Journal Club

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Under What Circumstances Do Rostral Ventrolateral Medulla Neurons Support Blood Pressure?

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Review of Wenker et al.

Arterial blood pressure is tightly regulated to ensure that tissues are optimally perfused in accordance with metabolic demand. Chief among the effectors that acutely regulate arterial pressure are a class of sympathetic postganglionic neurons that innervate vascular smooth muscle; the tonic activity of these neurons underlies the vascular tone that maintains arterial pressure (Guyenet, 2006). This group of sympathetic efferents is highly sensitive to stimuli that threaten tissue perfusion and oxygenation, such as acute fluctuations in arterial pressure and disturbances in blood-gas status (i.e., too little O_2 or too much CO_2). Unraveling the CNS circuits responsible for setting sympathetic vasomotor tone at rest and in response to homeostatic perturbations (e.g., hypotension, respiratory disturbance, and dehydration) remains an ongoing goal of autonomic neuroscience.

One brain region that has been intensively studied with respect to cardiovascular regulation is the rostral ventrolateral medulla (RVLM). RVLM neurons project to spinal sympathetic preganglionic neurons and have discharge properties consistent with a role in blood pressure control: they fire in phase with the cardiac cycle and respond to hypoxia (low O_2) and hypercapnia (high CO_2) (Brown and Guyenet, 1985; Koshiya et al., 1993; Moreira et al., 2006). The majority of spinally projecting ("presympathetic") RVLM neurons are both glutamatergic and peptidergic, and some (C1 neurons) also synthesize catecholamines (Stornetta, 2009). In anesthetized animals, bilateral pharmacological inhibition of RVLM neurons substantially reduces (by −50 mmHg) arterial pressure and eliminates most sympathetic reflexes, strongly suggesting that the RVLM is a critical hub for the generation of sympathetic vasomotor tone and integration of autonomic reflexes (Guertzenstein and Silver, 1974; Reis et al., 1989; Schreihofer et al., 2000). However, it is unclear whether this high level of control is maintained in the conscious state and also whether C1 and non-C1 neurons are differentially involved.

In a recent article published in *The Journal of Neuroscience*, Wenker et al. (2017) used loss-of-function optogenetics to investigate the role RVLM C1 and other RVLM neurons play in the acute regulation of arterial pressure. The authors used two different viral vectors to introduce ArchaerhodopsinT3.0 (ArchT), an inhibitory light-gated proton pump (Han et al., 2011), into overlapping populations of neurons in the ventrolateral medulla oblongata. In one cohort of rats, C1 neurons were targeted with a lentivirus that expressed ArchT under the PRSx8 promoter (PRSx8-ArchT), which preferentially targets neurons that express the Phox2b transcription factor (Abbott et al., 2009; Kanbar et al., 2010), ~60% of which are also C1 neurons. This vector transduced ~50% of the total number of RVLM C1 neurons but also transduced nearby non-catecholamingeric neurons, which were presumably Phox2b neurons in the retrotrapezoid nucleus. Another cohort of rats received an adeno-associated virus that expressed ArchT under the control of the CaMKII promoter (CaMKII-ArchT). This vector produced a less selective transduction pattern, with reporter expression detected in many more neurons overall, including glutamatergic and GABAergic neurons, but far fewer C1 neurons (only ~23% of the total population). Thus, both vectors transduced a combination of C1 and non-C1 neurons, but the PRSx8-ArchT cohort contained a smaller overall number of transduced neurons, a high proportion of which were C1 cells, whereas the CaMKII-ArchT cohort contained a larger overall population, fewer of which were C1 cells.

Wenker et al. (2017) first examined the contribution of C1 and other RVLM neurons to resting sympathetic vasomotor tone by delivering a 10 s light stimulus to the RVLM region in conscious rats and quantifying the peak change in arterial

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pressure, which acts a proxy measure of sympathetic vasomotor tone over this short time-scale. Photoinhibition of RVLM neurons in PRSx8-ArchT-treated animals only slightly (−4 mmHg) decreased arterial pressure, qualitatively similar to the effect of neurotoxic lesion of C1 neurons in normotensive animals (Madden and Sved, 2003; Menuet et al., 2017). Although it is possible that the relatively low number of C1 neurons transduced might have contributed to the small blood pressure decrease reported by Wenker et al. (2017), these data nevertheless support the view that C1 neurons are not major contributors to sympathetic vasomotor tone in conscious, unchallenged animals. Photoinhibition also produced a relatively small reduction in blood pressure in animals that received the less-selective CaMKII-ArchT vector. Much larger drops in blood pressure (~14 mmHg for both PRSx8- and CaMKII-ArchT cohorts) were seen under isoflurane anesthesia, however. Wenker et al. (2017) suggest that the exaggerated blood pressure drop observed under anesthesia might reflect a direct excitatory action of isoflurane on presympathetic RVLM neurons and/or an anesthetic-induced suppression of the arterial baroreceptors, a fundamental source of inhibitory input to these neurons.

Together, these data indicate that excitatory drive to preganglionic sympathetic neurons is far less dependent on RVLM neurons in conscious than in anesthetized settings, and that the contributions made by C1 and non-C1 neurons is probably comparable. They therefore raise the possibility that other presympathetic sites located in the brainstem and/or the hypothalamus (Strack et al., 1989) exert a greater role in determining the resting level of sympathetic vasomotor tone than previously thought (Dampney et al., 2003). Among the other presympathetic regions of the brain, the paraventricular nucleus of the hypothalamus also appears to make a modest contribution to resting sympathetic vasomotor tone in the conscious state, with one study reporting decreases in arterial pressure of ~10 mmHg following administration of inhibitory compounds to the paraventricular nucleus in conscious rats (Martins-Pinge et al., 2012). Nevertheless, it seems unlikely that the RVLM and paraventricular nucleus generate the entirety of sympathetic vasomotor tone at rest, unless perhaps a synergistic interaction exists, which is conceivable given that these two nuclei are reciprocally connected and both express numerous neuromodulators (Guyenet, 2006; Stornetta, 2009).

Previous work has shown that, in addition to regulating resting sympathetic vasomotor tone, RVLM neurons mediate the sympathoexcitatory effect of hypoxia and hypercapnia in anesthetized animals (Koshiya et al., 1993; Takakura et al., 2011), but whether this function is mediated by C1 or other RVLM neurons is unresolved, with C1 lesion studies yielding conflicting results (Madden and Sved, 2003; Malheiros-Lima et al., 2017). To investigate this function, Wenker et al. (2017) acutely exposed PRSx8-ArchT- and CaMKII-ArchT-treated conscious rats to low ambient levels of O_2 or high CO_2 and analyzed the change in arterial pressure during light activation. They found that, regardless of what vector rats were treated with, illumination of the ventrolateral medulla produced marked reductions in arterial pressure during hypoxia and, to a lesser extent, during hypercapnia. The interpretation is that C1 and other RVLM neurons function to maintain sympathetic vasomotor tone during respiratory disturbance. However, an important caveat is that the PRSx8-ArchT vector also transduced neurons in the neighboring retrotrapezoid nucleus, a region responsible for sensing CO_2 and coordinating respiratory and sympathetic responses (Abbott et al., 2009; Takakura et al., 2011). Indeed, given that light activation during hypercapnia evoked respiratory depression in PRSx8-ArchT, but not CaMKII-ArchT, treated rats, it is likely that a functionally relevant number of retrotrapezoid nucleus neurons were transduced with this vector. Hence, it is unclear whether the destabilization of arterial pressure following light activation in hypercapnic PRSx8-ArchT rats is attributable to inhibition of C1 or retrotrapezoid nucleus neurons. As well as mediating the physiological response to hypoxia, C1 neurons are thought to coordinate a concurrent arousal response (Abbott et al., 2012). In the future, the authors might consider using a similar loss-of-function optogenetic approach to examine this possibility.

In conclusion, Wenker et al. (2017) show that C1 and other RVLM neurons make a surprisingly small contribution to the generation of sympathetic vasomotor tone in conscious steady-state homeostatic conditions but are recruited to stabilize arterial pressure during anesthesia and hypoxia. Recent evidence implicates C1 neurons in the development of neurogenic hypertension (Menuet et al., 2017). Given that the results reported by Wenker et al. (2017) suggest that C1 and other RVLM neurons function similarly in the regulation of arterial pressure in health, it would be valuable to examine whether non-C1 neurons also undergo maladaptive changes with the onset of hypertension.

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