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Differentiated Pressor and Sympathetic Responses to Dual Brain Stimulation: Ventromedial Hypothalamus versus Locus Coeruleus[1] (41988)

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Abstract. Sensitivity of the ventromedial hypothalamus (VMH) to electrical stimulation was compared with that of the locus coeruleus (LC) in urethane-anesthetized rats. Based not only on current strengths required to elicit threshold effects, but also on magnitude of pressor responses to suprathreshold stimulation, the LC was consistently more sensitive than the VMH. Despite this greater pressor sensitivity, splanchnic nerve firing increased almost equally upon stimulation of either brain area. Similar comparisons made in other rats following bilateral adrenalectomy or pretreatment with a vasopressin antagonist showed no significant alteration of pressor and sympathetic responsiveness to stimulation of either the LC or the VMH. When frequency of neural firing was recorded from a lumbar sympathetic trunk instead of the splanchnic nerve, increases in sympathetic nerve activity produced by LC stimulation were significantly larger than those produced from the VMH. The results suggest that greater pressor sensitivity of the LC is due, at least in part, to stronger constriction in vascular beds innervated by the lumbar sympathetic chains.

Seemingly identical increases in blood pressure have been elicited from different brain areas by electrical stimulation, yet neither the neural pathways nor the underlying mechanisms are likely to be the same. Two brain regions which may elevate blood pressure mainly by increasing sympathetic vasomotor activity are the ventromedial hypothalamus (VMH) and the locus coeruleus (LC). Electrical stimulation of the VMH, which has profuse neural connections to other brain areas including the LC (1), produces pressor responses accompanied by tachycardia and increased sympathetic nerve firing (2). On the other hand, similar stimulation of the LC, which consists almost entirely of noradrenergic neurones (3–5), elevates blood pressure by activating neural pathways descending through the ventrolateral medulla (6). It is, however, difficult to identify the mechanisms causing pressor responses from each site because either site alone has usually been stimulated in different rats.

To allow quantitative comparisons of sensitivity in the present studies, pressor and sympathetic nerve responses were recorded while the VMH and the LC were stimulated alternately in the same rats. Regional differences in sympathetic nerve activity were recorded from multifiber preparations of either the splanchnic or lumbar nerves. Additional experiments were done using rats that had been either adrenalectomized or pretreated with a vasopressin antagonist to determine whether ensuing pressor responses could in part be due to increased release of endogenous catecholamines or vasopressin.

Materials and Methods. *Rat groups.* Experiments were done on 46 male Sprague–Dawley rats, weighing 299 ± 4 g, purchased from SASCO Inc. (Omaha, Nebr.). Results from 21 rats that did not show significant pressor responses to electrical stimulation of either VMH or LC were discarded. Of the remaining 25 rats, 11 were used to characterize cardiovascular and splanchnic sympathetic evoked responses to stimulation of two different brain areas successively in the same rat. Subsequently, similar responses were recorded in three other groups consisting of 4

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rats each following either pretreatment with a vasopressin antagonist or bilateral adrenalectomy, and 6 rats prepared for recording lumbar sympathetic nerve activity.

Cardiovascular and sympathetic responses to brain stimulation. All rats were initially anesthetized with sodium pentobarbital (6 mg/100 g ip) for implantation of chronic electrodes into the VMH and LC. Two concentric stainless-steel electrodes 0.5 mm in diameter (NE-100; custom-made by Rhodes Medical Instruments, Woodland Hills, Calif.) were implanted on opposite sides of the brain: one in the ventromedial hypothalamus at stereotaxic coordinates anteroposterior 6.0, lateral 1.0, and dorsoventral −3.7; and the other in the locus coeruleus at stereotaxic coordinates anteroposterior −1.4, lateral 0.8, and dorsoventral −2.5 (7). Electrodes were fixed to the skull using stainless-steel screws and dental cement (8). Three days later, each rat was again anesthetized with urethane (100 mg/100 g ip) and separate catheters were inserted into a femoral artery for recording blood pressure and into a femoral vein for drug injections. In four rats both adrenal glands were removed at this time through bilateral flank incisions.

Phasic arterial pressure was recorded by connecting the aortic catheter through Tygon tubing to a pressure transducer (Statham P23Gb). The phasic signal from the transducer was used to trigger a biotachometer for simultaneous registration of heart rate on another channel of the recorder.

Sympathetic activity in the major splanchnic nerve was recorded by placing the nerve bundle immediately above the coeliac ganglion over a bipolar stainless-steel electrode (uninsulated tips 1 mm apart). To record sympathetic activity from the lumbar sympathetic chain, the bipolar electrode was placed on the left nerve bundle which was exposed at the level of the third and fourth lumbar vertebrae. Nerves and electrode tips were immersed in mineral oil. All rats were paralyzed with decamethonium bromide (Syncurine, 0.2 mg/100 g iv) to eliminate movement artifacts and then ventilated artificially to maintain blood gas pH and CO_2 normal. Monophasic spike potentials from either the splanchnic or lumbar sympathetic nerves were amplified (Grass P15AC amplifier bandpass 30–3000 cycles/sec) and recorded continuously on magnetic tapes which were later played back into an amplitude analyzer (F. Haer and Co., Brunswick, Maine) to convert individual waves into uniform pulses. Because ganglion blockade would be equivalent to crushing the nerve to obtain zero activity, pentolinium tartrate (Ansolysen, 0.5 mg/100 g iv) was injected routinely at the end of every experiment; during playback, the low-level control of the window discriminator was set to filter background noise persisting after ganglion blockade. The number of individual pulses per second was counted with a rate analyzer whose output was recorded as a histogram on an ink-writing recorder (9).

Pulsatile femoral pressure and sympathetic nerve activity were then recorded continuously during progressive increases in intensity of electrical stimulation of the two brain areas. In some rats the VMH was stimulated before the LC, while in others the sequence was reversed with the LC being stimulated first and the VMH second. For electrical stimulation, 5-sec trains of 1-msec biphasic pulses at a frequency of 100/sec were applied at current strengths ranging from 10 to 150 μA. Current strength was graded in 10-μA increments until a threshold pressor response (an increase in mean femoral pressure of at least 4 mm Hg) was elicited, after which 50-μA increments were added up to a peak of 150 μA. In rats pretreated with the vasopressin antagonist, pressor responses to intravenous injection of vasopressin were also recorded before and after administration of the antagonist.

Brain histology, drugs, and statistics. At the end of each experiment a 0.5-mA direct current was passed through the chronically implanted electrodes for 10 sec to produce a small lesion. Two similar lesions, one in the VMH and the other in the LC, were thus produced on opposite sides of each brain. The whole brain was then removed and stored in Formalin (containing 1% potassium ferricyanide) until sectioning. Transverse sections (10 μm) stained with cresyl violet were compared with the atlas by Pellegrino *et al.* (7) to locate lesion sites.

Drugs used were arginine vasopressin (Parke–Davis), 2.5 mU; and the vasopressin

antagonist [1-(β-mercapto-β,β-cyclopentamethyleneproprionic acid)-2-(o-methyl) tyrosine]-Arg^8-vasopressin, 3 μg. All doses are expressed in terms of the respective salts per 100 g body wt. The vasopressin antagonist was purchased from Bachem Inc. (Torrance, Calif.) and injected intravenously as described by Crofton *et al.* (10).

Data were routinely expressed as averages ± SEM and analyzed using two-tailed *t* tests for comparing means of dependent or independent samples (11); differences at a 5% level ($P < 0.05$) were considered significant. An analysis of variance was used to examine results from more than two groups and for *F* ratios significant at 5% or less, Duncan's multiple range test (12) was applied to determine significance of differences between pairs of means. When splanchnic and lumbar nerve data were expressed as percentage changes, since a normal distribution cannot be assumed, neural activity was analyzed using either the Wilcoxon sign test for related samples or the Mann–Whitney *U* test for independent samples (11) to determine significant differences between pairs of means.

Results. *Threshold sensitivity of the ventromedial hypothalamus and locus coeruleus.* Electrical stimulation of either the VMH or the LC with 5-sec current trains produced essentially the same qualitative effects: blood pressure and sympathetic nerve activity increased while heart rate was unaltered or slightly diminished. Upon stimulation of either brain area with effective currents, frequency of splanchnic nerve firing rose within 1 sec and this was followed by a slower increase in blood pressure which peaked within 2–3 sec after the 5-sec train stopped (Fig. 1). Stimulus threshold, defined as the current strength required to elicit barely detectable responses of at least 4 mm Hg in mean blood pressure or 5 spikes/sec in sympathetic neural firing, was consistently lower for the LC than for the VMH. The threshold for elevating blood pressure was 40 ± 3 μA for the LC as compared with 81 ± 5 μA for the VMH ($P < 0.001$) with corresponding average pressor responses of 10 ± 2 mm Hg and 7 ± 1 mm Hg, respectively. Thresholds for producing detectable increases in sympathetic nerve firing were almost invariably lower than those for blood pressure, averaging

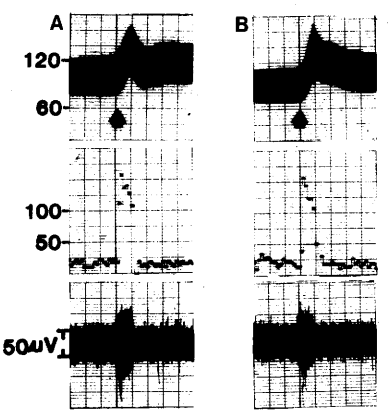

FIG. 1. Effects on splanchnic nerve firing and blood pressure of stimulating the ventromedial hypothalamus (A) and the locus coeruleus (B) in a urethane-anesthetized rat. Upper trace of phasic femoral arterial pressure (mm Hg), middle trace a histogram of frequency of neural firing (spikes/sec), and lower trace original analog signal of neural firing. Large arrows indicate onset of 5-sec periods of stimulation with 150-μA currents. Recorder speed 125 mm/min.

39 ± 2 μA for the LC and 69 ± 6 μA for the VMH ($P < 0.001$), with corresponding average responses of 11 ± 2 and 10 ± 1 spikes/sec respectively.

For currents above threshold strength magnitude of pressor and splanchnic nerve responses increased progressively as stronger currents were used for stimulation. Currents ranging from 50 to 150 μA consistently caused larger pressor effects when applied to the LC than when applied to the VMH, but attendant increases in splanchnic nerve firing were not different (Fig. 2; Table I). Because this indicated that the greater sensitivity of the LC was not accompanied by a greater increase in splanchnic vasomotor activity, additional experiments were done to explore the possible involvement of different humoral pressor mechanisms.

Reproducibility of responses to successive stimulation of the ventromedial hypothalamus and locus coeruleus. To determine whether prior stimulation of one brain area affected responses to subsequent stimulation of the other area, sequence of stimulation was alternated so that the VMH was stimulated first followed by the LC in 7 rats, and then the sequence was reversed (i.e., LC first and VMH second) in 4 others. Regardless of the

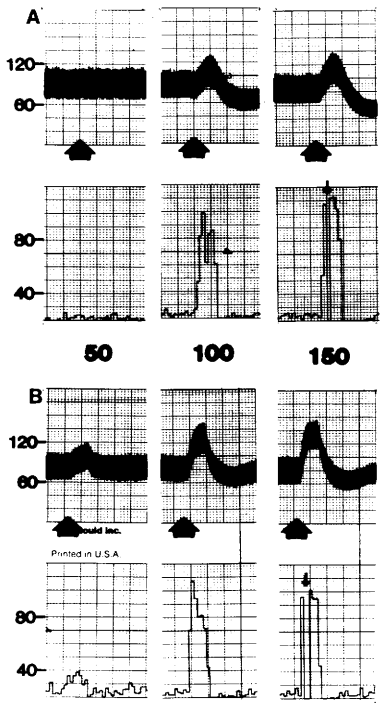

FIG. 2. Responses to graded electrical stimulation of the ventromedial hypothalamus (A) and the locus coeruleus (B) in a urethane-anesthetized rat. In each panel upper trace of femoral pressure and lower trace a histogram of splanchnic nerve firing. Center numbers indicate three intensities expressed as current strengths (μA) used for electrical stimulation. Small arrows indicate where histogram went off-scale because of very high increases in firing frequency. Units as in Fig. 1. Recorder speed 1 mm/sec.

sequence of stimulation, magnitude of pressor responses to stimulation of either area with 50- to 150-μA currents remained the same; consequently, data from all 11 rats were pooled (Table I).

Does greater pressor responsiveness to the locus coeruleus result from stronger activation of humoral pressor mechanisms? The possibility that greater pressor responses to LC stimulation were partly mediated by release of endogenous vasopressin was studied in four rats following pretreatment with a vasopressin antagonist. Mean arterial pressure fell by 26 ± 6 mm Hg within 5 min after intravenous injection of the antagonist. Intravenous injection of 2.5 mU of vasopressin elevated mean arterial pressure by 21 ± 2 mm Hg initially, but following administration of the antagonist this was markedly reduced to 3 ± 1 mm Hg ($P < 0.01$). Pressor and splanchnic nerve responses to subsequent electrical stimulation of the LC and VMH in these rats were almost identical to those recorded previously in untreated control rats. Similarly, stimulation of both brain areas also elicited the same pressor and splanchnic nerve responses in four other rats whose adrenal glands had been removed (Table II). In both rat groups, current thresholds (μA) were lower for the LC (35 ± 5 in rats pretreated with the vasopressin antagonist and 40 ± 6 in those that were adrenalectomized) than for the VMH (73 ± 10 and 83 ± 5 respectively; $P < 0.001$ for both comparisons). As was shown previously in control rats, in both these groups pressor responses to LC stimulation were also consistently larger than those to VMH stimulation, while corresponding increases in splanchnic nerve firing were almost the same regardless of the brain site stimulated (Table II).

Lumbar sympathetic nerve activity during stimulation of the ventromedial hypothalamus and locus coeruleus. Because sympathetic fibers in the splanchnic nerve supply mainly the abdominal organs, it was considered possible that VMH and LC stimulation may affect vasoconstrictor tone in other sympathetic pathways differently. To test this possibility, responses to graded VMH and LC stimulation were quantified in 6 other rats, but sympathetic nerve firing was recorded from a lumbar sympathetic trunk instead of the splanchnic nerve. Baselines for mean blood pressure (91 ± 5 mm Hg) and heart rate (367 ± 14 bpm) were essentially like those from control rats studied earlier, but frequency of neural firing (spikes/sec) in the lumbar sympathetic chain (7 ± 1) was significantly lower than that in the splanchnic nerve (averaging 20 ± 2 in 11 rats; $P < 0.01$). Upon graded electrical stimulation of the VMH and LC, mean pressor responses were likewise of the same magnitude as those in control rats. However, corresponding increases in lumbar nerve firing were considerably smaller than those recorded previously from splanchnic nerves, and for currents of 100 and 150 μA the differences were statistically significant (Table III; Fig. 3).

TABLE I. CARDIOVASCULAR AND SPLANCHNIC NERVE RESPONSES TO SUCCESSIVE VMH AND LC STIMULATION IN URETHANE-ANESTHETIZED RATS

Current strength (μA)	VMH stimulation			LC stimulation		
	VMH 1st (n = 7)	VMH 2nd (n = 4)	Combined (n = 11)	LC 1st (n = 4)	LC 2nd (n = 7)	Combined (n = 11)
	A. Mean pressure response (mm Hg)					
50	1 ± 1	0 ± 1	1 ± 1	13 ± 7	15 ± 3	14 ± 3[a]
100	17 ± 3	20 ± 7	18 ± 3	55 ± 8	50 ± 3	52 ± 3[a]
150	36 ± 3	30 ± 6	33 ± 3	64 ± 7	61 ± 3	62 ± 3[a]
	B. Heart rate response (bpm)					
50	0	0	0	4 ± 3	2 ± 1	3 ± 1
100	−2 ± 5	−5 ± 3	−3 ± 3	5 ± 3	1 ± 2	3 ± 2
150	−6 ± 5	−8 ± 4	−7 ± 3	4 ± 4	3 ± 4	−1 ± 3
	C. Splanchnic nerve response (spikes/sec)					
50	4 ± 2	3 ± 2	3 ± 1	23 ± 11	27 ± 4	25 ± 4
100	86 ± 11	73 ± 18	81 ± 9	113 ± 13	92 ± 9	100 ± 8
150	116 ± 8	110 ± 12	114 ± 7	118 ± 13	111 ± 13	114 ± 9

Note. Data expressed as average changes from baselines of 79 ± 6 mm Hg for mean pressure, 395 ± 7 bpm for heart rate, and 20 ± 2 spikes/sec for splanchnic nerve activity.

[a] $P < 0.05$ as compared with corresponding average for the VMH using a paired t test.

TABLE II. CARDIOVASCULAR AND SPLANCHNIC NERVE RESPONSES TO SUCCESSIVE VMH AND LC STIMULATION IN CONTROL, VASOPRESSIN-ANTAGONIST PRETREATED (ANT-VP), AND ADRENALECTOMIZED (ADREX) RATS

Current strength (μA)	VMH stimulation			LC stimulation		
	Control (n = 11)	anti-VP (n = 4)	ADREX (n = 4)	Control (n = 11)	anti-VP (n = 4)	ADREX (n = 4)
	A. Mean pressure response (mm Hg)					
50	1 ± 1	−2 ± 1	1 ± 1	14 ± 3[a]	21 ± 6[a]	22 ± 9[a]
100	18 ± 3	21 ± 7	16 ± 6	52 ± 3[a]	51 ± 7[a]	49 ± 5[a]
150	33 ± 3	33 ± 7	28 ± 7	62 ± 3[a]	64 ± 1[a]	60 ± 5[a]
	B. Heart rate response (bpm)					
50	0	−3 ± 2	0	3 ± 1	2 ± 2	0 ± 1
100	−3 ± 3	−9 ± 4	−10 ± 2	3 ± 2	−5 ± 6	−13 ± 8
150	−7 ± 3	−11 ± 3	−10 ± 2	−1 ± 3	−15 ± 5	−24 ± 4
	C. Splanchnic nerve response (spikes/sec)					
50	3 ± 1	6 ± 3	5 ± 4	25 ± 4	47 ± 15	33 ± 5
100	81 ± 9	82 ± 24	76 ± 26	100 ± 8	93 ± 13	98 ± 17
150	114 ± 7	121 ± 17	98 ± 23	114 ± 9	118 ± 7	111 ± 16

Note. Data expressed as average changes from baselines for 11 control rats of 79 ± 6 mm Hg for mean pressure, 395 ± 7 bpm for heart rate, and 20 ± 2 spikes/sec for splanchnic nerve activity; with corresponding baselines for 4 vasopressin-antagonist pretreated rats of 75 ± 2 mm Hg, 371 ± 6 bpm, and 31 ± 5 spikes/sec, and for 4 adrenalectomized (ADREX) rats of 81 ± 6 mm Hg, 364 ± 34 bpm, and 19 ± 3 spikes/sec.

[a] $P < 0.05$ as compared with corresponding average for the VMH using a paired t test.

TABLE III. COMPARISON OF RESPONSES TO BRAIN STIMULATION OBTAINED DURING SPLANCHNIC AND LUMBAR SYMPATHETIC NERVE RECORDING

Current strength (μA)	Splanchnic nerve (n = 11)		Lumbar chain (n = 6)	
	VMH	LC	VMH	LC
	A. Mean pressure response (mm Hg)			
50	1 ± 1	14 ± 3[a]	0	13 ± 5[a]
100	18 ± 3	52 ± 3[a]	13 ± 2	46 ± 6[a]
150	33 ± 3	62 ± 3[a]	30 ± 4	54 ± 5[a]
	B. Neural firing response (spikes/sec)			
50	3 ± 1	25 ± 4	1 ± 0	6 ± 2[b]
100	81 ± 9	100 ± 8	5 ± 1[b]	30 ± 7[a,b]
150	114 ± 7	114 ± 9	14 ± 5[b]	45 ± 5[a,b]
	C. Neural firing response (percentage increase)			
50	18 ± 7	119 ± 25	15 ± 7	75 ± 28
100	334 ± 53	496 ± 69	74 ± 21[d]	379 ± 93[c]
150	519 ± 65	563 ± 78	203 ± 69[d]	564 ± 61[c]
	D. Ratio of Δ% neural firing/Δmm Hg pressor response			
50	—	—	—	—
100	21.5 ± 4.3	9.3 ± 1[c]	6.3 ± 1.9[d]	8.0 ± 1.5
150	17.0 ± 2.8	9.0 ± 1.3[c]	6.9 ± 2.2[d]	11.6 ± 2.6

Note. Data expressed as average changes from baselines of 79 ± 6 mm Hg for mean pressure and 20 ± 2 spikes/sec in rats with splanchnic nerve recording, and of 91 ± 5 mm Hg and 7 ± 1 spikes/sec in those with lumbar nerve recording.

[a] $P < 0.05$ as compared with corresponding average for the VMH using a paired t test.

[b] $P < 0.05$ as compared with corresponding average for splanchnic nerve recording using an unpaired t test.

[c] $P < 0.05$ as compared with corresponding average for the VMH using the Wilcoxon sign test for related samples.

[d] $P < 0.05$ as compared with corresponding average for splanchnic nerve recording using the Mann–Whitney U test for independent samples.

Absolute increases in lumbar nerve firing, unlike those in splanchnic nerve firing which increased equally regardless of the site of brain stimulation, were more pronounced during LC than during VMH stimulation, again with significant differences during stimulation with 100- and 150-μA currents (Table IIIB). Because initial baselines differed widely, the same data expressed as percentage changes were also analyzed. During VMH stimulation percentage increases in lumbar nerve firing were also smaller than those in splanchnic nerves, but corresponding differences during LC stimulation were no longer significant. By contrast, percentage increases in lumbar nerve firing still remained significantly higher during LC than during VMH stimulation (Table IIIC). Since lumbar nerve firing during LC stimulation, whether expressed as absolute or percentage increases, was consistently higher than that during VMH stimulation, these results suggest that stronger pressor responses to LC stimulation may be caused, at least in part, by greater differential increases in sympathetic vasoconstrictor activity transmitted through the lumbar, but not the splanchnic, nerves.

An important implication of the findings just described is that increases in splanchnic nerve firing produced by VMH stimulation may not be totally devoted to blood pressure regulation. This possibility was tested by calculating ratios to determine how much of an increase in sympathetic nerve firing occurred with every mm Hg rise in mean arterial pressure. Upon dividing percentage increases

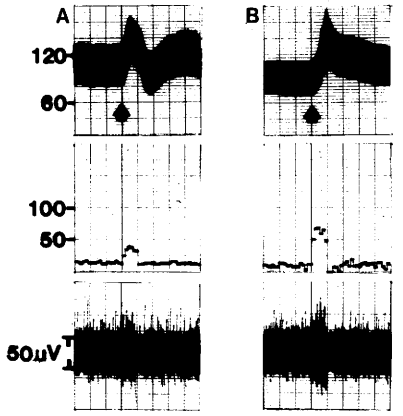

FIG. 3. Effects on lumbar nerve firing and blood pressure of stimulating the ventromedial hypothalamus (A) and the locus coeruleus (B) in a urethane-anesthetized rat. Arrangement, labeling, and recorder speed as in Fig. 1.

in splanchnic nerve firing by corresponding increases in mean arterial pressure, the ratios obtained during VMH stimulation with 100- and 150-μA currents (similar correlations were not attempted for other currents because VMH stimulation with 50 μA had no appreciable pressor effects) were consistently higher than those produced by any other combination of experimental conditions (e.g., lumbar nerve recording during VMH or LC stimulation, or splanchnic nerve recording during LC stimulation; see Table IIID). For every millimeter Hg increase in mean pressure produced by VMH stimulation, splanchnic neural firing was increased much more than that in the lumbar nerve, or in either nerve during comparable LC stimulation.

Verification of brain electrode placement. Transverse sections prepared from brains removed postmortem were examined with a microscope to verify where the tip of the stimulating electrode was located. For the VMH, electrode tips were invariably found in the VMH (Fig. 4A), adjacent to the fornix, median forebrain bundle, and anterior and lateral hypothalamic areas. Average stereotaxic coordinates (mm) obtained in six rats by comparison with the rat atlas (6) were anteroposterior 5.9 ± 0.1, lateral 1.0 ± 0.1, and dorsoventral −3.1 ± 0.2. For the LC, all electrode tips were located in the LC (Fig. 4B), adjacent to the periventricular gray, dorsal tegmental nucleus, and parabrachial nucleus. Average stereotaxic coordinates ob-

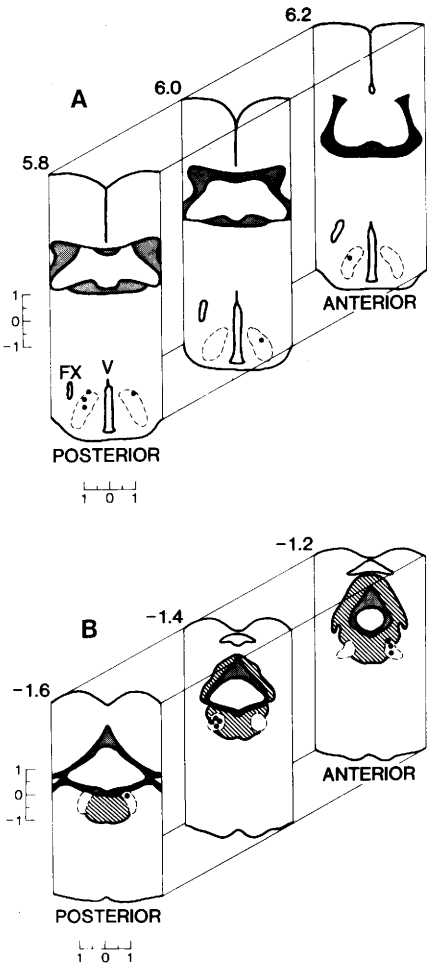

FIG. 4. Top diagram A showing sites of electrode implantation for the VMH in six rats. Numbers at the top indicate anteroposterior coordinate at which each cross section was made using the third cerebral ventricle (V) and fornix (FX) as landmarks. The stippled area represents the lateral and third ventricles. VMH delineated by dotted lines with center of electrode site marked by a black circle for each rat. Bottom diagram B showing sites of electrode implantation for the LC in eight rats. Electrode sites and anteroposterior coordinates marked as in diagram A. Stippled areas represent the aqueduct of Sylvius while the striped areas show the periventricular gray including the dorsal tegmental nucleus on coordinates −1.2 and −1.4. Vertical and horizontal scales for both diagrams in mm.

tained in eight rats were anteroposterior −1.3 ± 0.1, lateral 0.8 ± 0.1, and dorsoventral −2.3 ± 0.1.

Discussion. The locus coeruleus is obviously more sensitive to electrical stimulation at 100 Hz than the ventromedial hypothalamus. Hence it had a lower stimulus threshold and responded more strongly to suprathreshold currents (Table I) than the VMH. This difference in pressor sensitivity cannot be due to a disproportionate constriction in vascular beds innervated by the major splanchnic nerve since stimulation at either brain site increased neural firing frequency almost equally (Table I). Differences in amounts of circulating catecholamines or vasopressin released by brain stimulation are also unlikely because pressor responses were unaffected by bilateral adrenalectomy or pharmacologic blockade of vasopressin (Table II). Because LC stimulation produced larger increases in lumbar neural firing (Table III) its greater pressor sensitivity must be due, at least in part, to a stronger constriction in vascular beds supplied by the lumbar sympathetic trunk.

Alterations in splanchnic nerve activity occurring during electrical stimulation of different parts of the central nervous system have been analyzed previously. Increases in spike potentials recorded from the splanchnic nerve during spinal cord stimulation in anesthetized rats suggest the presence of both pre- and postganglionic fibers, but how this influences the cardiovascular system was undetermined (13). A direct cause-and-effect relationship between changes in splanchnic nerve firing and in blood pressure produced either by stimulating various medullary areas electrically (14, 15) or by changing baroreceptor input through different cardiovascular manipulations (16) has been shown in unanesthetized decerebrate cats. Based on the consistent demonstration that blood pressure rose when splanchnic nerve activity was increased, and conversely fell when splanchnic nerve activity was diminished, Gootman and Cohen (16) concluded that splanchnic nerve discharge is a reliable indicator of central sympathetic regulation of blood pressure. That conclusion also seems applicable to the hypothalamus since pressor responses to hypothalamic stimulation are almost invariably preceded by increased splanchnic nerve firing in normotensive as well as in hypertensive rats (2, 9, 17). Our present findings suggest, however, that increased splanchnic nerve activity alone cannot account for the blood pressure elevation caused by LC stimulation.

The two nerves from which we recorded sympathetic activity could differ in many ways. Notwithstanding subtle species differences in anatomical distribution of sympathetic nerves (18), in rats the major splanchnic nerves, like those in man, do not innervate the same structures as the lumbar sympathetic chains. While the splanchnic nerves supply not only nonvascular smooth muscles but also blood vessels going to visceral organs (including the adrenal medulla), the lumbar chains mainly supply vessels to skeletal muscles. Another difference could be in fiber composition since electrical activity recorded from any given nerve bundle would include spike potentials from both vasodilator and vasoconstrictor fibers (19), and without concurrent recording of regional blood flow the extent to which each type contributes to the total is unknown. Apart from differences in size of the vascular bed innervated or in number of vasoconstrictor fibers contained, lumbar chain activation could conceivably also cause a different pattern of regional blood flow redistribution from that produced by the major splanchnic nerve. But regardless of all these variables, it seems reasonable to assume that since ensuing increases in neural firing did not differ significantly, the same splanchnic nerve fibers were activated upon stimulation at either brain site. By the same token, the greater increase in lumbar nerve firing during LC stimulation implies that more fibers, especially those causing vasoconstriction, may have been activated than by VMH stimulation.

Though central mechanisms for cardiovascular regulation are best studied in conscious animals, we studied urethane-anesthetized rats so that sympathetic nerve responses to electrical stimulation of two discrete brain areas could be compared. Both brain areas were stimulated in the same rat to reduce variability from rat to rat, and identical electrodes were used for stimulation to keep the

size of the stimulated areas constant. Despite these precautions, neither the number of neurones stimulated at each brain site nor the number of nerve fibers firing in each multifiber preparation can be estimated. Consequently, the greater increases in frequency of lumbar nerve firing produced by LC stimulation could have resulted from activation of more neural elements at either stimulation or recording sites, or at any points of connection between them.

Assessment of neural mechanisms for cardiovascular regulation becomes progressively more difficult at higher levels of the neuraxis because of the increasing structural and functional complexity as one ascends from the spinal cord to the brain (20). Electrical stimulation aggravates the difficulty further because of the inability to distinguish at the stimulation site between neurones and fibers of passage. Instead of isolated brain centers or pathways for each response Hilton (21) suggests a longitudinally organized system extending through the brain stem, from the hypothalamus to the medulla, which integrates a highly differentiated response pattern. For instance, to produce a fully integrated defense reaction including its cardiovascular response, he proposes that in conscious animals specific regions of the hypothalamus, midbrain, pons, and medulla must act in concert. Perhaps the LC represents another extension of this integrating system since it is connected, structurally as well as functionally, to various hypothalamic areas. The LC is anatomically located within the pontomedullary area representing the caudal extension of the hypothalamic "defense center" in cats (22). Aside from autoradiographic demonstration of fibers connecting the VMH to the LC (1), adrenergic nerve endings in different hypothalamic nuclei are known to originate from cell bodies located in the LC (3). A functional link has also been suggested by the finding in anesthetized cats that pressor responses to electrical stimulation of the LC were markedly inhibited following destruction of the posterior hypothalamus (23).

1. Saper CB, Swanson LW, Cowan WM. The efferent connections of the ventromedial nucleus of the hypothalamus of the rat. J Comp Neurol **169**:409–442, 1976.
2. Buñag RD, Tomita T, Sasaki S. Streptozotocin diabetic rats are hypertensive despite reduced hypothalamic responsiveness. Hypertension **4**:556–565, 1982.
3. Kobayashi RM, Palkovits M, Kopin IJ, Jacobowitz DM. Biochemical mapping of noradrenergic nerves arising from the rat locus coeruleus. Brain Res **77**: 269–279, 1974.
4. Cedarbaum JM, Aghajanian GK. Afferent projections to the rat locus coeruleus as determined by a retrograde tracing technique. J Comp Neurol **178**:1–16, 1978.
5. Grzanna R, Molliver ME. The locus coeruleus in the rat: An immunohistochemical delineation. Neuroscience **5**:21–40, 1980.
6. Ward DG, Gunn CG. Locus coeruleus complex: Elicitation of a pressor response and a brain stem region necessary for its occurrence. Brain Res **107**:401–406, 1976.
7. Pellegrino LJ, Pellegrino AS, Cushman AJ. A Stereotaxic Atlas of the Rat Brain. New York, Appleton–Century–Crofts, 1979.
8. Buñag RD, Riley E, Montello M. Sustained pressor responsiveness to prolonged hypothalamic stimulation in awake rats. Amer J Physiol **231**:1708–1715, 1976.
9. Takeda K, Buñag RD. Sympathetic hyperactivity during hypothalamic stimulation in spontaneously hypertensive rats. J Clin Invest **62**:642–648, 1978.
10. Crofton JT, Rockhold RW, Share L, Wang BC, Horowitz ZP, Manning M, Sawyer WH. Effect of intracerebroventricular captopril on vasopressin and blood pressure in spontaneously hypertensive rats. Hypertension **3**(Suppl II):71–74, 1981.
11. Bruning J, Kintz B. Computational Handbook of Statistics. Glenview, Ill., Scott, Foresman, pp10–13, 24–27, 1977.
12. Duncan DB. Multiple range and multiple F tests. Biometrics **11**:1–42, 1955.
13. Celler BG, Schramm LP. Pre- and postganglionic sympathetic activity in splanchnic nerves of rats. Amer J Physiol **241**:R55–R61, 1981.
14. Kahn N, Mills E. Centrally evoked sympathetic discharge: A functional study of medullary vasomotor areas. J Physiol **191**:339–352, 1967.
15. Gootman PM, Cohen MI. Evoked splanchnic potentials produced by electrical stimulation of medullary vasomotor regions. Exp Brain Res **13**:1–14, 1971.
16. Gootman PM, Cohen MI. Efferent splanchnic nerve activity and systemic arterial pressure. Amer J Physiol **219**:897–903, 1970.
17. Buñag RD, Takeda K, Riley E. Spontaneous remission of hypertension in awake rats chronically exposed to shaker stress. Hypertension **2**:311–318, 1980.

18. Baljet B, Drukker J. The extrinsic innervation of the abdominal organs in the female rat. Acta Anat **104**:243–267, 1979.
19. Ninomiya I, Judy WV, Wilson MF. Hypothalamic stimulus effects on sympathetic nerve activity. Amer J Physiol **218**:453–462, 1970.
20. Smith OA, Astley CA, Hohimer AR, Stephenson RB. Behavioral and cerebral control of cardiovascular function. In: Hughes MJ, Barnes CD, eds. Neural Control of Circulation. New York, Academic Press, pp1–21, 1980.
21. Hilton SM. Ways of viewing the central nervous control of the circulation—old and new. Brain Res **87**:213–219, 1975.
22. Coote JH, Hilton SM, Zbrozyna AW. The ponto-medullary area integrating the defence reaction in the cat and its influence on muscle blood flow. J Physiol **229**:257–274, 1973.
23. Przuntek H, Philippu A. Reduced pressor responses to stimulation of the locus coeruleus after lesion of the posterior hypothalamus. Naunyn-Schmiedeberg's Arch Pharmacol **276**:119–122, 1973.

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