Tonic glutamatergic drive of RVLM vasomotor neurons?

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THE ROSTRAL VENTROLATERAL MEDULLA (RVLM) plays a critical role in the maintenance of baseline arterial pressure (AP) in all species in which it has been examined, and it appears to be the primary site responsible for the tonic supraspinal drive of sympathetic vasomotor outflow and AP, at least in unanesthetized animals. For example, inhibition of the RVLM in anesthetized rats, cats, and rabbits reduces AP to the same extent as cervical spinal cord transection (6, 24), and this is accompanied by a marked reduction in sympathetic nerve activity (SNA) (6). RVLM neurons projecting to the region of sympathetic preganglionic neurons in the spinal cord are tonically active and respond to a variety of stimuli that impact AP (8). Although during the past 20 years many inputs to these RVLM vasomotor neurons have been described, the input or inputs responsible for their tonic baseline activity have remained uncertain (5, 24).

One of the initial hypotheses regarding the generation of tonic activity of RVLM-spinal vasomotor neurons must have been that tonically active glutamatergic inputs to the RVLM contributed to the activity of these cells, simply reflecting the fact that the vast majority of excitatory synapses in the mammalian central nervous system (CNS) use glutamate as a neurotransmitter. This hypothesis was quickly rejected because blockade of glutamate receptors in the RVLM by local injection of the glutamate receptor antagonist kynurenic acid (Kyn) had no effect on baseline AP in either anesthetized or conscious rats (1, 9, 15). Still, Kyn injected into RVLM did block key excitatory cardiovascular reflexes mediated through the RVLM, such as the chemoreceptor reflex and somatic pressor response (14, 16). Thus it became accepted that glutamate in the RVLM was not essential for the maintenance of the tonic activity of RVLM vasomotor neurons (at least in rats). However, Ito and Sved (13) suggested that maybe glutamate in RVLM had tonic but opposing actions, both exciting RVLM vasomotor neurons and exciting neurons that resulted in the inhibition of RVLM vasomotor neurons. To test this hypothesis, they first removed the known inhibitory input to the RVLM from the caudal ventrolateral medulla (CVLM) (20, 22) by injecting muscimol (Mus) into the CVLM, and then after AP increased they injected Kyn into RVLM. After injection of Kyn into the RVLM, AP decreased rapidly, falling substantially below the initial baseline values. These results were interpreted as indicating that glutamatergic inputs to the RVLM are tonically active and support both excitatory and counterbalancing inhibitory inputs to RVLM-spinal vasomotor neurons (13, 23), although it is possible that these glutamatergic inputs become active only after inhibition of the CVLM. This general response was observed in Sprague-Dawley rats, Wistar-Kyoto rats, and spontaneously hypertensive rats (11, 13). Ito and Sved suggested that the marked fall in AP resulted from decreased sympathetic vasomotor outflow after decreased activity of RVLM vasomotor neurons, but SNA was not measured directly in their experiments.

Now, Horiuchi, Killinger, and Dampney (10) have examined the impact on renal SNA (RSNA) of inhibiting the CVLM and then injecting Kyn into RVLM of anesthetized rats. The results of their study in this issue of the American Journal of Physiology-Regulatory, Integrative and Comparative Physiology (10) markedly conflict with the results of Ito and Sved, questioning the role of glutamate in the RVLM in the tonic maintenance of sympathetic vasomotor tone and AP in the rat. In contrast to the dramatic results reported by Ito and Sved (13), Horiuchi et al. (10) report that injection of Kyn bilaterally into RVLM after injection of Mus into CVLM does not reduce AP below the initial baseline values; although mean AP (MAP) decreased ~60 mmHg within 5 min after Kyn injection (from a level of ~175 mmHg), the resulting MAP was only ~25 mmHg less than that in rats injected with vehicle instead of Kyn and was still substantially above the initial baseline. Importantly, this decrease in AP produced by Kyn was not associated with a decrease in RSNA. Thus there are two noteworthy observations in this key experiment by Horiiuchi et al. (10). First, there is the failure to confirm the extent of the Kyn-evoked decrease in AP reported by Ito and Sved. Second, the decrease in AP that was observed was not associated with a decrease in RSNA.

There are several differences between the two studies that could account for the differential effects on AP. For example, the anesthetic regimen differed between the studies; Horiuchi et al. (10) used intraperitoneal urethane, whereas Ito and Sved used intravenous chloralose or intravenous urethane after induction of anesthesia with halothane. It is worth noting that intraperitoneal urethane produces a marked increase in plasma osmolality because it draws fluid into peritoneal cavity, and changes in plasma osmolality (and the resulting hormonal responses) may impact central neural vasomotor control (4). Possibly as a result of the different anesthetics, baseline MAP values differed significantly between studies (~90 mmHg in Horiuchi et al. vs. ~120 mmHg in Ito and Sved), as did baseline heart rates. Furthermore, most microinjection studies are done on artificially ventilated animals, and the respiratory state of the animal is often not well documented and might influence evoked cardiovascular responses. This highlights the fact that the starting conditions of the rats in the two studies were quite different, possibly reflecting different states of central neural control, and this may account for the different AP responses observed. As with any microinjection study, the anesthetic state and physiological condition of the animal may significantly impact the results and in terms of the hypothesis presented by Ito and Sved (13, 23) may change the relative balance of the excitatory and inhibitory drives.

Another difference between the two studies is the timing of the injections. Does it make a difference that injections are spaced closer together or farther apart? As this variable, like many variables in microinjection experiments, has not been
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systematically evaluated, it is impossible to know. Across the various laboratories conducting microinjection experiments, there are many subtle differences (often not described in the methods) that may influence the results. For example, the speed of injection is likely to be an important factor in the distribution of injected drug (19), but, as in the Horiuchi et al. (10) report, this parameter is often not described. Ideally, the physiological responses would be robust enough, like depressor and pressor responses evoked by glutamate injection into CVLM and RVLM, respectively, that the general observation is reproducible across all laboratories independent of subtle differences in technique. However, even with the well-established observation that glutamate injected into RVLM increases AP, the magnitude of that response varies greatly across reports in the literature. Of course there are many small differences in experimental protocol that may account for the quantitative differences in results. One that may not receive enough attention is the strain or substrain of rat. We have observed that the magnitude of the pressor response evoked by the injection of 1 nmol glutamate into the RVLM of chloralose-anesthetized Sprague-Dawley rats can vary from ~10 mmHg to >40 mmHg based solely on the source of the rat (i.e., different commercial suppliers) (C. J. Madden and Sved, unpublished observations).

Possibly the most critical difference between the two studies is the exact sites of the microinjections. In both studies the CVLM and RVLM sites were functionally identified by depressor and pressor responses, respectively, produced by microinjection of glutamate. However, even if the center of the microinjection sites is identical, differences in the angle of the head or the angle of the pipette or the design of the pipette tip or many parameters related to the injection itself may produce differences in the exact area impacted by the injection. Thus, although the center of the microinjection sites in the CVLM and RVLM may be the same in the two studies (and in the absence of exact landmarks it is impossible to know), the precise area over which neuronal function is inhibited by Mus injection or glutamate receptors antagonized by Kyn injection may vary significantly across studies. Injectate often moves up along the pipette track, and the pipette angles relative to the brain stem are very different for the CVLM injections in the two studies. Although the same dose of Mus was injected into the CVLM in both studies, the time course of the pressor response seemed to differ; Horiuchi et al. (10) comment that MAP peaked 3–8 min after placement of the injection on the second side, whereas in the Ito and Sved (13) experiment, MAP peaked within 3 min. Furthermore, although detailed data were not included in the reports, it appears that the increase in MAP evoked by Mus injection into CVLM was more sustained in the Ito and Sved study (13).

With any experiment, it is of concern when two laboratories do a very similar experiment and get very different results, but this is often the way that science moves forward. Certainly, until all the critical issues are identified and tested, the role of glutamate in maintaining the activity of RVLM vasomotor neurons will remain a question. Regarding this specific experiment, two laboratories have now weighed in. However, it is possible that many laboratories have tried this experiment and not reported the results because simple confirmatory or "failure to confirm" data sets are typically not published.

Horiuchi et al. (10) suggest that it is not possible to inhibit all sympathoinhibitory neurons in the CVLM by microinjecting Mus without affecting at least some RVLM vasomotor neurons. Diffusion of drugs to neighboring heterogeneous regions is an issue that cannot be ignored in microinjection studies and may have contributed to the different results obtained by Horiuchi et al. (10) and Ito and Sved (13). It may very well be that the precise area impacted by the CVLM microinjections is at the core of the different results between the two studies. The CVLM remains a rather poorly described heterogeneous area (20, 22), and the model proposed by Ito and Sved (13, 23) incorporated a new and rather unexplored aspect of this heterogeneity, a tonically active sympathoexcitatory component.

Horiuchi et al. (10) also performed the related experiment of blocking GABAergic inhibitory inputs to RVLM with local injection of bicuculline (Bic) before injection of Kyn; a similar experiment was previously reported by Miyawaki et al. (17). Bic injected into RVLM produced a large increase in AP, and then the subsequent injection of Kyn reduced MAP by 30 mmHg from ~160 mmHg (n = 7) in the study by Miyawaki et al. (17) but only by ~20 mmHg in three of five rats in the Horiuchi et al. report (10). Based on the model proposed by Ito and Sved (13, 23), the decrease in AP in this experiment should be less than that when Kyn is injected into the RVLM after inhibition of the CVLM; this appears to be what was observed. Ito and Sved also conducted an experiment using Bic to inhibit GABA inputs to RVLM before injection of Kyn, and the AP results were similar to those reported by Miyawaki et al. (17) (Ito and Sved, unpublished observation). Thus, after combined blockade of glutamate and GABA receptors in the RVLM a substantial level of sympathetic vasomotor tone appears to remain. Therefore, either a powerful tonic excitatory non-glutamatergic input remains, the RVLM vasomotor neurons exhibit autoactivity under these unusual conditions, or the microinjections do not sufficiently block all glutamate and GABA receptors on RVLM vasomotor neurons. The Ito and Sved data (13, 23) would suggest that the critical excitatory input to the RVLM arises from the vicinity of the CVLM and was not inhibited by the Mus injections in the Horiuchi et al. study (10).

Horiuchi et al. (10) also report that the decrease in AP that they do observe in response to Kyn injection into RVLM after Mus injection into CVLM is not accompanied by a decrease in RSNA, and they conclude therefore that the change in AP may be independent of a change in sympathetic vasomotor outflow. However, the observation by Ito and Sved that heart rate decreased along with AP (at least with urethane anesthesia) after Kyn injection into the RVLM of CVLM-inhibited rats is consistent with a decrease in cardiac SNA. Maybe these cardiovascular responses were not mediated by the renal nerve, but that is not to say that all vasomotor responses will be reflected by changes in RSNA. As one notable example, Deering and Coote (7) reported that the increase in AP elicited by stimulation of the hypothalamic paraventricular nucleus was accompanied by an increase in splanchnic, cardiac, and adrenal SNA but a decrease in RSNA. It is clear that recording only a single sympathetic nerve can lead to markedly erroneous conclusions regarding sympathetic outflow. Interestingly, Miyawaki et al. (17) observed that the fall in AP that occurred with injection of Kyn into the RVLM after Bic injection was accompanied by
decreases in lumbar and splanchnic SNA, although RSNA was not measured in that study. It must be noted that all sympathetic vasomotor nerves are not controlled by identical neural pathways (18, 21). Thus, as noted by Horiuchi et al. (10), it is possible that the tonic activity of RVLM neurons regulating sympathetic outflow to non-renal vascular beds may be more dependent on glutamatergic inputs than is the case for RVLM neurons controlling RSNA.

So where does this leave us regarding understanding the role of glutamate in maintaining the tonic activity of RVLM vasomotor neurons? Obviously all the answers are not available and further examination of this issue, using more than just micro-injection approaches, is essential. The Horiuchi et al. (10) report highlights how complicated the system is and how much more we still have to understand. However, whatever the role of glutamate in the maintenance of sympathetic vasomotor tone may be in anesthetized rats under baseline conditions, it is becoming increasingly clear that glutamate in the RVLM is important for the maintenance of AP in various rat models of hypertension (2, 11, 12) and physiological states (3, 4), as simple injection of Kyn into the RVLM lowers AP under these conditions.

REFERENCES