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C1 neurons: the body’s EMTs

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Guyenet PG, Stornetta RL, Bochorishvili G, DePuy SD, Burke PG, Abbott SB. C1 neurons: the body’s EMTs. Am J Physiol Regul Integr Comp Physiol 305: R187–R204, 2013. First published May 22, 2013; doi:10.1152/ajpregu.00054.2013.—The C1 neurons reside in the rostral and intermediate portions of the ventrolateral medulla (RVLM, IVLM). They use glutamate as a fast transmitter and synthesize catecholamines plus various neuropeptides. These neurons regulate the hypothalamic pituitary axis via direct projections to the paraventricular nucleus and regulate the autonomic nervous system via projections to sympathetic and parasympathetic preganglionic neurons. The presympathetic C1 cells, located in the RVLM, are probably organized in a roughly viscerotopic manner and most of them regulate the circulation. C1 cells are variously activated by hypoglycemia, infection or inflammation, hypoxia, nociception, and hypotension and contribute to most glucoprivic responses. C1 cells also stimulate breathing and activate brain stem noradrenergic neurons including the locus coeruleus. Based on the various effects attributed to the C1 cells, their axonal projections and what is currently known of their synaptic inputs, subsets of C1 cells appear to be differentially recruited by pain, hypoxia, infection/inflammation, hemorrhage, and hypoglycemia to produce a repertoire of stereotyped autonomic, metabolic, and neuroendocrine responses that help the organism survive physical injury and its associated cohort of acute stress responses such as blood loss. C1 cells may also contribute to glucose and cardiovascular homeostasis in the absence of such physical stresses, and C1 cell hyperactivity may contribute to the increase in sympathetic nerve activity associated with diseases such as hypertension.

C1 neurons; blood pressure; brain stem

Best known for their contribution to the control of arterial pressure (AP), the C1 neurons have also been implicated in many other physiological processes ranging from neuroendocrine responses to infection and inflammation, glucose homeostasis, reproduction, breathing, thermoregulation, hypothalamo-pituitary axis (HPA)-mediated stress responses, and food consumption. The purpose of this review is to summarize the most salient information concerning the C1 cells, to point out some of the remaining gaps in our current knowledge, and to suggest a few unifying physiological principles that could account for these seemingly disparate observations.

Based on the various effects attributed to the C1 cells and what is currently known of their synaptic inputs, we propose that these neurons are, figuratively speaking, the body’s “emergency medical technicians.” By this we imply that these neurons produce stereotyped autonomic, metabolic, and neuroendocrine responses designed to help the organism survive major acute physical stresses such as accidental, pathological, or dive-related hypoxia or physical injury and its associated cohort of acute infection, blood loss, and hypotension. These emergency responses include, in the short term and depending on the stress, vasoconstriction, cardioinhibition, or acceleration, breathing stimulation, anti-diuresis, changes in metabolism, and gastrointestinal (GI) functions designed to conserve peripheral glucose supplies for selective use by the brain, adjustment of body temperature via regulation of brown adipose fat, and positive or negative stimulation of food intake.

Identification and Definition of the C1 Neurons

The ability of brain tissue to synthesize epinephrine from norepinephrine enzymatically was demonstrated by Barchas et al. in 1969 (38). The responsible enzyme phenylethanolamine N-methyl transferase (PNMT) was purified by Joh and Goldstein (87) in 1973, and PNMT was soon after detected by immunohistochemistry in the brain of rats (74, 75). The largest cluster of PNMT immunoreactive (ir) neurons, called C1, was found in the ventrolateral aspect of the medulla oblongata (VLM) roughly from the caudal end of the facial motor nucleus to the rostral third of the lateral reticular nucleus corresponding to the level of the area postrema in the transverse plane...
The C1 cells represent the rostral half of a column of VLM catecholaminergic neurons that extends down to the spinomedullary junction. This cell column was originally identified as catecholaminergic using the Falck-Hillarp fluorescence method and was called the A1 group (45). The term A1 group is now used exclusively in reference to the VLM noradrenergic neurons (catecholaminergic neurons that do not make PNMT). The location of the A1 neurons and of other lower brain stem noradrenergic neurons is also shown in Fig. 1, A and B. The C1 group contains around 72% of the medullary PNMT-ir neurons in rats (124). The only other PNMT-ir neurons, the C2 and C3 clusters, are located respectively in the rostral portion of the nucleus of the solitary tract and in the rostral part of the dorsomedial medulla (Fig. 1). They contain 13% (C2) and 15% (C3) of the PNMT-ir neuron population in rats (124). The C2 and C3 clusters are often lumped together in the literature as the C2 group. Almost all C1 neurons also contain tyrosine-hydroxylase (TH) and dopamine-

hydroxy-

lase (D\textsubscript{H}), and a large majority (75%) possess detectable levels of aromatic amino acid decarboxylase (139). Accordingly, the C1 neurons should in theory be able to synthesize both norepinephrine and epinephrine.

In the early 1970s Hökfelt et al. (74, 75) provided a first-pass description of the collective axonal projections of the C1–3 PNMT-ir neurons. The identified targets consisted of the dorsal vagal complex, including the dorsal motor nucleus of the vagus (DMV), the intermediolateral cell column (IML), the locus coeruleus, the periaqueductal gray matter, the hypothalamic paraventricular nucleus (PVH), the perifornical region, and dorsomedial nucleus of the hypothalamus. Based on this projection pattern and the location of the somata, Hökfelt et al. (75) speculated that central nervous system (CNS) adrenergic neurons might control AP and respiration, food and water intake, gonadotrophin and oxytocin secretion, and body temperature. A role of the CNS adrenergic neurons in the control of vigilance was also hypothesized based on the presence of PNMT-ir terminals within the locus coeruleus.

**Signaling by the C1 Neurons**

Although most C1 neurons contain all the enzymes required to synthesize catecholamines, there is no direct evidence (e.g., electrochemical or electrophysiological) that they actually release such compounds and, if so, which (norepinephrine, epinephrine, or both), where (dendrites, subsets of terminals), and how (action potential-dependent release, receptor-mediated intracellular calcium release). Neurons innervated by the C1 cells (e.g., sympathetic preganglionic neurons, locus coeruleus, and DMV) typically respond to exogenously applied catecholamines via \(\alpha\) (1 and/or 2) and/or \(\beta\) adrenergic receptors (6, 82, 116), but these receptors could be activated by norepinephrine released by nor-

![Fig. 1. Location and principal axonal projections of the C1 neurons.](http://ajpregu.physiology.org.org by 10.220.33.1 on October 15, 2017)
adrenergic neurons that also target the regions innervated by the C1 cells (e.g., A5 neurons in the intermediolateral cell column). Most C1 cells (90%) lack a plasmalemmal monoamine transporter suggesting that these cells, unlike their noradrenergic counterparts, lack the means of replenishing their catecholamine stores via reuptake (40, 107).

At present, the best documented aspect of C1 cell communication is their “wiring transmission” (201), which operates via conventional ionotropic glutamatergic synapses and likely accounts for the short-term effects that have been attributed to C1 cell activation in vivo; i.e., short time-scale sympathetic reflexes and acute AP stabilization (66, 128). The axonal varicosities of the C1 cells typically form conventional synapses; e.g., in the IML, locus coeruleus, rostral ventrolateral medulla (RVLM), and DMV (5, 52, 121–123). These synapses are usually (75%) asymmetric and the C1 neurons express vesicular glutamate transporter-2 (VGLUT2) mRNA and lack markers of inhibitory neurons such as glutamic acid decarboxylase (GAD), and glycine transporter 2 (GlyT2) (39, 160, 176, 178). VGLUT2 is a protein whose presence is necessary and sufficient for neurons to release glutamate by calcium-dependent exocytosis (183). For these and other reasons of a pharmacological nature, the C1 neurons have long been suspected to signal via conventional glutamatergic transmission. This hypothesis has now been verified in the case of the C1 projections to the DMV using electron microscopy and optogenetics (52).

The C1 neurons express various combinations of neuropeptides; e.g., neuropeptide Y (NPY), substance P, enkephalins, thyrotropin-releasing hormone (TRH), cocaine- and amphetamine-regulated transcript (CART), and pituitary adenylate cyclase-activating polypeptide (PACAP) (141, 174). TRH and substance P receptors are Gq-linked and typically produce excitatory effects, whereas enkephalin and NPY receptors are Gi/o-linked and cause pre- and postsynaptic inhibition. Therefore, in theory, the C1 neurons should be capable of exerting complex pre- and postsynaptic effects depending on the types of peptides that they release and the composition of the nearby neuropil. These peptides do indeed have distinctive actions when injected intrathecally or into the RVLM (e.g., 27, 57 and other papers from this group), but their cellular mechanisms of action and the physiological context of their release are unknown.

The PVH has been investigated in greater detail and provides a possible model of how catecholamines and NPY, two transmitters presumably released by the C1 cells and other lower brain stem catecholaminergic neurons, operate at the cellular level. The NPY innervation of the PVH originates from three sources, adrenergic neurons (primarily C1 and to a lesser degree C3 for a total of ~42% of the NPY-positive terminals in the PVH), noradrenergic neurons (primarily A1 and a small fraction of A2 for a total of 21%) while the remaining 36% of PVH NPY terminals originate from agouti-related peptide (AgRP)-synthesizing arcuate neurons (59). The A1 neurons target primarily the magnocellular component, whereas the C1 and A2 neurons innervate predominantly the parvocellular division of the PVH (59). As a result, the lower brain stem, particularly the C1 group, is the main source of NPY-ir terminals present within the parvocellular division of the PVH. All known NPY receptors are Gi/o coupled and are therefore assumed to cause presynaptic and/or postsynaptic inhibition by activating inwardly rectifying potassium conduc-

tances or by inhibiting calcium channels (187). Accordingly, any excitatory effect of NPY ought to be mediated by disinhibition. NPY released along with GABA by the nerve terminals of arcuate neurons in the PVH appears to stimulate food intake by inhibiting a subset of melanocortin-4 receptor (MC4R)-expressing oxytocinergic neurons that innervate the dorsal medulla and the parabrachial region (16, 60, 61). The same mechanism could conceivably contribute to glucoprivic feeding, a response that is mediated by the C1 and or the A1 neurons and is attenuated by silencing NPY expression in these cells, albeit only if DBH is also downregulated (97). The effects of catecholamines on bulbospinal presympathetic PVH neurons have been investigated in rat brain slices. The most conspicuous effect of exogenously applied norepinephrine on these PVH cells is an increased excitatory postsynaptic currents (EPSC) frequency (α1-adrenoceptor mediated) and a reduced inhibitory postsynaptic currents (IPSC) frequency (α2-adrenoceptor mediated) (35, 98). In other words, in vitro, catecholamines exert a globally excitatory effect on the presympathetic PVH neurons, and this effect seems largely mediated by presynaptic modulation of other PVH inputs.

In summary, conventional ionotropic glutamatergic transmission mediates the short-term effects of the C1 cells. C1 cell-derived peptides and catecholamines have detectable effects in the regions innervated by the C1 cells. However, the only physiological deficit that has been associated with the downregulation of a peptide (NPY) made by the C1 cells is a reduction of the glucoprivic response, and even in this case, the deficit was observable only when catecholamine synthesis was simultaneously downregulated (97). The conditions under which the C1 cells release peptides or catecholamines and the effects that these substances produce need clarification.

Anatomical Nomenclature

In simple terms, the ventrolateral medulla (VLM) describes the ventrolateral quadrant of the medullary reticular formation. This region extends from the level of the facial motor nucleus to the spinomedullary junction. The C1 neurons are interspersed with various other neurons within the rostral half of the VLM. Approximately one-third of the C1 cells are presympathetic; i.e., innervate sympathetic preganglionic neurons, and these C1 cells are concentrated at the rostral pole of the VLM in a region described here as the RVLM (Fig. 1C). This region corresponds to the VLM “pressor area” defined by Sapru and colleagues (196), where injection of excitatory amino acids increases AP. As Fig. 1C emphasizes, C1 neurons are not restricted to the RVLM “pressor area.” C1 neurons are found within the VLM down to the level of the area postrema. Thus C1 neurons are also located in the “depressor area” of the VLM where injection of excitatory agents decreases AP in anesthetized rats (62, 196), presumably due to the activation of GABAergic interneurons that relay the baroreflex (see C1 Cells, Hypotension, and Baroreflexes). We define the VLM region that harbors these GABAergic neurons as the IVM (Fig. 1C), where the input to the rostral pole of the VLM (IVM) (195), and reserve the term caudal VLM (CVLM) to describe the region of the VLM located between the IVM and the medullospinal junction (Fig. 1C). With the use of this nomenclature, the CVLM contains the A1 noradrenergic neurons and VLM regions where injections of excitatory agents increases AP in anesthetized rats (62). In the
aggregates, the RVLM and IVLM are coextensive with the C1 cells. For reference, Fig. 1C also describes the location of four major functional subdivisions of the VLM defined by respiratory physiologists (for review see Ref. 166). The three most rostral regions reside dorsal to the C1 cell column, the fourth is approximately coextensive with the CVLM.

**Projections of the C1 Neurons**

The global reach of the C1 cells is extensive, well-described using viral vectors, and includes numerous brain stem and diencephalic structures typically associated with autonomic responses and stress behavior (Fig. 1, A and B) (1, 30). The projections and functions of the C2 group are essentially unknown. The C3 group (Fig. 1B) seems to have many of the same connections and properties as the C1 cells and may be an ectopic cluster of C1 cells (164). The axonal projections of individual C1 neurons are not precisely known but there is a strong presumption that these cells, including the presympathetic ones, are highly collateralized. In rats for example, at least 43% of RVLM bulbo spinal barosensitive neurons could be antidromically activated from at least one of several structures such as the lateral parabrachial nucleus and the periaqueductal gray matter (70). In agreement with anatomical data (186) at least 10% of physiologically identified RVLM presympathetic neurons also innervate the hypothalamus (70, 175). This population includes NPY-expressing C1 neurons (70, 175). According to Llewellyn-Smith and colleagues, the presympathetic C1 neurons may contribute no more than 2% of the total synaptic input to sympathetic preganglionic neurons (102).

The terminal fields of C1 neurons are found throughout the VLM and innervate both catecholaminergic and noncatecholaminergic neurons. The noncatecholaminergic neurons targeted by the C1 neurons may mediate the stimulation of breathing produced by C1 neuron activation (see later section). Some C1 neurons are interconnected by recurrent collaterals (123). The presence of PNMT-ir synapses within the RVLM/IVLM was first described by Milner et al. (123) and has been recently confirmed (5). These connections may contribute to the tendency of sympathetic vasomotor efferents to discharge synchronously in vivo, even in the absence of baroreceptor input and respiratory network activity (19). Both recurrent excitation and inhibition can theoretically produce network synchronization. The nature of the recurrent interactions between C1 cells is unclear. The recurrent collaterals identified by Agassandian et al. (5) were ultrastructurally asymmetric and filled with small clear vesicles and could represent excitatory glutamatergic synapses. However, ionotropic glutamate transmission does not seem to contribute much to maintaining the on-going activity of the vasomotor C1 cells in vivo (182). An alternative possibility, supported by physiological evidence, is that the recurrent collaterals between C1 cells release catecholamines and are inhibitory. Indeed, the C1 neurons express α2-adrenergic receptors whose activation inhibits these cells in various ways (100) and iontophoretic application of α2-adrenergic receptor antagonists increases the activity of the C1 cells in vivo (10).

In brief, the C1 neurons are highly collateralized and influence many brain regions simultaneously. The C1 cells have recurrent interactions that may synchronize, amplify or restrain the discharge of subsets of these neurons.

**C1 Cells, Hypotension, and Baroreflexes**

The contribution of the C1 cells to the baroreflexes (the sympathetic, respiratory, and neuroendocrine responses elicited by hypotension via a reduction in the activity of arterial baroreceptors) is summarized in Fig. 2. Seminal experiments carried out in the late 1970s and early 1980s demonstrated that the RVLM is the source of a dense projection to the intermediolateral cell column (IML) and suggested that this region contained neurons whose on-going activity was required for the maintenance of AP and sympathetic vasomotor tone in anesthetized mammals (12, 46, 66, 150). Because the RVLM is approximately coextensive with a subset of C1 neurons that directly innervate sympathetic preganglionic neurons (Fig. 1C), these investigators, chief among them D. J. Reis, proposed

![Fig. 2. Contribution of the C1 cells to the autonomic responses to hypotension. This and the following three figures are color-coded as follows: C1 neurons in pale blue, autonomic neurons and hormone releasing PVH neurons in orange, excitatory glutamatergic neurons (except C1 cells) in green, inhibitory presumably GABAergic neurons in magenta. Hypotension disinhibits (activates) many C1 cells via a pathway that consists minimally of three neurons: baroreceptor afferent neurons (baroreceptor), glutamatergic neurons located in the NTS and projecting to the ventrolateral medulla and, finally, IVLM GABAergic interneurons innervating the C1 cells (158). Barosensitive C1 neurons located in the rostral ventrolateral medulla (RVLM) are predominantly bulbo spinal. Barosensitive C1 neurons located in the IVLM innervate the hypothalamus among other places. The spinally projecting C1 cells innervate preganglionic sympathetic neurons located in the intermediolateral cell column (IML) that control a variety of vascular beds and the heart plus the norepinephrine (NE)-releasing chromaffin cells of the adrenal medulla (Adr). Hypotension also activates breathing by stimulating the central respiratory pattern generator (CRG). There is no direct evidence that this effect is mediated by axonal collaterals of presympathetic barosensitive C1 neurons as depicted, Hypotension also triggers the CRH/ACTH/corticosterone cascade, presumably through direct projections from IVLM C1 neurons to the paraventricular nucleus of the hypothalamus (PVH). Direct projections of barosensitive C1 cells to the PVH may also activate magnocellular vasopressin (VP)-releasing neurons and preautonomic PVH neurons that control renal sodium excretion. C1 cells, directly and/or indirectly (i.e., via A1 noradrenergic neurons) also activate the release of oxytocin (OT) from the supraoptic nucleus (SO). Barosensitive C1 cells also activate subsets of CNS noradrenergic neurons (not illustrated, see Fig. 5).
that the bulbo spinal C1 neurons drive vaso- and cardiomotor sympathetic efferents and stabilize AP under the regulatory influence of the arterial baroreflex (63, 148, 149). In support of this interpretation, the RVLM was found to contain spinally projecting neurons that were active at rest and inhibited by baroreceptor stimulation to a degree roughly commensurate with the change in sympathetic vasoconstrictor or cardiomotor outflow (e.g., 18, 25, 66). A large fraction of these neurons were later identified as C1 cells by intracellular and juxtacellular labeling methods, but these studies also identified a class of bulbo spinal baro-inhibited neurons devoid of detectable catecholamine-biosynthetic enzyme (101, 156). These “non-C1” presympathetic neurons were unaffected by injecting the ribosome-inactivating toxin anti-DβH-saporin (anti-DβH-sap) into the spinal cord (161) which gave additional credence to the notion that they were not catecholaminergic. Anti-DβH-sap binds selectively to the DβH enzyme when it is exteriorized on the plasma membranes of catecholaminergic neurons during exocytosis (140). After internalization and retrograde transport to the cell bodies, the toxin kills the DβH-expressing neurons that project to the site of toxin injection (140). Neurons that are resistant to anti-DβH-sap are therefore presumed to be neither noradrenergic nor adrenergic. Based on juxtacellular labeling, 72% of the population of bulbo spinal barosensitive (putative presympathetic) neurons of the rat RVLM could be C1 cells (154, 156). This proportion was estimated at only 26% when cell labeling was performed by intracellular recording (101). The higher estimate is in closer agreement with retrograde tracer anatomical experiments (e.g., 57) but each method has biases and the exact proportion of C1 versus non-C1 presympathetic neurons is unknown.

Both C1 and non-C1 presympathetic neurons express VGLUT2 and are therefore presumably glutamatergic (161). The non-C1 presympathetic neurons and about 40% of the C1 presympathetic neurons have lightly myelinated axons and axonal conduction velocity in the 1–8 m/s range, whereas the rest of the RVLM bulbo spinal C1 cells have unmyelinated axons and axonal conduction velocity of less than 1 m/s (130, 156). Morphological differences have been observed between RVLM presympathetic neurons, but the distinguishing features (dendritic complexity, prominent axonal initial segment, abundance of enkephalin-ir synapses, lack of NPY mRNA) seem to correlate best with the degree of axonal myelination of these neurons (i.e., their axonal velocity) rather than with the presence of a catecholaminergic marker (TH or PNMT) (101, 103, 175). The C1 neurons of the IVLM that innervate the hypothalamus do not have myelinated axons (189). A unique diagnostic marker of the noncatecholaminergic RVLM presympathetic neurons has not been found. These neurons may be identified using a combination of markers such as VGLUT2, PACAP-, or preproenkephalin-mRNA (57, 177) but the presence of spinal projections and the absence of TH remain the key to their histological identification. These cells have not been characterized in species other than the rat.

The specific contribution of the C1 neurons to AP control in conscious rats was first tested by exploring the consequences of their destruction with anti-DβH-sap (110, 111). Despite very large reductions in C1 cell numbers (>80%) and substantial collateral damage to the A5 noradrenergic neurons, only modest reductions of resting mean AP were observed in conscious rats (<10 mmHg) (111). In these rats, plasma catecholamine levels were only marginally lower than in controls, corresponding to a small drop in resting sympathetic tone. However, there was a substantial reduction in the rise of plasma norepinephrine, vasopressin, and oxytocin following hydralazine-induced hypotension (respectively −40%, 73%, and 40%), indicating permanent deficits in the neuroendocrine adjustments to baroreceptor unloading (110). In other experiments, the presympathetic C1 neurons (and A5 neurons) were destroyed by injecting the same toxin into the spinal cord (157, 161). These lesions spared the bulbo spinal barosensitive non-C1 RVLM neurons (161) and, as in the above-mentioned studies of Madden and Sved (110, 111), had inconsequential effects on resting AP (157). However, sympathoexcitatory responses evoked by electrical stimulation of the RVLM were severely attenuated as were the baroreflex and the increase in sympathetic nerve activity (SNA) elicited by carotid body stimulation. Together, these studies demonstrate that chronic lesions of the C1 neurons with anti-DβH-sap only modestly decreases resting AP and resting SNA, but permanently and substantially reduces the neuroendocrine response to hypotension, hypoxia, and noceception.

Interpreting the effects of chronic lesions of the C1 neurons is complicated by the potential development of compensatory mechanisms. For example, volume expansion by the kidney, baroreflex compensation, peripheral catecholaminergic receptor supersensitivity, and compensation by non-C1 presympathetic neurons may explain why C1 neuron lesions produce such modest hypotension at rest (110). The slow compensatory mechanisms that may be maintaining AP despite the absence of C1 neurons may be circumvented by applying rapid and selective loss of function approaches. Using the allatostatin receptor (R) method introduced by Callaway (184), Marina et al. (114) showed that acutely inhibiting the C1 cells produced large reductions of SNA, (~50%) and AP (~25 mmHg) in anesthetized rats and in an arterially perfused midcollicular transected preparation of the same species. These results, contrary to those of chronic lesions, support the view that the C1 cells make a large contribution to resting SNA and AP as first hypothesized by Reis and colleagues. A few caveats must be evoked however. The rat preparations used by Marina et al. (114) to test the effects of C1 cell inhibition were barodenervated and anesthetized or hypothermic midcollicular transected arterially perfused preparations. Additionally, the lentiviral vector used in this study to introduce the allatostatin R into RVLM neurons is not entirely selective for the C1 neurons. The selectivity of this vector relies on the artificial promoter PRSx8, which is a binding site for transcription factors Phox2a and b (79). Phox2 is expressed by other RVLM neurons besides the C1 cells, notably by the retrotrapezoid nucleus and by subsets of cholinergic neurons (3). Also, the construct is somewhat leaky, which means that viral transcription may occur even in Phox2-negative neurons exposed to PRSx8-lenti- or adeno- virus, albeit at a slower rate (see Ref. 79, Guyenet PG and Stornetta RL, unpublished results). In our experience (1) a more selective way to target the C1 neurons is to use the Cre-Lox technology with rodents transgenically engineered to express Cre under a catecholamine promoter in combination with directed injections of a floxed viral vector (31). Finally, the reduction of SNA observed in the study by Marina et al. (114) was still much smaller than that produced by adminis-
tation into the RVLM of muscimol, a nonselective inhibitor of neuronal activity. Accordingly, the contribution of the C1 cells versus other RVLM neurons to resting AP in conscious mammals is still uncertain.

RVLM presympathetic neurons are probably organized in a roughly viscerotopic manner (skeletal muscles, viscera, heart, etc.). The principal evidence is that the sympathetic nerves to these organs can be differentially activated by microinjecting glutamate into separate regions of the RVLM of anesthetized cats (118). Similarities between the discharge pattern of individual presympathetic neurons and regional SNA in anesthetized rats also suggest that there exist at least two broad types of RVLM presympathetic neurons that differentially control skeletal muscle versus visceral vascular beds (e.g., 71, 153 and, for review, see Ref. 66). Cutaneous arterioles are preferentially controlled by supraspinal drives that originate from the medullary raphe (131). Although, the viscerotopic organization of the RVLM is an attractive theory, there is also anatomical evidence that supports the existence of C1 neurons with very broad target specificity that could potentially mediate the type of generalized sympathoactivation envisioned by Cannon (85).

RVLM presympathetic neurons are presumably inhibited by arterial baroreceptors via a chain of at least two CNS neurons (Fig. 2) but the monosynaptic nature of the connections between the postulated links has not been established (e.g., 33, 159 and, for reviews see Refs. 66 and 158). The first CNS neuron of the baroreflex is glutamatergic, resides in the dorsolateral aspect of the intermediate nucleus of the solitary tract (NTS), and activates GABAergic interneurons located in the IVLM (Figs. 1C and 2). The projection from NTS to IVLM is largely unilateral. There is almost complete overlap between the location of the baro-activated GABAergic neurons and the caudal portion of the C1 cell column (Figs. 1C and 2). The baro-activated GABA neurons of the IVLM innervate the RVLM bilaterally. Many C1 neurons located in the IVLM are also barosensitive (189) and presumably also receive input from the comingled baroactivated GABAergic interneurons like their presympathetic counterparts (Fig. 2). The baroinhibited C1 neurons located in the IVLM innervate the hypothalamus (189) and presumably regulate the secretion of oxytocin and vasopressin (Fig. 2).

In anesthetized rodents, C1 and other presympathetic neurons are extremely active even in the absence of cardiopulmonary (including baroreceptor) or supracollicular input (up to 35 Hz) (66, 76). This high level of activity is apparently not driven by conventional ionotropic glutamatergic inputs and is absent in dissociated C1 cells, which typically lack dendrites (66). The high activity of C1 cells present in vivo could possibly be partly intrinsic to these neurons, perhaps of dendritic origin, or it could be under the control of unconventional and as yet unidentified transmitters (66, 76). The basal discharge rate of various subsets of C1 cells could be regulated, directly or via the glia, by local factors such as hypoxia, hypoglycemia, inflammatory cytokines (IL1), blood flow restriction, and circulating steroid hormones (32, 146, 163).

Although respiratory control was originally listed as a probable function of the brain adrenergic system (75), the close proximity between the C1 cells and the respiratory column (Fig. 1, B and C) has made it difficult to assess whether these neurons control breathing. In mouse slices, fictive respiration is strongly activated by α1-adrenergic receptor stimulation, but unresolved species differences persist and these experiments have not demonstrated whether the C1 neurons or other catecholaminergic neurons regulate breathing (190, 199). Using optogenetics, we have shown that selective C1 neuron stimulation activates breathing in conscious mice (1). Barosensitive C1 neurons presumably contribute to the ventilatory baroreflex; i.e., the breathing stimulation elicited by hypotension, well described in conscious humans (173).

The parvocellular division of the PVH contains many types of neurons including presympathetic neurons thought to regulate renal function, the heart, and regional blood flows (Fig. 2) (23, 60). C1 neurons located in the IVLM densely innervate the PVN and likely control the circulation albeit via a more circuitous route than their presympathetic (bulbospinal) counterparts (Fig. 2). The evidence is twofold. The C1 cells densely innervate the parvocellular region of the PVH (1, 30) and norepinephrine produces presynaptic facilitation in presympathetic PVH neurons (35, 98). This evidence is still circumstantial because the C1 cells have not been shown to actually release catecholamines in the PVH but the fact that the C1 cells are glutamatergic renders the hypothesis that they activate PVH presympathetic neurons more plausible.

The extent to which the C1 cells contribute to sympathetic tone and AP in conscious mammals is still debatable because the consequences of a complete and instantaneous inhibition of these cells in the conscious state are unknown. The notion that these cells are critically important to AP maintenance in conscious mammals is still an extrapolation of data acquired under anesthesia or in reduced preparations in which resting SNA and the resting discharge of the C1 cells is abnormally high due to surgery and pharmacological issues (anesthetics). C1 lesion experiments (110, 111) suggest the C1 neurons contribute modestly to AP under normal resting conditions and are most important during acute hypotension. Such a drop in blood pressure would likely only occur in a pathological situation (e.g., hemorrhage).

**Regulation of the Parasympathetic Outflow by the C1 Neurons: A Gastrointestinal, Cardiovascular, or Immune Connection?**

The regulation of the parasympathetic nervous system by the C1 cells is rarely mentioned, perhaps because the bulk of the work dedicated to these cells has been focused on their role in controlling sympathetic vasomotor efferents and AP. Yet, the high density of PNMT-ir terminals within the dorsal vagal complex, especially within the DMV, was identified in the first paper dedicated to the immunohistochemical distribution of this enzyme (74). This observation was replicated by Siaud et al. (165) who suggested a direct PNMT innervation of the DMV neurons that control the stomach and pancreas. Loewy and coworkers used the retrograde migration of pseudorabies virus (PRV) from the pancreas to find that the PNMT innervation comes primarily from C1 cells with lesser contributions from the C2 and C3 neurons (104). This work was performed in rats subjected to a spinal cord transection at the C8 level, therefore, the C1 neurons could not have been labeled retrogradely via the sympathetic nerves. According to these authors, at least 25% of the C1 neurons, located in what appears to be the IVLM, innervate the DMV (104). A projection of the C1 cells to the DMV was also observed by Card and Sved (30) in
rats. Using a Cre-dependent viral vector in both TH-Cre transgenic rats and DβH-Cre mice, we confirmed that the C1 cells innervate the DMV massively and we demonstrated that the connection between the C1 cells and parasympathetic preganglionic neurons is monosynaptic and excitatory (52). In this study 70% of the electrophysiologically sampled DMV neurons received input from C1 neurons. This high percentage may not be representative of the entire DMV but it suggests that C1 neurons probably target DMV neurons that innervate multiple organs besides the pancreas. Given the excitatory nature of this connection, C1 neurons have the potential of accelerating GI transit, secretion rate and blood flow within exocrine GI glands by activation of parasympathetic innervation to these targets. The potential role of this connection in glucoprivic responses or hypoxic adjustments is examined later.

The C1 cell projection to the DMV could also produce some form of cardioinhibition (rate and or ventricular contractility) or modulate immune responses via the spleen (36, 134, 170). Under most physiological circumstances cardiovagal tone and vasomotor sympathetic activity vary in opposite directions (e.g., exercise, baroreflex) but there are exceptions. Coactivation of these outflows occurs during diving, a behavior that triggers Fos activation throughout the A1/C1 group (119, 135). The most rostral of these activated neurons are most likely presympathetic neurons that contribute to the muscle and gut vasoconstriction associated with diving (119, 135). However, the rest of the C1 cells activated by diving do not innervate the spinal cord and may contribute to other autonomic responses associated with diving such as hepatic glucose release, epinephrine release favoring anaerobic metabolism and, possibly, bradycardia. Hypoxia is a condition under which coactivation of the cardiovagal and sympathetic outflows have also been observed (91, 92, 137).

**C1 Neurons and Responses to Hypoxia**

The contribution of the C1 cells to the cardiorespiratory, endocrine and thermoregulatory effects of hypoxia are summarized in Fig. 3. The level of evidence supporting each of the represented mechanisms varies considerably. The primary response of most RVLM presympathetic barosensitive neurons to carotid body stimulation (cyanide or brief hypoxia) in rodents is a vigorous activation (93, 144, 179), although this response can be modified by the baroreflex (93) and central respiratory generator drive (71, 113). Consistent with this observation, a large proportion of C1 neurons express Fos in conscious mammals exposed to hypoxia (54, 73), and sympathetic nerve activation elicited by carotid body stimulation is severely depressed after selective lesions of the C1 neurons (157). The pathway between the carotid bodies and the C1 cells may involve a single interneuron located within the commissural part of the NTS (7, 37, 151). Hypoxia also activates Fos in many IVLM C1 neurons suggesting that the effect of this stimulus is not restricted to the presympathetic neurons.

The Cushing response is the neurogenic increase in AP induced by cerebral ischemia (44) and is likely the direct consequence of extreme brain stem hypoxia. This response is independent of the carotid bodies and is largely mediated by the activation of the barosensitive neurons of the RVLM including the C1 cells (47, 48, 67, 180). Their depolarization, like that of the pre-Bötzinger complex, could be partly a cell-autonomous response, for example, an increase in persistent sodium current or calcium permeability (69, 136) or could result from the release of gliotransmitters such as ATP (65, 115, 169, 181, 202). Reis and collaborators championed the idea that the C1 cells are oxygen sensors, implying that these neurons are activated by small “physiologically relevant” reductions in brain PO2 and then trigger countervailing responses including an increase in AP and a neurogenic rise in cerebral blood flow without concomitant change in brain metabolism (180). This appealing theory has been difficult to prove or refute but new evidence detailed later does suggest that the C1 cells could be regulating cortical blood flow via the locus coeruleus and its effect on the glia. In the respiratory field the general consensus is that hypoxia does not activate breathing in the absence of the carotid bodies although, in their presence, CNS hypoxia does stimulate breathing somewhat (42). One could therefore argue that the cardiorespiratory depressant effect of even moderate hypoxia would be more profound were it not for the countervailing influence of CNS oxygen “sensors.” These sensors could be conceivably be the C1 neurons or surrounding glial cells that secondarily activate the C1 cells via ATP or some other gliotransmitter (115). How the C1 neurons activate breathing has yet to be determined. A direct connection between the C1 cells and elements of the central respiratory generator is plausible (Figs. 2 and 3) because the C1 cells have axonal collaterals within the VLM
that contact non-aminergic hence putatively respiratory cells (5, 101). Indirect connections between the C1 cells and the respiratory centers may occur via C1 projections to the retrotrapezoid nucleus, the lateral parabrachial region, the perigluteal gray matter or even via hypothalamic nuclei such as the dorsomedial nucleus/perifornical region (1, 30).

Hypoxia is a condition under which coactivation of the cardiovagal and sympathetic outflows has been observed (91, 92, 137) even though cardiac rate is typically increased by hypoxia in conscious mammals. Cardiovagal activation may improve the efficiency (90) and reduce the metabolic cost (191) of blood pumping during periods of high sympathetic drive to the heart. The projections of the C1 cells to the DMV could in theory underlie this increase in cardiovagal tone. C1 cell projections to the cardiovagal motoneurons located in the VLM are also plausible on anatomical grounds but have not been characterized.

Sustained hypoxia produces hypothermia in rodents. The hypothermia is caused by an active suppression of shivering and brown adipose tissue (BAT) SNA, which eventually leads to a reduced core temperature and metabolic rate. Similar responses are produced by hypoglycemia (Figs. 3 and 4). Hypoxia- and or hypoglycemia-sensitive C1 cells participate in these responses by inhibiting raphe pallidus presympathetic neurons that control BAT SNA. The pathway includes a direct projection to the raphe pallidus and, possibly, an indirect pathway via the PVH (109, 112). The inhibition of the presympathetic neurons that regulate BAT SNA may be mediated by a catecholamine released by the C1 cells (112), although an inhibitory interneuron could be interposed (Fig. 5). Finally, hypoxia is a powerful stimulus for the release of adrenal epinephrine, a response which, in the adult as opposed to the neonate, is neurogenic (24). Hypoxia-activated presympathetic C1 cells are likely to contribute to this response since these cells demonstrably contribute to the activation of other sympathetic efferents (Fig. 3) (157).

In summary (Fig. 3), a high proportion of C1 neurons are vigorously activated by hypoxia via carotid body stimulation and, possibly, via direct effects of hypoxia on the ventrolateral medulla. These C1 neurons contribute to the sympathetic, respiratory, neuroendocrine and thermoregulatory consequences of hypoxia.

Acute hypoxia is often a pathological or accidental situation and brain stem hypoxia of a magnitude that would cause the Cushing response qualifies as a critical situation. Hypoxia only produces hypothermia if severe and sustained. These considerations underlie the present concept that the C1 neurons intervene under extreme (emergency) conditions. This view does not exclude the possibility that the hypoxic sensitivity of these cells also contributes to cardiorespiratory regulation under more physiological circumstances. Strenuous exercise comes to mind since the carotid bodies and SNA are greatly activated under such conditions.

Control of the CRH-ACTH-Corticosterone Cascade by the C1 Neurons

Based on Fos expression and unit recording data, the C1 neurons are activated by the same physical stresses that elicit...
the CRH-ACTH-corticosterone surge, for example, interleukin-1 (IL-1), lipopolysaccharide (LPS), pain, hypotension, hemorrhage, hypoxia, and hypoglycemia (66, 143, 146, 155) (Figs. 3 and 4). In two well-documented cases, IL-1 administration and hypoglycemia, the integrity of the C1 neurons was shown to be required for the CRH-ACTH-corticosterone response to occur (146, 155). The corticosterone surge is attributed to the direct projection of the C1 neurons to the parvo-cellular division of the PVH (59) but this plausible interpretation has not been strictly demonstrated. To test the contribution of the C1 cells to the release of CRH, the tool of choice has been to lesion the catecholaminergic neurons that innervate the PVH by injecting anti-DβH-sap into this nucleus (146, 155). This procedure also destroys a substantial proportion of A1, A2, A5, and locus coeruleus neurons. Accordingly, the hormonal deficits induced by administering the toxin in the PVH region cannot yet be attributed exclusively to the loss of the C1 neurons (as opposed to other catecholaminergic cells) and cannot yet be exclusively attributed to the direct projections of these catecholaminergic neurons to the PVH as opposed to their projections elsewhere in the brain.

The mechanism by which LPS triggers the CRH-ACTH-corticosterone cascade probably involves the production of IL-1 in blood, the activation of vascular endothelial IL-1 receptors in the brain stem, and the production of prostaglandin E2 (PGE2) by the endothelium and perivascular macrophages. PGE2 presumably reaches the C1 neurons by diffusion and activates these cells via EP3 receptors (155, 163). Lesions of the lateral parabrachial nuclei attenuate the activation of the A1 neurons by systemic administration of IL-1β but, based on Fos expression, these lesions have no effect on the activation of C1 and PVH neurons elicited by the same stimulus (26). This observation reinforces the notion that some of the C1 cells are a primary target of circulating IL-1 and that these cells rather than the A1 neurons mediate the activation of CRH-producing parvocellular PVH neurons by an immune challenge. Hypotension, hypoxia, hypoglycemia, and nociception could conceivably elicit the corticosterone surge by activating a common set of C1 neurons that directly innervate the CRH-releasing neurons of the PVH. Alternately, the CRH neurons may receive input from multiple modality-specific subsets of C1 cells (i.e., glucose sensitive, baro-sensitive, hypoxia sensitive).

Bacterial infections, hemorrhage, and pain are acute pathophysiological events that are associated with trauma. In keeping with the theme of this review, it appears that the C1 cells are especially implicated in these emergency situations.

Contribution of the C1 Neurons to Glucoprivic Responses

The C1 neurons contribute to nutrient intake in a general sense by regulating sympathetic vasomotor tone to GI organs (152) but C1 cells seem to be more specifically implicated in the homeostatic responses to glucose deficiency (glucoprivic responses, Fig. 4). Glucoprivic responses include the release of epinephrine, glucagon, and corticosterone, increased food consumption and GI activity, and decrease in BAT activity (108, 147, 188). The following evidence implicates the C1 neurons in all of these responses. The strong excitatory input from the C1 cells to DMV neurons suggests that subsets of C1 cells produce GI stimulation (52). In theory, some of these C1 neurons could be hypoglycemia responsive since glucoprivic activation is a strong signal for the initiation of GI contractions (120). C1 cell lesions reduce the plasma epinephrine increase caused by administration of 2-deoxyglucose by ~75% (110). A group of C1 neurons located predominantly in the IVLVM is activated by 2-deoxyglucose administration and their activation causes glucose release (147, 188). Some of these hypoglycemia-sensitive C1 neurons are bulbospinal and likely to be a source of excitatory drive to the sympathetic efferents that regulate adrenal epinephrine release (147). Like the latter, these C1 neurons respond to hypoglycemia but not to baroreceptor activation (129, 188). Food intake is stimulated by administering 2-deoxyglucose into the IVLVM, the hot spots being similar to the region that produced an increase in epinephrine release (145). Administration of TRH into the VLM also produces hyperglycemia and hyperinsulinemia (13). The hyperglycemia is mediated by the sympathetic outflow (epinephrine release) whereas the hyperinsulinemia is mediated via parasympathetic activation of the pancreas, consistent with the direct projection of the C1 cells to the DMV. Last, but not least, feeding behavior is stimulated by injecting norepinephrine or NPY into the PVH, two transmitters presumably released by the C1 cells (172), NPY administration into the PVH elicits a selective increase in carbohydrate consumption with little or no effect on protein or fat consumption (171), and the combined downregulation of NPY and catecholamines in the RVLM reduces glucoprivic feeding (97). Hypoglycemia could conceivably stimulate food intake by inhibiting a subset of PVH MC4R-expressing oxytocinergic neurons that innervate the dorsal medulla (nucleus of the solitary tract) and the parabrachial region (16, 60, 61) (Fig. 4). These PVH neurons are most likely different from the presympathetic ones (23).

There is also evidence that the glucose-activated C1 cells reduce BAT activity (Fig. 4). For example, administration of the glucoprivic agent 5-thioglucose into the IVLVM inhibits BAT (108). The inhibitory effect of the C1 cells on BAT activity could be partially mediated via the PVH since activation of this nucleus also suppresses BAT thermogenesis (109) (Fig. 4). C1 neuron-mediated inhibition of BAT activity may also occur via direct projections to the raphe pallidus (1, 30) (Fig. 4). Conceivably, BAT SNA is regulated by a single subset of C1 cells that respond to both hypoglycemia and hypoxia (Figs. 3 and 4).

ACTH release is another consequence of hypoglycemia. Glucose production is increased by corticosterone which, as indicated above, is partly under the control of C1 neurons. Corticosterone promotes gluconeogenesis and shifts the metabolism of non-neural tissues away from glucose utilization to fatty acids and protein utilization. Under hypoxia, glucose release is adaptive by facilitating anaerobic metabolism.

In brief, C1 neurons seem implicated in every aspect of the glucoprivic response including its hormonal and appetitive component (Fig. 4). The C1 cells influence glucoprivic responses at the level of sympathetic and parasympathetic preganglionic autonomic neurons and the PVH but other structures are also implicated. For example, glucoprivation may reduce BAT activity by increasing the activity of C1 cells innervating the raphe pallidus and stimulate appetite by increasing the activity of C1 cells innervating the NTS and parabrachial region (these demonstrated projections are not represented in Fig. 4). Several important knowledge gaps
The C1 cells because subsets of these neurons are vigorously mediated by hypoglycemia, hypotension, and hypoxia in conscious mammals (43, 54, 127). These effects are likely mediated via glutamatergic close appositions with the A1 noradrenergic neurons (2, 30) and the A2 neurons (Holloway BB, Stor-netta RL, and Guyenet PG, unpublished results) (Fig. 5). This pathway is poised to help preserve brain function during systemic challenges such as hypotension, hypoxia, and hypoglycemia or any form of severe somatic stress. Such a regulation must be somehow superimposed and integrated with the local reactive hyperemia and glial metabolic adjustments elicited by increases in neuronal activity (17, 64). The A5 neurons whose function is still elusive but are activated by hypoxia and hypotension may exert similar types of “housekeeping” effects at the level of the IML under conditions when sympathetic efferents are strongly activated.

The potential regulation of the A1 neurons by the C1 cells can also be viewed in the light of an integrated physiological response to acute injury. The A1 cells preferentially innervate the magnocellular cells of the PVH and supraoptic nuclei, whereas the C1 cells preferentially target the parvocellular division of the PVH (59). These anatomical observations and much physiological evidence indicate that the A1 neurons provide an important excitatory drive to hypothalamic magnocellular neurons, which is mediated by NPY, catecholamines, and perhaps ATP (e.g., 22, 50, 197). As previously mentioned, C1 cell lesions that spare the A1 neurons severely attenuate the release of vasopressin and oxytocin elicited by hypotension (110). These deficits could be partly explained by the loss of the excitatory input from the C1 cells to the A1 neurons (Fig. 5). This pathway is poised to help preserve blood volume in case of traumatic injury involving hypoxia, hypotension, and blood loss because each of these stimuli vigorously activates the C1 cells.

C1 Cells and Disease

C1 cells degenerate in multiple system atrophy (MSA) and advanced Parkinson’s disease (PD), two conditions associated with postural hypotension (20, 21). Congenital central hypoventilation syndrome (CHHS) is a developmental disease caused by mutations of the transcription factor Phox2b and characterized by sleep apnea and the loss of the respiratory chemoreflexes (194). A defect in the number or connectivity of the C1 neurons may contribute to the dysautonomias also experienced by CHHS patients because the development of the C1 neurons is Phox2b dependent (49).

Resting SNA is mildly elevated in human diseases associated with hypertension such as essential hypertension, obesity,
and obstructive sleep apnea (51, 56) and severely elevated in congestive heart failure (55, 96). An increase in the resting activity of the C1 cells and other presympathetic neurons of the RVLM could plausibly contribute to the increased SNA in such diseases (9, 78). Chronic baroreceptor stimulation, which presumably decreases the activity of the C1 cells in the conscious state as under anesthesia, produces a sustained AP reduction in experimental animals and in humans with drug-refractory essential hypertension (106). In human heart failure, the overflow of catecholamine metabolites from the brain is enhanced suggesting that the release of CNS norepinephrine, and possibly epinephrine, is elevated (94).

In theory, a pathological increase in C1 neuron activity could have three causes. The most plausible is a change in the activity of the neurons that regulate the C1 cells. Indeed, abnormal activity within the PVH, the NTS, and the carotid bodies contributes to various forms of hypertension (4, 8, 192) and heart failure is associated with excessive carotid body activity and changes in the activity of cardiopulmonary and muscle afferents (e.g., 142). A second possible cause of C1 cell hyperactivity, also supported by experimental evidence, may be some change in the local environment of these neurons, perhaps because of microvascular inflammation or glial activation (81, 83, 89, 117, 200). For example, endothelial nitric oxide (NO) synthase (eNOS) overexpression in RVLM, which plausibly increases local blood flow causes an especially large hypotension in spontaneously hypertensive rats of the stroke prone variety (SHRSP) (88), whereas excessive levels of radical oxygen species in the RVLM seem to contribute to hypertension in this strain (89). In these models, there is little evidence that the changes in NO or reactive oxygen species (ROS) are specifically associated with the C1 cells and, in heart failure, signs of CNS inflammation are widespread and involve many regions that control the RVLM, e.g., the PVH (58). The “selfish brain hypothesis” of hypertension is a variation on the theme of the Cushing response (32). The idea is that flow restriction within the brain stem vasculature, including the VLM and NTS, caused by compression of hypertrophied vessels or by an inflammatory process that reduces the arteriolar lumen, produces local hypoxia or some other biochemical change leading to neuronal activation and ultimately activation of the sympathetic outflow, in other words a graded Cushing response that restores brain blood flow (32). Finally, the activity of the C1 neurons could theoretically be upregulated by a change in their intrinsic properties. Consistent with this possibility the RVLM seems hyperreactive to angiotensin II in several hypertension models (9, 83). This last evidence implicates the C1 cells perhaps more directly in the pathological process because, in the RVLM, angiotensin-1A receptors (AT1R) are predominantly expressed by these neurons (34).

In brief, C1 cell damage may contribute to the dysautonomias present in several degenerative diseases (MSA and PD) and, possibly, to one developmental disease (CCHS). C1 cell hyperactivity is a plausible albeit not directly demonstrated factor contributing to the sympathetic hyperactivity associated with hypertension and heart failure. The postulated hyperactivity of the C1 neurons could be caused by an altered balance of synaptic inputs. Local factors such as vascular inflammation and increased local ROS production and changes in the intrinsic properties of these cells (e.g., increased AT1R expression) may also contribute to their hyperactivity in hypertension.

In conclusion, the C1 neurons regulate the HPA axis via direct projections to the PVH and possibly by more circuitous routes. The C1 neurons also regulate both the sympathetic and parasympathetic nervous systems via direct projections to the preganglionic neurons. Only a third of the C1 cells are presympathetic and a large proportion of these presumably regulate the circulation. The presympathetic C1 neurons target several pontomedullary and midbrain regions in addition to the IML. Therefore, in the conscious state, their effects on the circulation and other physiological functions probably result from their action on a network of interconnected structures. Finally, the C1 cells that do not innervate the spinal cord likely also regulate AP and SNA via their projections to the dorsomedial nucleus of the hypothalamus, the midbrain central gray area, the parabrachial nuclei, and the PVH. The pre-sympathetic C1 cells that regulate epinephrine release and glucocorticoid responses presumably also regulate networks rather than just pre-sympathetic autonomic or PVH neurons.

The control of the parasympathetic system is the least understood aspect of the function of the C1 cells. The C1 projection to the DMV is dense and presumably originates from the IVLM. These C1 neurons likely regulate GI motility and GI hormonal secretions and could conceivably also influence cardiac performance and the immune system.

The predominant short-term synaptic effect of the C1 cells is an excitation that is mediated by conventional ionotropic glutamatergic transmission. The C1 cells synthesize catecholamines and inhibitory as well as excitatory neuropeptides but the circumstances of their release and the resulting cellular effects need clarification.

Based on neuroanatomical evidence, Loewy and colleagues (85) proposed that many C1 cells have widely divergent projections and are wired to produce generalized increases in SNA that are not limited to any particular vascular bed or even peripheral organ. Remarkably, the C1 cells are also wired to produce a generalized increase in CNS noradrenergic release. CNS noradrenergic neurons have both a support role (astrocyte stimulation, blood flow control, microglia stimulation) and a neuroeffecter role (modulation of neuronal activity) (132). The same duality (metabolic role and circulatory control) applies to catecholamines released by the peripheral sympathetic system, for example, in skeletal muscles (72, 84). Activation of the locus coeruleus by the C1 cells may therefore help preserve forebrain function during hypotension, hypoxia, and hypoglycemia by stimulating glial metabolism and cerebral blood flow above and beyond the level that would be required by local neuronal activity in the absence of such systemic perturbation (185). This level of regulation could potentially complicate the interpretation of functional magnetic resonance imaging. Forebrain neuron activation, the other expected consequence of activating the locus coeruleus by the C1 cells, may help heighten arousal or attention in situations associated with injury or other bodily harm (15). Additionally, activation of the C1 cells, e.g., by LPS or circulating interleukin-1 as would happen during injury and sepsis presumably has adaptive immunological consequences that are mediated by the central noradrenergic system (microglia stimulation) (132), by the release of ACTH and, possibly, by activation of the parasympathetic system. Finally, the activation of selected lower brain stem noradrenergic neurons by the C1 cells may have more specialized effects such as to enhance the release of oxytocin.
and vasopressin from magnocellular neurons under conditions such as hypotension and hemorrhage.

The number of functional subtypes of C1 cells is not known. Figure 6 is a highly hypothetical attempt to illustrate how the differential recruitment of 12 subsets of C1 cells by hypotension, hypoxia, diving, or hypoglycemia might produce a response pattern adapted to each situation. The number of C1 cell groups is arbitrary. There could be fewer subsets than represented. For example, the CNS noradrenergic neurons may receive collaterals from glucosensitive and or barosensitive C1 cells and breathing could also be activated by collaterals of baro- and hypoxia-sensitive presympathetic neurons. On the other hand Fig. 6 does not consider the pattern elicited by infection and inflammation, which may engage additional cell types. From a neurophysiological standpoint, the presympathetic C1 neurons include at least four types of neurons that have variable influence over the heart, skeletal muscle arterioles, gut arterioles, the kidney, and the adrenal medulla. Three of them (muscle, gut, and cardiac types, Fig. 6) are barosensitive and are differentially activated or inhibited by such factors as GI hormones (e.g., CCK) and the respiratory pattern generator (66). These “cardiovascular” neurons are activated by carotid body stimulation but are presumably not directly influenced by hypoglycemia. A fourth type of presympathetic C1 neurons may be dedicated to the control of epinephrine-releasing chromaffin cells. These neurons are probably less numerous than those that control the circulation. They respond to hypoglycemia and likely to hypoxia but not to hypotension (Fig. 6). With exceptions (at least 10% of the total), the C1 neurons that innervate the hypothalamus are different from the presympathetic ones and one would think that they are as functionally diverse as the presympathetic C1 neurons but there is little evidence of this yet. Figure 6 depicts ACTH release as being triggered by a single class of C1 neurons that respond indifferently to hypotension, hypoxia, hypoglycemia but this assumption has not been tested. The number of C1 cell subtypes that innervate the DMV (two are represented in Fig. 6) is equally uncertain.

The A1 cells are likely postsynaptic to the C1 neurons and these interactions are not reciprocal because the A1 cells do not seem to innervate the C1 region (111). Also, VGLUT2 is not detectable in the terminals of the A1 neurons (52), whereas the norepinephrine transporter is generally absent from the C1 cells (11).

The SNA hyperactivity observed in hypertension, heart failure, and other diseases is of CNS origin and plausibly partly mediated by an increase in the rate of discharge of the C1 cells but the evidence is still circumstantial. In human heart failure, CNS catecholaminergic systems do seem hyperactive (94), although the measured metabolites probably originate from the quantitatively dominant noradrenergic terminals rather than from the C1 cells. A comparable increase in CNS catecholaminergic turnover is produced in humans by ganglionic blockade (125) a stimulus that almost certainly activates the C1 cells via baroreceptor unloading. These observations are consistent with our hypothesis that the C1 cells upregulate the activity of the CNS noradrenergic neurons.

**Perspectives and Significance**

The C1 cells are activated in response to physical stresses such as pain, hypoxia, hemorrhage, infection, inflammation, hypotension, and hypoglycemia and many C1 cells are also activated by psychological stress (99). C1 cell activation increases the release of norepinephrine both peripherally and centrally with broad and presumably adaptive consequences on the metabolism of various tissues and on the membrane polarization of excitable cells (neurons, vascular smooth muscle and glands). How severe these stresses need to be for the C1 cells to produce such responses is uncertain. The physical stresses to which experimental animals are typically subjected to reveal changes in C1 cell activity, for example, with the Fos expression method, are severe by human standards. Tissue injury, hemorrhage, pain, severe hypoxia, and bacterial infection (LPS) are clearly accidental or pathological events. The physiological responses elicited by C1 cell activation facilitate survival by preventing cardiovascular collapse, maintaining oxygenation of essential organs, and providing tissues with a readily usable energy substrate, glucose, hence the concept of C1 neurons as EMTs proposed in this review. The postulated
“emergency” nature of their physiological role is also in keeping with the fact that very extensive lesions of the C1 cells produce only minor effects on AP but impair bodily responses to severe acute hypotension.

Many fundamental questions regarding the biology of the C1 neurons remain unanswered. The developmental lineage of these neurons is unexplored. By analogy with on-going work on the serotonergic system (86), specific combinations of transcription factors presumably govern the differentiation of subsets of medullary catecholaminergic neurons. Such studies may clarify whether the RVLM presympathetic C1 and non-C1 neurons, which appear to have many common structural and physiological features, are developmentally related. The catecholaminergic phenotype of the presympathetic C1 neurons could conceivably be a vestige of evolution that may have disappeared altogether from the presympathetic neurons referred to as the non-C1 cells. Indeed the latter neurons have the same location and transmitters (glutamate, enkephalin, PACAP, lack of NPY), similar projections and inputs as a subset of C1 presympathetic cells (174). The only notable difference is that they lack (or express undetectably low levels of) catecholamine biosynthetic enzymes.

The functional differences between A1 and C1 neurons, especially between the A1 and the caudal C1 cells, are still incompletely delineated. There appear to be fundamental differences between the biology of these neurons (e.g., A1 cells lack VGLUT2), but the work of Lipski and others (40) suggests that transitional phenotypes between A1 and C1 neurons exist (e.g., neurons that express both norepinephrine transporter, NET, and PNMT). Also there is very little information concerning the role of the C2 and C3 adrenergic neurons besides evidence that they target some of the same CNS neurons as the C1 cells (e.g., 105).

RVLM presympathetic neurons can discharge up to 35 Hz under anesthesia if AP is low. A reduction in GABAergic inhibition contributes to this activation and presumably underlies the tremendous increase in SNA that is unleashed by hypotension in conscious mammals, humans included. However, disinhibition does not explain why neurons become active and the source of the excitatory inputs to RVLM presympathetic neurons is an enduring mystery. The use of retrograde transynaptic vectors (rabies, pseudorabies) may help identify these excitatory synaptic inputs (28). Further understanding of the local factors that regulate the activity of the C1 neurons (glutamatergic, oxygen, blood vessel-derived factors) will probably also be critical.

One would also want to find out how many subtypes of C1 neurons there are and which brain neurons are targeted by each C1 cell subtype. However, this information must be complemented by a thorough understanding of the inputs of each subtype of C1 cell. Based on Fos studies, a very large fraction of the C1 cells can be activated by a single stimulus such as hypoxia, pain, LPS, and psychological stress suggesting that each of these stimuli may cause a broad activation of the sympathetic system and of the brain’s noradrenergic system. Other stimuli may selectively activate much smaller subsets of C1 cells thereby enabling organ-specific changes in SNA.

In this review we proposed that the C1 cells intervene mostly under “emergency” situations. This view largely derives from the nature of the stimuli known to increase their activity (pain, hemorrhage, bacterial infection, hypoxia, hypoglycemia). However, subsets of C1 cells may also contribute to homeostasis under more physiological conditions. This could be the case of the highly barosensitive RVLM presympathetic C1 cells that control the vasoconstrictor tone, the heart, and ultimately AP. Opto/pharmacogenetic experiments in conscious animals may provide answers to these questions. Finally, the increased SNA associated with hypertension and heart failure (94) could be caused in part by an increase in the discharge rate of RVLM presympathetic C1 cells. This also remains to be demonstrated in conscious animals.

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REFERENCES


97. Llewellyn-Smith LJ, Minson JB, Filowsky PM, Chalmers JP. There are few catecholamine- or neuropeptide Y-containing synapses in the...
intermedio-lateral column of rat thoracic spinal cord. 


