Tonic drive to sympathetic premotor neurons of rostral ventrolateral medulla from caudal pressor area neurons

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Campos, R. R., and R. M. Mcallen. Tonic drive to sympathetic premotor neurons of rostral ventrolateral medulla from caudal pressor area neurons. Am. J. Physiol. 276 (Regulatory Integrative Comp. Physiol. 45): R1209–R1213, 1999.—The responses of sympathetic premotor neurons in the rostral ventrolateral medulla (RVLM) to activation or inactivation of neurons in the caudal pressor area (CPA) were studied in urethane-anesthetized rats. Extracellular recordings were made from 32 barosensitive single units in the RVLM, of which 26 were antidromically activated from the cervical cord. Unilateral microinjections of L-glutamate (0.5–5 nmol) into the CPA increased firing in 13 of 14 premotor neurons by 90 ± 30% while raising blood pressure. Both ipsilateral and contralateral injections were effective. Unilateral or bilateral inhibition of CPA neuron activity by microinjecting glycine (5–200 nmol/side) lowered blood pressure, while it reduced firing in 9 of 10 and 16 of 17 premotor neurons, respectively, by 45 ± 9 and 39 ± 6%. A significant proportion of tonic activity in RVLM sympathetic premotor neurons is thus driven, directly or indirectly, by neurons in the CPA.

SYMPATHETIC VASOMOTOR TONE depends critically on the tonic activity of a restricted group of bulbospinal neurons, the sympathetic premotor neurons of the rostral ventrolateral medulla (RVLM) (5, 9). When they are inhibited, blood pressure falls to low levels (5, 9, 19, 20), whereas when they are activated, they cause widespread increases in sympathetic activity to the heart and blood vessels (16, 19). These and other observations have led to the view that their activity is essential for the maintenance of vasomotor tone.

What is controversial, however, is the question of what supports the tonic basal activity of these neurons. Recently, two major views were proposed. The first is that their resting activity is derived from intrinsic membrane properties, which support pacemaker spiking even in the absence of synaptic drive (9, 11, 13). The second is that these neurons are driven by others in the brain stem, perhaps acting as part of an oscillating network (7). New evidence from intracellular recordings made in vivo has prompted the view that normal ongoing spiking activity of these neurons is largely or completely driven by synaptic inputs (15). Whether or not this proves to be the whole story, the sources of that excitatory synaptic drive are an important matter to be resolved.

Most vasomotor drive evidently originates in the brain stem (1, 5). Yet although inhibiting neurons in the RVLM itself causes a strong fall in vasomotor tone, this cannot be said for most other brain stem regions. Many can phasically excite vasomotor pathways, but few appear to provide resting tone. An exception, however, is the caudal pressor area (CPA) at the extreme caudal part of the ventrolateral medulla (CVLM). Bilateral inactivation of its neurons was recently found to lower blood pressure by almost as much as inhibiting the RVLM (4, 18). Possas and colleagues (18) went on to suggest that CPA neurons may act by tonically driving the premotor neurons of the RVLM. The present paper addresses that issue.

METHODS

Experiments were performed in 24 male Sprague-Dawley rats (250–400 g). They were approved by the Animal Experimentation Ethics Committee of the Howard Florey Institute and carried out according to National Health and Medical Research Council guidelines. Animals were first anesthetized with thiopental sodium (50 mg/kg ip), given a tracheostomy, and then ventilated artificially with 2% isofluorane in oxygen. Rectal temperature was kept close to 37°C with a heating blanket. The right carotid artery and jugular vein were cannulated, the former being connected to a blood pressure transducer.

A pneumatic cuff was placed around the lower thoracic aorta, via a left intercostal incision. The rat was then mounted prone in a stereotaxic frame with the head mildly ventroflexed (bite bar at −11 mm). The occipital bone was opened over the dorsal medulla and caudal cerebellum, and the dura was retracted. The cervical spine was held horizontal and under mild tension by a thoracic spinal clamp. A laminectomy then exposed three lower cervical cord segments into which a concentric bipolar stimulating electrode was inserted by a micromanipulator to stimulate the left dorsolateral funiculus. The mandibular branch of the left facial nerve was exposed via a small incision, and a second concentric bipolar electrode was positioned to stimulate it.

After surgery, the isofluorane anesthesia was gradually withdrawn while being replaced by urethane (given intrave-
nously as a 25% solution). The urethane was administered a small quantity at a time, titrating the dose necessary to suppress withdrawal reflexes to noxious pinching (final dose 1–1.4 g/kg in different animals). After ensuring that this level of anesthesia had stabilized under urethane, the animal was then paralyzed with pancuronium bromide (1 mg/kg iv). The anesthetic level was retested when paralysis were off 1–2 h later, and extra urethane (0.1–0.3 g/kg iv) was given if needed. Only after adequate anesthesia had been confirmed were repeat doses of pancuronium given.

Glass recording micropipettes with a long shank were pulled from 1.5-mm diameter borosilicate tubing and bumped to tip sizes of 5 µm (for field potentials) or 1–3 µm (for single units). They were filled with either 2 M NaCl or 2% Pontamine sky blue dye in 0.5 M sodium acetate. Micropipettes were inserted vertically via the cerebellum into the brain stem with a micromanipulator. Neural activity from the pipette was amplified and filtered (high pass 100–500 Hz, low pass 3 kHz). A time window discriminator detected unitary spike potentials, while spike waveform and discrimination were continuously monitored. The filtered signal was led, along with the blood pressure transducer, to an instrumentation tape recorder. Discriminated spike pulses and blood pressure were also recorded and analyzed on computer (“1401plus” interface and “Spike2” program, Cambridge Electronic Design, Cambridge, UK).

The caudal pole of the facial nucleus (CP7) was located from its antidromic field potential on facial nerve stimulation, as described by Brown and Guyenet (2). Sympathetic premotor neurons were recorded from ~0–400 µm caudal to CP7 and 1.6–1.9 mm lateral to the midline. Recording sites were later marked by passing 5–10 nA of negative current for 5–10 min through the recording electrode to deposit Pontamine blue dye. Spinal axons were demonstrated by electrical stimulation in the spinal cord (bipolar, 0.2-ms stimuli at 5–10 Hz). The caudal pressor area (CP7) was located 1–2 mm caudal to the calamus scriptorius, 1.5–1.6 mm lateral to the midline, and 2 mm deep to the dorsal medullary surface. If a site proved ineffective, micropipettes were moved either rostrally or caudally until an effective site was located. They were then either left in situ for the remainder of the experiment or (in most cases) removed between injections but reinserted to the same locus for subsequent tests. Injection sites were marked either by including a small admixture of rhodamine-tagged latex microspheres (LumaFluor, Naples, FL) to the injectate or by subsequently injecting 30 nl of 2% Pontamine sky blue solution into the same site.

The Spike2 program generated histograms of the spike count of the neurons in 5- or 10-s time bins. The cumulative sum test was first used to detect whether the bin count diverged from control levels, taking P < 0.01 to indicate significance (17). The percentage change was then calculated from a 10-s stretch during the response peak (or nadir) and compared with the prestimulus control period. Injections were generally repeated, and the largest percentage response of the neuron was taken. Linear regression was used to correlate responses of neurons with their background firing rates, taking P < 0.05 to indicate a significant relationship.

At the end of the experiment, animals were killed by an overdose of pentobarbital sodium (100–200 mg iv). The brain stem was then removed and fixed by immersion for at least 24 h in 4% paraformaldehyde solution. Transverse 40-µm frozen sections were cut and mounted. Marks of injection and recording sites were located by conventional microscopy on unstained sections, using dark-field optics to define structures. They were traced from projected images at ×30 magnification and mapped onto sections drawn from the appropriate brain stem level.

RESULTS

Extracellular single-unit recordings were made from 32 single barosensitive neurons in the retrofacial part of the RVLM. The experimental arrangement and marked recording sites are shown in Fig. 1, A and B, respectively. All 32 showed a pronounced decrease in firing rate during the pressor response to brief aortic constriction (Fig. 2A), and 26 of 32 were antidromically
activated from the lower cervical cord (Figs. 2B and 3B). Additionally, pulse-triggered histograms of their resting activity showed cardiac periodicity (Figs. 2C and 3C). Basal firing rates ranged from 0.9 to 46 Hz (mean 14.8 ± 11.7; SD; n = 32) and conduction velocities from 0.5 to 6.3 m/s (mean 3.2 ± 1.7; SD, n = 26): six were <2 m/s. All tested properties were in line with those previously established for this neuronal population in rats (2, 5, 9).

L-Glutamate (0.5–5 nmol) injected unilaterally into the CPA (marked sites shown in Fig. 1C) significantly increased the firing rates of 13 of 14 RVLM neurons to 90.0 ± 30.4% above baseline (mean ± SE, range 6–424%, n = 13) while blood pressure increased by 14.4 ± 1.7 mmHg. Figure 2 shows an example of this alongside the inhibitory response of the neuron to a similar rise in blood pressure produced by aortic constriction. Significant excitatory responses were obtained in six RVLM neurons from L-glutamate injections into the ipsilateral CPA, five from the contralateral CPA, and two from both sides. Lateral differences were not systematically investigated, however. A single neuron (not shown) gave the reverse response, decreasing its firing rate by 7% after L-glutamate injection into the contralateral CPA.

Ten RVLM neurons (9 also tested with CPA L-glutamate) were tested for their response to unilateral injections of 5–200 nmol glycine into the CPA (7 ipsilateral, 3 contralateral). The firing rate in 9 of 10 was significantly reduced by 44.8 ± 9.0% (mean ± SE, range 16–96%, n = 9), while blood pressure fell 9.6 ± 1.4 mmHg. An example is shown in Fig. 2A. The slow decline in baseline blood pressure before the injection is attributable to passive diffusion of glycine from the recently inserted pipette. The single exceptional premotor neuron (which also responded anomalously to glutamate) increased its firing rate by 13% when glycine was injected into the ipsilateral CPA.

In a separate experimental series, 17 RVLM neurons (all bulbospinal) were tested for their response to bilateral injection of glycine into the CPA. Firing rate was significantly reduced in 16 of 17 neurons by a
maximum of $38.6 \pm 6.2\%$ (mean ± SE, range 8–95%, $n = 16$), while blood pressure decreased by $21.0 \pm 2.9$ mmHg. An example is shown in Fig. 3. Here, again, a slow downward drift in blood pressure followed the insertion of glycine-filled pipettes into the CPA, but a rapid decline in both blood pressure and premotor neuron firing followed when 50 nl were injected bilaterally. Not shown is the response of the single exceptional neuron that increased its activity by 39% in response to this procedure.

The largest percentage responses to manipulations of the CPA were shown by slower-firing RVLM neurons. The correlation was statistically significant, although weak, in the case of responses to CPA glycine (unilateral and bilateral data combined: $r^2 = 0.16, P < 0.05; n = 27$) but not for responses to CPA L-glutamate ($r^2 = 0.16, P = 0.16; n = 14$).

As a control for the potential spread of injectate to more rostral regions, microinjections of 50–100 nmol glycine were also made bilaterally at sites ~1 mm rostral to the CPA, in the “depressor” region of the CVLM (9, 20). Three RVLM neurons that were previously inhibited by glycine injections into the CPA were all excited (by $117 \pm 65\%$, while blood pressure rose by $33 \pm 16$ mmHg).

**DISCUSSION**

It is now generally accepted that sympathetic premotor neurons of the RVLM are critical for conveying the ongoing excitatory drive that supports the activity of preganglionic vasomotor neurons (5, 9). What has been actively debated, however, is what determines the resting activity of these premotor neurons (and thereby most vasomotor tone). Important new findings indicate that, at least under experimental conditions in vivo, fast excitatory synaptic inputs are prominent in these neurons and appear to trigger their spiking activity (15). This may be contrasted with the situation in brain slices, where slow pacemaker potentials are dominant (9, 11, 13). This discovery begs the obvious question of where is the source, or sources, of that synaptic drive? This paper presents evidence that a significant proportion may derive from neurons in the CPA.

Although a number of brain stem regions, when activated, drive sympathoexcitatory pathways via the RVLM (5), only rarely have such regions been shown to support any tonic vasomotor drive. The critical evidence that blood pressure falls when their cell bodies are inactivated is lacking. Two exceptions, however, are the pontine reticular formation and the CPA. Hayes and Weaver (10) found that unilateral injections of glycine into a diffuse region of the pontine reticular formation caused the blood pressure of anesthetized rats to fall by ~30 mmHg. The effect was transient, lasting ~1 min; yet, surprisingly, repeat injections were ineffective (10) for a long time. Pontine neurons were inferred to provide tonic excitatory drive to premotor neurons in the RVLM via a glutamatergic synapse (12), but other mechanisms seem able to rapidly compensate for their function. The significance of this region for the support of vasomotor tone has thus been hard to assess, given the ephemeral nature of the response and its lack of a clear anatomic substrate.

The CPA was discovered in cats by Feldberg and Guertzenstein (6) and in rats by Gordon and McCann (8), although the latter investigators noted no fall in blood pressure after injections of inhibitory substances, perhaps because these did not cover enough of the region. An additional complication was that in cats, deep anesthesia was necessary to demonstrate the actions of the CPA (6). In normally anesthetized rats, however, Guertzenstein and colleagues (4, 18) later showed that bilateral inhibition of the CPA by either glycine or GABA lowered blood pressure by 30–40 mmHg and that the effect was similar in decerebrate, unanesthetized rats. In contrast to that from the pontine reticular formation, the hypotension evoked from the CPA lasted ~5 min and was repeatable (4). These findings were interpreted as showing that a significant proportion of vasomotor tone depends on the activity of CPA neurons. Because the pressor effect of activating CPA neurons could be blocked at the RVLM (by injecting glycine there bilaterally), the further suggestion was made that CPA neurons act via RVLM sympathetic premotor neurons (18). The present findings add direct support for that conjecture.

In the present study, sympathetic premotor neurons were inhibited on average by ~40% when neurons in the CPA were inactivated, whether unilaterally or bilaterally. Technical factors may have made this an underestimate of the tonic drive of the CPA neurons, however. First, the glycine microinjected in these experiments may have missed the optimum CPA site on either or both sides. It may have spread rostrally to involve some of the depressor neurons of the CVLM (9, 20), whose actions directly oppose those of the CPA (see RESULTS). Second, slow leakage of glycine while pipettes were left in situ could have already lowered CPA neuron activity before the deliberate microinjection was made. Third, no allowance was made for the responses of the premotor neurons being offset by baroreceptor unloading during the fall in blood pressure. This may be why the mean premotor neuron responses to unilateral and bilateral glycine injections into the CPA were similar, whereas the falls in blood pressure differed by a factor of two. Baroreceptor reflexes may also have contributed to the anomalous responses shown by 2 of the 32 premotor neurons.

It is perhaps not surprising that the more slowly firing premotor neurons showed a greater percentage drop in activity when the CPA was inhibited by glycine. Possibly the faster firing neurons receive more excitatory drive from sources other than the CPA, and this may contribute to their higher resting activity.

We cannot yet say whether CPA neurons drive sympathetic premotor neurons monosynaptically, via indirect projections, or both. Few anatomic details are known about the connections of this caudalmost region of the medulla. But a direct glutamatergic connection seems unlikely: bilateral injections of the nonselective glutamate antagonist kynurenate into the RVLM block...
neither pressor nor depressor actions elicited from the CPA (4). Nor, significantly, do they have much effect on resting vasomotor tone and blood pressure (9). Nonglutamatergic synapses may thus be implicated in both cases. Nor can we eliminate other (nonexclusive) possibilities, such as that CPA neurons raise the excitability of RVLM neurons by means other than fast excitatory postsynaptic potentials or that their mode of action is by tonic disinhibition, mediated perhaps by the depressor neurons of the CVLM (4). These details await direct study.

In summary, a significant fraction of the resting activity of sympathetic premotor neurons in the RVLM is removed when neuronal cell bodies in the CPA are inhibited. Neurons in the CPA are therefore strong candidates for one of the sources of the synaptic drive that supports the resting activity of sympathetic premotor neurons of the RVLM and thereby vasomotor tone.

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