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TONIC VASOMOTOR CONTROL BY THE ROSTRAL VENTROLATERAL MEDULLA: EFFECT OF ELECTRICAL OR CHEMICAL STIMULATION OF THE AREA CONTAINING C1 ADRENALINE NEURONS ON ARTERIAL PRESSURE, HEART RATE, AND PLASMA CATECHOLAMINES AND VASOPRESSIN[1]

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Abstract

We have studied the responses to electrical and chemical stimulation of the ventrolateral medulla in the chloralose-anesthetized, paralyzed, artificially ventilated rat. Locations of most active pressor responses were compared to regions containing neurons labeled immunocytochemically for phenylethanolamine *N*-methyltransferase (PNMT), the enzyme catalyzing the synthesis of adrenaline.

Elevations of arterial pressure (+81.6 ± 2.5 mm Hg) and cardioacceleration (+73 ± 13.6 bpm) were elicited with low current (5 times threshold of 9.5 ± 1.1 μA) electrical stimulation in a region of rostral ventrolateral medullary reticular formation we have termed the nucleus reticularis rostroventrolateralis (RVL). Electrical stimulation of the RVL increased plasma catecholamines (16.8-fold for adrenaline, 5.3-fold for noradrenaline, and 1.9-fold for dopamine) and vasopressin (1.7-fold before spinal transection, 4.7-fold after).

The location of the most active pressor region in the ventrolateral medulla corresponded closely with the location of C1 adrenaline-synthesizing (PNMT-containing) neurons. In addition, the location of the most active pressor region in the dorsomedial medulla corresponded with the location of a bundle of PNMT-containing axons.

Unilateral injections into the RVL of the excitatory amino acid monosodium L-glutamate (50 pmol to 10 nmol), but not saline, caused transient dose-dependent and topographically specific elevations (maximum +71.6 ± 4.9 mm Hg) of arterial blood pressure and tachycardia. Injections of the rigid structural analogue of glutamate, kainic acid, caused large, prolonged (at least 15 min) pressor responses and tachycardia.

Unilateral injections of the inhibitory amino acid γ-aminobutyric acid (GABA) into the RVL caused transient dose-dependent hypotension (maximum −40.8 ± 6.6 mm Hg) and bradycardia, whereas the specific GABA antagonist bicuculline caused prolonged (10 to 20 min) elevations (+64.2 ± 6.8 mm Hg) of arterial pressure and tachycardia. By contrast, injections of the glycine antagonist strychnine had no significant effect. Bilateral injections of the neurotoxin, tetrodotoxin, dropped arterial pressure to low levels (51.7 ± 4.7) not changed by subsequent spinal cord transection at the first cervical segment (52.5 ± 6.2).

We propose the following. (*1*) Neurons within the RVL, most probably C1 adrenaline-synthesizing neurons, exert an excitatory influence on sympathetic vasomotor fibers, the adrenal medulla, and the posterior pituitary. (*2*) These neurons are tonically active and under tonic inhibitory control, in part via GABAergic mechanisms—perhaps via the nucleus of the solitary tract (NTS). (*3*) Tonic activity of these neurons is responsible for the maintenance of resting arterial tone. (*4*) Since these

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neurons receive a direct projection from the NTS (Ruggiero, D. A., C. A. Ross, and D. J. Reis (1982) Soc. Neurosci. Abstr. *8:* 723), these same neurons of the RVL, probably C1 adrenaline neurons, may also be an integral part of the baroreceptor reflex arc.

It has long been recognized that the integrity of the medulla oblongata is necessary for maintaining resting levels of arterial pressure (AP). Observations that transection of the spinal cord at the first cervical segment resulted in a collapse of AP and abolition of tonic sympathetic nerve activity, while lesions of the brainstem just rostral to the medulla failed to alter it, gave rise to a concept that the medulla contains "tonic vasomotor neurons"—neurons whose activity is necessary for maintaining background discharge of preganglionic sympathetic neurons, and thus a normal AP (Owsjannikow, 1871; Dittmar, 1870, 1873; Alexander, 1946). Until recently, attempts to localize the tonic vasomotor neurons by placing focal lesions in the medulla were without success (Wang and Ranson, 1939; Manning, 1965), suggesting that the tonic vasomotor neurons were widely distributed in the medulla, and not restricted to a single neuronal group.

Over the past several years, however, several studies have demonstrated that focal lesions at two sites in the medulla result in falls of AP to levels comparable to those produced by spinal cord transection. One of these regions is in the dorsomedial medulla, in the region of the nucleus reticularis parvocellularis (Kumada et al., 1979). The other area is in the rostral ventrolateral medulla just caudal to the facial nucleus of the pons. Several groups found that bilateral chemical or electrolytic lesions of this area resulted in collapse of AP to spinal levels (Guertzenstein and Silver, 1974; Guertzenstein et al., 1978; Bousquet et al., 1980; Dampney and Moon, 1980). Electrical stimulation of either the dorsomedial or ventrolateral regions causes elevations of AP (Dampney et al., 1979; Dampney and Moon, 1980). Since the ventrolateral area, but not the dorsomedial area, contains neurons projecting directly to the thoracic intermediolateral column (Amendt et al., 1978, 1979; Martin et al., 1981), it was proposed that it is the ventrolateral area which contains tonic vasomotor neurons, and that axons of these neurons traverse the dorsomedial medulla before descending to the spinal cord (Dampney and Moon, 1980; Dampney, 1981; Dampney et al., 1982).

Within a region of the rostral ventrolateral medulla (RVL) very similar to the ventrolateral vasomotor center lie neurons containing the enzyme phenylethanolamine *N*-methyltransferase (PNMT). These neurons were termed the C1 group by Hökfelt et al. (1974). Since these neurons also contain immunoreactive tyrosine hydroxylase and dopamine β-hydroxylase, they are believed to synthesize the neurotransmitter adrenaline (Hökfelt et al., 1974; Saavedra et al., 1974; Howe et al., 1980; Armstrong et al., 1982). We have recently mapped out the location of the C1 neurons in detail in the rat, and demonstrated that they represent a substantial proportion of the neurons in the RVL projecting to the intermediolateral column (Armstrong et al., 1982; Ross et al., 1981a, b, 1982, 1984).

These observations therefore raise the possibility that it is the C1 neurons which may be the purported medullary tonic vasomotor neurons. In the present study we sought to characterize, using electrical and chemical stimulation techniques, the effects upon cardiovascular function and release of vasoactive hormones of neurons lying within the RVL. Moreover, by combining electrical stimulation and immunocytochemical techniques, we examined the hypothesis that the tonic vasomotor neurons in the RVL may correspond to the C1 adrenaline-synthesizing neurons. Portions of these data have been presented in preliminary form (Ross et al., 1982, 1983).

Materials and Methods

General procedures

Experiments were performed on 105 male Sprague-Dawley (350 to 450 gm) rats. Anesthesia was induced with halothane (2% in a mixture of 50% O_2, 50% N_2); polyvinyl cannulas (Tygon microbore, 0.015-inch inside diameter, 0.030-inch outside diameter) were inserted in the left femoral vein and artery, and α-chloralose (60 mg/kg, i.v.) was administered. After the trachea was cannulated, the animal was paralyzed with curare (0.3 mg/kg, i.v.) and maintained on intermittent positive pressure ventilation with 50% O_2, 50% N_2 at a ventilation rate of 80 breaths/min and a volume of 2.0 to 3.5 ml. Halothane (0.7%) was continued until surgical procedures were completed. Chloralose (20 mg/kg) and curare were readministered hourly. Body temperature was maintained between 36° and 37°C with a thermostatically controlled heating pad.

Arterial pressure was recorded via a femoral catheter connected to a strain-gauge transducer (Statham P23Db) and recorded on a polygraph (Grass model 7). Mean pressure was derived using a low pass filter, and heart rate was computed from the pulse wave and displayed on the polygraph by a tachograph preamplifier (Grass 7P4).

The animal was mounted in a stereotaxic apparatus (Kopf) with the bite bar 12 mm below the interaural line. The occipital bone and atlanto-occipital membrane were exposed after retraction of the dorsal neck muscles. After separation from the dura, the occipital bone was removed. The atlanto-occipital membrane was cut, and the dura overlying the cerebellum was retracted. The caudal half of the cerebellum was removed by suction, exposing the floor of the fourth ventricle as far rostrally as the caudal aspect of the inferior cerebellar peduncle.

In some animals, after a parietal craniotomy, the midbrain was transected with a specially machined inverted L-shaped knife, avoiding damage to the midsagittal sinus. The knife cuts were made between 3 and 4 mm

rostral to the ear bars and passed through the rostral midbrain at the level of the red nucleus as subsequently verified histologically. In some animals, the midbrain was hemitransected 1 to 2 weeks before the acute experiments, and methicillin (10 mg/kg, i.m.) was administered. These animals were returned to their cages and tube fed daily with 10% sucrose until used.

In some rats, the spinal cord was transected with a scalpel or cautery instrument after exposing the first cervical segment by laminectomy. The spinal cord, 1 mm on either side of the cut, was removed by suction and the wound was packed with Gelfoam. The completeness of the transection was verified post mortem. In some experiments, AP was maintained within normal range after spinal transection by intravenous infusion of norepinephrine (approximately 2 μg/min, i.v.).

Bilateral nephrectomy and adrenalectomy were performed via a dorsal approach.

Chemical sympathectomy was produced in some rats by systemic administration of the neurotoxin 6-hydroxydopamine (6-OHDA) (Thoenen and Tranzer, 1968; Del Bo et al., 1983b), 24 to 48 hr before experimentation. 6-OHDA, while functionally destroying sympathetic terminals, spares the adrenal medulla (Finch and Leach, 1970; DeChamplain and Nadeau, 1971). Twenty-four to 48 hr before the experiment, each of these rats was anesthetized with halothane (2%), and a polyvinyl cannula was inserted into a superficial branch of the jugular vein, pulled subdermally to exit via an incision in the back, and plugged. After termination of the anesthesia, 6-OHDA (100 mg/kg as the hydrobromide, in saline plus 1% ascorbate) was slowly injected into the cannula. This treatment produced effective functional sympathectomy as shown by absence of a pressor response to injection of tyramine (Del Bo et al., 1983b).

Receptors for arginine vasopressin were blocked by administration of the synthetic arginine vasopressin antagonist, 1-β-mercapto-β,β-cyclopentamethylenepropionic acid), 2-(*O*-methyl)tyrosine arginine vasopressin (10 μg/kg, i.v.), kindly provided by Dr. Maurice Manning of the Medical College of Ohio (Kruszynski et al., 1980).

Drugs were administered intravenously, including atropine or methylatropine (Sigma, 0.3 mg/kg), phentolamine (Regitine, Ciba, 2.5 mg/kg), and propranalol (Ayerst, 1 mg/kg).

Electrical stimulation

In order to compare the location of the most active pressor sites in the ventrolateral medulla with the location of C1 neurons, we systematically explored the ventrolateral medulla with low current electrical stimulation. For mapping studies, the electrode was lowered vertically into the medulla under visual guidance, using a surgical microscope, and stimulated with 10-sec trains at 100 Hz and 25 μA every 0.2 mm. For each track, the electrode was lowered until it just touched bone below the ventral surface of the brain. A territory from the level of the calamus scriptorius to 3.0 mm rostral to it and from the midline to 3.0 mm lateral to it was explored, using tracks separated by 0.4 mm in the mediolateral direction and by 1.0 mm in the rostrocaudal direction. The locations of the most active pressor responses were plotted onto schematic drawings of sections through the medulla at 0, +1, +2, and +3 mm rostral to the calamus scriptorius (these levels were chosen to correspond to those mapped in a coordinate anatomical study; Ross et al., 1984), and compared to the location of neurons immunocytochemically labeled for PNMT at those levels.

In experiments designed to characterize the response, the electrode was directed at the most active pressor region in the RVL. Coordinates were approximately 2.0 mm rostral to the calamus scriptorius, 2.0 mm lateral to the midline, and 3.0 to 3.8 mm below the floor of the fourth ventricle.

In experiments designed to determine the dependence of the response on stimulus frequency, current was held at 2 or 3 times threshold, and frequency varied. In experiments designed to determine the dependence of the response on stimulus current intensity, the frequency was kept at 100 Hz and current was varied in multiples of threshold current.

Chemical stimulation and lesions

Drugs were injected into the medulla via glass micropipettes broken back to a tip diameter of approximately 50 μm. In each animal, the site in the RVL most responsive to electrical stimulation using low current (20 to 25 μA) was chosen as the site for injection. The electrode was withdrawn, and the micropipette was inserted the same distance down the same track. The injection volume in all experiments was 100 nl, delivered by hand over a period of approximately 2 sec.

Since the ventrolateral medulla is known to be a chemosensitive region (Mitchell et al., 1963), care was taken in drug experiments that solutions were not of unphysiological pH or tonicity. In each experiment the response to physiological saline (160 mM NaCl in 10 mM phosphate buffer, pH 7.4) was tested before injecting any drug. (In about 10% of the animals studied, injections of saline into the RVL caused rises in AP greater than 20 mm Hg; these animals also usually had elevated base line pressures and evidence of cerebral edema and were not used for drug injection experiments.) In some experiments, the response to injecting different concentrations of saline (0.1 to 1 M) was also tested. All drugs were dissolved in phosphate-buffered physiological saline, pH 7.4.

In experiments designed to test the response to different concentrations of drugs, saline was tested first, then ascending concentrations of the drug, and finally saline again. Injections were separated by 5 min. At the end of the series, 100 nl of a 1% solution of fast green dye was injected in the same spot.

In order to examine the cardiovascular effects of interruption of nerve impulse activity, tetrodotoxin (10 pmol in 100 μl of water) was injected bilaterally into the C1/RVL region. Tetrodotoxin inactivates both cell bodies and fibers by blockade of fast sodium channels (Takata et al., 1966).

Measurement of plasma catecholamines and vasopressin

To measure the effects of stimulation of the RVL on the release of catecholamines, the animal was prepared

as usual, except that two femoral arteries were cannulated. Blood samples (0.5 ml) were withdrawn from an arterial cannula into heparinized syringes and replaced with an equal volume of saline. Plasma was separated in a refrigerated centrifuge (3000 × *g* for 10 min). A 200-μl aliquot of the plasma supernatant of each sample was frozen and stored at −70°C. Plasma catecholamines were assayed using a modification of the radioenzymatic method (Da Prada and Zurcher, 1976; Peuler and Johnson, 1977).

Approximately ½ hr following craniotomy, a base line sample was obtained. Fifteen to 20 min later, the RVL was stimulated for 1 min (100 Hz at 5 times threshold), and blood was slowly withdrawn during the last 20 sec of the stimulation period. Fifteen to 20 min later, a third (poststimulus) sample was obtained.

In separate experiments, plasma vasopressin was assayed using a modification of a radioimmunoassay method (Merkelbach et al., 1975). Blood (0.5 ml) was withdrawn into heparinized tubes, and plasma was separated by centrifugation. Samples were refrigerated and assayed within 2 weeks. Samples were drawn: (*a*) approximately ½ hr after craniotomy, (*b*) during a 1-min stimulation of the RVL as above, (*c*) 15 min after cervical spinal transection, and (*d*) during a second 1-min stimulation period.

Histological procedures

Localization of electrodes. At the end of selected electrical stimulation experiments, each rat was perfused through the heart by saline followed by 10% phosphate-buffered formalin with 1% potassium ferrocyanide and potassium ferricyanide to identify stimulation sites by the Prussian blue reaction. Frozen 50-μm sections were cut through the region of the stimulation sites and counterstained with cresyl violet or neutral red. The locations of stimulation sites were plotted onto drawings of the sections, using a microprojector. Immediately after chemical stimulation experiments, brains were removed, and the locations of the fast blue injection sites were ascertained by cutting the medulla transversely through the region of the RVL with a razor blade and examining with a dissecting microscope.

Immunocytochemical labeling of PNMT. Rats were anesthetized with pentobarbitol (50 mg/kg) and perfused through the heart with 4% paraformaldehyde in 0.1 M phosphate buffer. Brains were postfixed in the same fixative for 30 min, and 40-μm sections through the medulla were cut on a sliding microtome. Sections were labeled immunocytochemically for PNMT using the peroxidase-antiperoxidase technique (Sternberger, 1974; Pickel et al., 1979). Antibodies to PNMT were prepared by injecting PNMT purified from bovine adrenal medulla into rabbits, as previously described (Joh and Goldstein, 1973; Park et al., 1982; Joh and Ross, 1983). Controls included preimmune serum and antisera blocked by incubation with purified PNMT (Armstrong et al., 1982). Sections were examined using light and darkfield illumination. Projection drawings of selected sections, at levels corresponding to those of the electrical stimulation experiments, were made using a microprojector, and the locations of the immunoreactive profiles were plotted onto them using a camera lucida microscope attachment.

In some experiments, electrical stimulation was combined with PNMT labeling. Electrical stimulation was carried out as usual, and the site of the most active pressor response in the RVL was marked with a small lesion. Immediately afterward, while the rat was still being artificially ventilated, it was perfused with 4% paraformaldehyde, and sections were labeled for PNMT as usual.

Data analysis

Statistical analysis of the data was undertaken using two-tailed Student's *t* tests for paired data, except for the comparisons of intact animals with those with chronic hemitransection, which involved unpaired data.

Results

Cardiovascular responses to electrical stimulation of the RVL

Characteristics of the pressor response to RVL stimulation

Electrical stimulation of the RVL with a 10-sec stimulus train (100 Hz, 10 to 50 μA) invariably resulted in an elevation of AP and, usually, heart rate (HR) (Fig. 1*A*). Ninety percent of the peak response was obtained within 2 sec of the onset of the stimulus, and in about 70% of the animals, the response decayed within 1 or 2 sec following its termination. In 30% of the rats, a smaller secondary peak of AP appeared 2 to 12 sec following the termination of the stimulus train (e.g., Fig. 3*a*) with the response decaying rapidly thereafter.

The stimulus-locked rise in AP was accompanied by pure tachycardia in about 75% of the animals (Fig. 1*A*). In the remaining 25%, the acceleration of HR was preceded by bradycardia (e.g., Fig. 3*a*). At the end of stimulation, the HR either remained elevated, gradually returning to control values, or was replaced by bradycardia. The pressor response to stimulation of the RVL was stable and could be reproduced over several hours with repeated stimuli.

Relationship to stimulus frequency and current

The magnitudes of the rises in AP and HR elicited from the RVL were dependent upon stimulus frequency and intensity (Fig. 1, *B* and *C*). Both the AP and HR responses appeared at stimulus frequencies of 5 to 10 Hz and were maximal between 150 and 300 Hz (Fig. 1*B*). At no frequency was a depressor response seen. The stimulus current required to elicit a rise in AP of 10 mm Hg (threshold current) at 150 Hz was 9.5 ± 1.1 μA ($n = 6$). The pressor response increased linearly with respect to the logarithm of the stimulus current to reach a maximum at 5 to 6 times threshold (Fig. 1*C*).

The increase in HR was also graded with respect to current strength, reaching a maximum at 5 times threshold. Therefore, to achieve reproducible, near-maximal responses, the RVL was stimulated in most experiments with a 10-sec train at 100 Hz and at stimulus intensities of 5 times threshold.

Interestingly, in approximately 10% of rats, stimula-

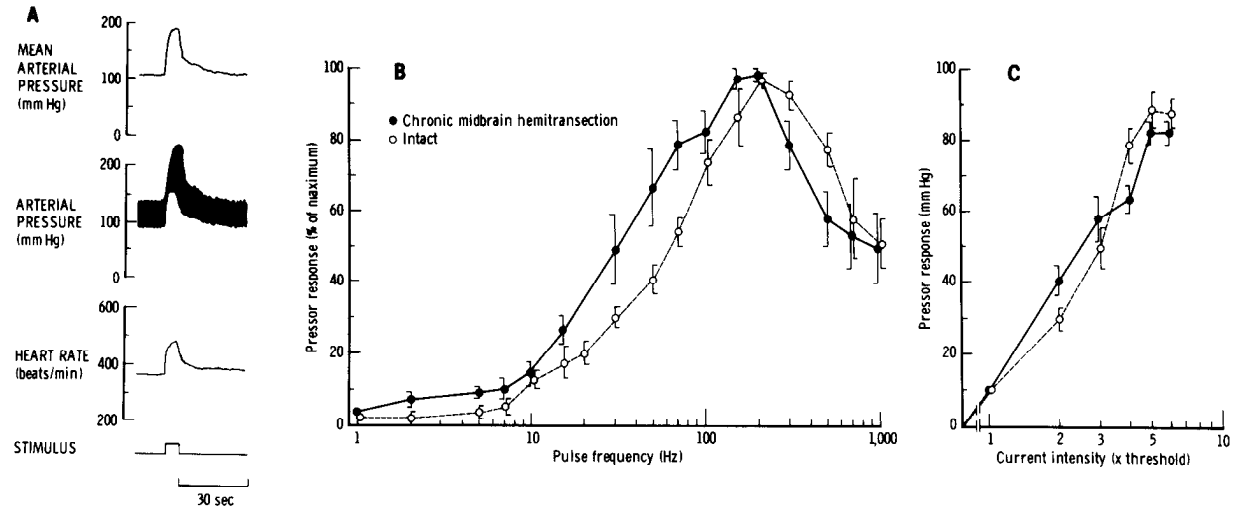

Figure 1. *A*, Pressor response and cardioacceleration elicited by focal electrical stimulation (40 μA, 100 Hz, 0.5 msec, 10-sec train) of the RVL in an untreated rat. *B*, Frequency response: Pressor responses to electrical stimulation of the most active pressor site in the RVL with stimulus frequencies of 1 to 1000 Hz. *C*, Current response: Pressor responses to stimulation of the RVL at 100 Hz, varying the current in multiples of threshold current (defined as that current necessary for a 10-mm Hg rise in AP).

tion of the RVL with relatively high current intensities (greater than 5 times threshold) was followed by a marked and sustained fall in mean AP (Fig. 2), leading, within 2 to 3 min, to fulminating pulmonary edema and, in about 15 min, death.

Effects of RVL stimulation on plasma catecholamines and vasopressin

Stimulation of the RVL in the intact rat increased plasma dopamine 2-fold, noradrenaline 5-fold, and adrenaline 17-fold (Table IA), and nearly doubled the amount of plasma vasopressin (AVP) (Table IB). After spinal transection, stimulation of the RVL caused a nearly 5-fold increase in plasma AVP.

Efferent mechanisms

Intact rats

The stimulus-locked pressor response to electrical stimulation of the RVL was not affected by atropine (0.3 mg/kg, i.v.) (Fig. 3, *a* and *b*, Table II), bilateral nephrectomy, bilateral adrenalectomy, or midbrain transection (Table II), but was virtually abolished either by combined treatment with phentolamine (2.5 mg/kg) and propranolol (1 mg/kg) or by transection of the spinal cord at the first cervical segment (Table II).

However, spinal cord transection unmasked a small pressor response differing from that observed in intact rats (Fig. 3, *c* and *d*). This response appeared approximately 5 sec after the end of the 10-sec stimulus train and persisted for 20 to 40 sec. This residual pressor response (residual because it appears after interruption of outflow from the brainstem to sympathetic neurons and adrenal medulla) was associated with a release of vasopressin greater than in the intact animals (Table IB). Circulating vasopressin was responsible for the elevation in AP since administration of the vasopressin

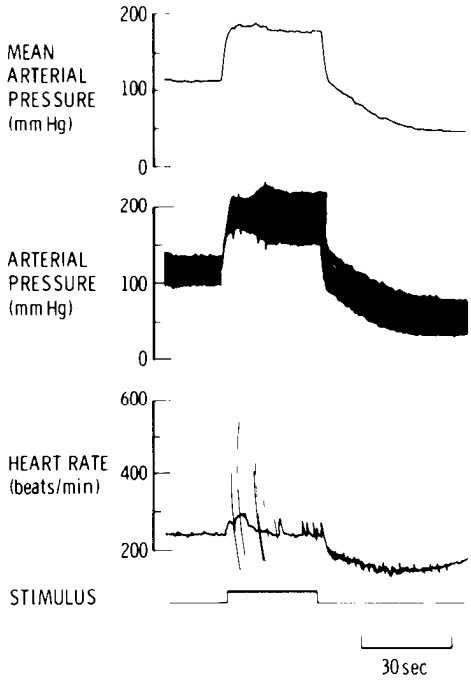

Figure 2. Profound hypotension following increase in blood pressure after electrical stimulation (at 5 times threshold for 30 sec) of the RVL. This response occurred in about 10% of rats, was accompanied by pulmonary edema and arterial hypoxemia, and led to death of the animal.

antagonist (Kruszynski et al., 1980) blocked the residual pressor response (Fig. 3*e*, Table II).

The elevation in HR appearing during stimulation of the RVL was reduced 40 to 50% by atropine (Fig. 3, *a* and *b*, Table II). Following atropine, the tachycardia was not altered by nephrectomy, adrenalectomy, or midbrain transection (Table II), but was eliminated by combined treatment with phentolamine plus propranolol or by

TABLE I

Effect of electrical stimulation of the RVL on plasma catecholamine and vasopressin levels in untreated rats[a]

	Prestimulation	Stimulation	Poststimulation	Times Increase[b]
		A. Catecholamines		
Adrenaline	29.1 ± 2.7	499 ± 79	95.6 ± 15.6	16.8[c]
Noradrenaline	61.8 ± 7.0	330 ± 54	106 ± 61	5.3[c]
Dopamine	45.3 ± 9.4	98.3 ± 13.3	59.5 ± 6.2	1.9[d]
		B. Arginine Vasopressin		
Before spinal transection	13 ± 1	22 ± 4		1.7[d]
After spinal transection	14 ± 1	66 ± 10		4.7[c]

[a] All concentrations are in picograms per milliliter of plasma, mean ± SEM.

[b] Stimulation level compared to prestimulation level; $n = 12$ for catecholamines, $n = 6$ for vasopressin.

[c] $p < 0.001$.

[d] $p < 0.01$.

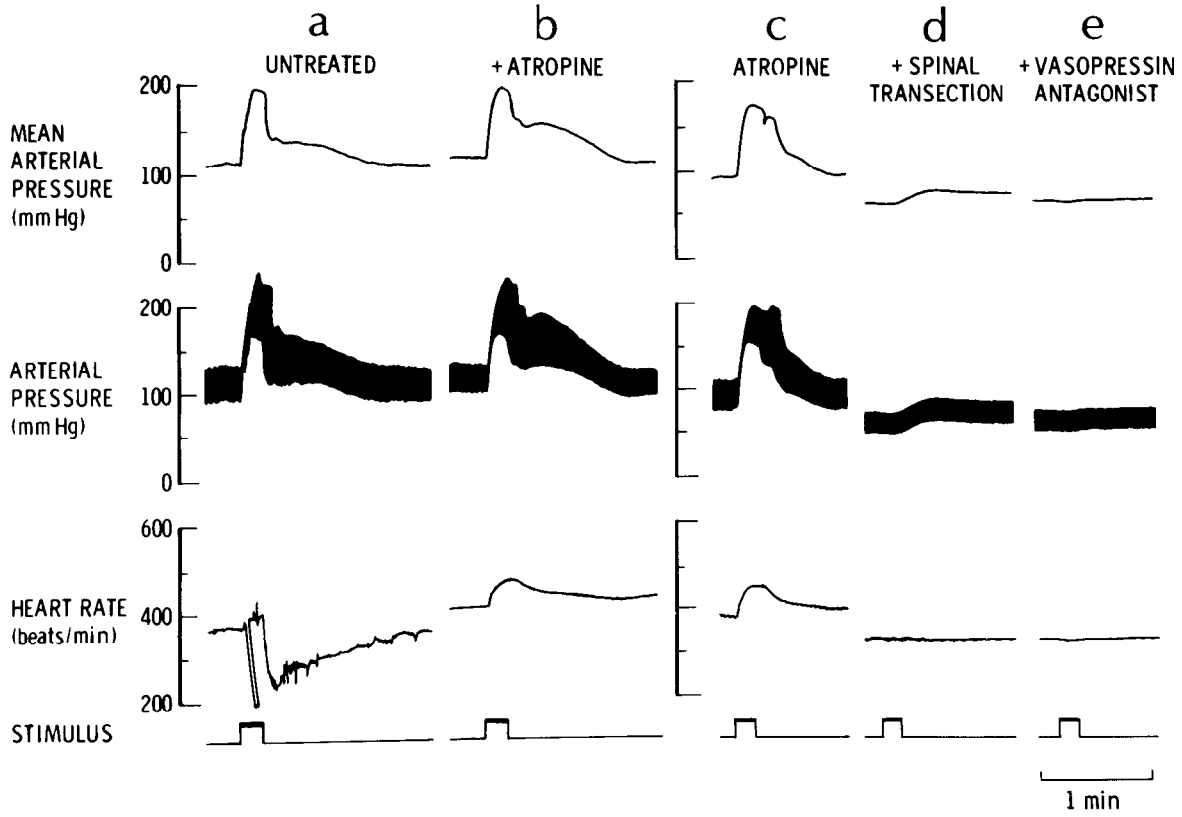

Figure 3. Effect of pharmacological and surgical manipulations on the response to electrical stimulation of the RVL. *a*, Untreated rat. Stimulation (5 times threshold, 100 Hz, 10 sec) elicits an immediate stimulus-locked pressor response, followed by a slow decay to base line. There is a brief stimulus-locked bradycardia followed by tachycardia, followed after the end of the stimulus by bradycardia. *b*, After atropine (0.4 mg/kg), the triphasic response is converted to uniphasic tachycardia, and the poststimulus peak of AP is increased. Methylatropine (0.4 mg/kg) has the same effect. *c*, Separate experiment using atropine alone. *d*, Same rat as in *c*, after cervical spinal transection. The stimulus-locked pressor response and the tachycardia are abolished. A small residual pressor response begins several seconds after the end of the stimulus is unmasked. *e*, Same rat, plus vasopressin antagonist (10 μg/kg, i.v.). The vasopressin antagonist abolishes the residual response.

spinal cord transection (Table II). The stimulus-locked tachycardia was therefore a consequence of excitation of sympathetic nerves to the heart coupled with inhibition of the cardiac vagus. The fact that HR was elevated in the face of an elevated AP suggests that, during stimulation, baroreceptor reflex responses are blocked. However, the poststimulus bradycardia was entirely due to vagal excitation since it was abolished by atropine or methylatropine. Presumably it reflected the release from inhibition of baroreflex responses so that the elevated AP which persisted at the termination of stimulus was then capable of stimulating baroreceptors.

Following adrenalectomy

Adrenalectomy did not modify the latency, duration, or magnitude of the pressor or HR responses elicited by stimulation of the RVL (Table II). Nor did it modify the frequency/response or stimulus intensity/response char-

acteristics of the evoked elevation in AP (Fig. 4, *B* and *C*).

Following chemosympathectomy

The sympathetic nerves were functionally destroyed 24 to 48 hr after administration of 100 mg/kg of 6-OHDA (Del Bo et al., 1983b). Such treatment reduced base line AP from 114 ± 2.5 to 69.2 ± 2.5 mm Hg ($p < 0.001$; $n = 6$).

Chemosympathectomy abolished the stimulus-coupled pressor response but unmasked another, delayed response, which appeared about 5 sec after termination of the 10-sec stimulus train, reached a peak 15 to 20 sec later, and persisted for 2 to 3 min (Fig. 4*A*). The magnitude of this delayed pressor response was as large as, or occasionally greater than, that in intact animals.

During stimulation, the HR variably increased or decreased. However, at the end of the stimulus, coincident

TABLE II

Effects of surgical or pharmacological manipulations on cardiovascular responses to electrical stimulation of the RVL

Treatment	*n*	Arterial Pressure (mm Hg)			Heart Rate (bpm)		
		Before Treatment	After Treatment	$\frac{\text{After}}{\text{Before}} \times 100$	Before Treatment	After Treatment	$\frac{\text{After}}{\text{Before}} \times 100$
Atropine							
Basal	6	119 ± 4	120 ± 5	101 n.s.[a]	408 ± 24	466 ± 13	114[b]
Stim.	6	+81.6 ± 2.5	+84.2 ± 6.2	103 n.s.	+73.3 ± 13.0	+40.8 ± 6.5	56[b]
Nephrectomy[c]							
Basal	4	97.5 ± 4.3	96.3 ± 6.6	99 n.s.	440 ± 17	430 ± 18	98 n.s.
Stim.	4	+86.3 ± 6.3	+85.0 ± 5.4	98 n.s.	+22.5 ± 10.3	+22.0 ± 9.1	91 n.s.
Adrenalectomy[c]							
Basal	6	108 ± 6	105 ± 5	97 n.s.	450 ± 15	452 ± 13	100 n.s.
Stim.	6	+78.3 ± 2.8	+82.5 ± 2.5	105 n.s.	+26.7 ± 5.0	+25.0 ± 5.8	94 n.s.
Midbrain transection[c]							
Basal	5	111 ± 14	109 ± 4	98 n.s.	470 ± 28	465 ± 23	99 n.s.
Stim.	5	+91.0 ± 6.4	+88.0 ± 4.1	98 n.s.	+30.0 ± 14.0	+16.6 ± 2.4	55 n.s.
Phentolamine + propranolol[c]							
Basal	4	101 ± 14	61.3 ± 13.0	61[b]	475 ± 16	335 ± 45	71[b]
Stim.	4	+75.0 ± 3.5	+12.5 ± 3.2	17[d]	+42.5 ± 11.8	0.0 ± 0.0	0[e]
Cervical spinal transection[c]							
Basal	6	123 ± 5	64.2 ± 5.4	52[d]	460 ± 17	373 ± 10	81[e]
Stim.	6	+80.8 ± 2.4	+11.7 ± 2.5[f]	14[d]	+40.0 ± 10.0	+1.7 ± 1.7	4[b]
Vasopressin antagonist after spinal transection[c]							
Basal	5	76.0 ± 2.9	71.0 ± 2.9	93 n.s.	354 ± 7	352 ± 5	99 n.s.
Stim.	5	+14.4 ± 0.6	+1.0 ± 1.0[f]	7[e]	0.0 ± 0.0	0.0 ± 0.0	

[a] n.s., not significant.
[b] $p < 0.05$.
[c] Animals pretreated with atropine or methylatropine (0.3 mg/kg).
[d] $p < 0.001$.
[e] $p < 0.01$.
[f] Beginning of response delayed until after end of stimulus train, peak response 5 to 20 sec after end of stimulus train.

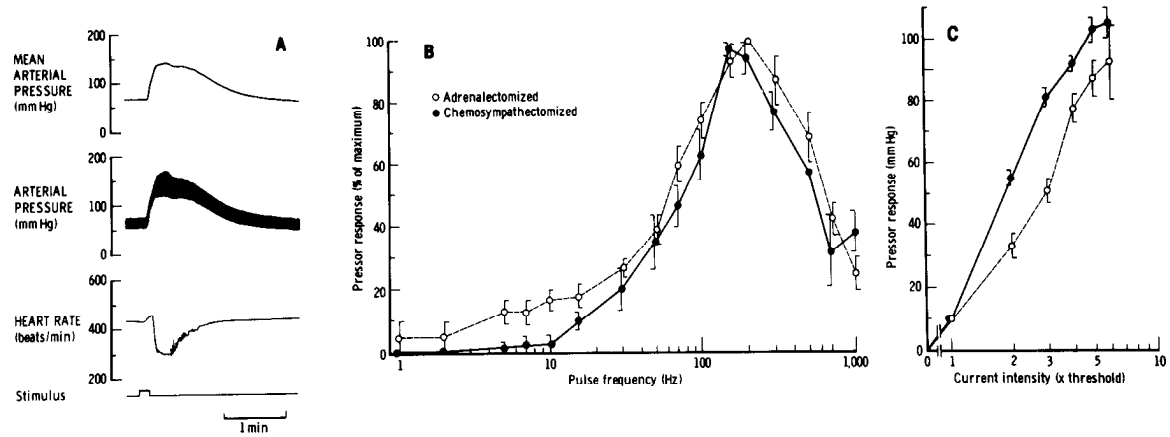

Figure 4. A, Response to stimulation of the RVL in a rat chemosympathectomized by pretreatment 24 hr earlier with 100 mg/kg of 6-OHDA. The stimulus-locked pressor response is abolished. Instead, there is a delayed pressor response of long duration accompanied by tachycardia beginning during the stimulus train, and bradycardia afterwards. Frequency (*B*) and current dependence (*C*) of the RVL pressor response in chemosympathectomized (*solid circles*) and adrenalectomized (*open circles*) rats.

with the elevation of AP, there was a pronounced bradycardia (Fig. 5*a*). The delayed pressor response had the same frequency optimum as in intact animals and was evoked at similar threshold currents (7.8 ± 0.7 μA, n = 6) and was graded with respect to current intensity (Fig. 4, *B* and *C*). There was no difference in the frequency/response curve nor the shape of the stimulus current/response curve in chemosympathectomized rats compared with untreated or adrenalectomized animals (Fig. 4, *B* and *C*). The magnitude of the pressor response was increased by atropine or methylatropine, as the bradycardia was converted to tachycardia (Fig. 5*b*, Table III).

The magnitude of the delayed response was reduced by 70 to 80% by spinal cord transection, adrenalectomy, or combined treatment with phentolamine and propranolol (Fig. 5, *c* and *d*, Table III) but was unaffected by nephrectomy. The delayed pressor response was predominantly, but not entirely, the result of release of catecholamines from the adrenal medulla. Following adrenalectomy in chemosympathectomized rats, stimulation of the RVL still produced a small elevation of AP—the residual response (Fig. 5*d*), similar to that appearing in otherwise intact rats following cervical spinal cord transection (see above). As in normal animals, the residual response was completely eliminated by treatment with the vasopressin antagonist (Fig. 5, *e* and *f*, Table III) or, in two rats (not shown), by midbrain transection. Thus the residual pressor response was a consequence of the release of vasopressin.

Relationship of pressor responses elicited from the RVL to PNMT-containing neurons and axons

In separate groups of animals, a careful comparison was made between the distribution of neurons and processes labeled with PNMT (Fig. 6, *left side*) in the medulla and the distribution of the active pressor sites defined by electrical stimulation (Fig. 6, *right side*).

Distribution of PNMT-labeled neurons and fibers in the medulla

Neurons staining for PNMT (Fig. 6) were located in the ventrolateral medulla in the C1 group, and in the dorsal medulla in the C2 group, as described previously (Hökfelt et al., 1974). In agreement with Hökfelt et al. (1974), C1 neurons were located in the ventrolateral medulla from about the level of the calamus scriptorius (Fig. 6*a*) to the level of the caudal facial nucleus (Fig. 6*d*). They were most dense in the region of the ventrolateral medullary reticular formation—an area we have termed the nucleus reticularis rostroventrolateralis (RVL) Ross et al., 1984) at a level about 2.0 mm rostral to the calamus scriptorius (Fig. 6*c*). A more detailed description of the anatomical location of medullary PNMT-containing neurons is presented elsewhere (Armstrong et al., 1982).

With darkfield microscopy, fine PNMT-labeled axons were visible in the medulla. A dense bundle of longitudinally traveling fibers was present in the dorsomedial medulla at all medullary levels (Figs. 6 and 7). At the most caudal medullary level, this bundle appeared to turn ventrally, and descended toward the spinal cord. At all levels of the medulla, labeled fibers arose from PNMT-labeled neurons in the ventrolateral medulla and coursed (within the plane of the coronal sections) toward the dorsomedial bundle (Fig. 6). Axons also appeared to arise from the C2 group in the region of the nucleus of the solitary (NTS) and enter the dorsomedial bundle (not shown).

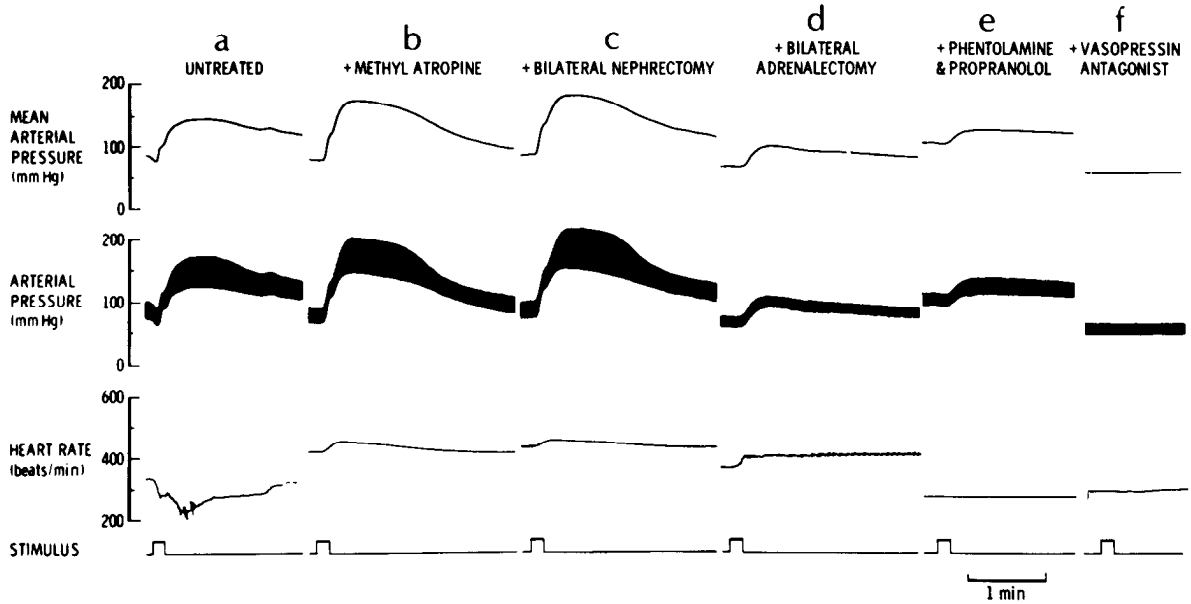

Figure 5. Effect of pharmacological and surgical manipulations on the response to stimulation of the RVL in a rat chemosympathectomized with 6-OHDA. *a*, Untreated; *b*, after methylatropine; *c*, plus bilateral nephrectomy; *d*, plus adrenalectomy; *e*, plus α- and β-receptor blockade; *f*, plus vasopressin antagonist. These data demonstrate that the delayed pressor response seen in 6-OHDA-treated rats is predominantly due to adrenal catecholamines but is partly due to vasopressin, presumably released by the posterior pituitary.

TABLE III

Effect of surgical or pharmacological manipulations on cardiovascular responses to electrical stimulation of the RVL in 6-OHDA-chemosympathectomized rats

Treatment	n	Arterial Pressure (mm Hg)			Heart Rate (bpm)		
		Before Treatment	After Treatment	After / Before × 100	Before Treatment	After Treatment	After / Before × 100
Atropine or methylatropine							
Basal	6	84.3 ± 6.4	85.0 ± 6.5	101 n.s.[a]	414 ± 16	460 ± 10	111[b]
Stim.	6	+61.7 ± 3.8	+83.3 ± 2.1	135[c]	+88.6 ± 22.0	+29.6 ± 3.4	33[d]
Nephrectomy[e]							
Basal	5	83.0 ± 3.4	86.0 ± 3.3	104 n.s.	460 ± 16	470 ± 18	102 n.s.
Stim.	5	+78.0 ± 7.8	+75.0 ± 7.6	96 n.s.	+25.0 ± 12.5	+23.0 ± 10.7	92 n.s.
Midbrain transection							
Basal	6	82.5 ± 6.1	75.0 ± 9.6	91 n.s.	428 ± 15	426 ± 16	100 n.s.
Stim.	6	+75.0 ± 6.1	+60.8 ± 7.6	81[c]	+21.0 ± 6.4	+25.0 ± 9.7	119 n.s.
Spinal transection[e]							
Basal	6	72.5 ± 6.0	52.8 ± 3.8	73[b]	495 ± 15	435 ± 18	88[b]
Stim.	6	+90.8 ± 7.4	+16.7 ± 3.6	18[d]	+40.0 ± 15.2	0.0 ± 0.0	0[b]
Phentolamine + propanolol[e]							
Basal	6	86.7 ± 7.4	72.5 ± 8.5	84 n.s.	472 ± 14	336 ± 18	71[c]
Stim.	6	+78.3 ± 2.8	+18.3 ± 4.6	23[d]	+33.3 ± 5.6	+6.6 ± 4.9	20[c]
Adrenalectomy[e]	6	81.7 ± 2.5	67.5 ± 4.0	83[c]	440 ± 30	417 ± 29	95 n.s.
Basal	6	+85.8 ± 5.7	+25.0 ± 6.2	29[d]	+30.5 ± 4.5	+31.7 ± 6.0	104 n.s.
Stim.							
Phentolamine + propanolol after adrenalectomy[e]	6	72.5 ± 4.0	71.0 ± 5.2	98 n.s.	445 ± 28	326 ± 22	73[b]
	6	+30.0 ± 5.3	+23.3 ± 3.1	78 n.s.	+34.0 ± 5.1	+6.0 ± 6.0	18[d]
Basal							
Stim.							
Vasopressin antagonist after phentolamine, propanolol, and adrenalectomy[e]							
Basal	6	75.0 ± 34	65.0 ± 5.6	87 n.s.	313 ± 14	315 ± 15	101 n.s.
Stim.	6	+23.3 ± 3.1	+2.5 ± 1.1	11[c]	+10.0 ± 6.3	+8.3 ± 5.4	83 n.s.

[a] n.s., not significant.
[b] $p < 0.05$.
[c] $p < 0.01$.
[d] $p < 0.001$.
[e] In animals pretreated with atropine or methylatropine (0.3 mg/kg).

Distribution of pressor responses in relationship to C1 neurons

In 20 rats, the medulla was explored with a stimulating electrode in 0.2-mm steps over an area corresponding to the extent of the C1 neurons and their fiber paths, extending from the calamus scriptorius to a site 3 mm rostral to it, and across the entire lateral extent of the medulla. Stimuli consisted of 10-sec trains of pulses of 25 μA delivered at 100 Hz.

The distribution of sites yielding pressor responses greater than 30 mm Hg are indicated on the *right side* of Figure 6. Pressor responses greater than 50 mm Hg were encountered in two areas within the medulla. One was in the ventrolateral and the other was in the dorsomedial medulla (Fig. 6). At the level of the calamus scriptorius (Fig. 6*a*), these two pressor regions were only partially distinct, with responses also observed between them. At this level, the ventrolateral region lay immediately dorsal to the lateral reticular nucleus. One millimeter rostral to the calamus scriptorius, the ventrolateral pressor region lay just dorsal to the region between the two divisions of the lateral reticular nucleus, while the dorsal pressor region lay in an area containing many longitudinally running PNMT-labeled fibers, ventrolateral to the nucleus of the tractus solitarius and the hypoglossal nucleus (Figs. 6*b* and 7).

Two millimeters rostral to the calamus, the ventrolateral pressor region lay within the RVL just dorsal to the ventral surface of the brain (Fig. 6*c*). This region yielded the most active pressor responses and also contained the greatest number of C1 neurons. The close relationship between the stimulating electrode and C1 neurons is illustrated in Figure 8, where the relationship of an electrode track to neurons in the same animal stained with PNMT is shown. In this experiment, the threshold

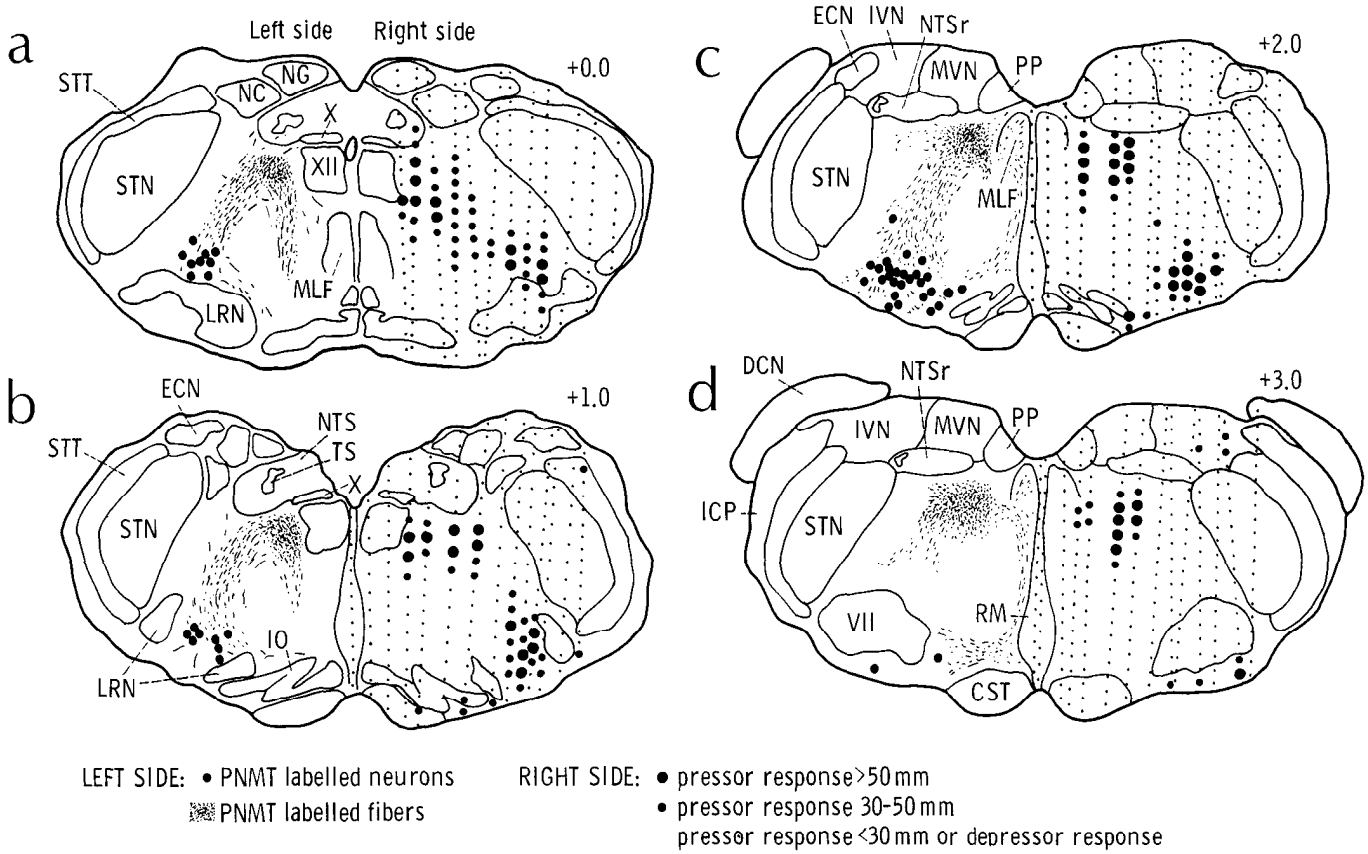

Figure 6. Locations of the most active medullary pressor regions to constant current electrical stimulation compared to locations of C1 cells and fibers immunocytochemically labeled for PNMT. *Left side of each section*, PNMT-labeled C1 neurons and PNMT-labeled fibers. *Right side of each section*, Pressor responses to electrical stimulation with 25 μA (100 Hz, 0.5 msec, 10-sec train). Responses between 30 and 50 mm Hg are indicated by *small solid circles*, whereas those greater than 50 mm Hg are indicated by *larger solid circles*. Responses less than 30 mm Hg or depressor responses are indicated by *small dots*. The location of the pressor region in the ventrolateral medulla corresponds to the location of the C1 neurons, and the location of the pressor region in the dorsomedial medulla corresponds to the location of the PNMT-labeled fiber bundle. *CST*, corticospinal tract; *DCN*, dorsal cochlear nucleus; *ECN*, external cuneate nucleus; *ICP*, inferior cerebellar peduncle; *IO*, inferior olive; *IVN*, inferior vestibular nucleus; *LRN*, lateral reticular nucleus; *MLF*, medial longitudinal fasciculus; *MVN*, medial vestibular nucleus; *NC*, nucleus cuneatus; *NG*, nucleus gracilis; *NTS*, nucleus tractus solitarius; *NTSr*, nucleus tractus solitarius pars rostralis; *PP*, nucleus prepositus; *RM*, raphe magnus; *STN*, spinal trigeminal nucleus; *STT*, spinal trigeminal tract; *TS*, tractus solitarius; *VII*, facial nucleus; *X*, dorsal motor nucleus of vagus; *XII*, hypoglossal nucleus.

for a 10-mm rise in AP was 8 μA, indicating that the elements being excited were very close to the electrode tip (Ranck, 1975).

Three millimeters rostral to the calamus, only a few pressor responses were encountered, in the region ventral to the facial nucleus (Fig. 6*d*). At 2 and 3 mm rostral to the calamus, the dorsomedial pressor region maintained approximately the same location, similar to that of the PNMT-labeled fibers.

Occasional or inconsistent pressor responses were encountered in other locations, including the ventromedial medullary surface ventral to the inferior olivary nucleus, the spinal trigeminal tract, and the inferior vestibular nucleus. Depressor responses (not shown) were also encountered in several locations, including caudal portions of the nucleus of the tractus solitarius, the solitary tract, the raphe pallidus and the area just lateral to it, and, less consistently, in the spinal trigeminal tract, the ventral medulla ventral to the inferior olivary nucleus, and a region near the spinal trigeminal nucleus, lateral to the ventrolateral pressor region, 2 or 3 mm rostral to the calamus.

These experiments therefore demonstrate overall a very close anatomical correspondence between the pressor sites in the medulla oblongata and the distribution of cell bodies and axonal pathways containing PNMT.

Role of intrinsic neurons in the RVL in mediating the pressor response

Pressor responses after chronic midbrain hemisection

To rule out the possibility that stimulation of axons passing through the ventrolateral medulla originating in neurons in the ipsilateral hypothalamus or amygdala (Saper et al., 1976; Hopkins and Holstege, 1978) contributed to the pressor response elicited by electrical stimulation of the RVL, experiments were conducted in five rats with chronic ipsilateral midbrain hemisections.

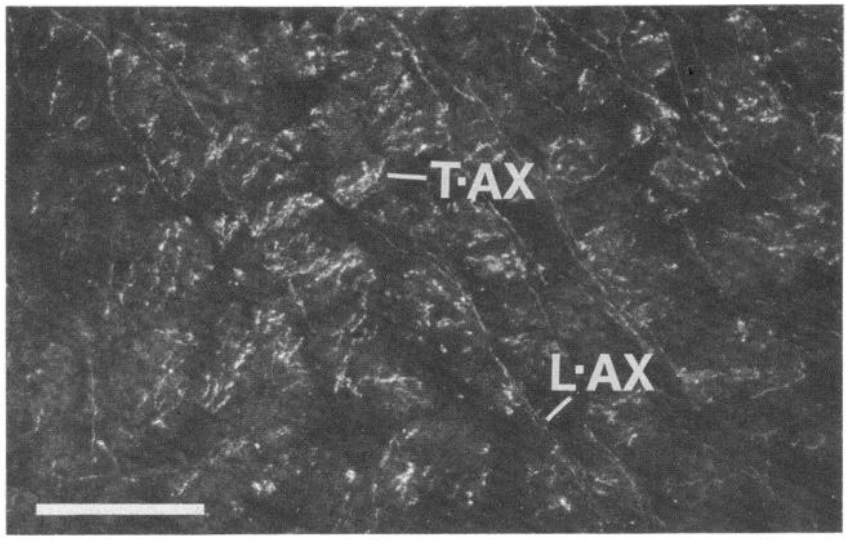

Figure 7. Photomicrograph of PNMT-labeled axons, cut transversely in the bundle in the dorsomedial medulla. The midline is toward the *left*; dorsal is toward the *top*. *T-AX*, PNMT-labeled axons, cut at right angles to the plane of the (coronal) section. *L-AX*, PNMT-labeled axons, traveling within the plane of the section.

The pressor responses elicited by stimulation of the RVL in hemisected rats had threshold currents similar to those of controls (8.8 ± 2.6 μA, versus 9.5 ± 1.1 in normals; $p < 0.05$), showed comparable frequency optima of 150 to 200 Hz (Fig. 1*B*), and were similarly graded with respect to stimulus current (Fig. 1*C*).

Effects of chemical stimulation and lesions of RVL

L-Glutamate and kainate. We examined the effects of microinjection into the RVL of small amounts of the excitatory amino acids, L-glutamate or its more potent stable analogue, kainic acid. In low doses, L-glutamate or kainic acid excites most neurons in the CNS, whereas in higher doses, they may produce depolarization blockade (McGeer et al., 1978; Puil, 1981). L-Glutamate excites only perikarya and not fibers of passage (Zieglgansberger and Puil, 1973; Fries and Zieglgansberger, 1974).

Microinjections of 100 nl of L-glutamate, but not saline, into the RVL consistently elicited a pressor response and tachycardia (Fig. 9, Table IV). Comparable volumes of saline elicited only a small rise in pressure or, more commonly, no response (Fig. 9*c*, Table IV). The minimal effective dose was approximately 50 pmol (Fig. 10*A*), and the effect was dose dependent. The maximal rise in AP produced by 10 nmol was over 90 mm Hg, which was comparable to the maximal effect elicited by electrical stimulation. The pressor and HR responses to L-glutamate were rapid in onset, beginning within 1 or 2 sec from the start of the injection and reaching a peak within 10 sec. The time of offset was dependent on dose and was within 10 sec at lower doses, and lasting up to 5 min with higher doses. In selected experiments, 1% fast blue dye was co-injected with L-glutamate in order to establish the diffusion sphere. Fast blue dye was restricted to the RVL and hence the effect of L-glutamate can be considered to be highly localized.

To assess the anatomical specificity of the response, 10 nmol of L-glutamate were injected 1 mm dorsal (four rats), 1 mm rostral (five rats), and 1 mm medial (three rats) to the RVL. The responses to injections in the dorsal and rostral sites, and in two of the five instances in medial sites, resulted in responses no different from those produced by saline alone (Fig. 9, *d* and *e*). Injections 1 mm medial to the RVL in three of five animals caused depressor responses as large as 35 mm (Fig. 9*f*). Similar depressor responses were also seen with injections in the region of the raphe pallidus in the midline.

The responses to L-glutamate could not be ascribed to mechanical distortion since the same volume of saline elicited virtually no response. Nor could they have been dependent on pH, which was 7.4 in both the saline and L-glutamate solutions. Hypertonic sodium chloride (1 M) injected in a volume of 100 nl did not produce a response greater than that of physiological saline.

In six animals, kainic acid (5 nmol), a stable analogue of L-glutamate, was injected into the rostral ventrolateral medullary pressor area. Kainic acid resulted in very large pressor responses and, in five of six animals, tachycardia. The mean pressor response was more than 90 mm Hg, and the tachycardia was about 100 bpm. This response lasted at least 10 to 15 min. In several cases, mean pressure began to fall after this period of time, but since the animals may have been approaching cardiac failure after such greatly elevated pressures, they were followed no longer.

Inhibitory amino acids and their antagonists. In contrast to L-glutamate, injection of the inhibitory amino acid GABA consistently resulted in a dose-dependent fall of AP and, most commonly, bradycardia (Fig. 11*a*). The smallest effective dose of GABA was between 20 and 50 pmol, and a maximal depressor response occurred between 5 and 10 nmol of the agent (Fig. 10*B*). The depressor response to GABA appeared within 1 to 2 sec

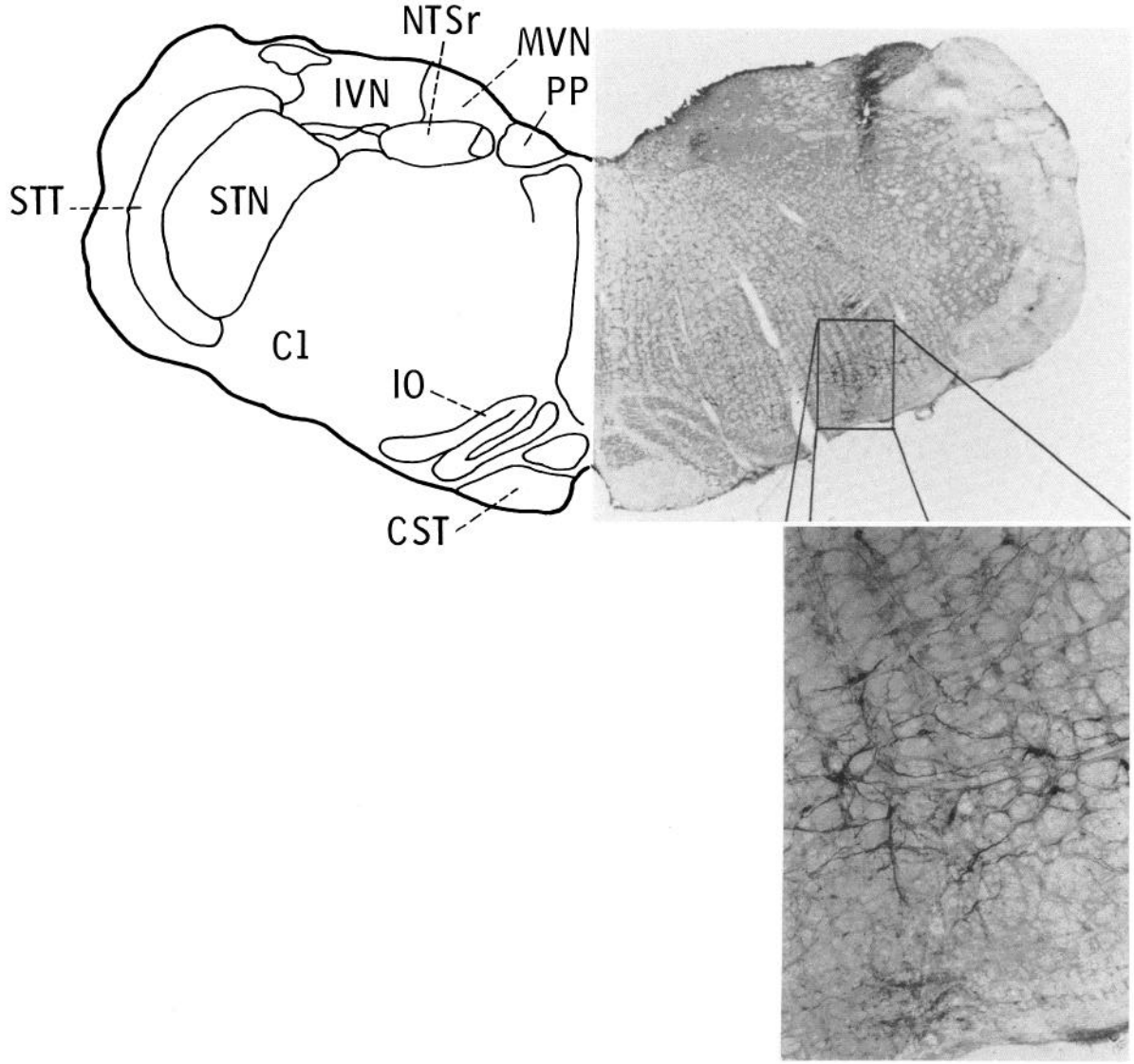

Figure 8. Location of an electrode track in close proximity to PNMT-labeled C1 neurons. The animal was prepared as usual for AP recording and the RVL was stimulated electrically. Then the animal was perfused with paraformaldehyde, and sections through the medulla were labeled immunocytochemically for PNMT. The electrode track can be seen entering the brain through the dorsolateral reticular formation. *Inset*, Neurons labeled for PNMT, with the bottom of the electrode track just ventral to them. The electrode passed directly through the C1 group. The threshold (for a 10-mm rise in AP) from the ventrolateral medulla in this rat was 10 μA, demonstrating that the elements being excited were very close to the electrode tip. *C1*, PNMT cell body system of ventrolateral medulla; *CST*, corticospinal tract; *IO*, inferior olive; *IVN*, inferior vestibular nucleus; *MVN*, medial vestibular nucleus; *NTSr*, nucleus tractus solitarius pars rostralis; *STN*, spinal trigeminal nucleus; *STT*, spinal trigeminal tract.

and reached a peak in about 10 sec. The time of fall-off varied with the dose, ranging from less than a minute to nearly 5 min. With a 10-nmol dose, the mean fall in AP was about 40 mm Hg (Table IV). In two of six rats, GABA elicited no change in HR or tachycardia. In the remaining four animals, 10 nmol of GABA elicited a dose-dependent slowing of the heart with bradycardia of 50 to 60 bpm.

Injection into the RVL of the GABA antagonist bicuculline (50 pmol) produced a marked elevation of AP (Fig. 11*b*). The response was slower to develop than the response to L-glutamate, reaching a peak about 1 min after injection and persisting for 20 min. The mean rise in AP was about 70 mm Hg (Table IV). The changes in HR were more variable, with four animals showing bradycardia and two showing tachycardia (Table IV).

To determine whether the pressor response to bicuculline was specific to this agent or would be elicited by any inhibitory amino acid antagonist, the putative glycine antagonist strychnine was also tested. Injection of glycine itself elicited a depressor response (Fig. 12*a*). However, strychnine, even at 10 times the concentration used for bicuculline (500 pmol versus 50 pmol), had nearly no effect on AP or HR (Fig. 12*b*, Table IV).

Tetrodotoxin. Bilateral injection of tetrodotoxin into the RVL caused a profound fall in resting AP and HR. In six rats, AP and HR were 121 ± 4 mm Hg and 420 ± 11 bpm before injection, and 51.7 ± 4.7 ($p < 0.001$) mm

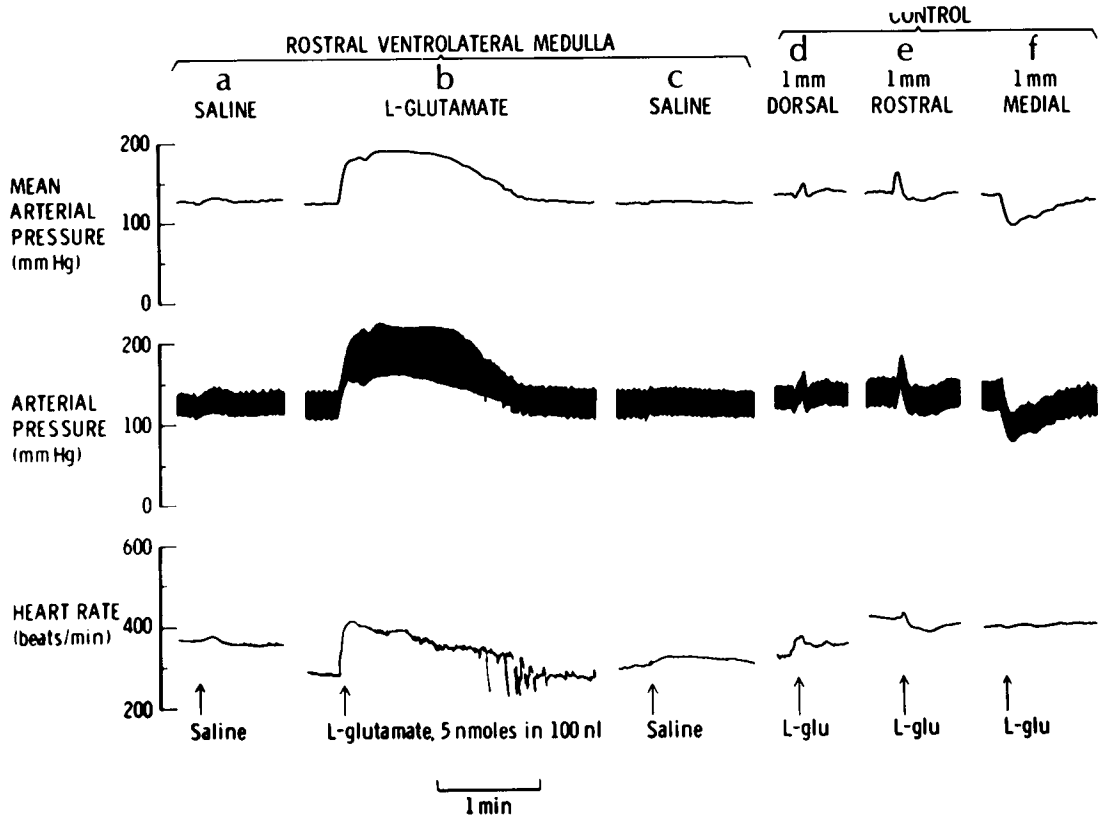

Figure 9. Cardiovascular responses to unilateral injections of sodium L-glutamate into the RVL and control regions. L-Glutamate, which stimulates only perikarya and dendrites, and not axons, caused a pressor response when injected into the RVL, but not when injected into adjacent areas. Each injection consisted of 100 nl. At least 5 min elapsed between injections. *a*, Control injection of saline into the RVL. *b*, Injection of L-glutamate, 5 nmol. *c*, Second control injection of saline 5 min after L-glutamate. *d*, Injection of L-glutamate 1 mm dorsal to the RVL. *e*, L-Glutamate, 1 mm rostral. *f*, L-Glutamate, 1 mm medial.

TABLE IV

Cardiovascular effects of unilateral injections of excitatory and inhibitory amino acids into the RVL and control regions

	n	Mean Arterial Pressure		Heart Rate	
		Base Line	Change	Base Line	Change
		A. Injections of L-glutamate into the RVL			
Saline	6	125 ± 5	+8.0 ± 3.4[a]	360 ± 23	+10.0 ± 4.5[a]
L-Glutamate (10 nmol)	6	123 ± 3	+71.6 ± 4.9	343 ± 24	+76.0 ± 13.8
Kainate	6	120 ± 4	+93.3 ± 3.3	388 ± 25	+98.0 ± 34.2
		B. Injections of L-glutamate (10 nmol) into control regions			
1 mm dorsal to RVL	4	127 ± 4	+10.0 ± 6.1[a]	392 ± 33	+23.3 ± 14.3
1 mm rostral to RVL	5	125 ± 6	+14.0 ± 1.0	350 ± 26	−22.5 ± 2.5[a]
1 mm medial to RVL	5	126 ± 3	−25.0 ± 11.0[b]	334 ± 32	+22.0 ± 12.0
		C. Injections of inhibitory amino acids and antagonists into the RVL			
GABA (10 nmol)	6	120 ± 4	−40.8 ± 6.6	408 ± 7	−34.0 ± 9.4
Bicuculline (50 pmol)	6	113 ± 6	+64.2 ± 6.8	348 ± 14	−87.5 ± 26.9[b]
Strychnine (500 pmol)	5	109 ± 4	+6.0 ± 1.9	350 ± 11	+4.0 ± 4.0

[a] The change in one animal was in the opposite direction and that datum was not included.

[b] The changes in two animals were in the opposite direction and those data were not included.

Hg and 352 ± 19 bpm ($p < 0.01$) 10 min after injection. Subsequent cervical spinal cord transection led to no further fall, with AP and HR 52.5 ± 6.2 mm Hg and 383 ± 16, bpm, 10 min following transection.

Discussion

Integrative cardiovascular mechanisms produced by excitation of the ventrolateral medulla

The present study demonstrates that focal electrical or chemical stimulation of a highly restricted region of the rostral ventrolateral medulla in the rat elicits an elevation of AP, tachycardia, and release of adrenal medullary catecholamines and vasopressin. The active region appears comparable in its distribution to the pressor region defined in the ventrolateral medulla of the rabbit by Dampney and Moon (1980).

The stimulus-locked rise in AP produced during electrical stimulation of the RVL in intact rats is attributable to excitation of sympathetic vasomotor fibers, since the response was almost entirely blocked by cervical spinal

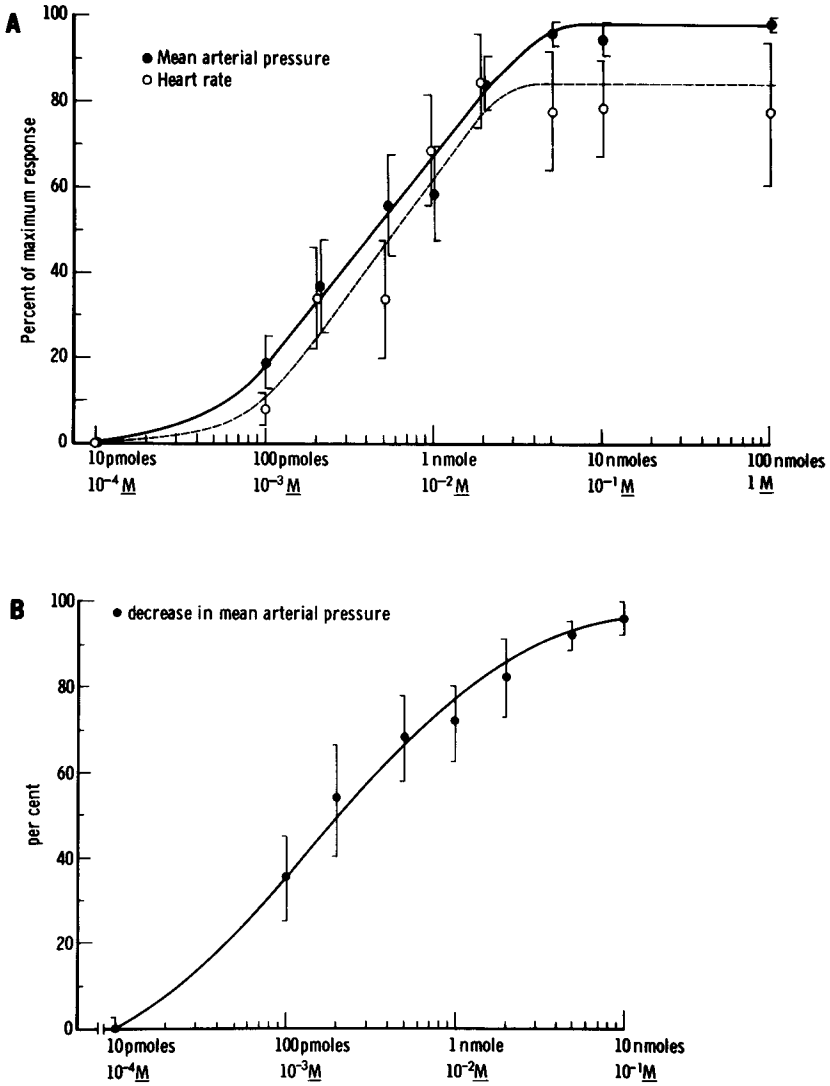

Figure 10. *A*, Dose response curves for the pressor response and cardioacceleration elicited by injection of L-glutamate into the RVL (response to L-glutamate minus response to control injection of saline). *Solid circles*, elevation of mean arterial pressure as percentage of maximum; *open circles*, tachycardia as percentage of maximum. Maximum for pressor response was 71.6 ± 4.9 mm Hg; for tachycardia the maximum was 76.0 ± 13.8 bpm. *B*, Dose response curves for the depressor response elicited by injection of GABA into the RVL. *Solid circles*, decrease in mean arterial pressure as percentage of maximum decrease. Maximum response: −40.8 ± 6.6 mm Hg.

cord transection, pharmacological blockade of α- and β-adrenergic receptors, or destruction of sympathetic nerves with 6-OHDA. In most cases, stimulation also excited sympathetic cardioacceleratory fibers and inhibited the cardiac vagus. In some animals, however, stimulation elicited co-excitation of the vagus nerve, probably as a consequence of direct stimulation of the perikarya of cardiovagal neurons located in the vicinity of the RVL in the rat (Nosaka et al., 1979). HR responses to electrical stimulation of the RVL in the rat differed from those of the rabbit (Dampney and Moon, 1980), in which no HR response was seen. The basis for the species difference is not certain.

Stimulation of the RVL also released hormones acting to elevate AP—in particular, catecholamines from the adrenal medulla, and vasopressin presumably from the posterior pituitary. In intact rats, the catecholamines and vasopressin did not contribute to changes in AP or HR. However, following destruction of the sympathetic nerves, adrenal catecholamines elicited an elevation of AP which appeared after a longer latency than the neurally mediated (stimulus-locked) response, was prolonged in duration, and was of a magnitude equal to or greater than that of the stimulus-locked response.

Three mechanisms probably contributed to the unmasking of a delayed, augmented, and persistent pressor response by catecholamines released from the adrenal medulla after the sympathetic nerves have been destroyed. (*a*) There may have been more catecholamines released in the 6-OHDA-treated rats (Mueller et al., 1967; Thoenen et al., 1969; Gautier et al., 1972; Del Bo et al., 1983b). (*b*) There may have been an increased

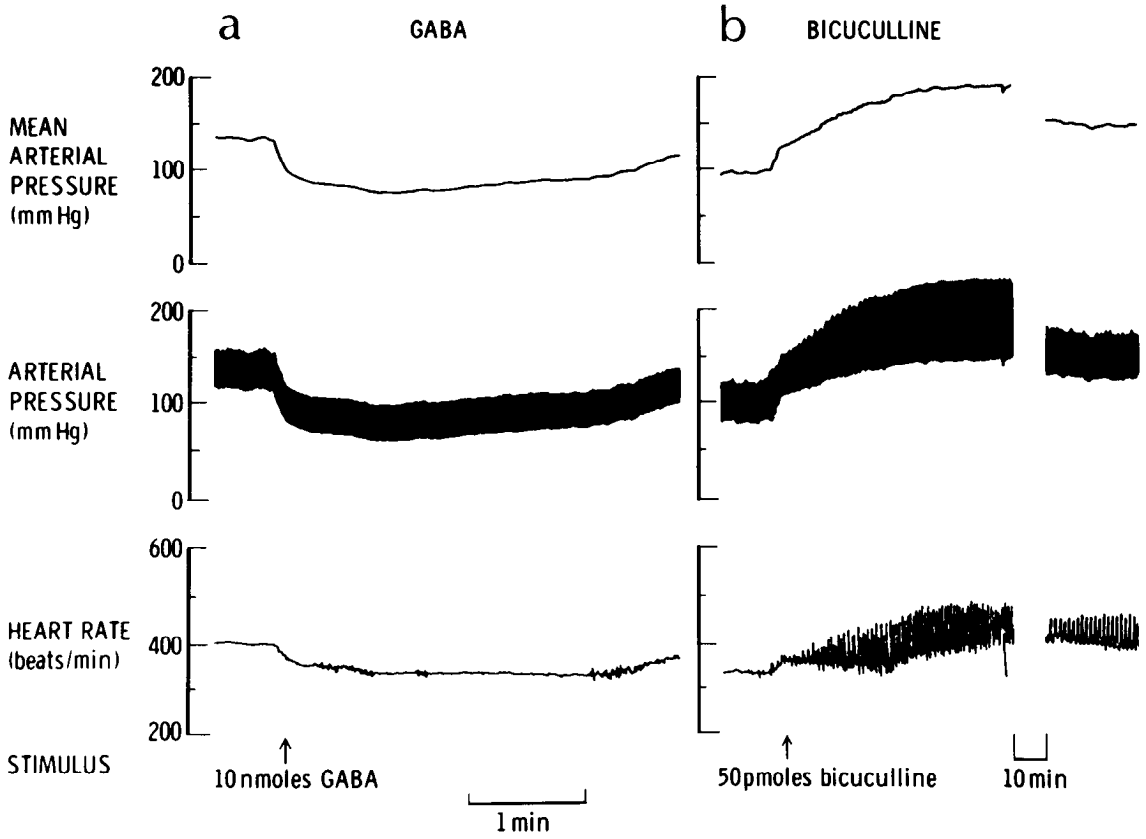

Figure 11. Cardiovascular responses to unilateral injections into the RVL of (*a*) GABA (10 nmol) and (*b*) bicuculline (50 pmol). GABA caused a decrease in AP and heart rate, whereas the specific GABA antagonist bicuculline caused a long-lasting elevation of AP and tachycardia.

effect of circulating catecholamines due to postsynaptic receptor sensitivity (Haeusler et al., 1969), or decreased reuptake after destruction of presynaptic terminals (Finch and Leach, 1970; Del Bo et al., 1983b). (*c*) Finally, interruption of the efferent vasomotor limb of the baroreceptor reflex arc reduced the counterbalancing sympathoinhibitory response secondary to the direct pressor effect of circulating catecholamines.

The increase in plasma dopamine was about 16% of the absolute increase of noradrenaline, a value which is greater than would be anticipated (3 to 10%) if the dopamine released had been present in the adrenal only as a precursor for noradrenaline (Bell and Gillespie, 1981; Bell, 1982); this finding is in accord with the view that dopamine may be released into the periphery as a neuromediator (Bell, 1982).

Adrenal medullary catecholamines were not responsible for the entire pressor response observed after chemosympathectomy. When the adrenals were subsequently removed, or when the spinal cord was transected (thereby interrupting sympathetic outflow from the brainstem to excite the adrenal medulla), stimulation of the RVL unmasked a small and delayed residual rise in AP which was blocked by a specific vasopressin antagonist.

The appearance of a vasopressin-mediated pressor response after spinal cord transection or adrenalectomy plus chemosympathectomy may have been due to interference with baroreceptor reflex mechanisms. In untreated animals, elevations of AP would reduce both release of vasopressin and its peripheral effectiveness, via the baroreceptors (Share and Levy, 1962; Share, 1965; Rothballer, 1966; Del Bo et al., 1983a). After blockade of the sympathoadrenally mediated pressor response, these inhibitory effects would be eliminated.

The pathways by which RVL stimulation elicits release of vasopressin from the hypothalamus are unknown. Electrical stimulation of the caudal brainstem can release vasopressin via ascending pathways to the diencephalon (Chang et al., 1937; Mills and Wang, 1964a, b). Moreover, chemical stimulation of the ventrolateral surface of the brain with nicotine or convulsant drugs also releases vasopressin (Bisset et al., 1975; Feldberg and Rocha e Silva, 1978), although the region so stimulated appears to be caudal to the region stimulated in our present experiment.

Relationship of the pressor responses to intrinsic neurons in the RVL

Phasic control of arterial pressure. The pressor response elicited by electrical stimulation of the RVL appears for two reasons to be the consequence of excitation of intrinsic neurons in the region rather than fibers of passage.

First, the response persists after chronic ipsilateral midbrain hemitransection, demonstrating that it does not depend on the integrity of fibers which originate from the hypothalamus and central nucleus of the amyg-

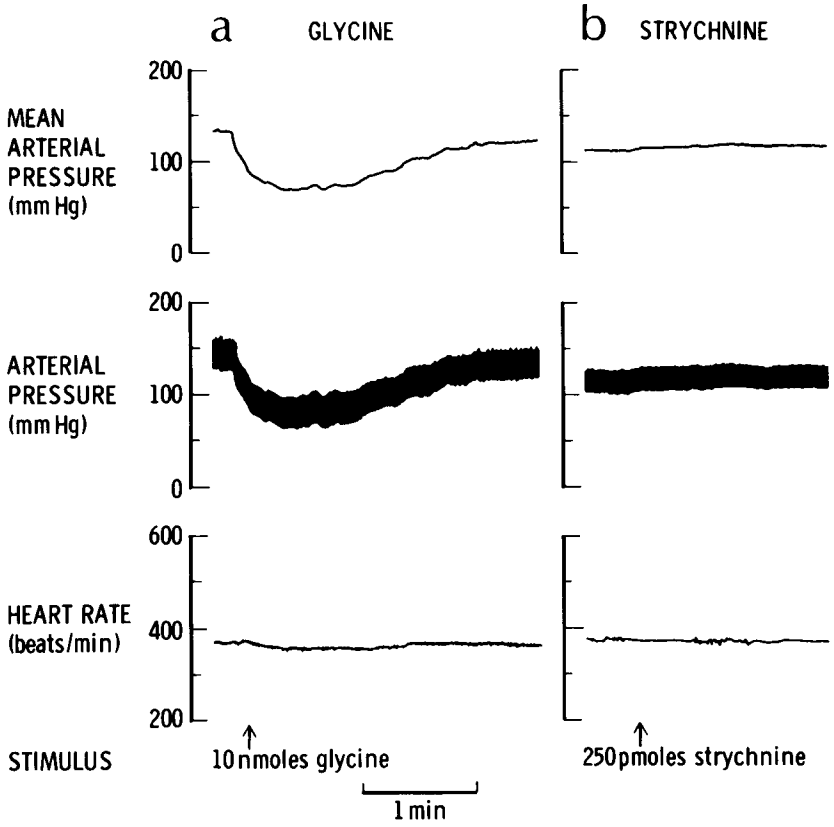

Figure 12. Cardiovascular responses to unilateral injections of (*a*) glycine (10 nmol) or (*b*) strychnine (500 pmol) into the RVL. Whereas glycine caused depressor responses, the glycine antagonist strychnine had little or no effect on AP, even at 10 times the dose used for bicuculline.

dala and pass through the ventrolateral medulla (Saper et al., 1976; Hopkins and Holstege, 1978). Moreover, the persistence of the pressor response from the RVL after acute total midbrain transection demonstrates that the response does not depend on excitation of fibers *ascending* from the ventrolateral medulla to rostral brainstem and secondarily exciting neurons projecting to the cord.

Second, and more direct, is the evidence that microinjection directly into the RVL of very small amounts of the excitatory amino acid L-glutamate elicits a dose-dependent and anatomically selective pressor response of a magnitude comparable to that produced by electrical stimulation. L-Glutamate when applied locally in brain excites neurons (Curtis et al., 1960; Krnjevic and Phillis, 1963; Cotman et al., 1981; Puil, 1981) but not axons (Zieglgansberger and Puil, 1973; Fries and Zieglgansberger, 1974; Nicoll et al., 1980; Miller and Armstrong-James, 1982). Thus the autonomic effects of electrical stimulation of the RVL are presumably a consequence of stimulation of intrinsic neurons, a finding recently supported by Dampney et al. (1982), who also have observed that injection of higher concentrations of L-glutamate into the ventrolateral medulla of the rabbit also elicits a pressor response.

Tonic control of arterial pressure. That electrical or chemical stimulation of intrinsic neurons in the RVL elevates AP indicates that such neurons, when *phasically* excited, will, in turn, excite preganglionic neurons. However, evidence also suggests that these neurons *tonically* excite sympathetic neurons. The observations that bilateral chemical or electrolytic lesions within the ventrolateral medulla of rabbit (Dampney and Moon, 1980) or cat (Guertzenstein and Silver, 1974; Guertzenstein et al., 1978; Bousquet et al., 1980) produce a fall of AP similar to that seen following spinal cord transection support this contention. Electrolytic lesions just rostral to the RVL do not cause falls in AP (Dampney and Moon, 1980), suggesting that these effects are not due to interruption of projections descending along the ventrolateral brainstem.

The fall of AP to levels comparable to that produced by spinal cord transection following bilateral injection of tetrodotoxin into the region or, as we have recently demonstrated, by bilateral electrolytic lesions of the C1 region (Granata et al., 1983b) demonstrates that the integrity of the C1/RVL region is necessary for the maintenance of resting vasomotor tone. Although these experiments do not exclude the possibility that such manipulations interfere with fibers of passage rather than intrinsic neurons, the fact that GABA microinjected into the RVL produces a marked and dose-dependent reduction of AP, whereas local administration of the GABA antagonist bicuculline elevates it, strongly suggests that intrinsic neurons in the RVL are tonically active. Moreover, these neurons presumably have GABA receptors. Thus it seems probable that GABA terminals in the RVL continuously inhibit the discharge of vasopressor neurons. The origin of the GABAergic innerva-

tion to the RVL is unknown; however, it may arise from neurons in the NTS which project directly to the RVL (Ruggiero et al., 1982).

Relationship to C1 adrenaline neurons

The question arises whether the neurons in the ventrolateral medulla which mediate the pressor responses are the C1-adrenergic neurons. Three lines of evidence would support the contention. First, a large proportion of those neurons in the RVL which directly innervate the intermediolateral column of the cord contain PNMT and thus are presumably adrenergic (Ross et al., 1981a, 1982). We have shown, using anterograde transport of tritiated amino acids, that the RVL gives rise to a highly specific projection to all regions of the thoracic intermediolateral column including those which contain preganglionic neurons innervating the adrenal medulla (Ross et al., 1982, 1983, 1984). Whether different bulbospinal neurons of the RVL provide innervation of different segments of the intermediolateral column or whether individual neurons in the RVL innervate via collaterals several levels of the intermediolateral column is unknown. The fact that in our studies the vasomotor and adrenal medullary responses could not be dissociated according to electrode position, thresholds, or stimulus frequency or current/response characteristics suggests at least that similar groups of neurons provide integrated sympathetic and hormonal responses to stimulation. Dampney et al. (1982) have shown that the pressor area in the ventrolateral medulla corresponds closely to the area projecting directly to thoracic spinal cord.

Second, the sites from which pressor responses of the greatest magnitude were elicited were concentrated in the areas containing C1 cell bodies in the RVL. In addition, pressor responses were elicited along the trajectories of fibers stained for PNMT which course into the dorsomedial medulla and then appear to descend to innervate the spinal cord. These findings would lend support to speculations (Dampney and Moon, 1980; Dampney, 1981) that the pressor responses elicited from the dorsomedial medulla were a consequence of excitation of axons of neurons residing in the ventrolateral medulla. Our findings would add to this inference the possibility that such axons are derived from the adrenaline cells of the C1 group.

Third, the response appeared to be selective for the C1 group. Thus, in our experiments, pressor responses were not elicited by low current electrical stimulation of the lateral reticular nucleus, a cellular group which has been proposed as a vasomotor center in the medulla (Henry and Calaresu, 1974; Ciriello and Calaresu, 1977; Thomas et al., 1977). Nor did pressor responses arise with high specificity from the region containing the A1 noradrenergic cell group (Neumayr et al., 1974). Pressor responses were inconsistently elicited from the region of the ventrolateral subpial group (Ross et al., 1981b) or nucleus interfascicularis hypoglossi (Loewy and Neil, 1981; Loewy and McKellar, 1981). In some experiments, stimulation of these regions elicited only bradycardia.

The evidence therefore suggests that it is the adrenaline-containing neurons of the C1 region which constitute the neuronal population of the RVL controlling the circulation. When excited, these neurons elevate, and when silenced, they profoundly reduce AP. Nevertheless, it should be emphasized that the results are at best correlative and do not as yet *prove* that the adrenaline neurons are entirely responsible for the responses. Conceivably, other perikarya in the region could be projecting in parallel to the cord and thus might be responsible for the physiological effect.

Yet even assuming that it is the adrenaline neurons which are responsible for the cardiovascular responses, it is also not certain that the neurotransmitter released from these neurons, which excites preganglionic neurons in the spinal cord, is adrenaline: adrenaline when microiontophoresed into the intermediolateral column usually inhibits the discharge of sympathetic preganglionic neurons (Coote et al., 1981; Guyenet and Cabot, 1981). However, these iontophoretic studies must be interpreted with care. First, it is quite possible that microiontophoretically applied adrenaline may act on receptors at sites other than those on the preganglionic neuron which, in turn, are turned on by the descending pathway—for example, receptors upon adjacent interneurons or on presynaptic terminals. Second, exogenously applied adrenaline may act upon receptors normally innervated by other descending aminergic systems, particularly those arising from the noradrenergic projections (Westlund and Coulter, 1980; Westlund et al., 1981). Third, stimulation of C1 neurons may co-release other agents which are, in fact, the pharmacologically active ones. For example, adrenaline neurons appear to contain neuropeptide Y (Hökfelt et al., 1983), although the role of this peptide in vasomotor control is not known. Finally, it is also not known whether all adrenaline neurons are functionally homogeneous: for example, in this study it appeared that the more active pressor responses were elicited from the lateral rather than from the medial portions of the C1 group.

The apparent paradox that stimulation of chemically identified cells in the medulla produces one action on preganglionic sympathetic neurons, with the purported transmitter applied locally having an opposite effect, is not unique. Indeed, a comparable contradiction is seen with respect to the descending serotonergic projections from the brainstem to the spinal cord. Whereas iontophoresis of serotonin into the intermediolateral column usually results in excitation of sympathetic preganglionic neurons (DeGroat and Ryall, 1967; Coote et al., 1981), electrical stimulation of the caudal raphe nuclei, which give rise to the descending serotonergic innervation of the intermediolateral cell column (Basbaum et al., 1978; Amendt et al., 1979; Loewy, 1981), causes sympathoinhibition (Coote and Macleod, 1974; Neumayr et al., 1974; Cabot et al., 1979; Gilbey et al., 1981). Until further studies are available, the consistent evocation of a pressor response by stimulation of C1 perikarya or axons would appear to offer evidence as direct as possible that the role of these neurons in controlling AP is excitatory.

Relationship to vasoactive centers represented on the ventral surface of the medulla oblongata

The present studies raise the question of whether neurons in the RVL, in particular, C1 neurons, may be

responsible for mediating the vasomotor and other autonomic effects produced by chemical stimulation of the ventral surface of the medulla. Previous studies have mapped several "chemosensitive zones" responsive to altered cerebrospinal fluid, pH, and PO_2 (Mitchell et al., 1963; Loeschcke et al., 1970; Trouth et al., 1973; Fukuda and Honda, 1976; Pokorski, 1976; Lioy et al., 1981; Wennegren and Oberg, 1980). Other studies have found that the application of drugs to the ventrolateral medullary surface has dramatic cardiovascular effects (Guertzenstein, 1973; Guertzenstein and Silver, 1974; Bisset et al., 1975; Feldberg and Rocha e Silva, 1978, 1981; Wennegren and Oberg, 1981). Physiological experiments, mostly in the cat, have mapped three areas on the ventrolateral medullary surface: a rostral area M, a middle area S, and a caudal area L (see Trouth et al., 1973; Ross et al., 1981b). Respiration and blood pressure were increased by electrical stimulation of the surface of the brain at areas L and M, or by perfusion of these areas with cerebrospinal fluid with decreased pH or elevated PCO_2 (Mitchell et al., 1963; Loeschcke et al., 1970; Schlafke et al., 1970; Trouth et al., 1973). Cooling or application of procaine to area M causes apnea (Mitchell et al., 1963). Cooling of area S caused respiratory arrest and a fall in blood pressure (Schlafke and Loeschcke, 1967). Although it is difficult to compare studies in the cat and rat, there appears to be a correspondence between the C1/RVL region mapped in the present study and the areas M and S. However, another area on which nicotine and other drugs act to induce vasopressin secretion (Bisset et al., 1975; Feldberg and Rocha e Silva, 1978, 1981) appears to lie further caudally.

Since PNMT-containing neurons frequently have dendrites extending near the ventrolateral surface (Armstrong et al., 1982), the C1 neurons may directly serve a chemoreceptor function. A few of the C1 neuronal perikarya themselves lie close to the surface and may have been included in the neurons termed the ventrolateral subpial group (Ross et al., 1981b), which project directly to the thoracic spinal cord. On the other hand, it is possible that other chemosensitive neurons lying close to the surface (Pokorski, 1976; Fukuda and Honda, 1976) may relay signals to the C1 neurons or project directly to the spinal cord (Ross et al., 1981b).

Summary and conclusions: The role of adrenergic and noradrenergic neurons of the ventrolateral medulla in cardiovascular control

The present study adds further evidence that neurons of the ventrolateral medulla which synthesize, store, and release catecholamines play a vital role in cardiovascular control. These catecholamine groups reside within a region we have termed the nuclei rostroventrolateralis and caudoventrolateralis (RVL and CVL; Ross et al., 1984) and are biochemically, anatomically, pharmacologically, and functionally differentiated.

Neurons in the A1 group of the caudal ventrolateral medulla (nucleus caudoventrolateralis or CVL) synthesize, store, and release noradrenaline. These neurons do not project to the spinal cord (Blessing et al., 1981a, b; Ross et al., 1981a), but rather project rostrally to innervate the hypothalamus (Blessing et al., 1982a, b; Sawchenko and Swanson, 1982). Stimulation of the region with low frequency electrical stimuli or glutamate produces a fall of AP (Blessing and Reis, 1982), whereas bilateral lesions, produced electrolytically, or by kainic acid, or by the application of GABA, elevates AP (Blessing et al., 1981c; Blessing and Reis, 1983) and releases vasopressin (Blessing et al., 1982b). This region appears to receive projections from NTS (Ruggiero et al., 1982), but lesions of the A1 area do not block baroreceptor reflexes (Granata et al., 1983b).

In contrast, the rostral portion of the ventrolateral medulla (nucleus reticularis rostroventrolateralis or RVL) contains neurons, the C1 group, which synthesize adrenaline, since they contain all of the enzymes, including PNMT, required for its biosynthesis (Armstrong et al., 1982). These neurons project to autonomic centers of the spinal cord (Ross et al., 1981a, 1983, 1984). Electrical or chemical stimulation of the region produces elevations of AP, tachycardia, inhibition of baroreceptor reflexes, and release of adrenal medullary catecholamines and posterior pituitary vasopressin. The neurons appear tonically active, since bilateral lesions of the RVL, lesions of their axons (Granata et al., 1983b), or local application of tetrodotoxin or GABA reduce AP, whereas local injection of bicuculline elevates it. These observations suggest that it is the C1 adrenaline neurons of the RVL which exercise tonic vasomotor control of the circulation and represent the tonic vasomotor neurons of the brain.

The rostral ventrolateral medulla has been implicated in mediating several other important cardiovascular responses besides those elicited by drugs applied to the ventrolateral surface. Inactivation of this region greatly reduces the cerebral ischemic reflex (Dampney and Moon, 1980), the response to systemic injection of clonidine (Bousquet and Guertzenstein, 1973· Bousquet et al., 1975), and the response to hypothalamic stimulation (Bloch et al., 1977; McAllen et al., 1982). Whether C1 neurons mediate these responses is unknown.

Finally, neurons in the RVL, in particular the C1 neurons, may be the elements in the RVL which mediate baroreceptor reflexes. The rostral ventrolateral medulla receives dense projections from cardiovascular portions of the nucleus of the tractus solitarius (Loewy and Burton, 1978; Ricardo and Koh, 1978; Dampney et al., 1982; Ruggiero et al., 1982). Using double label techniques, we have also found that projections from the cardiovascular NTS directly overlap the regions of the RVL containing C1 neurons which innervate the thoracic spinal cord (Ruggiero et al., 1982). Inactivation of the RVL reduces or abolishes the baroreceptor reflex (Dampney, 1981; Feldberg and Rocha e Silva, 1981; McAllen et al., 1982; Granata et al., 1983a, b). These data, taken together, suggest that neurons in the RVL, in particular C1 adrenaline-synthesizing neurons, may reflexly as well as tonically control the circulation.

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