHg), chemical stimulation of the Arc resulted in increases in MAP and GSNA (section 6). Our recent studies suggest that baroreceptor unloading may play a role in the direction of blood pressure response (increase or decrease) elicited from the Arc. Indeed, bilateral barodenervation converted decreases in MAP and GSNA elicited by chemical stimulation of the Arc to increases in MAP and GSNA; these responses may be mediated via the release of alpha-MSH and/or ACTH and glutamate from the Arc neurons projecting to the PVN (unpublished observations).

## 9. Tachycardic responses elicited from the Arc

Chemical stimulation of the Arc always elicited tachycardic responses regardless of the level of baseline BP or heart rate (HR). When the baseline values for MAP and HR were 87–94 mmHg and 396–412 bpm, respectively, in urethane-anesthetized rats, microinjections (50 nl) of NMDA (1–10 mM) into the Arc elicited increases HR (24–75 bpm). The maximal increases in HR were elicited by 10 mM concentration of NMDA. Bilateral vagotomy significantly attenuated the tachycardic, but not pressor, responses to microinjections of NMDA into the Arc. This experiment indicated that tachycardia elicited by the chemical stimulation of the Arc may be partially mediated by a decrease in vagal input to the heart. However, tachycardic responses to Arc-stimulation were not abolished after bilateral vagotomy suggesting sympathetic pathways are also involved in mediating these responses. Direct application of iGLUR antagonists (NBQX and D-AP7) at T1–T4 attenuated the tachycardic response elicited by Arc-stimulation. When the bilateral vagotomy was combined with direct application of iGLUR antagonists applied to T1–T4, the tachycardic responses elicited by stimulation of the Arc were abolished (Nakamura et al., 2009). The increases in HR elicited by microinjections of NMDA into the Arc were not altered by the blockade of $GABA_A$Rs, NPY1Rs, and OPRs in the PVN. The pathways within the brain that mediate tachycardic responses elicited from the Arc remain to be elucidated.

## 10. Tonic inhibition of excitatory Arc neurons

Pressor and tachycardic responses were elicited by the microinjections of gabazine ($GABA_A$R antagonist) into the Arc. Moreover, microinjection of gabazine into the Arc converted the decreases in MAP and GSNA elicited by microinjections of NMDA into the Arc to increases in MAP and GSNA, while the tachycardic responses to NMDA were not changed (Kawabe et al., 2012). These results indicated that GABAergic neurons in the Arc (e.g., NPY/AgRP neurons) may tonically inhibit other neurons in this nucleus that may contain excitatory neurotransmitters (e.g., POMC/CART neurons) (Fig. 6). As mentioned in section 4, NPY/AgRP neurons in the Arc contain GABA (Ghamari-Langroudi, 2012; Sanchez-Lasheras et al., 2010) and there are GABAergic synapses on POMC/CART neurons located in the Arc. POMC/CART neurons in the Arc contain excitatory neurotransmitters such as alpha-MSH and glutamate (Dicken et al., 2012; Hentges et al., 2009). NPY/AgRP neurons have been reported to inhibit POMC/CART neurons by direct synaptic inhibition (Belgardt et al., 2009).

## 11. Leaky blood-brain barrier in the ventromedial Arc

The Arc extends into the median eminence (one of the circumventricular organs lacking blood-brain barrier) which is located ventral to the third ventricle in the proximal portion of the pituitary stalk. The capillaries of the hypophyseal portal system are located in the median eminence which has high capillary density and high capillary blood flow. These capillaries have fenestrated endothelium and lack blood-brain barrier (Gross, 1992; Schulz and Engelhardt, 2005). It has been suggested that the proximity of the Arc to the median eminence may favor the accessibility of the Arc neurons to physiologically active substances circulating in the blood. This notion is not universally accepted because tanycytes, located in the floor of the third ventricle in the region where Arc extends into the median eminence, possess tight junctions forming an impermeable barrier (Mullier et al., 2010). Although circulating molecules diffuse in the parenchyma of the median eminence because of the lack of blood-brain barrier in this structure, the tanycytes located in the floor of the third ventricle restrict the flow of these molecules into the Arc. On the other hand, the tanycytes located in the lateral walls at the base of the third ventricle in the Arc do not possess efficient tight junctions. This arrangement may allow diffusion of molecules circulating in the CSF into the parenchyma of the Arc (Mullier et al., 2010).

Some anatomical features allude to the possibility that parts of the Arc may have a leaky blood-brain barrier. For example, highly permeable microvessels have been identified in the ventromedial Arc of experimental animals (Ciofi, 2011; Ciofi et al., 2009; Norsted et al., 2008). These vessels may allow entry of circulating substances to a subpopulation of Arc neurons. Thus ventromedial Arc shares some features that are common in classical circumventricular organs (Ciofi, 2011). Consistent with this notion is the observation that when horse radish peroxidase (HRP) was injected intravenously, it leaked across the blood-brain barrier and labeled the neurons in the ventromedial Arc (Broadwell and Brightman, 1976). Pre-stalk injections of HRP in the median eminence resulted in labeling of rostral but not caudal Arc neurons (Wiegand and Price, 1980). Based on this information, it appears that blood-borne molecules may have access to ventromedial Arc neurons. Some of the blood-borne substances that may act in the Arc to elicit cardiovascular response are leptin and insulin (sections 12 and 15). Although the Arc is involved in mediating the cardiovascular responses to intravenously injected Ang II and Ang-(1-12), these effects may not be due to leakage of these peptides across the blood-brain barrier in the Arc; instead, circulating angiotensin may act on neurons in the SFO (which lacks blood-brain barrier) and mediate cardiovascular actions via the projections of SFO neurons to the Arc (sections 13.2 and 14.3).

## 12. Effect of leptin in the Arc

### 12.1. Leptin

The information regarding effects of leptin has been accumulated mostly from investigations on energy homeostasis (Reviews: Penicaud et al., 2012; Tartaglia, 1997). Leptin is a hormone consisting of 167 amino acids produced by adipocytes in white fat tissue and its concentration in the blood increases with accumulation of fat and decreases with fasting and leanness. It acts in the brain for long-term regulation of body weight. Leptin is the product of ob gene and the receptor mediating its actions is the product of db gene. Mice with mutations in the gene for leptin (ob/ob mice) have a deficiency of leptin while mice with a mutation in the gene for leptin receptor (db/db mice) are deficient in leptin receptor activity. Both ob/ob and db/db mice are obese and have an increased food intake.

### 12.2. Leptin receptors

So far six variants of leptin receptor (OB-R), including long (OB-$R_L$) and short forms (OB-$R_s$), have been identified. The longest form of leptin receptor is believed to be the primary receptor involved in energy homeostasis (Banks et al., 2000). The extracellular domains of both long and short forms of leptin receptor are identical (816 amino acids). Both types of receptors have a transmembrane domain of 23 amino acids. The intracellular domain of the long form of leptin receptor consists of 303 amino acids while it terminates shortly after amino acid 29 in short forms of the leptin receptor.

### 12.3. Cardiovascular effects leptin in the Arc

Intravenous or intracerebroventricular injections of leptin have been reported to increase SNA and BP (Dunbar et al., 1997; Haynes et al., 1997; Montanaro et al., 2005; Shek et al., 1998; Rahmouni and Morgan, 2007). Leptin crosses blood-brain barrier via a saturable transport system (Banks et al., 1996; Burguera et al., 2000). The short form of leptin receptor is believed to be involved in the transport of leptin into the brain (Chen et al., 1996: Kastin et al., 1999; Lee et al., 1996). Because leptin can cross blood-brain barrier, it is expected that it may act on different CNS structures to increase SNA and BP. The Arc is one of the sites where leptin may act because OB-$R_L$ is expressed in high levels in this nucleus (Elmquist et al., 1999; Sanchez-Lasheras et al., 2010). As mentioned earlier, ventromedial Arc has been reported to have a leaky blood-brain barrier (Ciofi, 2011; Ciofi et al., 2009; Norsted et al., 2008). Therefore, there is a possibility that circulating leptin may gain access to neurons in the ventromedial Arc. This possibility has not been tested although direct microinjections of leptin into the Arc have been reported to elicit cardiovascular and/or sympathetic responses. For example, Montanaro et al. (2005) reported that microinjections of leptin (100 ng/100 nl) directly into the Arc in anesthetized rats elicited increases in lumbar sympathetic activity (LSNA) but the increases in BP and HR were not significant. Rahmouni and Morgan (2007) reported that direct microinjections of leptin (500 ng/200 nl) into the Arc of alpha-chloralose anesthetized rats increased brown adipose tissue nerve activity and RSNA as well as BP. Collectively, the results of Rahmouni and Morgan (2007) and Montanaro et al. (2005) suggest the Arc is involved in mediating the sympathetic and cardiovascular responses of leptin. Leptin has been reported to stimulate CART neurons in the Arc projecting to the spinal cord (Cowley et al., 2001; Elias et al., 1998; Ghamari-Langroudi, 2012).

## 13. Effect of Ang II in the Arc

### 13.1. Microinjections of Ang II in the Arc

We studied the effect of microinjections of Ang II into the Arc for comparison of our results with Ang-(1-12) (Arakawa et al., 2011; see section 13). Microinjections of Ang II (1 mM) into the Arc elicited increases in MAP and HR. The onset of cardiovascular effects of Ang II was shorter than that of Ang-(1-12) which is consistent with the notion that Ang-(1-12) has to be converted to Ang II by angiotensin converting enzyme (ACE) and chymase.

### 13.2. Role of Arc in mediating cardiovascular responses of circulating Ang II

Intravenous injections of Ang II (300 pmol/kg) elicited increases in MAP which were significantly greater than those elicited by intravenous injections of the same dose of Ang-(1-12). However, there were no significant differences in the HR responses elicited by these two peptides (Arakawa et al., 2011).

In order to test if the Arc played a role in mediating the responses to intravenous injections of Ang II, muscimol (1 mM) was injected bilaterally into the Arc. In the rat, the Arc is about 2.6 mm long. The diameter of the diffusion sphere of a 100 nl microinjection has been

estimated to be about 1 mm (Nicholson, 1985). Therefore, 2 microinjections (100 nl each) of muscimol (1 mM) were made into the Arc on each side to ensure that most of the neurons in the Arc were inhibited. The coordinates for rostral and caudal sites were 2.5 mm caudal to the bregma, 0.1–0.3 mm lateral to the midline and 9.8–10.2 mm deep from the dura and 3.6 mm caudal to the bregma, 0.1–0.3 mm lateral to the midline and 9.8–10.2 mm deep from the dura, respectively. No change in the basal MAP and HR were observed after bilateral inhibition of Arc neurons using muscimol microinjections suggesting that, unlike the RVLM (Willette et al., 1983; Willette et al., 1984), the Arc may not be normally involved in the maintenance of systemic BP. Lack of responses to microinjections of NMDA (10 mM) indicated the inhibition of the Arc was complete. Subsequent intravenous injections of Ang II (300 pmol/kg) elicited attenuated increases in MAP and HR. However, in a separate group of rats, bilateral blockade of angiotensin type 1 receptors (AT1Rs) in the Arc by microinjections of losartan (10 mM) did not attenuate the increases in MAP and HR to subsequent intravenous injections of Ang II.

Attenuation of cardiovascular responses of the circulating Ang II by bilateral inhibition of the Arc using muscimol microinjections suggested that the Arc does play a role in mediating the responses to the circulating Ang II. Increase in Fos-immunoreactivity in the Arc of rabbits during intravenous infusions of Ang II is consistent with the notion that Arc is involved in mediating these cardiovascular responses (Davern and Head, 2007). However, bilateral blockade of AT1Rs in the Arc did not attenuate the Ang II-induced responses suggesting that circulating Ang II did not reach Arc neurons via a leaky blood-brain barrier. The following pathways could be speculated to mediate the activation of Arc neurons by circulating Ang II. The circulating Ang II could act on AT1Rs in the SFO which lacks blood-brain barrier (Ferguson, 2009). Activation of neurons in the SFO could then stimulate neurons in the Arc via direct or indirect projections and elicit pressor and tachycardic responses. The transmitter at the terminals of the projections from the SFO to the Arc does not appear to be Ang II because bilateral blockade of AT1Rs in the Arc did not attenuate cardiovascular responses to intravenously administered Ang II. In this context, it may be noted that the existence of a projection from the SFO to the Arc has been reported (Gruber et al., 1987).

Baseline BP and HR were not altered by the bilateral blockade of AT1Rs in the Arc in normal rats. However, decrease in these variables following AT1R blockade may be elicited in pathological states. Similar observations have been reported in the PVN; bilateral blockade of AT1Rs in the PVN had no significant effect on resting SNA in normal situations but sympathoexcitation induced by hyperosmolality or heart failure was significantly reduced when AT1Rs were blocked in the PVN (Chen and Toney, 2003; Zheng et al., 2009).

## 14. Effect of Ang-(1-12) in the Arc

### 14.1. Ang-(1-12)

Nagata et al., (2006) have recently identified a new endogenous angiotensin [Ang-(1-12)]. It is a C-terminal extended form of angiotensin I and consists of 12 amino acid residues. In the rat, intravenous injection of Ang-(1-12) elicited an increase in BP which was blocked by previously administered ACE inhibitor suggesting that the actions of Ang-(1-12) were mediated via its rapid conversion to Ang II; for this reason this new angiotensin was also named as proangiotensin-12 (Nagata et al, 2006; Varagic et al., 2008). The actions of Ang-(1-12) are mediated via AT1Rs (Nagata et al, 2006; Varagic et al., 2008).

In terms of the physiological significance, the identification of Ang-(1-12) may provide an alternate substrate for synthesis of angiotensin peptides in the brain. Although there is a general consensus that Ang II can be synthesized in the brain (Grobe et al., 2008), some

controversies still persist in this field of research. For example, angiotensinogen is widely distributed in the brain regions involved in cardiovascular regulation but the levels of renin in the brain are low (Grobe et al., 2008). Despite the low levels of renin, Ang II is present in neurons located in many brain regions including the SFO, PVN, RVLM and NTS (Huang et al., 2003; Lind et al., 1985; Palkovits et al., 1995; Pickel and Chan, 1995). The mismatch between the levels of angiotensinogen and renin in the brain has prompted the hypothesis that Ang II may be synthesized in the brain via a renin-independent pathway. The enzymes involved in the biosynthesis of Ang-(1-12) remain to be identified. However, it is known that renin is not involved in the formation of Ang-(1-12) (Ferrario et al., 2009; Trask et al., 2008). Thus, Ang-(1-12) may be generated in the brain via a renin-independent pathway and it may be converted to Ang II. In this context, it should be pointed out that the levels of Ang-(1-12) in the brain are about five times greater than those of Ang II (Nagata et al., 2006). We and others have reported that both ACE and chymase may be involved in the conversion of Ang-(1-12) to Ang II (Arakawa et al., 2011; Arakawa et al., 2012; Arnold et al., 2010; Chitravanshi and Sapru, 2011; Chitravanshi et al., 2012; Nagata et al., 2006). Hyperactivity of the brain renin-angiotensin system (RAS) plays a crucial role in mediating hypertension (Veersingham and Raizada, 2003). Studies on transgenic mice support the role of increased brain Ang II in hypertension (Lochard et al., 2003; Morimoto et al., 2001; Morimoto et al., 2002; Sakai et al., 2007). Ang-(1-12) may eventually emerge as important component of RAS in the Arc.

### 14.2. Cardiovascular effects of Ang-(1-12) microinjections in the Arc

Several components of the RAS have been identified in the Arc. For example, angiotensinogen, ACE, and ATRs have been identified in the Arc (Donadio et al., 2006; He et al., 1999; Isa et al., 2009; Zhu et al., 2004). We hypothesized that the Arc may be one of the sites of cardiovascular actions of angiotensins and carried out experiments in urethane-anesthetized, artificially ventilated, adult male Wistar rats (Arakawa et al., 2011). Microinjections (50 nl) of Ang-(1-12) (0.25–2 mM) into the Arc, which was previously identified by microinjections of NMDA (10 mM), elicited dose-dependent increases in MAP, HR and GSNA (Fig. 4). Bilateral microinjections of Ang-(1-12) into the Arc elicited significantly greater increases in MAP and HR when compared with the responses elicited by unilateral microinjections of this peptide into this nucleus. No desensitization of responses was observed with repeated microinjections of Ang-(1-12) when the interval between two injections was at least 20 min; therefore, the interval between two intravenous injections of Ang-(1-12) was at least 20 min to avoid tachyphylaxis. Bilateral vagotomy attenuated the increases in HR elicited by Ang-(1-12). Microinjections of AT1R antagonists (losartan) into the Arc (Fig. 4), but not an AT2R antagonist (PD123319) (not shown), attenuated cardiovascular and GSNA responses elicited by microinjections of Ang-(1-12) (1 mM) into the Arc. Bilateral blockade of AT1Rs in the Arc by losartan did not alter the basal MAP and HR. Simultaneous inhibition of ACE by captopril (200 mM) and chymase by chymostatin (10 mM) in the Arc abolished Ang-(1-12)-induced responses suggesting that both ACE and chymase are involved in the conversion of Ang-(1-12) into Ang II. We have reported similar effects of captopril and chymostatin on Ang-(1-12)-induced responses in the NTS, RVLM, and PVN (Arakawa et al., 2012; Chitravanshi and Sapru, 2011; Chitravanshi et al., 2012).

An explanation for the mechanism of increases in MAP, HR, and GSNA elicited by microinjections of Ang-(1-12) into the Arc can be summarized as follows. Microinjections of Ang-(1-12) into the Arc may cause excitation of the Arc neurons involved in cardiovascular regulation via pre- and/or post-synaptic effects. The projections that could mediate these effects include direct or indirect inputs from the Arc to the PVN, raphe nuclei, RVLM and IML (Cechetto and Saper, 1988; Ciriello et al., 2003; Li et al., 2009; Sim and

Joseph, 1991). Consistent with this notion is our report in which the pressor responses elicited by microinjections of NMDA into the Arc were mediated via activation of iGLURs in the spinal cord (Nakamura et al., 2009). Both inhibition of vagal outflow to the heart and activation of spinal cord iGLURs mediate the tachycardic responses to microinjections of Ang-(1-12) into the Arc (Nakamura et al., 2009).

### 14.3. Role of Arc in mediating responses to circulating Ang-(1-12)

The dose-response of cardiovascular actions of intravenously administered Ang-(1-12) showed that maximal increases in MAP were elicited by 300–400 pmol/kg dose of Ang-(1-12). Desensitization of responses was not observed after repeated intravenous injections of Ang-(1-12) (300 pmol/kg) at 20–40 min intervals.

There is a possibility that intravenously administered Ang-(1-12) could reach the ventromedial part of the Arc because it has a leaky blood-brain barrier (see section 11). This possibility was tested as follows. The neurons in the Arc were inhibited bilaterally by microinjections of muscimol (1 mM) (see section 13.2). Subsequent intravenous injections of Ang-(1-12) (300 pmol/kg) elicited attenuated increases in MAP and HR (Fig. 5A & B). These results indicated that the Arc is involved in mediating the cardiovascular responses to intravenously administered Ang-(1-12). However, in a separate group of rats, bilateral blockade of AT1Rs in the Arc by microinjections of losartan (10 mM) did not attenuate the increases in MAP and HR to subsequent intravenous injections of Ang-(1-12) (Fig. 5C & D). This observation indicated that AT1Rs in the Arc did not mediate the cardiovascular effects of intravenously administered Ang-(1-12). The pathways mediating the effects of circulating Ang-(1-12) may involve projections from the SFO to the Arc (see section 13.2).

## 15. Effects of insulin in the Arc

Insulin has been reported to increase SNA via its actions in the CNS. For example, intracerebroventricular injections of insulin in the rat have been reported to increase SNA and increase baroreflex control of SNA and HR (Morgan et al., 1993; Muntzel et al., 1994; Okada and Bunag, 1994; Pricher et al., 2008). Recently, the Arc has been demonstrated to be one of the sites where insulin can act to elicit cardiovascular responses (Cassaglia et al., 2011). These authors injected insulin (0.6–60 nU) into the Arc of alpha-chloralose anesthetized female Sprague-Dawley rats and observed an increase in basal LSNA and baroreflex gain of LSNA. These effects are likely to be mediated via insulin receptors which have been reported to be present in the Arc (Marks et al., 1990; Werther et al., 1987). The role of Arc in circulating insulin was tested in hyperinsulinemic-euglycemic clamped rats. Insulin was infused (15 mU/kg/min) and blood glucose was measured at 2 min intervals. When the blood glucose levels were decreased by 10 mg/dl, 50% dextrose was infused at the rate of 0.25–0.5 ml/hour in order to achieve euglycemia. Glucose infusion was continued for 2 hours. The basal LSNA and the gain of baroreflex control of LSNA were increased following 60 min of intravenous infusion of insulin. Then muscimol (1 mM) was injected bilaterally into the Arc in order to inhibit these neurons; the effects of intravenous insulin on LSNA were reversed. The authors concluded that the Arc is one of the sites where intravenously injected insulin acts to increase SNA and these effects are mediated via the PVN.

## 16. Role of the Arc in cardiovascular effects of acupuncture

Manual or electroacupuncture (EA) is being increasingly accepted as an alternative and/or adjunctive therapy for the treatment of cardiovascular diseases such as hypertension (Review: Zhou and Longhurst, 2012). The CNS is known to play a critical role in the genesis and maintenance of hypertension. Increase in sympathetic nervous system activity is

associated with hypertension in humans (Schultz et al., 2007; Esler et al., 2001) as well as experimental animal models of hypertension, such as the Dahl salt-sensitive rat, deoxycorticosterone (DOCA)-salt rat, spontaneously hypertensive rat (SHR) and renin transgenic rat [TGR (mREN2)27] (Gyurko et al., 1993; Lark and Weyhenmeyer, 1992; Nishimura et al., 1992; Pochiero et al., 1983).

Longhurst and colleagues (Zhou and Longhurst, 2012) have extensively studied the role of Arc in acupuncture-induced attenuation of pressor and sympathoexcitatory responses. Essential information acquired from these studies can be summarized as follows. In anesthetized cats, application of bradykinin to the gallbladder or electrical stimulation of the splanchnic nerve afferents elicited a pressor and sympathoexcitatory reflex response. This reflex response was attenuated by applying EA bilaterally at the P5-6 acupoints. These acupoints overly the median nerve and are located 1.5–2.0 and 2.5–3.0 cm above the wrist between the ligaments of the flexor carpi radialis and the palmaris longus (Li et al., 2006). EA was applied by delivering electrical pulses (1–4 mA, 0.5 msec duration, 2 Hz) via needles placed at P5–6 acupoints. A simplified explanation of the circuits involved in the attenuation of sympathoexcitatory responses to application of bradykinin to the gallbladder or electrical stimulation of the splanchnic nerve afferents is as follows. EA activated long-loop pathways including the Arc neurons, vlPAG, raphe nuclei and the RVLM (Zhou and Longhurst, 2012). EA activated Arc neurons, which, in turn activated vlPAG neurons via a direct glutamatergic projection. Inhibition of GABA release in the vlPAG by activation of presynaptic cannabinoid type 1 receptors also resulted in the disinhibition of vlPAG neurons (Tjen-A-Looi et al., 2009). Excitation of vlPAG neurons resulted in the excitation of medullary raphe nuclei (raphe pallidus and obscurus), which, in turn, inhibited RVLM neurons; release of opioids and GABA mediated the inhibition of RVLM neurons (Tjen-A-Looi et al, 2007). Inhibition of RVLM neurons resulted in attenuation of sympathoexcitatory responses to the application of bradykinin to the gallbladder or splanchnic nerve afferent stimulation (Li et al., 2010).

## 17. Conclusions

Studies conducted in this and other laboratories indicate that the Arc does play a role in cardiovascular regulation. Our studies indicate that both pressor (sympathoexcitatory) and depressor (sympathoinhibitory) responses can be elicited by the chemical stimulation of the Arc depending on the baseline BP. Pressor (sympathoexcitatory) responses are mediated via spinal iGLURs. HR responses are mediated via excitation of sympathetic input as well as inhibition of vagal input to the heart. The depressor (sympathoinhibitory) responses to the chemical stimulation of the Arc are mediated via the release of GABA, NPY and opiates in the PVN. The role of the PVN in mediating the pressor responses has not been reported. The Arc projections relevant to our studies described in this review are shown in Fig. 6.

There are many questions that remain unanswered regarding the role of the Arc in cardiovascular regulation. For example, our results show that the Arc, unlike the RVLM, may not be normally involved in the regulation of systemic BP. The physiological or pathophysiological situations in which the Arc is involved in cardiovascular regulation remain to be identified. We have shown that both pressor and depressor responses can be elicited from the Arc depending on the baseline BP. Although the mechanism by which baseline BP determines the direction of the cardiovascular response elicited from the Arc remains to be established, our unpublished results indicate that baroreceptor unloading may contribute to the pressor responses observed at lower baseline BP. The role of PVN and the neurotransmitters involved in mediation of pressor (sympathoexcitatory) responses elicited from the Arc have not been studied.

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## Selected abbreviations

| | |
|---|---|
| **Arc** | hypothalamic arcuate nucleus |
| **ACE** | angiotensin converting enzyme |
| **ACTH** | adrenocorticotropin |
| **AgRP** | agouti-related peptide |
| **Alpha-MSH** | alpha-melanocyte stimulating hormone |
| **AMPA** | ±-α-amino-3-hydroxy-5-methyl-isoxazole-4-propionic acid hydrobromide (non-NMDA receptor agonist) |
| **AMPK** | adenosine monophosphate-activated protein kinase |
| **AT1R** | angiotensin II type 1 receptor |
| **AT2R** | angiotensin II type 2 receptor |
| **CART** | cocaine and amphetamine-regulated transcript |
| **CVLM** | caudal ventrolateral medullary depressor area |
| **D-AP7** | D(−)-2-amino-7-phosphono-heptanoic acid (NMDA receptor antagonist) |
| **DMN** | hypothalamic dorsomedial nucleus |
| **GSNA** | greater splanchnic nerve activity |
| **IML** | Intermediolateral cell column of the spinal cord |
| **LSNA** | lumbar sympathetic nerve activity |
| **nAmb** | nucleus ambiguus |
| **NBQX** | 2,3-dioxo-6-nitro-1,2,3,4-tetrahydrobenzo-[f]quinoxaline-7-sulfonamide (non-NMDA receptor antagonist) |
| **NMDA** | N-methyl-D-aspartic acid |
| **NPY** | neuropeptide Y |
| **NPY1R** | neuropeptide type 1 receptor |
| **NTS** | nucleus tractus solitarius |
| **OPR** | opiate receptor |
| **PAG** | periaqueductal gray |
| **POMC** | proopiomelanocortin |
| **PVN** | hypothalamic paraventricular nucleus |
| **RSNA** | renal sympathetic nerve activity |
| **RVLM** | rostral ventrolateral medullary pressor area |
| **SFO** | subfornical organ |
| **VMH** | ventromedial hypothalamus |

**VMN** hypothalamic ventromedial nucleus

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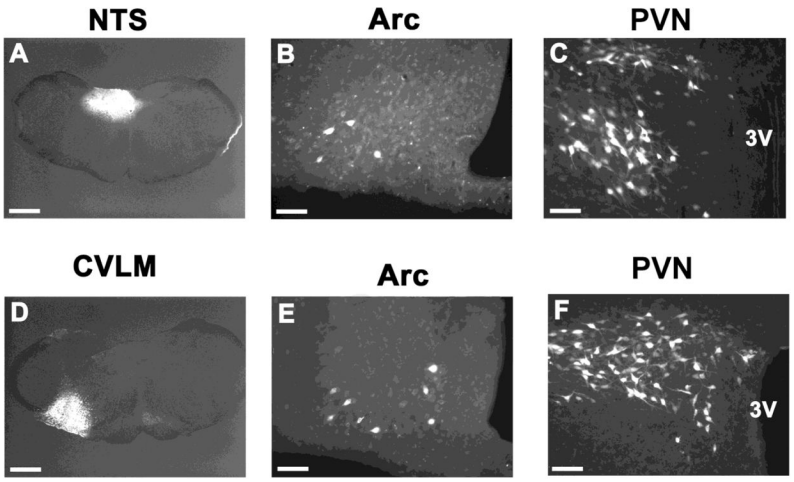


**Fig. 1.**

Projections from the Arc and PVN to the NTS and CVLM.

A: Microinjection site of the retrograde tracer (FG) into the left NTS (bar = 1 mm). B: Sparse and scattered retrogradely labeled neurons in the ipsilateral Arc (bar = 100 μm). C: In the same rat, numerous retrogradely labeled neurons in the ipsilateral PVN (bar = 100 μm). D: Microinjection site of FG into the left CVLM (bar = 1 mm). E: Few and scattered retrogradely labeled neurons in the ipsilateral Arc (bar = 100 μm). F: In the same rat, robust retrograde labeling of ipsilateral PVN neurons (bar = 100 μm). FG: Fluoro-Gold (4% dilution, 5–10 nl volume).

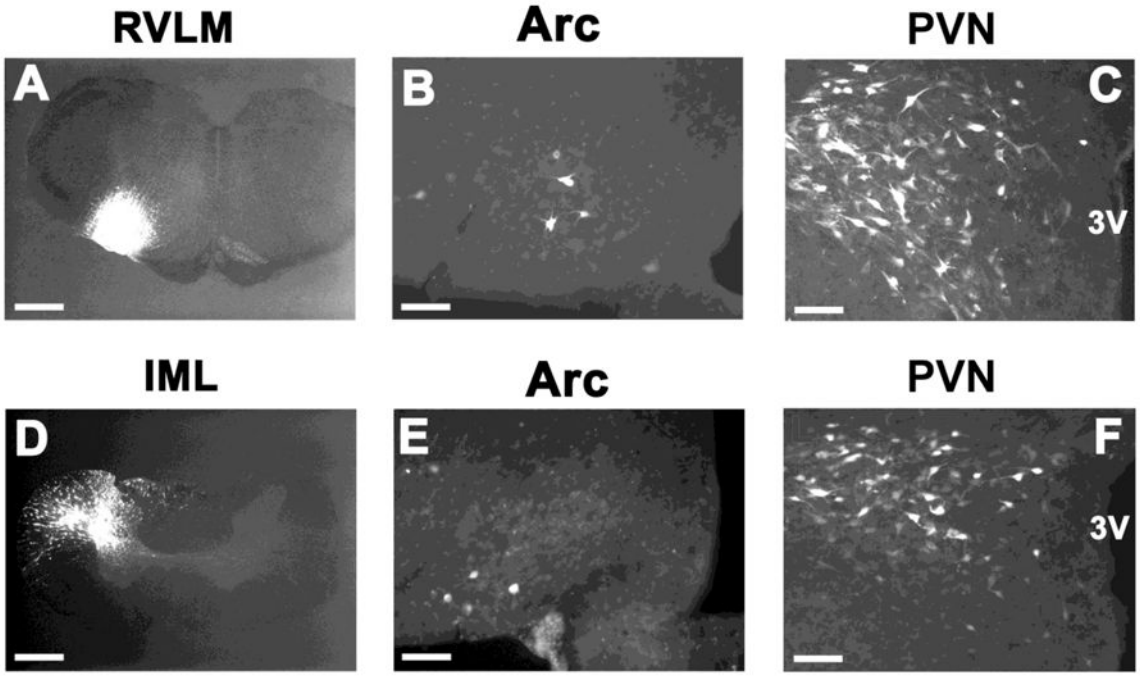


**Fig. 2.**

Projections from the Arc and PVN to the RVLM and IML.

A: Microinjection site of FG into the RVLM (bar = 1 mm). B: Few and scattered retrogradely labeled neurons in the ipsilateral Arc (bar = 100 μm). C: Robust retrograde labeling of ipsilateral PVN neurons in the same rat (bar = 100 μm). D: Microinjection site of FG into the IML (bar = 1 mm). E: Few and scattered retrogradely labeled neurons in the ipsilateral Arc (bar = 100 μm). F: Robust retrograde labeling of ipsilateral PVN neurons in the same rat (bar = 100 μm). FG: Fluoro-Gold (4% dilution, 5–10 nl volume).

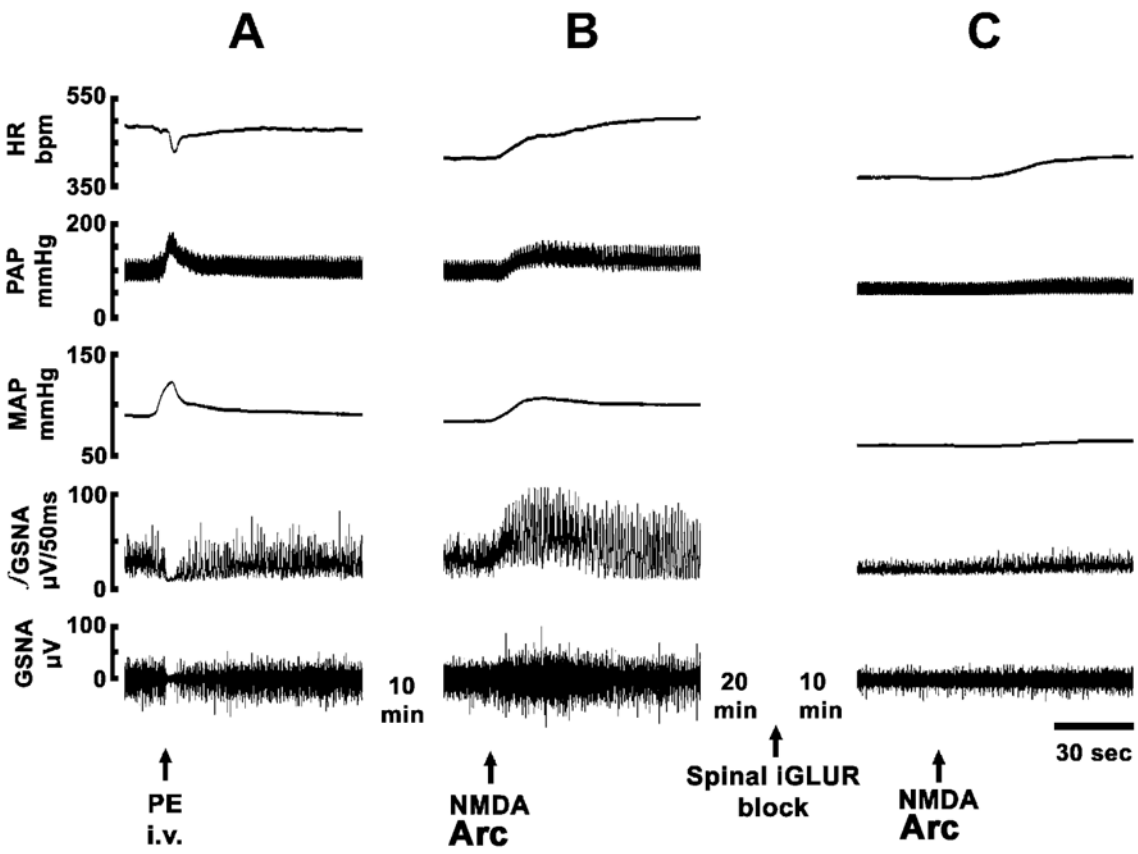


**Fig. 3.**

Increase in GSNA elicited by chemical stimulation of the Arc. Top trace: HR (bpm), 2nd trace: PAP (pulsatile arterial pressure, mmHg), 3rd trace: MAP (mmHg), 4th trace: integrated GSNA (∫GSNA, μV/50 ms), and bottom trace: whole GSNA (μV). A: The pressor response elicited by phenylephrine (PE; 10 μg/kg, i.v.) produced reflex inhibition of GSNA and bradycardia indicating barosensitivity of GSNA. B: Ten min later, microinjection of NMDA (10 mM) into the Arc increased HR, PAP, MAP, integrated GSNA and whole GSNA. C: Twenty min later, intrathecal injection (20 μl) of ionotropic glutamate receptor (iGLUR) antagonists (NBQX, 2 mM and D-AP7, 5 mM) at T9–T10 decreased the baseline HR, PAP, MAP, integrated GSNA and whole GSNA which reached a minimum within 10 min. At this time, microinjection of NMDA failed to elicit an increase in PAP, MAP, integrated GSNA and whole GSNA responses while the increase in HR was attenuated. (Reproduced from Nakamura et al., 2009, Hypertension 54, 1369–1375).

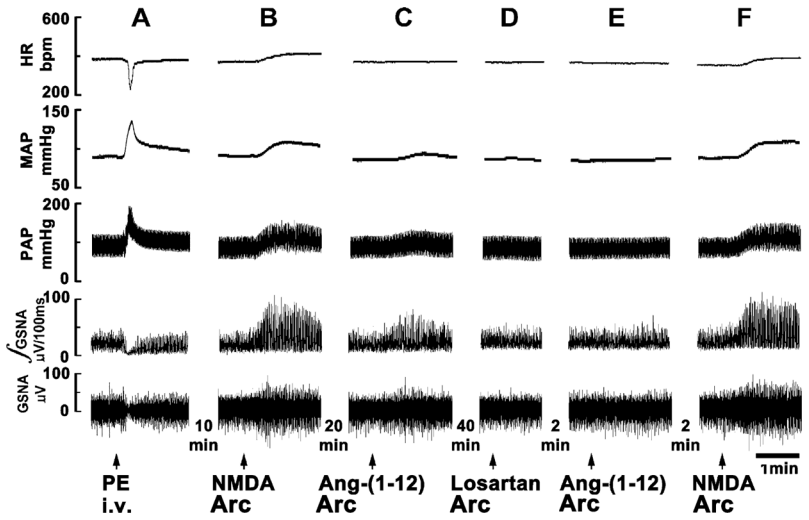


**Fig. 4.**

Cariovascular responses to Angiotensin-(1-12) in the Arc. A. Top trace: HR (bpm), 2nd trace: MAP (mmHg), 3rd trace: PAP (pulsatile arterial pressure, mmHg), 4th trace: integrated GSNA (∫GSNA, μV/100 ms), and bottom trace: whole GSNA (μV). A: PE (10 μg/kg, i.v.) elicited reflex inhibition of GSNA and bradycardia. B: After an interval of 10 min, microinjection of NMDA (10 mM) into the Arc resulted in an increase in HR, MAP, PAP, integrated GSNA and whole GSNA. C: Twenty min later, microinjection of Ang-(1-12) (1 mM) into the Arc increased the HR, MAP, PAP, integrated GSNA and whole GSNA. D: Forty min later, losartan (10 mM) was microinjected into the Arc; no response was elicited. E: Two min later, microinjection of Ang-(1-12) (1 mM) at the same site failed to elicit a response. F: Two min later, microinjection of NMDA (10 mM) continued to elicit usual cardiovascular responses. (Reproduced from Arakawa et al., 2011, Am. J. Physiol. 300, H951–H960).

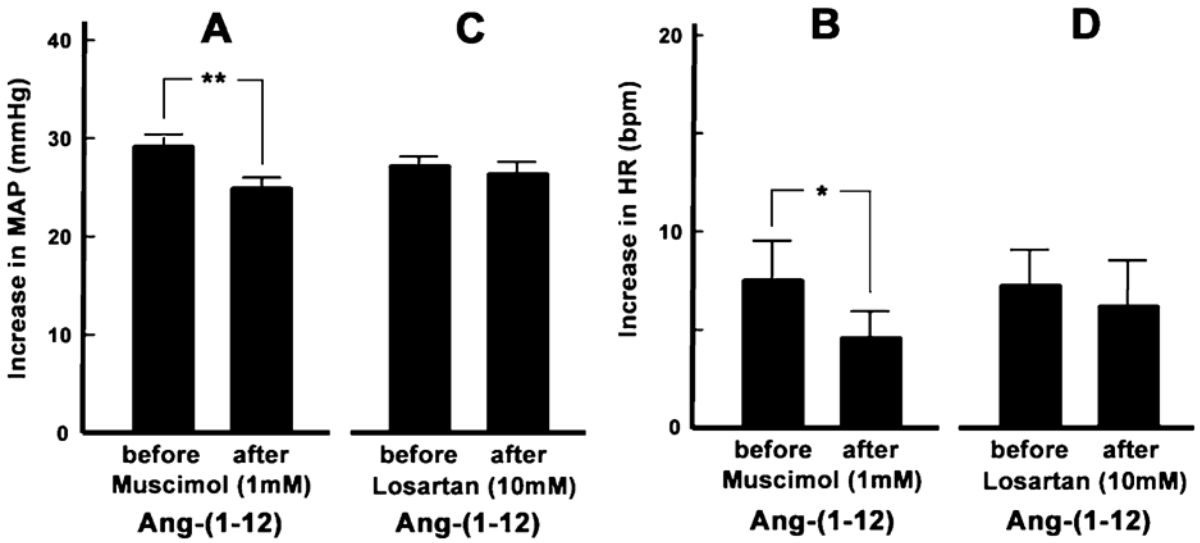


**Fig. 5.**

The Arc plays a role in mediating responses to intravenously administered Ang-(1-12). A: The increases in MAP elicited by intravenously injected Ang-(1-12) (300 pmol/kg) before and after bilateral inhibition of Arc by muscimol (1 mM) were 29.5 ± 1.2 and 25.1 ± 1.1 mmHg, respectively; the pressor response after the inhibition of Arc was significantly attenuated ($^{**}P < 0.001$) (n = 10). B: In the same group of rats, the increases in HR produced by the same injections of Ang-(1-12) before and after bilateral inhibition of Arc by muscimol were 7.5 ± 2.0 and 4.6 ± 1.3 bpm, respectively; the tachycardic response after the inhibition of Arc was significantly attenuated ($^{*}P < 0.05$). C: In a different group of rats (n = 6), the increases in MAP induced by intravenously injected Ang-(1-12) (300 pmol/kg) before and after bilateral blockade of AT1Rs in the Arc by microinjections of losartan (10 mM) were 27.0 ± 1.9 and 26.3 ± 1.0 mmHg, respectively; the pressor responses were not significantly different ($P > 0.05$). D: In the same group of rats, the increases in HR elicited by the same injections of Ang-(1-12) before and after bilateral blockade of AT1Rs in the Arc were 7.3 ± 1.8 and 6.2 ± 2.2 bpm, respectively; the tachycardic responses were not significantly different ($P > 0.05$). (Reproduced from Arakawa et al., 2011, Am. J. Physiol. 300, H951–H960).

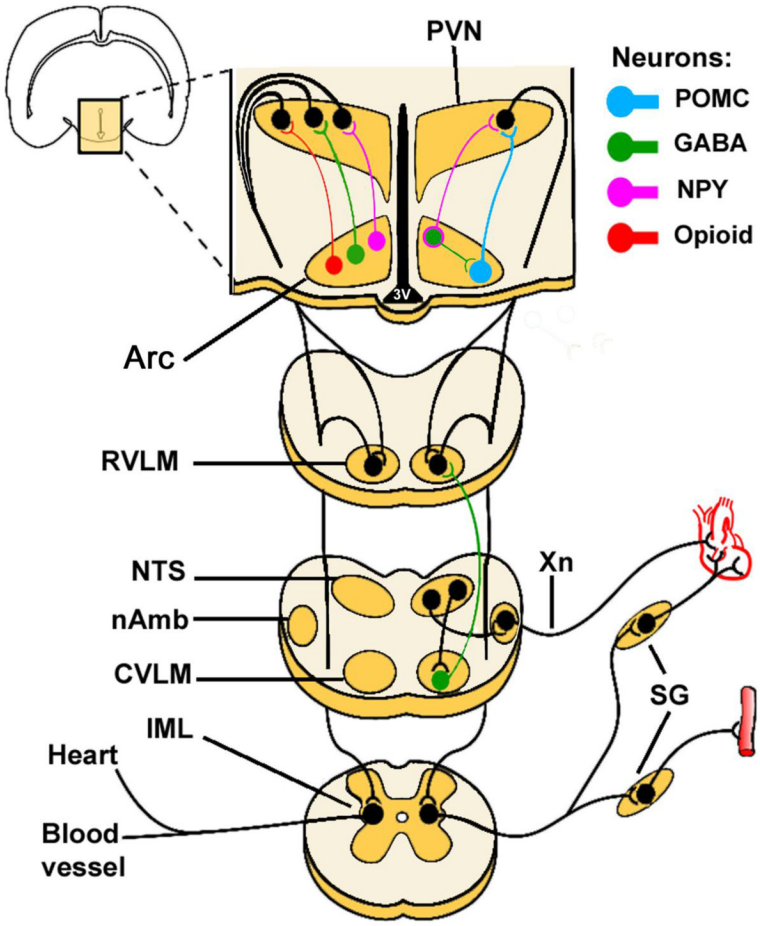


**Fig. 6.**

Pathways mediating the depressor and sympathoinhibitory responses elicited from the Arc. At normal baseline BP levels (e.g., 98–100 mmHg), microinjections of NMDA (10 mM) into the Arc resulted in decreases in BP and GSNA and increases in HR. Activation of GABA, NPY and opiate containing neurons in the Arc (shown on left side of the top panel) may result in the release of GABA, NPY and opiates in the ipsilateral PVN. GABA and NPY may be co-released by NPY/AgRP neurons located in the Arc. GABA and opiates (e,g., beta-endorphin) may be co-released by POMC neurons located in the Arc. Inhibition of PVN neurons by these neurotransmitters may cause a decrease in BP and GSNA via decrease in the activity of the projections from the PVN to the ipsilateral RVLM and IML. The pathways mediating the increases in HR are not known, although inhibition of vagal input to the heart is involved in this response. Unilateral blockade of $GABA_A$Rs in the Arc results in an increase in BP and HR indicating that excitatory neurons (probably POMC neurons) in the Arc are under tonic GABAergic inhibition (shown on the right side in the top panel). All the circuits shown in the diagram are located bilaterally but are shown on one side only for clarity. Arc: hypothalamic arcuate nucleus; CVLM: caudal ventrolateral medullary depressor area; GABA: gamma aminobutyric acid; IML: intermediolateral cell column; nAmb: nucleus ambiguus; NPY: neuropeptide Y; NTS: nucleus tractus solitarius; POMC: proopiomelanocortin; PVN: hypothalamic paraventricular nucleus; RVLM: rostral ventrolateral medullary pressor area; SG: sympathetic ganglion; Xn: tenth nerve (vagus); 3V: third ventricle. (Diagram adapted from Sapru, 2004).