The caudal pressor area of the rat: its precise location and projections to the ventrolateral medulla

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Sun, Wei, and W. Michael Paneton. The caudal pressor area of the rat: its precise location and projections to the ventrolateral medulla. Am J Physiol Regul Integr Comp Physiol 283: R768–R778, 2002; 10.1152/ajpregu.00184.2002.—Investigators have demonstrated pressor areas in the medullas of various species. The present study precisely localized the pressor area in the caudal medulla of the rat and determined its projections to the caudal and rostral ventrolateral medulla. The caudal medulla first was mapped grossly in rats with injections (30 nl) of glutamate (30-, 15-, and 7.5-nmol doses) placed 0.5, 1.0, and 1.5 mm caudal to the calamus scriptorius, 1.0, 1.5, and 2.0 mm lateral to the midline, and 1.8, 1.7, and 1.6 mm ventral to the dorsal medullary surface, respectively, and their arterial pressures were recorded. One of these nine injections showed significant increases in arterial pressure. We micromapped this area with a total of 27 injections (30 nl) of glutamate (30-, 15-, and 7.5-nmol doses) placed 0.5, 1.0, and 1.5 mm caudal to the calamus scriptorius, 2.0 mm lateral to the midline, and 1.7 mm ventral from the dorsal surface of the medulla, induced significant increases in arterial pressure. The neuronanatomic connections of neurons in the CPA to the ventrolateral medulla were then investigated with iontophoretic injections of either the anterograde tracer biotinylated dextran amine (BDA) made into the CPA or the retrograde tracer FluoroGold (FG). BDA injections resulted in bouton-laden fibers throughout both caudal and rostral portions of the ventrolateral medulla. Either of the FG injections resulted in numerous spindle-shaped neurons interspersed between the longitudinal fiber bundles running through the CPA area. The proximity of the CPA neurons to the A1 catecholaminergic cell group is discussed.

cardiovascular; glutamate; micromapping; blood pressure; caudal medulla

It is well known that neurons located within the ventrolateral medulla constitute central elements for the generation and maintenance of vasomotor tone. For example, neurons located in the rostral ventrolateral medulla (RVLM) are important for the maintenance of blood pressure (13, 14, 27, 36, 53). Two views have been proposed concerning the basal tonic activity of RVLM neurons (16). One is that their resting activity is derived from intrinsic membrane properties, which support pacemaker spiking even in the absence of synaptic drive (27, 32, 37). The other is that these neurons are driven by other brain stem neurons (4, 39, 46).

Several reports have suggested a pressor area in the ventrolateral medulla located caudal to the obex, called herein the caudal pressor area (CPA), which when stimulated induces increases in blood pressure (6, 8, 10, 11, 19, 22, 31, 41, 47). Bilateral inactivation of CPA neurons with glycine, GABA, or muscimol induces a hypotension as does inhibiting the RVLM (8, 9, 30, 41, 47), but this counters data from another group (22). The CPA also might provide a tonic drive to the RVLM (7–9, 22, 31, 47) and thus be involved in supporting arterial pressure. In this regard, data have demonstrated that the cardiovascular responses to CPA stimulation depend on the functional state of RVLM neurons; increases in arterial pressure in response to CPA stimulation can be abolished by functional inactivation of the RVLM while stimulation of the RVLM increases arterial pressure during CPA inhibition (22, 47). Thus data gathered to date indicate that CPA effects are mediated through the RVLM and suggest that the CPA may be a major modulator of RVLM activity under physiological conditions (8, 9, 30, 41, 47). Natarajan and Morrison (41) recently concluded that the pathway from CPA to the RVLM involves an obligatory glutamatergic activation of sympathoexcitatory neurons in the vicinity of the caudal ventrolateral medulla (CVLM).

However, the neurons of the CPA have not been identified. We used small injections (10–30 nl) of the excitatory amino acid glutamate first to localize precisely the CPA in the caudal third of medulla of the rat. These data were used to guide our injections of biotinylated dextran amine (BDA) into the CPA to document projections into the ventrolateral medulla. Injections of the retrograde tracer FluoroGold (FG) into the RVLM and CVLM allowed us to determine the exact location and morphology of CPA neurons. Our data demonstrate that the CPA neurons exist in a rather narrow band between the caudal pressor area (CPA) and the RVLM.
nal end of the lateral reticular nucleus (LRN) and the medullary dorsal horn (MDH) at the level of the pyramidal decussation and that most CPA neurons are spindle shaped and oriented along a dorsomedial to ventrolateral axis. Preliminary reports of these data have appeared in abstract form (56, 57, 60).

MATERIALS AND METHODS

Forty-one adult Sprague-Dawley rats (275∼300 g; Harlan) were used in this study. All experiments were approved by the Animal Care Committee at St. Louis University.

Mapping studies. The rats were first anesthetized with an injection of ketamine hydrochloride (100 mg/kg ip), and then their femoral veins were cannulated with polyethylene tubing for the administration of drugs and further anesthesia. Anesthesia was maintained with urethane (100 mg·kg⁻¹·h⁻¹ after an initial dose of 300 mg/kg; Sigma) so that there were no responses to tail pinch and the blink reflex was absent. The femoral artery was cannulated, and through it arterial pressure was recorded with a Gould P23 strain-gauge transducer and amplified (Grass 7P122) on a Grass 7D physiograph. Heart rate was determined by counting systolic peaks on the arterial pressure trace. The trachea was transected, and a caudal-facing tracheal cannula was placed into it for spontaneous ventilation. Respiration was monitored with a low-pressure volumetric transducer (Grass PT5) and amplified (Grass 7P122). The animals then were placed in a stereotaxic device (Kopf Instruments). The brain stem was exposed via a dorsal incision through the atlantooccipital membrane, and injections were made into the caudal medulla to locate the CPA. The calamus scriptorius was used as our zero point reference.

In the gross mapping study, injections were made in 18 rats via a glass micropipette (25 μm OD) cemented to a 0.5-μl Hamilton syringe inserted into the stereotaxic unit at a 24-degree angle. This angle approximated the sloping caudal medulla and was relatively orthogonal to the brain stem, allowing for easier reconstruction of the micropipette tracks in the transverse sections. Nine injections of glutamate in saline (30 nl of either 30-, 15-, or 7.5-nmol doses), usually mixed with red fluorescent beads, were made per animal, with six rats in each group. Injections were placed in random order in a gridlike arrangement 0.5, 1.0, and 1.5 mm caudal to the calamus scriptorius at a depth 1.8, 1.7, and 1.6 mm (from the rostral to caudal row, respectively) ventral from the dorsal surface of the brain stem, and 1.0, 1.5, and 2.0 mm lateral to the midline (Fig. 1A). The interval between injections varied between 10 and 25 min, depending on when arterial pressure returned to control levels. Ten minutes after the injections, we injected 30 nl of either glutamate or saline into the spot showing the greatest pressor response in five of the rats. In three rats, reinjection of glutamate induced a pressor response similar to that seen previously, and these cases served as positive controls. In the two other rats, injection of saline induced no pressor response.

In the micromapping study, we used a pneumatic pico-pump (PV830 Pneumatic PicoPump, WPI) to make pressure microinjections of 5 nmol of glutamate (10 nl) in five additional rats. The volume injected in this group was based on the movement of the meniscus along the micropipette. The

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**Fig. 1.** The gross mapping study. A: schematic for glutamate injections (30 nl) used in the caudal third of the medulla of the rat to find pressor areas. Injections were made along 3 rows, each injection spaced 500 μm from its neighbor and either 1.8, 1.7, or 1.6 mm deep to the dorsal surface of the brain stem (from rostral to caudal, respectively). The most significant increase in blood pressure was at injection F, shown in red. “X” marks the calamus scriptorius. B: tracings of blood pressure (BP; top rows) and respiration (Resp; bottom rows) after a series of injections (arrows) of 15 nmol of glutamate into the caudal medulla of R1548. Note the large rise in BP after the injection in the panel labeled F (in red). C: photomicrograph of merged bright-field and fluorescent images of an effective injection of glutamate into spot F in case R1530. The injection appears reddish (outlined by oval) because of the fluorescent microspheres mixed with the glutamate solution. This injection was centered between the medullary dorsal horn (MDH) and caudal pole of the lateral reticular nucleus (LRN).
micropipette had a 0.58-mm ID with a tip of 20-μm OD; the glutamate was injected by calibrating the air pressure (≈20 psi) and the duration of injection (12 ms) of the piezopump. We focused on the area where arterial pressure was significantly increased in the gross mapping study. A series of 27 injections, nine per level separated by 300 μm, was made at the depths 1.1, 1.4, and 1.7 mm ventral from the dorsal surface of the brain stem (see Fig. 3).

After the experiments, the rats were perfused transcardially with phosphate-buffered saline and then a fixative of phosphate-buffered 4% paraformaldehyde. The brains were removed, soaked in 20% sucrose and fixative overnight, and then sectioned (50 μm), mounted on gelatinized slides, and counterstained for Nissl substance with either thionin or neutral red. Injection sites were visualized using a fluorescent and bright-field microscope (Nikon E-800). The physiological signals for heart rate, arterial pressure, and respiration had been transmitted to a computer through an interface [1401 plus; Cambridge Electronic Design (CED)] and were stored and analyzed using Spike 2 software (CED). In these mappings the interval were looking for immediate changes in arterial pressure after the glutamate injection. Arterial pressure and heart rate thus were measured for 5 s within a 10-s period both before and after the injections, and these data were compared. The data were analyzed using GB-Stat software with one-way ANOVA and followed by post hoc Fisher test, taking $P < 0.05$ to indicate significance.

**Neuroanatomic experiments.** Rats were first anesthetized with intramuscular injections of ketamine (100 mg/kg) and prepared for aseptic surgery. Anesthesia was maintained with intraperitoneal injections of pentobarbital sodium (40 mg/kg), as required. The animals were secured in a stereotaxic device (Kopf Instruments) in the prone position, and their brains were exposed via a dorsal incision. Micropipettes (20- to 25-μm OD) were filled with either 10% BDA (Molecular Probes; mol wt 10,000) or 2% FG (Fluorochrome, Englewood, CO) in saline. The CPA was approached using coordinates determined from the micromapping study (see above), confirmed with an injection of glutamate, and then iontophoresically injected with BDA in eight rats. The RVLM (just caudal to the facial nucleus; –2.8 mm to interaural zero) was injected with FG in six rats while the CVLM near the obex was injected in two rats. Both the BDA and FG solutions were injected iontophotically using 5-μA positive current pulses (7 s on/off) for 10–15 min using a Midgard constant-current device. The micropipette remained in place for 5 min to help prevent spread of the tracer. The wound was washed with sterile saline and closed with silk. After 5–7 days, the animals again were deeply anesthetized with pentobarbital sodium and perfused through the heart using a peristaltic pump (Masterflex), first with 300 ml of phosphate-buffered saline (pH 7.4), followed immediately by fixative of 4% paraformaldehyde in 0.1 M phosphate buffer. Brains and spinal cords were removed and stored in the fixative solution with 20% sucrose overnight at 4°C. The next day, frozen transverse sections were cut (40–50 μm) through the brain stem on a freezing microtome and serially collected in phosphate buffer. To visualize the BDA or FG, a 1:3 series was reacted. For the BDA cases, one series of sections was washed three times in 0.1 M phosphate and then soaked in buffer with 0.2% Triton for at least 5 min. The sections then were incubated in Vectastain ABC Elite solution (1:200) for 1 h, rinsed in phosphate buffer, and the BDA was visualized using diaminobenzidine dihydrochloride (DAB) intensified with nickel ammonium sulfate and reacted with 0.0003% hydrogen peroxide. For the FG cases, one series of sections was soaked in rabbit primary anti-FG antibody (Chemicon; 1:20,000) overnight at room temperature. The following morning the sections were washed three times in phosphate buffer with 0.2% Triton and then incubated 1 h in biotinylated goat anti-rabbit immunoglobulin (Sigma; 1:200). The sections were then washed, incubated in Vectastain ABC Elite solution for 1 h, and reacted with DAB per the BDA reaction. Also, two rats were perfused as above, and their brains were removed, sectioned, and similarly processed for immunoreactivity to tyrosine hydroxylase (Diasorin; 1:10,000) as described above. All sections were arranged serially, mounted on gelatinized slides, air-dried, dehydrated, counterstained with either neutral red or thionin, and placed under a coverslip with Permount. Results were viewed with a Nikon E-800 microscope and photos taken with a digital camera (Microimager II, QImaging) and Northern Eclipse software.

**RESULTS**

**Gross mapping studies.** Since cardiorespiratory neurons are located throughout the rostrocaudal extent of the ventrolateral medulla, we originally mapped the caudal half of the medulla, i.e., from the obex caudally. However, rostral to the level of the calamus scriptorius, the glutamate injections often caused depressor responses that often resulted in shifts of baseline arterial pressure. Because the series of injections was done in a random order with respect to their placement, such shifts were deemed problematic. We thus mapped only the most caudal third of the medulla, i.e., caudal to the calamus scriptorius, where the immediate increases in arterial pressure induced by glutamate injections were noted, and only these results are reported herein.

In the gross mapping study, 30 nl of glutamate injected at spot F1 (Fig. 1A) consistently induced immediate increases in arterial pressure when either 1.0 or 0.5 M concentrations were used. Mean arterial blood pressure (MAP) increases ranged from 11.4 ± 3.8 to 48.0 ± 8.3 mmHg (Fig. 2) after the nine injections of 30 nmol glutamate with the highest being at spot F1 ($P < 0.05$). Injections of 15 nmol glutamate induced MAP increases from 0.6 ± 5.6 mmHg to the largest increase of 20.5 ± 3.0 mmHg at the same spot (spot F; Figs. 1B and 2). This location also showed significant increases in MAP over the other eight injections (Fig. 2; $P < 0.01$). Control injections of saline into spot F were done in two cases, but neither induced any changes in arterial pressure, heart rate, or respiration. However, a repeat injection of glutamate into spot F at the end of three other experiments induced responses similar to those seen previously. Although injections of 7.5 nmol of glutamate into spot F often induced increases in arterial pressure, the changes were not significant compared with the other eight injections of this group, and these data are not shown. However, the greatest increases in arterial pressure were directly related to the concentration of glutamate used (Fig. 2C).

There were no significant changes in heart rate when preinjection rate was compared with that after injections of spot F. For example, heart rate was 311 ± 28 beats/min before injection of 15 nmol glutamate and 315 ± 28 beats/min after the injections. After some of these injections, both depth and rate of respiration...
increased. The histological sections of the brains from the gross mapping revealed the injections for spot F were located in the longitudinally running fibers situated between the MDH and caudal pole of the LRN (Fig. 1C)

Micromapping study. The micromapping study served to pinpoint the CPA as well as help determine whether its neurons are clustered or scattered. We focused on spot F in this set of experiments, where the gross mapping study had shown a significant increase in arterial pressure after the glutamate injections. At level I, 1.1 mm ventral to the dorsal surface of the brain stem, or 0.6 mm dorsal to the presumptive CPA (Fig. 3), there were no significant changes in MABP after injections of 5 nmol glutamate (Fig. 4, level I). Similar injections of glutamate at the level just 300 μm dorsal to spot F induced a large and significant increase in arterial pressure (Fig. 4, level II), whereas other injections at this level did not. Our spot F was centered in level III (Fig. 4), which is 1.7 mm ventral to the dorsal surface of the brain stem, 1.0 mm caudal to the calamus scriptorius, and 2.0 mm lateral to the midline. Injections of 5 nmol glutamate into this spot showed the highest increase of MABP (Fig. 4, III: \( P = 0.005 \), 1-way ANOVA). Interestingly, the increases after these 10-nl injections were comparable to those seen after the 30-nl injections of a similar concentration of glutamate (Fig. 2C). With the post hoc Fisher test, injections into spot F showed significant \( (P < 0.01) \) increases in MABP over 24 of 26 of the other injections; the site immediately dorsal to spot F in level II as well as the site immediately caudomedial to it in level III also showed significant increases in MABP (Fig. 4).

Comparing heart rate before and after the glutamate injections in the micromapping study showed that there was a significant change only at spot F, while all the injections surrounding the spot were insignificant. The average heart rate increased from 288 to 297 beats/min after injections into spot F in the five animals. Respiration was unaltered by glutamate injections in the micromapping study.

The transverse sections through the caudal medulla in some cases showed the micropipette tracks of the injections, which helped us elucidate the precise location of the CPA. Figure 3 shows three such tracks and the nine injections making the middle (−1.0 mm from calamus scriptorius) group of injections from adjacent sections (case R1530). There were significant increases in arterial pressure of 20.7 and 16.4% (Fig. 3A) at points in column B, level II and in column B, level III (see Fig. 3B), respectively, while surrounding injections induced minimal changes.

Since we made nine injections of glutamate in the gross mapping study and as many as 27 shots of glutamate in the micromapping study, some consideration of baseline arterial pressure must be made. First, the interval between injections of 10 min or longer worked very well in our hands, and the arterial pressure always came back to nearly control levels (Table 1). Although baseline arterial pressure in the gross mapping study shifted more with the greatest glutamate concentration, the SE ranging from 3.8 to 6.8 mmHg, the baseline arterial pressure was better controlled after injections of 0.5 M glutamate, shifting <9% and the SE ranging 1.6 to 3.2 mmHg. Moreover, even though each rat had received 27 injections of 5 nmol glutamate in the micromapping study, and the whole process lasted ~8 h, the SE was <3.0 mmHg.

**Fig. 2.** Summary of results of gross mapping study after injections at 1.0 or 0.5 M glutamate. There was a significant increase of BP \( (P = 0.05 \text{ in } A; P = 0.01 \text{ in } B) \) after injections of either 30 nmol (A) or 15 nmol (B) of glutamate into spot F (1.0 mm caudal to the calamus scriptorius, 2.0 mm from midline, and 1.7 mm ventral to touch of dorsal medullary surface; shaded columns). The 9 injections of glutamate into the caudal medulla were done in random order with at least 10 min between injections. Each bar represents the results from 6 animals. C to R, caudal to rostral; M to L, medial to lateral. C: percent increase in BP after injections of different doses of glutamate into spot F. Responses were dependent on concentration administered such that the more concentrated solutions of glutamate induced the greatest increase in arterial pressure. Note that the 10-nl injections of glutamate yielded increases comparable to the 30-nl injections.
Neuroanatomic studies. BDA was injected into the CPA in eight rats. The coordinates were verified before the injections with an injection of glutamate. Although the animal was not cannulated to record arterial pressure, previous experiments had shown that the non-paralyzed yet completely anesthetized rat went into a writhing contraction of its body when the CPA was injected with 30 nmol glutamate, but not with lesser doses. This peculiarity was used to advantage to check the accuracy of our stereotaxic coordinates for the BDA injections into the CPA.

An injection of BDA is shown in Fig. 5A. It was centered between the MDH and caudal pole of the LRN and included the medial part of the CPA. Numerous labeled fibers left the injection site (Fig. 5A, arrow); we have refined our technique such that we can see labeled fibers of extremely small caliber as well as larger fibers (see Fig. 5B). Many such fibers ascended ipsilaterally through ventral parts of the ventrolateral medulla and just medial to the ventromedial spinal trigeminal nucleus. There were relatively numerous labeled fibers with boutons throughout the CVLM (Fig. 5B) compared with those seen more rostrally in the RVLM (Fig. 5C). It also was noted in this case that most of the boutons on labeled fibers in the RVLM were associated with the smallest fibers. There was only minimal labeling in the contralateral ventrolateral medulla in this case. Other brain stem autonomic areas, including the nucleus tractus solitarii, A5, and parabrachial nuclei, also were labeled after these injections (56) and will be described in detail later. Although all the injections were reasonably small in these cases, there was obvious spread into the reticular formation just dorsal to the CPA in many of them. When this occurred, the number of labeled fibers with boutons was greatly increased throughout the whole rostrocaudal extent of the ventrolateral medulla.

FG was injected into either the CVLM or RVLM in an effort to retrogradely label neurons in the CPA. An injection into the CVLM (Fig. 6A) was centered just medial to the rostral LRN near the level of the obex and included the LRN, nucleus ambiguus, medial edge of the spinal trigeminal nucleus, and reticular neuropil. Other injections were made more rostrally into the RVLM; the injection shown (Fig. 6B) was centered at the caudal edge of the facial nucleus and included this
motor nucleus, the nucleus ambiguus, and surrounding reticular neuropil. Numerous retrogradely labeled neurons were found among the fascicles of white matter between the MDH and LRN (Fig. 6, C and D) after either of these injections. Many of these neurons were spindle shaped and oriented in a dorsomedial-to-ven
trolateral direction. Our preliminary analysis of these spindle-shaped neurons had a long diameter of $18.6 \pm 2.8 \, \mu m$ and short diameter of $7.1 \pm 1.2 \, \mu m$.

Immunohistochemical techniques were used to localize the A1 catecholaminergic neurons in the caudal medulla. Such neurons were found mostly dorsal to the lateral part of the caudal LRN (Fig. 7C) and were more medial than the CPA we define (Fig. 7D, oval). An injection of glutamate into the A1 area (Fig. 7A) induced an increase of 9.7% in arterial pressure, but when the injections were centered in the CPA (i.e., Fig. 7B), the arterial pressure was much greater. The two injections of glutamate into the CPA in case R1548 induced 24 and 19% increases in blood pressure (Fig. 7B).

**DISCUSSION**

A CPA, which shows marked increases in arterial pressure when stimulated, has been reported in several studies, but neither its exact location nor its neurons have been identified. Microinjections of glutamate were used in the present study to first grossly define the area of the CPA near the medullary-spinal junction, and then other injections of lesser volume were used to micromap this area and further pinpoint these pressor neurons. The results show that CPA neurons are near the ventral surface of the brain between the MDH and the caudal pole of the LRN. Neuroanatomic experiments showed that CPA neurons are mostly spindle shaped, are interspersed among the longitudinal fascicles of white matter, and project to both the CVLM and RVLM.

**Technical considerations.** A purpose of the present study was to define accurately the location of the CPA using microinjections of glutamate into the caudal third of the medulla. We assumed that such injections chemically stimulate neurons but spare axons of passage (12, 20, 21), especially those involved in the control of arterial pressure in these cases. However, application of excitatory amino acids also causes large

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Mean and SE of blood pressures before the 9 injections of glutamate in cases for the gross mapping studies and the 27 injections in cases for the micromapping studies.
disturbances in the local concentrations of ions in the extracellular medium, mainly an increase of K\(^+\)/H\(^+\) and decrease of Ca\(^{2+}\)/H\(^+\), secondary to their depolarizing action. In extreme cases, these shifts may be irreversible, causing cell death (49).

The amount of glutamate used in our experiments (5–30 nmol) fell within the range of 10–100 nmol recommended by Goodchild and colleagues (21) as a technique to activate neurons in localized regions of the CNS. The pressor responses still could be reproduced after multiple injections into the CPA in our experiments, as well as those of others (8, 22). Moreover, the concentrations and volumes discussed by Lipski et al. (38) were larger than those we used. This suggests that a local depolarizing block of neurons with duration of minutes did not occur with the concentrations used in our cases.

Gross mapping studies. In the present study, we used relatively small volumes (10–30 nl) of glutamate in an effort to limit spread of the injection. According to the formulated equations from Nicholson (42), an injected volume of 30 nl affects a sphere of radius of ∼325 µm, although this number does not factor in concentration of the injectate nor tissue tortuosity. The injection pattern (Fig. 1A) and the volume (30 nl) of glutamate used in our gross mapping study were designed to canvas the caudal third of the medulla by sparing few neurons. The interval of 500 µm between the injections means there should have been little overlap between them.

The effective concentration of glutamate needed to stimulate neurons is also not known. When the increase in arterial pressure is compared between cases using injections of either 1.0 or 0.5 M glutamate, that induced by the more concentrated solution was considerably larger (Fig. 2). Also, the more concentrated solutions must have influenced a larger sphere of neurons. While injections of 0.25 M glutamate often showed increases in arterial pressure when placed near spot F, the SE was too great and these data were not significant. Thus a concentration of 0.5 M was considered best in our cases.

In our preliminary experiments (data not shown), we also made injections of glutamate up to 1.0 mm rostral to those reported herein. These injections were within the area of the brain stem considered by most as the caudal ventrolateral medulla, which contains depressor neurons associated with the baroreceptor reflex (13, 14, 27, 36, 53). Indeed, there were large shifts in baseline arterial pressure after such injections, mostly depressor, which we considered detrimental to our analysis. However, no such depressor responses were seen in the averages generated from the nine injections reported (Fig. 2). This suggests that the relay for the baroreceptor reflex is rostral to the calamus scriptorius of the rat (see 11).

Injection of 0.5 M glutamate into spot F (see Fig. 1), which is dorsolateral to the LRN and ventromedial to the MDH at the level of the pyramidal decussation, caused a significant increase in arterial pressure over the other eight injection sites (see Fig. 2). This spot generally confirmed the observations of Natarajan and Morrison (41) on the location of the CPA.

Micromapping studies. We micromapped this area further in other experiments (see Fig. 3), using injections of only 5 nmol glutamate in a 10-nl volume. The
micromapping experiments also support the work of Nicholson (42), who stated that the spread of an injection of 10 nl should be a sphere with a diameter between 134 and 225 μm, depending on whether the injectate remained as a bolus or infiltrated the reticular formation. The reticular neuropil in this area of the brain is far from uniform, however. There are numerous fiber tracts, including the spinothalamic tract, coursing through the area where CPA neurons are found. Such a substrate probably precludes that the injection actually diffuses away as a true sphere, but spreads amorphously.

The 27 different microinjections of 10 nl (Fig. 3) were placed greater (300 μm apart) than the diameter of either sphere postulated by Nicholson (42). The graphs of these data (Fig. 4) show significant increases in arterial pressure only in those areas near to spot F. Little increases in arterial pressure were seen with injections 600 μm away, and only relatively minor increases were seen with injections but 300 μm away. Indeed, the changes of MABP with the 27 microinjections resulted in a very significant increase in arterial pressure when spot F was injected. Even though the post hoc Fisher test did not show significance between the site of spot F with the one just 300 μm dorsal and the one caudomedial to it (see Fig. 5), the change of MABP increased >38% over either site. These data more precisely located the neurons of the CPA. It also suggests that CPA neurons are confined within a relatively small area.

There were no significant changes in heart rate when comparisons were made either before or after the injections of glutamate into spot F in the gross mapping study. Generally similar observations were made in the micromapping study; changes were not significant when the 10 injections surrounding spot F were compared with baseline rates. We were surprised however, that when heart rate before and after the injections into spot F were compared in the micromapping study, it was significantly higher after these injections. This perhaps could have been due to our rather small sample size (n = 5). Lesions of the CPA and surrounding neuropil cause a significant increase in resting heart rate, however (55).

CPA. Most vasomotor drive evidently originates in the brain stem (1), and the RVLM was initially recognized as the only region in the brain stem in which pharmacological inhibition produced a fall in arterial pressure. Other areas, such as the parabrachial complex (45) and the pontine reticular formation (29), have been demonstrated to have tonic vasomotor activity. Neurons located at the caudal edge of the CVLM also might be involved in supporting arterial pressure (8, 47). Topical application of leptazol on the caudal ventral medulla produced pressor responses (19). Stimulation of these CVLM sites with glutamate induces a rise in arterial pressure in anesthetized or decerebrate cats (18, 31) or rats (22) while depressor responses are seen when inhibitory neurotransmitters are injected (19, 41, 47). In rats, pressor responses also were obtained by stimulation of these caudal sites with angiotensin-(1–7) (52).

Although a CPA was first noticed in cats (19, 26, 61) and rats (22) several years ago, the precise location of
Fig. 7. Photomicrographs of sections cut through the CPA. A and B: injections (30 nl, 15 nmol; fluorescent red) of glutamate from sections photographed with combined fluorescent and bright-field illumination from case R1548, respectively. C: bright-field photomicrograph of a section immunohistochemically reacted against antibodies to tyrosine hydroxylase. The injection in A induced an increase of 9.7% in BP and apparently was centered in an area filled with A1 catecholamine neurons (compare arrows in A and C). However, when the injections were centered in the CPA (encircled by ovals, A–D), the increase in BP was much greater. For example, the 2 injections of glutamate into the CPA in case R1548 induced 24 and 19% increases in BP (see B). After analyzing all the data presented herein, we conclude that the CPA is represented by small- to medium-sized neurons intertwined among the longitudinal fiber bundles coursing between the MDH and caudal pole of the LRN. The CPA neurons (encircled in the bright-field picture of a section stained with thionin in D) have a rather limited rostrocaudal extent of ~500 μm; we suggest CPA neurons are different from the A1 catecholaminergic cell group (arrow in D). Scale bar in C represents 100 μm for A–D.

This is the first neuroanatomic demonstration of direct projections from the CPA to the RVLM and the caudal ventrolateral medulla. Small boutons generally were found on very small fibers in either area after BDA injections into the CPA, but the CVLM also had numerous larger boutons originating from larger fibers (Fig. 5). We used the few cells in the contralateral CPA after FG injections into the ventrolateral medulla to characterize the cell type in the area on the ipsilateral side. Only spindle-shaped neurons were found contralaterally with many more ipsilaterally. Although we cannot prove that these neurons are indeed the pressor neurons of the CPA, they were most abundant at the coordinates we had determined for the CPA in the mapping studies. Nevertheless, the pressor effect induced after stimulation of the CPA apparently is dependent on a relay through the caudal ventrolateral medulla (41).

The A1 catecholaminergic cell group is juxtaposed to the CPA (3), and some norepinephrine neurons of this group probably were included in many of our injections. These noradrenergic neurons are known to project to hypothalamic neurons (34, 35, 50, 59) involved in the release of ANG II (34) and arginine vasopressin (51), both of which can induce a pressor response. However, pressor responses still can be induced from stimulating the caudal part of the CVLM even in decerebrate animals (23, 24, 31, 47). Also, this suprabulbar pathway is used to elevate arterial press-
sure on a time scale of minutes (53), and we saw immediate increases in arterial pressure. Thus it appears that the circuitry of the pressor response seen herein is intrinsic to the brain stem.

Granata and colleagues (23, 24) have suggested that the A1 catecholamine group inhibits the neurons in the RVLM, but this has been discounted by others (6, 17, 48, 54). Data from the present study also do not support that stimulation of the A1 group induces increases in arterial pressure seen after the glutamate injections in the CPA. For example, although an injection of 15 nmol of glutamate just dorsal to the lateral edge of the LRN (Fig. 7A, arrow) induced an increase of arterial pressure of 9.7% in case R1548, we feel that this increase was due to spread into the nearby CPA (Fig. 7, ovals), because the two injections placed ~300 µm more laterally and centered within our CPA (Fig. 7B) induced increases of 24 and 19%. Similar findings were seen in our micromapping study. Eight of the nine medial injections (Fig. 4) did not show significant increases in arterial pressure compared with injections centered on spot F. Moreover, neuroanatomic studies have not shown double-labeled neurons in the A1 cell group after injections of retrograde tracers into either the RVLM or CVLM (5, 58). We thus conclude that the CPA is an area of neurons just lateral and ventral to the A1 group situated among longitudinally oriented bundles running between the MDH and caudal pole of the LRN (Fig. 7D). It is relatively small, ~500 µm in the anteroposterior direction plane and 300 µm wide, and causes a marked increase in arterial pressure when stimulated.

Several reports (10, 11, 30) contend that the increases in arterial pressure seen after stimulating the CPA is due to a disinhibition of the inhibitory pathway from the CVLM to the RVLM. However, Natarajan and Morrison (41) have suggested that CPA neurons excite sympathoexcitatory neurons in the CVLM via a glutamatergic synapse. While our experiments did not address this particular problem, we never saw an initial depressor response with our glutamate injections and have shown that lesions including the neurons of the CPA induce a significant and sustained increase in arterial pressure (60). However, we do show direct projections into both the CVLM and RVLM.

**Perspectives**

For the first time, we precisely located the neurons of the CPA, whose activation with glutamate causes an increase of arterial pressure. Our data showed that neurons of the CPA are located ventromedial to the MDH and lateral to the caudal pole of the LRN at the level of the pyramidal decussation (1.0 mm caudal to the calamus scriptorius, 2.0 mm lateral to the midline, and 1.7 mm ventral from the dorsal surface of the brain stem). The CPA eventually must influence neurons of the RVLM to modulate arterial pressure. We showed in our experiments that CPA neurons project to neurons in both the CVLM and RVLM.

The RVLM is a component of several circuits involved in the pressor responses of various reflexes, including the carotid occlusion, the baro- and chemoreceptor reflexes (13, 14, 25–28, 36, 53), the central ischemia reflex (15, 33), the exercise pressor reflex (2), and the diving response (40). While neurons in the area of the CPA are labeled after the transneuronal transport of herpes simplex viruses applied to a nerve important for the diving response (43), and after injections of BDA into areas of the MDH (43) where the response is inhibited (44), it is not essential for the increase in arterial pressure seen in the diving response (60). The role the CPA neurons play in modulating arterial pressure is still unknown.

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