The C1 Area of the Brainstem in Tonic and Reflex Control of Blood Pressure

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SUMMARY Recent studies have demonstrated that the neurons of the lower brainstem that are responsible for maintaining normal levels of arterial pressure reside in a specific area of the rostral ventrolateral medulla. In rat, the critical zone corresponds to a small region containing a subpopulation of the adrenergic C1 group, defined immunocytochemically by the presence of the epinephrine-synthesizing enzyme phenylethanolamine N-methyltransferase. Neurons of this region (the C1 area), possibly including the adrenergic neurons, directly innervate preganglionic neurons in the spinal cord, and are tonically active and sympathoexcitatory. The excitatory transmitter released into the spinal cord is unknown. The discharge of C1 area neurons is locked to the cardiac cycle and, in turn, leads to firing of sympathetic preganglionic neurons. The C1 area neurons are inhibited by baroreceptor input and mediate the vascular component of baroreceptor reflexes. They also mediate somatosympathetic pressor responses from skin and muscle and participate in reflex responses to hypoxia. The neurons are directly innervated by local neurons containing γ-aminobutyric acid, acetylcholine, enkephalin, and substance P, all of which modulate arterial pressure. The C1 area is the site of the hypotensive actions of clonidine. Clonidine appears to act on imidazole receptors in the C1 area to lower arterial pressure. The natural ligand for these receptors may be a newly defined substance in brain, clonidine-displacing substance. Neurons of the C1 area appear to be the critical neuronal group governing the normal resting and reflex control of arterial pressure. They may play a critical role in the maintenance of elevated arterial pressure in hypertension and as a site of action of antihypertensive drugs. (Hypertension 11 [Suppl 1]: I-8–I-13, 1988)

KEY WORDS • C1 area • blood pressure • baroreceptor reflexes • clonidine • imidazole receptors

ONE of the classic problems of autonomic physiology has been the identity of those neurons of the brainstem responsible for maintaining the discharge of spinal preganglionic neurons and, hence, establishing normal resting levels of arterial pressure (AP). While it has been recognized for over 100 years that the medulla oblongata is essential for such control — since removal of the brain above the medulla has no effect on resting AP but transection at the spinomedullary junction results in its collapse — the identity of the neural group(s) responsible has long eluded detection. Indeed, the failure of investigators to replicate, by localized lesions within the medulla, the effects of spinal cord transection upon AP led to a view that the essential neurons, rather than being concentrated in a single area, were distributed throughout the brainstem.

Over the past several years, two principal lines of evidence have demonstrated that a restricted area of the medulla oblongata, specifically, a zone lying in the rostral ventrolateral quadrant (RVL), functions as a tonic vasomotor center. First, the demonstration that the application of inhibitory neurotransmitters to a restricted zone on the ventral medullary surface underlying the RVL could lower AP to spinal levels indicated the presence within that region of synapses critical for pressure control. The second was the finding that electrolytic damage to the region of the RVL not only abolished the cerebral ischemic reflex but also dropped AP to spinal levels. Supporting a role for RVL in tonic vasomotor control were observations that neurons within the area project to the intermediolateral nucleus of thoracic spinal cord, the site of origin of preganglionic sympathetic neurons.

We proposed that the overlap between the location of the cardiovascular centers in the RVL and the
distribution of a subpopulation of catecholamine-containing neurons of the so-called adrenergic C1 group suggested a functional role for C1 neurons in cardiovascular control. The C1 group was first identified by Hokfelt et al., who demonstrated that a rostral component of the noradrenergic A1 group could be distinguished by the presence of the epinephrine-synthesizing enzyme phenylethanolamine N-methyltransferase (PNMT). In this paper we review the evidence implicating neurons of the rostral C1 area, conceivably the C1 adrenergic neurons themselves, in the resting and reflex control of AP.

Anatomical Considerations

The C1 area of the RVL in the rat and cat is localized within a zone lying caudal to the nucleus of the seventh nerve and rostral to most precerebellar relay neurons of the lateral reticular nucleus. It lies directly ventral to the retrofacial nucleus and is in close proximity to the ventral surface of the medulla upon which some processes of C1 cells terminate. The C1 area extends rostrocaudally as a cell column, representing a subdivision of the nucleus paragigantocellularis lateralis.

The rostral portion of the C1 area contains neurons, including those immunoreactive for PNMT, which project to the spinal cord. The C1 area is also characterized by a heavy terminal field derived from the nucleus tractus solitarii (NTS). Admixed within the C1 area are local neurons, some of which can be characterized immunocytochemically as containing γ-aminobutyric acid (GABA), or being cholinergic or enkephalinergic. Neuropeptide Y is colocalized with PNMT in some C1 neurons, while substance P, also found in the region, is only colocalized within a few PNMT-containing cells.

Ultrastructurally, the adrenergic neurons of the C1 group have several distinguishing features. They contain abundant mitochondria and not only are in proximity to capillaries but, in fact, often surround them. These features make these neurons ideal candidates as oxygen sensors, a function suggested by the importance of cells in this region in mediating the cerebral ischemic response and cerebral vasodilation in response to hypoxia.

Axons of the adrenergic neurons of the C1 area ascend dorsally through the medulla, contributing to the ascending and descending limbs of a major fiber tract, the principal tegmental adrenergic bundle (Figure 1). Local processes also project dorsally into the NTS, medially onto serotoninergic neurons of the raphe, and ventrally onto the subpial surface. It is the descending limb that appears to be of major importance in cardiovascular regulation, since these fibers project directly to the thoracic and lumbar spinal cord.

![Figure 1. Locations of the most active medullary pressor regions compared to locations of C1 adrenergic cells and fibers. Left side of each section: C1 neurons (solid circles) and fibers (lines) labeled with phenylethanolamine N-methyltransferase (PNMT). Right side of each section: pressor responses to electrical stimulation with 25 μA (100 Hz, 0.5 msec, 10-sec train). Responses between 30 and 50 mm Hg are indicated by small solid circles and those greater than 50 mm Hg are indicated by large solid circles. Responses less than 30 mm Hg or depressor responses are indicated by small dots. The location of the pressor region in the ventrolateral medulla corresponds to the location of the C1 neurons, and the location of the pressor region in the dorsomedial medulla corresponds to the location of the PNMT-labeled fiber bundle. CST = corticospinal tract; DCN = dorsal cochlear nucleus; ECN = external cuneate nucleus; ICP = inferior cerebellar peduncle; IO = inferior olive; IVN = inferior vestibular nucleus; LRN = lateral reticular nucleus; MLF = medial longitudinal fasciculus; MVN = medial vestibular nucleus; NC = nucleus cuneatus, NG = nucleus gracilis; NTS = nucleus tractus solitarii; NTSr = nucleus tractus solitarii pars rostral; PP = nucleus prepositus; RM = raphe magnus; STN = spinal trigeminal nucleus; STT = spinal trigeminal tract; TS = tractus solitarius; VII = facial nucleus; X = dorsal motor nucleus of vagus; XII = hypoglossal nucleus. (Reprinted from Ross et al. with permission of the Society for Neuroscience.)](http://hyper.ahajournals.org/)
where they exclusively innervate neurons within autonomic centers of the intermediolateral and intermediomedial columns bilaterally. The spinal projections of medullary adrenergic neurons consist of both myelinated and unmyelinated fibers.

Functions of C1 Neurons

Neurons of C1 Area Are Tonically Active and Sympathoexcitatory

That neurons in the C1 area are sympathoexcitatory has been demonstrated by the finding that chemical stimulation in the RVL excites sympathetic preganglionic neurons, elevates AP, accelerates the heart, and releases vasopressin and adrenal catecholamines (see Figure 1). Indeed, there is an extraordinarily close anatomical correlation between the sites in the medulla from which elevations of AP can be produced and the cytochemically demonstrated distribution of cells and fibers of the C1 group. In addition, the finding that electrolytic lesions or chemical interference with the activity of neurons in the C1 area results in a collapse of AP to that produced by spinal cord transection suggests that RVL sympathoexcitatory neurons are tonically active.

Electrophysiology

Recent electrophysiological studies of the behavior of neurons in the C1 area of the rat and corresponding areas in the cat have provided some insights as to why the cardiovascular neurons of the C1 area are so potent in regulating AP. We observed that neurons in the RVL, antidromically driven from the intermediolateral columns of the spinal cord, are tonically active and are inhibited by baroreceptor stimulation. That these cardiovascular neurons are contained exclusively within the C1 area has been demonstrated by marking their recording sites in sections stained for PNMT. Correlation analysis revealed that the discharges of reticulospinal neurons in the C1 area were synchronized to the peak excitations in the tonic discharge of the splanchnic sympathetic nerve both in the presence and absence of the baroreceptor input.

Thus, current evidence suggests that excitation of neurons in the C1 area that innervate the intermediolateral column determines the firing of large groups of sympathetic preganglionic neurons. Moreover, since C1 area vasomotor neurons are recorded at random, the finding that the discharges of each of them are synchronized with the activity of sympathetic nerves indicates that a large number discharge simultaneously. This implies that the relatively massive coincident discharge of C1 neurons results in widespread, potent, excitatory input to preganglionic sympathetic neurons. Withdrawal of this spinal input by lesions of the C1 area or by interruption of its spinal projection decreases the excitability of preganglionic neurons, resulting in the silencing of preganglionic discharge and collapse of AP. The extent to which other brain regions can compensate for the loss of this excitatory drive to preganglionic neurons and whether the tonic activity of RVL spinal sympathoexcitatory neurons is generated by neural circuits intrinsic to the RVL or imposed on these cells by afferent projections remain to be determined.

Role in Reflex Control of the Circulation

Not only do neurons of the C1 area participate in the tonic regulation of AP, but they are also critical in reflex circulatory control.

Arterial Baroreceptor Reflex

That C1 area neurons participate in baroreceptor reflexes was initially suggested by anatomical observations that neurons in the C1 area of the RVL receive dense input from the regions of the NTS containing terminals of afferent fibers from arterial baroreceptors and other cardiopulmonary afferents. The importance of this projection in the baroreceptor reflex is implied from the electrophysiological observations that the probability of firing of neurons in the C1 area is locked to the cardiac cycle and that baroreceptor input inhibits their discharge. Direct evidence that neurons of the C1 area mediate the vasodepressor response to baroreceptor stimulation has been demonstrated by the findings that bilateral chemical blockade of the C1 area, which does not affect fibers of passage, abolishes the reflex responses to electrical stimulation of the vagus nerve or stretch of the carotid sinus. Conversely, disinhibition of C1 area neurons produces the elevations of AP elicited by withdrawal of baroreceptor activity. This follows, since the elevations of AP produced by lesions of the NTS are also abolished by interruption of synaptic transmission in the C1 zone.

Evidence therefore suggests that the baroreceptor reflex arc (Figure 2), acting upon AP, consists of a projection from the baroreceptor afferents into the NTS which excites neurons in that nucleus. The activity along neural circuits from the NTS produces inhibition of sympathoexcitatory neurons in the C1 area that results in withdrawal of excitatory input to preganglionic sympathetic neurons and a fall of AP.

Somatosympathetic Reflexes

Another reflex that is mediated by C1 neurons is the elevation of AP arising from pain and other receptors on the limbs and body surface and mediated through afferent fibers synapsing within the spinal cord. Studies from this laboratory demonstrated that spinal afferents project directly into the C1 area from neurons located in the dorsal horn of the spinal cord. Elevations of AP elicited from electrical stimulation of the sural or sciatic nerves can be abolished by lesions or chemical interference within the contralateral, but not ipsilateral, C1 area. Furthermore, RVL spinal vasomotor neurons in the rat and rabbit are activated by sciatic stimulation, demonstrating that RVL neurons could mediate the pressor response to peripheral nerve stimulation.
Responses to Ischemia and Hypoxia

The C1 area neurons also participate in reflex responses to reduction of blood flow and oxygen perfusion of the brain. Lesions of the C1 area in rabbit abolish the potent pressor response elicited by cerebral ischemia. More recently, we demonstrated that lesions of the area also profoundly impair the diffuse cerebrovascular dilatation elicited by systemic hypoxia, without interfering with the vasodilation elicited by hypocarbia. These observations suggest that neurons within the C1 area respond to reductions of local blood flow or partial pressure of oxygen, or both, in such a manner as to engage various segments of the circulatory bed. That neurons of the C1 area may be involved in the reflex responses to reduction of blood flow and oxygen perfusion of the brain is consistent with the findings of the present study.

What Is The Transmitter Released by C1 Neurons Mediating Sympathoexcitation on Preganglionic Neurons?

Current evidence is consistent with a role for C1 area neurons responsible for the tonic and reflex control of circulation. Whether the sympathoexcitatory elements are C1 neurons or other neurons with pro-jection pathways identical to those of the C1 cells remains unresolved, as does the identity of the transmitter that is released by these cells to excite preganglionic sympathetic neurons in the spinal cord and raise AP. It is our belief that sympa-thoexcitation of RVL origin is not mediated solely by epinephrine. Although iontophoretically applied epinephrine inhibits rather than excites preganglionic sympathetic cells, it is conceivable that RVL neurons use another transmitter that may be coreleased with epinephrine to produce sympathoexcitation. Among the candidates are neuropeptide Y, substance P (although the evidence for coexistence in C1 cells suggests that few neurons in the C1 area contain this peptide) or conceivably, excitatory amino acid transmitters such as L-glutamate.

Pharmacology

Classic Neurotransmitters

The C1 area of RVL is sensitive to a variety of pharmacological agents, suggesting the richness of the transmitter regulation within the area’s functional microcircuitry involved in the control of the circulation. Locally applied to RVL, GABA is a potent sympatho-inhibitory agent. The fact that the local application of the GABA antagonist bicuculline results in a powerful elevation of AP and heart rate indicates that GABAergic mechanisms are tonically active. Anatomically, the presence of local GABAergic neurons, and the demonstration that these may produce direct synapses upon PNMT-containing cells, suggests strongly that GABAergic inhibition within the C1 area may arise from local neurons. These local neurons may, in fact, be transducers of impulses arising from elsewhere in the brainstem to mediate potent cardiovascular reflex responses. Noradrenergic input to the C1 area, possibly arising from more caudally situated cells, may also provide inhibition of C1 area neurons.

Excitatory neurotransmitters L-glutamate and kainic acid elevate AP when applied to the C1 area. It has been proposed that L-glutamate may be an important excitatory transmitter in this zone. The source of glutamatergic input to the region is unknown. Acetylcholine is also an important excitatory transmitter within the C1 area; it may arise from local neuronal perikarya within and adjacent to the C1 area. The C1 area contains muscarinic receptors that mediate the presor response secondary to the systemic administration of physostigmine.

Clonidine and the Imidazole Receptor

Not only is the C1 area of the RVL responsive to a number of putative neurotransmitters, but it is the major site of action at which clonidine, a clinically efficacious antihypertensive agent that is an imidazole, acts to lower blood pressure. The traditional view is that clonidine lowers AP by acting as an agonist of α2-adrenergic receptors in the brain as it does in the periphery. In support has been the finding that the
clonidine analogue [H]p-aminoclonidine ([H]PAC) can be totally displaced from membranes of the cerebral cortex by adrenergic ligands,\(^{42,44}\) and [H]PAC can be demonstrated by autoradiography to bind specifically within the RVL.\(^{43}\)

This view, however, has been challenged on the grounds that analysis of the structural relationships of a series of imidazoles or \(\alpha_2\)-adrenergic agents (or both) to their hypotensive actions suggests that their blood pressure–lowering actions relate more to their imidazole structure than to interaction with \(\alpha_2\)-adrenergic receptors. These findings have led to a concept that clonidine acts by an interaction with imidazole-prefering receptors to lower AP in RVL.

In recent studies\(^{44}\) we examined the binding of clonidine to imidazole receptors within the C1 area of the RVL. This new class of receptors may mediate the hypotensive actions of clonidine. We have found that in membranes of the bovine RVL: 1) [H]PAC binds to local membranes; 2) only 70% of the binding is displaceable by norepinephrine and other \(\alpha_2\)-adrenergic ligands; 3) the remaining 30% is displaced by imidazole-like substances with high selectivity; 4) the imidazole binding sites for clonidine are not localized to the cerebral cortex, thereby explaining why others, using the cerebral cortex as a test tissue, concluded that clonidine binds exclusively to \(\alpha_2\)-adrenergic receptors in brain; 5) the imidazole receptors are distinct from histamine receptors; and 6) the relationship between the hypotensive action of clonidine and allied substances relates more to their binding to imidazole than to \(\alpha_2\)-adrenergic receptors.\(^{41}\)

The question then arises: What is the natural ligand of the imidazole receptors? It is not histamine, the principal bioactive imidazole, since neither histamine nor its biosynthetic enzyme is found in the RVL.\(^{41}\) One possibility is a newly discovered substance in brain that displaces [H]PAC from membranes.\(^{46}\) This material, termed clonidine-displacing substance, appears to be a noncatecholamine of low molecular weight. That clonidine-displacing substance is biologically active is suggested by findings that it modifies blood pressure when injected into the RVL.\(^ {43,44}\) Although the findings of several groups have been discordant as to the direction of blood pressure change. Clonidine-displacing substance also produces contraction of the gastric fundus of the rat, which is not blocked by traditional antagonists.\(^{49}\)

To date, the structure of this material has been undefined. However, of considerable interest is that, like clonidine, clonidine-displacing substance binds to both \(\alpha_2\)- and imidazole receptors in the RVL. Clonidine-displacing substance remains a material of substantial interest in the central control of blood pressure.

It will be fascinating to determine whether variations in its concentration will relate to variations in blood pressure control in animals or humans with hypertensive disease.

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