Mid-Career Award for Research Excellence

Cardiovascular Regulation by the Arcuate Nucleus of the Hypothalamus
Neurocircuitry and Signaling Systems

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The importance of the central nervous system in cardiovascular regulation is well established. The brain is able to sense changes that occur in the peripheral circulation through several mechanisms, including afferent neural reflexes and humoral signals. The brain in turn can adjust various components of the cardiovascular system to maintain homeostasis by modulating the release of critical hormonal factors, chemical messengers, and neurotransmitters in concert with changing the activity of the sympathetic and parasympathetic branches of the autonomic nervous system subserving different organs and tissues throughout the body.

It has long been recognized that brain regulation of the cardiovascular system involves complex networks of neurons located in the brain stem, midbrain, and forebrain. Among the various brain structures involved in circulatory regulation, the hypothalamus plays a central role by integrating the information conveyed via various signal inputs with the neural networks involved to determine and coordinate the appropriate responses to maintain homeostasis. Much of the investigation of the cardiovascular regulation by the hypothalamus has focused on the paraventricular nucleus. Such reciprocal relationship is thought to represent a window for the ARC neurons to monitor changes in circulating factors and hormonal signals, particularly with regard to the control of energy homeostasis.

ARC neurons project directly to multiple areas of the hypothalamus, but with variable intensity. The highest fiber density from the ARC terminates in selected nuclei, such as the paraventricular nucleus, the dorsomedial hypothalamus, and the lateral hypothalamus (Figure 1). Moreover, anatomic and electrophysiological studies have demonstrated that fibers from the ARC have widespread projections to many extrahypothalamic areas, including those involved in cardiovascular regulation, such as the subfornical organ, the nucleus tractus solitarii, the parabrachial nucleus, the dorsal raphe nucleus, the locus coeruleus, the nucleus ambiguus, and the rostral ventrolateral medulla. Direct projections from the ARC to the sympathetic preganglionic neurons at the level of intermediolateral column of the thoracic spinal cord have also been documented. In addition to sending projections to different brain nuclei, the ARC neurons receive synaptic inputs from various brain regions, especially the hypothalamic nuclei, such as the posterior hypothalamic, ventromedial hypothalamus, and paraventricular nucleus. Such reciprocal relationship may enable the ARC to integrate neuronal signals with information conveyed by circulating hormones and other factors.

Retrograde tracing studies have linked the ARC to key cardiovascular organs. For instance, pseudorabies viruses injected into the kidney can be detected in the ARC with the infection occurring at later stages after the inoculation. Virally infected neurons in the ARC were also reported to appear late after pseudorabies inoculation of the left ventricular myocardium or hindlimb skeletal muscle. The late appearance of the infections in the ARC combined with the temporal distribution of viral labeling throughout the central nervous system after inoculation of the kidney, heart, or muscle is consistent with neurocircuities involving multiple synaptic connections, with the ARC being 3 or forth orders removed from these tissues.

Additional evidence linking the kidney to the ARC derives from studies that interfered with the activity of the anterior pituitary gland. In addition, presence of the arcuate nuclei of the ARC of tanycytes, special polarized ependymogliaial cells, that project to the fenestrated capillaries of the portal vessels in the palisade zone of the median eminence is thought to represent a window for the ARC neurons to monitor changes in circulating factors and hormonal signals, particularly with regard to the control of energy homeostasis.
renal nerves. Electric stimulation of the afferent renal nerves increased ARC neuronal activity as indicated by induction of Fos expression. Interestingly, assessment of hexokinase activity revealed altered metabolic activity in the ARC after renal denervation, but not after aortic baroreceptor denervation. These studies raise the possibility that the ARC is among the brain structures that receive the information carried by the afferent nerves. It is interesting to note that prolonged decrease in arterial pressure induced by sodium nitroprusside was found to cause a strong increase in Fos-positive neurons in the ARC along with other brain cardio regulatory nuclei. Alterations in blood volume also evoked robust Fos induction in the ARC, and chronic water deprivation affected gene expression and protein synthesis in the ARC. Furthermore, ARC neurons were found to be sensitive to hypertonic saline given systemically or centrally, and lesioning the ARC caused chronic hypovolemia and exaggerated hypernatremia during water deprivation. Altogether, these findings implicate the ARC in the compensatory responses to changes in arterial pressure and balance of body fluid and electrolytes.

Neuronal Populations of the ARC

The ARC contains several populations of neurons that are defined by virtue of the transmitters or neuropeptides they express and their function. Neuroendocrine neurons of the ARC have long been the focus of neuroendocrinologists because of their proven influence on the anterior pituitary gland hormone secretion. These ARC neuroendocrine neurons include those expressing dopamine, which regulate secretion of prolactin and gonadotropin-releasing hormone and those making growth hormone–releasing hormone that control the secretion of growth hormone. More recently, ARC neurons expressing kisspeptins have emerged as potent stimulators of the gonadotropin axis, with important implications for the ARC in the compensatory responses to changes in arterial pressure and balance of body fluid and electrolytes.

as one of its major targets brought this nucleus to the front stage of the neural control of energy homeostasis. The ARC was recognized as the site of first-order neurons in the neural circuits regulating metabolism. The information about the nutritional status of the organism received by these neurons from various sources is conveyed to different regions of the central nervous system.

The anorexigenic proopiomelanocortin (POMC) neurons and orexigenic Agouti-related protein (AgRP) neurons of the ARC have been studied extensively in the context of central control of food intake and energy expenditure. POMC is a large protein that does not seem to have any biological activity, but its breakdown produces β-endorphins and melanocortins that have various functions as hormones and neuropeptides. The melanocortin peptides consist of α-, β-, and γ-melanocyte-stimulating hormone and adrenal corticotrophin hormone. α-Melanocyte-stimulating hormone is a potent stimulator of the melanocortin-3 and melanocortin-4 receptors located predominantly in the second-order neurons. On the other hand, AgRP neurons produce the strong melanocortin-3 and melanocortin-4 receptors antagonist AgRP. Thus, POMC and AgRP neurons are considered a critical component of the melanocortin system.

It should be noted, however, that the relationship between POMC and AgRP neurons is more complex than a simple modulation of melanocortin-3 and melanocortin-4 receptors located in second-order neurons. This is because of the ability of AgRP neurons to directly influence the activity of POMC neurons. In addition, ARC AgRP and POMC neurons are able to modulate the activity of second-order neurons beyond the release of AgRP and α-melanocyte-stimulating hormone, respectively. This divergent capacity of ARC neurons is because of the fact that often they contain >1 biologically active molecule. Indeed, early studies have shown colocalization in the ARC of dopamine and neurotensin and sparse coexistence of dopamine and the inhibitory neurotransmitter γ-aminobutyric acid (GABA) and neuropeptide Y with somatostatin. We also know that the majority of POMC neurons express cocaine- and amphetamine-regulating transcript,
whereas a subset of these neurons produces GABA or the neurotransmitter acetylcholine. On the other hand, most AgRP neurons contain neuropeptide Y and GABA. This divergent signaling capacity allows ARC neurons to engage different transmitters for neuronal communication to control specific functions. For instance, AgRP neurons engage mainly GABA signaling to control nearby POMC neurons.27 In addition, loss of GABAergic transmission, but not AgRP or neuropeptide Y, was shown to underlie the dramatic starvation phenotype caused by ablation of ARC AgRP neurons in mice.30 This opens up the interesting possibility of targeting selective neurotransmitters in disease conditions implicating ARC neurons.

Recently, additional ARC neuronal populations expressing insulin-2 promoter,11 prodynorphin,12 or amylin precursor, amyloid polypeptide,33 have been identified as targets of leptin and implicated in the regulation of energy homeostasis, but the underlying molecular and cellular processes involved remain largely unknown. Other subsets of ARC neurons whose function is still unclear include those that produce ghrelin44 and those sensing glucose or lipids.35 Future studies are needed to determine the exact identity of these newly identified ARC neurons and how distinct they are from other well-defined cell types residing in this nucleus, such as those expressing dopamine, kisspeptins, POMC, or AgRP.

**Significance of the ARC in Cardiovascular Regulation**

An involvement of the ARC in cardiovascular regulation has long been suspected based on the anatomic connections between this nucleus and many brain structures influencing the cardiovascular system discussed above. This was further supported by early studies describing the hemodynamic and renal effects evoked by the melanocortins (reviewed in Gruber and Callahan38 and Versteeg et al40). However, the first direct evidence implicating the ARC in blood pressure control derives from the work of Brody et al.39 demonstrating that direct electrical stimulation of the ARC evoked a frequency-dependent increase in arterial pressure and in renal, mesenteric, and hindquarter vascular resistance in anesthetized and conscious rats. Chemical stimulation of the ARC by microinjection of the excitotoxin kainic acid, which activates glutamate receptors, also produced significant pressor and regional vasoconstrictor effects. These pressor and vasoconstrictor responses evoked by electric or chemical stimulation of the ARC were substantially enhanced after removal of the baroreflex by sinoaortic denervation and were abolished after vasopressin receptor blockade. A decrease in heart rate that was observed only in the conscious rats that had their ARC electrically stimulated was eliminated after sinoaortic denervation.38

Subsequent reports have shown that electric stimulation of the ARC elicits a biphasic change in arterial pressure, depressor response followed by a pressor response, and bradycardia.39,40 The reasons behind this biphasic arterial pressure response is not clear, but may involve different parts of the ARC with the depressor and bradycardic components postulated to originate from the caudal ARC and its projections to the dorsal motor nucleus of the vagus causing parasympathetic activation.17,40 Alternatively, this could be because of the stimulation of both excitatory (eg, POMC neurons) and inhibitory (eg, AgRP neurons) cells of the ARC.

In addition to the hemodynamic responses, it is established that broad stimulation or inhibition of the ARC alters sympathetic nerve activity (SNA). Studies from the Sapru laboratory have shown that when baseline arterial pressure is low, the pressor response and bradycardia evoked by nonspecific activation of the ARC with N-methyl-d-aspartic acid was associated with an increase in renal and splanchnic SNA in anesthetized rats.41 However, in subsequent studies, this group found that at normal baseline values of arterial pressure, ARC stimulation with N-methyl-d-aspartic acid led to sympathetic inhibition and a depressor response (reviewed in Sapru42). Baroreceptor unloading was proposed as a potential explanation for these contrasting changes in arterial pressure and SNA after ARC stimulation with N-methyl-d-aspartic acid.3

Cardiovascular and sympathetic responses were also elicited by pharmacological modulation of GABAergic signaling in the ARC. Administration of a GABA_A receptor antagonist, gabazine, into the ARC of rats increased arterial pressure, heart rate, and splanchnic SNA.3 Conversely, inhibition of the ARC with GABA_A receptor agonist, muscimol, was recently reported to decrease regional SNA, arterial pressure, and heart rate in rats.42 Taken together, the studies discussed above highlighted the fundamental significance of the ARC in cardiovascular regulation. This is fostered by the substantial evidence accumulated over the last several years, demonstrating that a vast number of peripheral signals affect the cardiovascular function through action in the ARC.

**Hemodynamic and Sympathetic Effects of Leptin Signaling in the ARC**

The documentation of the ability of leptin to increase arterial pressure and SNA, and its role in coupling obesity with hypertension and sympathetic overdrive, has led to intense effort to understand its mechanism of action. Our interest in the role of the ARC in mediating the sympathetic and cardiovascular effects of leptin was derived from the well-documented high density in this nucleus of the long signaling form of the leptin receptor (LepRb).43 This receptor belongs to the tyrosine kinase family of receptors and has a long intracellular domain able to activate several intracellular signaling pathways (Figure 2).

We reasoned that if the cardiovascular actions of leptin emanate from the ventromedial and dorsomedial nuclei as reported by Marsh et al,44 then injection of leptin into the ARC should spare the renal SNA and arterial pressure. On the other hand and given the predominant role of the ARC in underlying the metabolic actions of leptin,45 we postulated that this nucleus may underlie the SNA subserving thermogenic brown adipose (BAT). To test this, we combined site-specific micro-injection of leptin into the ARC with simultaneous recording of SNA subserving the kidney and BAT, as well as measurement of hemodynamic parameters in anesthetized rats.45 In contrast to our expectation, we observed that micro-injection of leptin (0.5 μg/0.5 μL) into the ARC increased sympathetic nerve outflow to both the kidney and the thermogenic BAT. This was associated with arterial pressure elevation. Montanaro et al46 reported that microinjection of
leptin (0.1 μg/0.1 nL) into the ARC caused lumbar sympathetic nerve activation and arterial pressure increase, although these responses displayed large variabilities that could be caused by the relatively low dose of leptin or the erraticism of the injection sites within the ARC. Interestingly, our data indicated that the sympathetic and pressor responses induced by ARC microinjection of leptin (0.5 μg) were qualitatively and quantitatively comparable to the responses obtained after cerebroventricular administration (10 μg) of this hormone. Thus, leptin action in the ARC is sufficient to cause regional sympathetic activation and pressor response. More recently, we found that leptin signaling in the ARC increases parasympathetic outflow to the liver, suggesting that ARC leptin regulates both arms of the autonomic nervous system. However, the cardiovascular consequences of ARC leptin–induced increase in the parasympathetic outflow are not clear.

To address the necessity of the ARC for the sympathetic and pressor actions of leptin, we examined the effect of selective deletion of the LepR from the ARC. This was achieved by ARC-restricted delivery of an adenovirus-expressing Cre recombinase (Ad-Cre) to mice expressing floxed alleles of the LepR (LepRflox/flox). The selectivity and efficiency of this strategy was verified through the demonstration of strong suppression of LepR expression and signaling capacity (using Stat3 as read out) in the ARC, but not in the adjacent nuclei, after Ad-Cre microinjection in LepRflox/flox mice. Notably, deleting leptin signaling in the ARC completely abolished leptin-induced sympathetic activation to both the kidney and BAT, whereas Ad-Cre microinjections that missed the ARC failed to alter the regional SNA responses evoked by leptin. These findings demonstrate that LepRs in the hypothalamic ARC are necessary for the renal and BAT sympathoexcitatory effects of leptin.

POMC neurons have emerged as the likely mediator of the cardiovascular effects of leptin action in the ARC. This is based on the work of John Hall and colleagues, demonstrating that selective deletion of the LepR from POMC neurons abolished the ability of leptin to increase arterial pressure. This is further supported by the demonstration that genetic ablation from POMC neurons of different components of the LepRb signaling pathways, such as Stat3, interferes with the pressor effects evoked by leptin. Implications of POMC neurons as first-order neurons in mediating the action of leptin on blood pressure is consistent with the requirement of downstream melanocortin-4 receptor and melanocortinergic drive from ARC to the paraventricular nucleus for the sympathetic and pressor effects of leptin. Neuropeptide Y signaling and glutamatergic transmission in the paraventricular nucleus have also been implicated in the sympathoexcitatory and pressor actions of leptin.

Insulin Acts in the ARC to Increase Sympathetic Outflow

Insulin action in the brain has been implicated in the regulation of various physiological processes. Pancreatic-derived insulin reaches the brain through a specific mechanism that involves active and saturable transport through the blood–brain barrier. Converging evidence from animal and human studies have shown that insulin is an important determinant of the activity of the sympathetic nervous system. Moreover and in line with the broad spectrum of physiological effects evoked by brain action of insulin, cerebroventricular administration of this hormone causes sympathetic nerve activation...
to numerous beds, including the hindlimb, BAT, kidney, and adrenal gland.\textsuperscript{56,57} In addition, central action of insulin increases baroreflex control of both heart rate and lumbar SNA\textsuperscript{58} and causes a modest rise in arterial pressure.\textsuperscript{57}

The earlier finding that lesioning the anteroventral third ventricle eliminated the ability of insulin to induce sympathetic nerve activation implicated a key role for the hypothalamus in the sympathoexcitatory effects of this hormone.\textsuperscript{59} This was further substantiated by the elimination of insulin-induced sympathetic nerve activation after inhibition of insulin receptor signaling pathways in the hypothalamus\textsuperscript{57} and the capacity of insulin to increase SNA when administered into the lateral ventricle, but not the fourth ventricle.\textsuperscript{58}

More recent studies have identified the ARC as the main site that mediates the sympathetic responses evoked by insulin.\textsuperscript{60,61} Cassaglia et al\textsuperscript{60} demonstrated that muscimol-mediated inhibition of the ARC or paraventricular nucleus completely reversed the lumbar sympathetic activation as well as the increase in the gain of the baroreflex control of lumbar SNA caused by peripheral hyperinsulinemia. On the other hand, these authors found that direct administration of insulin into the ARC, but not the paraventricular nucleus or dorsomedial hypothalamus, yielded a dose-dependent increase in lumbar SNA and baroreflex sensitivity. Together, these findings point to the sufficiency of insulin action in the ARC to increase SNA and baroreflex sensitivity through a neural network that involves the paraventricular nucleus.

Luckett et al\textsuperscript{61} used a different strategy taking advantage of the availability of a small molecule anti-insulin affibody to implicate the ARC in the sympathoexcitatory effects of insulin. They demonstrated that pretreatment with the anti-insulin affibody into the ARC interfered with the lumbar sympathetic activation induced by insulin when administered directly into the ARC, intracerebroventricularly, or systemically. Interestingly, microinjection of the anti-insulin affibody into the ventromedial hypothalamus did not impede the lumbar sympathetic activation to insulin. The rise in arterial pressure after systemic insulin was also eliminated when the anti-insulin affibody was microinjected into the ARC, but not into the ventromedial hypothalamus. Notably, pretreatment with the ARC anti-insulin affibody failed to interfere with the pressor and regional sympathetic responses evoked by gabazine, indicating that the effects observed with insulin are specific. Thus, insulin signaling in the ARC is necessary to affect sympathetic traffic and arterial pressure.

The insulin receptor is a member of the tyrosine kinase family consisting of 2 extracellular α- and 2 transmembrane β-subunits. Notably, although it is present throughout the central nervous system, the hypothalamus contains the highest density of the insulin receptor. This was demonstrated by in situ hybridization for insulin receptor messenger,\textsuperscript{13} binding assay with isotope-labeling insulin,\textsuperscript{11,12} and immunohistochemical labeling of the β-\textsuperscript{10} or α-subunit\textsuperscript{62} of the insulin receptor. Notably, among the hypothalamic nuclei, the ARC displayed the highest expression of the insulin receptor.\textsuperscript{62} Within the ARC, POMC and AgRP neurons are known to be equipped with the insulin receptor.\textsuperscript{63} These neurons may provide the melanocortinergic drive and glutamatergic neurotransmission to the paraventricular nucleus that have been implicated in mediating the sympathoexcitatory effects of insulin.\textsuperscript{64,65}

**Novel Mechanisms Underlying Cardiovascular Regulation by the ARC**

The mechanistic target of rapamycin (mTOR) is an evolutionary conserved protein that belongs to the phosphatidylinositol kinase–related kinase family.\textsuperscript{66} mTOR is critically involved in the regulation of several cellular functions, including gene transcription, protein synthesis, cell growth and proliferation, ribosome and mitochondria biogenesis, cytoskeleton organization, and autophagy.\textsuperscript{67–69} mTOR activity is modulated by various cues arising from growth factors, hormones, stress, nutrients, and energy status. mTOR functions largely as the catalytic subunit of large protein kinase complexes.\textsuperscript{56,60} mTOR interaction with 5 other proteins leads to the formation of a functionally distinct large complex, termed mTOR complex 1 (mTORC1, Figure 2). Well-characterized downstream targets of mTORC1 are 70S ribosomal protein S6 kinase and its downstream effector, the ribosomal protein S6.\textsuperscript{66}

Recently, we demonstrated that hypothalamic mTORC1 signaling is involved in the control of the sympathetic nerve drive and arterial pressure.\textsuperscript{70} We took advantage of the unique ability of leucine to activate mTORC1 signaling to show that leucine-mediated stimulation of mTORC1 signaling in the mediobasal hypothalamus increased arterial pressure and SNA subserving the kidney in rats.\textsuperscript{70} This was associated with a parallel, but uneven, decrease in blood flows recorded from the aortic, iliac, mesenteric, and renal arteries. Importantly, rapamycin-mediated hypothalamic mTORC1 inhibition abolished the increase in renal SNA and arterial pressure as well as the vasoconstriction induced by leucine.\textsuperscript{70} These sympathetic and cardiovascular responses evoked by mTORC1 seem to emanate from the ARC because inhibition of mTORC1 signaling selectively in this nucleus with an adenovirus expressing a dominant-negative S6 kinase decreased renal SNA and arterial pressure.\textsuperscript{70} Conversely, ARC-specific activation of mTORC1 signaling with an adenovirus expressing a constitutively active S6 kinase increases renal SNA and arterial pressure (K. Rahmouni, unpublished data). Collectively, these findings establish mTORC1 signaling in the ARC as a critical regulator of SNA and arterial pressure.

ARC mTORC1 has also emerged as a key downstream pathway mediating the regulation of sympathetic nerve traffic by leptin and insulin. Both leptin and insulin are able to stimulate mTORC1 signaling in the ARC through phosphatidylinositol-3-kinase (Figure 2). Blockade of ARC mTORC1 virtually eliminated the increase in renal SNA and arterial pressure caused by leptin.\textsuperscript{70} On the other hand, insulin requires ARC mTORC1 signaling to stimulate lumbar SNA.\textsuperscript{62} Thus, leptin and insulin engage selectively mTORC1 signaling in the ARC to cause sympathetic nerve activation to the kidney and hindlimb, respectively. However, the cellular and neuronal pathways implicated in ARC mTORC1 control of cardiovascular sympathetic function remain to be determined.

**Conclusions and Perspectives**

The ARC plays a prominent role in coordinating the regulation of multiple physiological functions to maintain homeostasis. Evidence discussed above demonstrates that this nucleus is an integral part of the neural network governing cardiovascular function. Hemodynamic and sympathetic changes can be
evoked from ARC, and neurons in this nucleus seem to play a critical role in sensing and relying signals emanating from the circulation to neuroendocrine and autonomic systems to mediate cardiovascular responses. The ARC neurons also receive neuronal signals from many brain regions and perhaps from peripheral organs, including those involved in cardiovascular control. Human studies implicating the melanocortin system in blood pressure regulation and obesity-associated hypertension and sympathetic overdrive\(^ {21,22}\) highlight the relevance of the ARC for cardiovascular health in humans. These findings also emphasize the need for studies in human subjects to translate laboratory discoveries regarding the cardiovascular regulation by the ARC to clinical applications.

Presence in the ARC of several components of the renin–angiotensin system—including angiotensinogen, renin, angiotensin-converting enzyme, and angiotensin type 1 receptor—points to a possible contribution to arterial pressure homeostasis of angiotensin II produced locally in the ARC.\(^ {73-75}\) This is further supported by the increase in arterial pressure and heart rate after direct ARC application of angiotensin II.\(^ {76}\)

Assessing the significance of the ARC renin–angiotensin system may provide clues about the role of this brain nucleus in cardiovascular health and disease, especially in disorders that compromise the integrity of the blood–brain barrier rendering the ARC accessible to circulating angiotensin II.\(^ {76}\)

Many of the details of the molecular and cellular processes, as well as the downstream neuronal pathways by which the ARC regulates the cardiovascular system, are emerging. Investigations of these processes and pathways have been hindered by the high degree of overlap in the mechanisms and networks underlying ARC control of various physiological processes. Dissection of the neural network emanating from the ARC that influences the cardiovascular system is central to our understanding of the role of the ARC in the cardiovascular complications of disease states, such as obesity.\(^ {49,77}\)

Finally, the contribution of ARC cells other than neurons such as microglia\(^ {39}\) to cardiovascular regulation remain largely untapped. Obtaining insights into the molecular and cellular mechanisms underlying cardiovascular regulation by the ARC will enhance our understanding how the brain controls circulation.

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