Effect of stimulation of nucleus raphe dorsalis on carotid blood flow. II. The cat

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GOADSBY, P. J., R. D. PIPER, G. A. LAMBERT, AND J. W. LANCE. *Effect of stimulation of nucleus raphe dorsalis on carotid blood flow. II. The cat.* Am. J. Physiol. 248 (Regulatory Integrative Comp. Physiol. 17) R263–R269, 1985.—The dorsal raphe nucleus (DRN) and surrounding midbrain of 74 cats were stimulated both electrically and chemically, and carotid flows were measured with electromagnetic flow probes. Stimulation of the DRN caused a frequency-dependent decrease in common carotid vascular resistance, which was abolished by bilateral section of the facial nerve intracranially. Injection of DL-homocysteic acid into the DRN reproduced the effect of electrical stimulation, indicating that the responses arose from excitation of cell bodies within the DRN, not from fibers of passage. The responses were mediated entirely within the brain stem since they remained intact after high spinal cord section. The vasodilator response was blocked by the intravenous administration of the nicotinic ganglion blocker hexamethonium but not by the α-adrenoceptor blocker phentolamine. The responses were unaffected by intravenous administration of methysergide but were markedly reduced after depletion of central serotonin by pretreatment with the serotonin depletor, p-chlorophenylalanine. A poststimulus constrictor response was mediated by release of catecholamines from the adrenal medulla and was blocked by the α-adrenoceptor antagonist phentolamine. No response involved supracollicular mechanisms since they persisted after decerebration.

cell bodies; serotonin; carotid vascular resistance

THE RAPHE NUCLEI of cat have been extensively mapped (17) and are largely similar to those of the monkey (8, 9, 14). We have shown (5) that electrical stimulation of the monkey DRN causes a frequency-dependent vasodilator response in both external and internal carotid vasculature, mediated by the facial nerve, and the external carotid response is followed (after high-frequency stimulation) by a poststimulus constrictor response. The present study was designed to answer the following questions. Is such an effect on the cranial vasculature seen in other species, and if so was a serotonergic synapse involved? Is the response entirely mediated within the brain stem, or did inframedullary or supratentorial mechanisms play a part?

MATERIALS AND METHODS

Seventy-four male or female cats, mean weight 2.8 ± 1.0 (SD) kg were anesthetized with a mixture of α-chloralose (20 mg·kg^{-1}) and urethan (500 mg·kg^{-1}). The animals were intubated, placed in a stereotaxic apparatus, paralyzed with gallamine triethiodide (Flaxedil M & B), and artifically respired to maintain a constant percent end-expiratory CO_2. A polyethylene catheter was inserted into the femoral artery and advanced into the thoracic aorta, and one was inserted into the femoral vein for administration of drugs.

Carotid flow. Both left and right common carotid arteries were exposed and fitted with appropriately sized electromagnetic flow probes. Flow and blood pressure signals were passed to an analog to digital converter and then to a Z80-based microcomputer (4).

Pharmacological studies. The following drugs were injected into the femoral vein catheter: *p*-chlorophenylalanine (PCPA, Sigma Chemical), hexamethonium bromide (Sigma), methyserigide (Sandoz), and phentolamine mesylate (CIBA-Geigy). To deplete central serotonin levels (10) 500 mg·kg^{-1} PCPA was injected intraperitoneally into four cats 3 days before the experiment. This injection was preceded by a 25 mg·kg^{-1} injection of desmethylimipramine hydrochloride (CIBA-Geigy), a norepinephrine uptake blocker, to isolate noradrenergic cell bodies from this manipulation.

Surgical manipulations. The cervical sympathetic trunk was located in the neck as it ran with the vagus nerve, and a piece of cotton was placed around it to facilitate its section during the experiments. The adrenal gland was approached from the loin, and after a preliminary skin incision the kidney was identified along with the renal vessels. The adrenal gland and its blood supply were carefully identified and mobilized so that a clamp could be placed across the hilum to achieve physiological adrenalectomy.

The spinal cord was sectioned at the C_1-C_2 level. After an incision along the line of the vertebral bodies the first and second cervical vertebrae were identified. The atlanto-axial membrane was cut and the cord cut through this opening. Shortly after section of the cord 20 ml fluid were usually administered to maintain blood pressure. Supracollicular decerebration was conducted through an extensive craniotomy. After opening the skull, a spatula was passed just rostral to the superior colliculus and the forebrain removed from the cranium. Facial nerve section was conducted by making a burr hole in the parietal bone just above the facial canal; with practice this gave a quick superior approach to the nerve. The nerve was

sectioned with a small scalpel blade under clear vision just as it entered the facial canal.

Stimulus site and modes of stimulation. The DRN in the cat was stimulated at coordinates corresponding to antero-posterior (AP) 0.2, lateral (L) 0.0, and height (H) −1.0 in the atlas of Berman (1) via a burr hole in the calvarium. A bipolar stainless steel electrode (Rhodes, SNEX-100), insulated except for 0.5 mm at the tip, was used to deliver paired opposite-polarity pulses (0.2–200·s^{-1}, 500 μa, 250 μs duration, 500 μs separation) for 15 s as described (5).

To differentiate between the effect of stimulation of fibers of passage and cell bodies, 240-nl injections of 1 M L-glutamate and DL-homocysteic acid (DL-H) were made into the DRN and surrounding brain stem. By using the same stereotaxic coordinates as those used for electrical stimulation, a micropipette filled with either glutamate or DL-H was lowered into the brain stem, and injections were delivered according to the method of Goodchild et al. (6). To localize these injections fast-green dye was mixed into the solution with either the glutamate or the DL-H.

Histology. The brains were fixed, removed, and sectioned according to methods previously described (5). Chemical injections were indentified as fast-green spots in the brain stem, and necessary corrections between theoretical and actual placement could be made before any computations were undertaken.

Statistics. Population means only are expressed as ±SD; all other means are expressed with their relevant SE. A three-way ANOVA was used on the raw data (16). Where applicable a simple unpaired two-tailed Student's *t* test was conducted. All tests were initially assessed at $P < 0.05$.

RESULTS

Resting state. In 74 cats mean arterial blood pressure was 109 ± 20 mmHg; common carotid flow was 28.0 ± 11.3 ml·min^{-1} on the right and 27.6 ± 11.9 ml·min^{-1} on the left. Common carotid resistances on the right and left were 4.5 ± 2.1 and 4.8 ± 2.9 mmHg·ml^{-1}·min^{-1}, respectively ($n = 74$).

TABLE 1. *Effect of DRN stimulation on common carotid resistance*

Intervention	*n*	Maximum Change in Carotid Resistance	
		%Dilatation	%Constriction
Control	74	14.1 ± 3.3	122.6 ± 9.3
Surgical			
Sympathectomy	14	14.3 ± 3.7	61.0 ± 11.0†
Adrenalectomy	3	14.8 ± 4.9	51.4 ± 10.7‡
Decerebration	4	12.2 ± 6.0	104.4 ± 17.9
Spinal cord section	9	12.8 ± 1.6	1.3 ± 0.7*
Facial nerve section	9	0.9 ± 0.3*	1.1 ± 0.6*
Pharmacological			
Hexamethonium	7	1.7 ± 0.4*	1.4 ± 0.3*
Methysergide	6	13.7 ± 2.8	115.6 ± 7.9
PCPA	4	5.7 ± 2.9*	9.9 ± 3.3*
Phentolamine	4	32.6 ± 3.3	1.8 ± 0.5*

Values are means ± SE. DRN, dorsal raphe nucleus. * Significant difference from control. † Not different from control as compared by paired *t* test with its own internal (same animal) control. ‡ Different from control as compared by paired *t* test with its own internal (same animal) control.

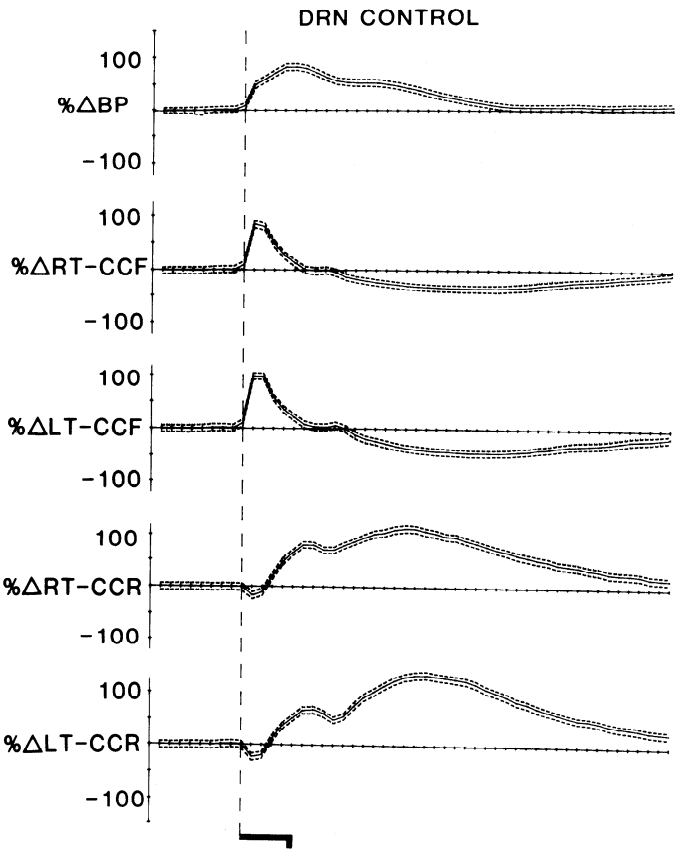

FIG. 1. Computer-processed plot for control responses to dorsal raphe nucleus (DRN) stimulation from 14 cats. Mean response (*solid line*) and SE (*broken lines*) are shown for percentage changes in blood pressure (BP), right common carotid flow (RT-CCF), left common carotid flow (LT-CCF), right common carotid resistance (RT-CCR), and left common carotid resistance (LT-CCR) during and after stimulation of the DRN at 50·s^{-1}. Stimulus lasted 15 s (*solid line*).

Localization and effects of DRN stimulation. Stimulation of the DRN in the cat resulted in a marked immediate pressor response. The carotid vascular resistance changes took the form of an immediate dilator response, mean maximum 14.1 ± 3.3%, which was superseded when the stimulus ceased by a prolonged poststimulus vasoconstriction, mean maximum 122.6 ± 9.3% (Fig. 1).

Electrical stimulation of the midbrain of the cat was conducted at various AP, L, and H coordinates, and the results are summarized in Figs. 2 and 3. These figures show the distribution of both the dilatations and constrictions that occurred in the carotid vasculature at the various brain stem sites stimulated. Each result plotted represents the mean of 4–12 animals depending on the site (Fig. 4). The delayed constrictor and pressor responses were obtained only from the region of the DRN, but the origin of the dilator response was not as well localized by electrical stimulation. Nevertheless, chemical stimulation with DL-H of the region in and around the DRN at AP 0.5 showed both the dilator and constrictor responses to originate in the DRN.

Chemical stimulation. Injection of DL-H into the DRN

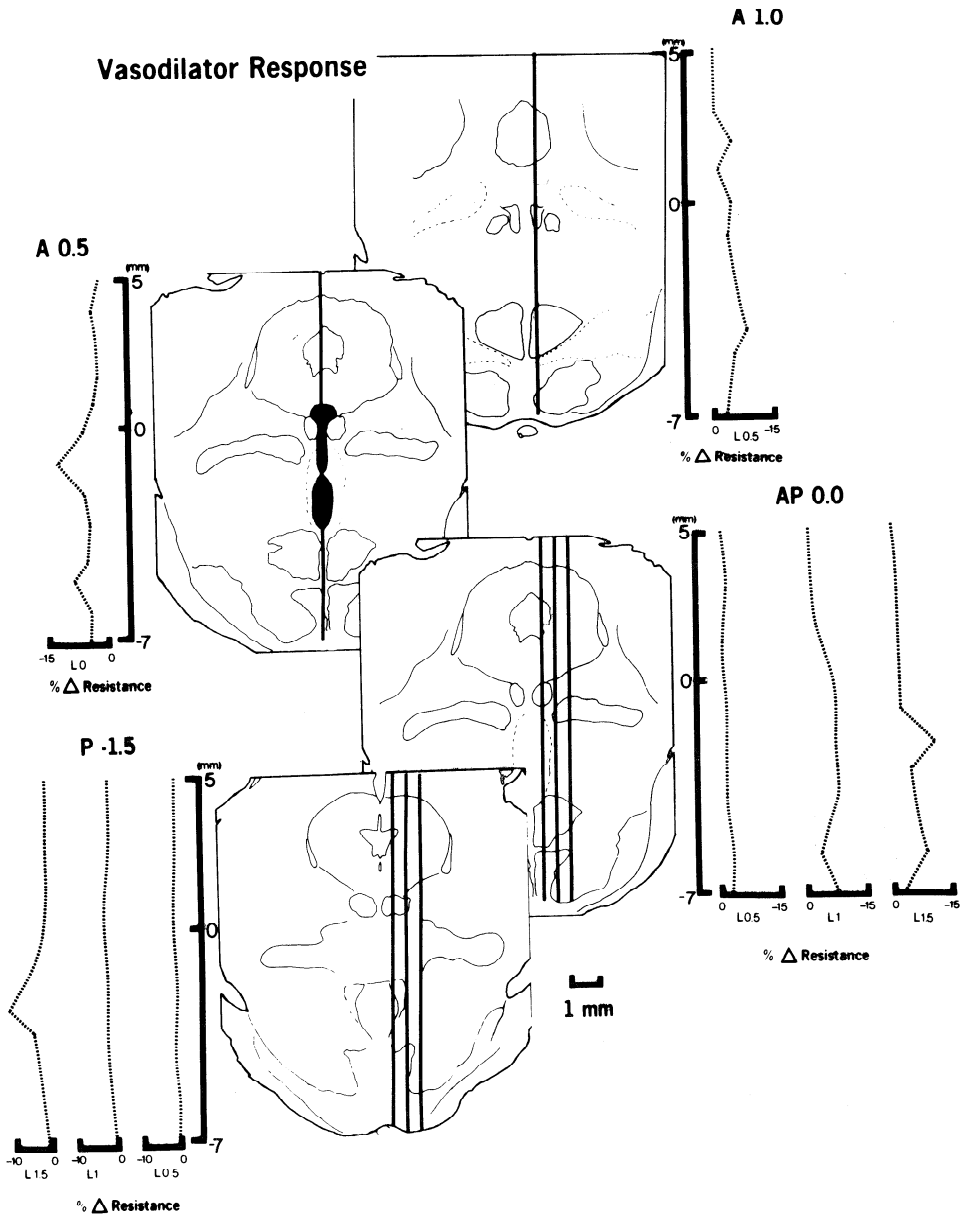

FIG. 2. Cross sections of brain stem of cat at various positions in region of dorsal raphe nucleus both anterior (A) and posterior (P) to stereotaxic zero and lateral (L) to midline. Each point represents mean percentage decrease (vasodilatation) in common carotid resistance from control for cohort of animals (4 12 cats) electrically stimulated for 15 s at $50 \cdot s^{-1}$ at a particular site. *Ordinate* of each graph represents height of point above and below stereotaxic zero in mm. Main area of dorsal raphe and raphe centralis superior is *shaded*. See Fig. 4 for more anatomic details.

of eight cats mimicked the effect of electrical stimulation, with a 16.0 ± 5.4% initial decrease in resistance followed by a 30.7 ± 9.4% poststimulus constriction (Fig. 5). L-glutamate, however, had little or no effect on the carotid vasculature when injected into the DRN ($n = 13$).

Surgical maneuvers. The results of the various surgical and pharmacological interventions are set out in Table 1. Bilateral cervical sympathectomy did not significantly affect the immediate dilator response nor the poststimulus constrictor response. The mean maximum dilatation after sympathectomy was 14.3 ± 3.7%, followed by a constriction of 61.0 ± 11.0% at a stimulation frequency of $50 \cdot s^{-1}$.

Bilateral clamping of the hilum of the adrenal medulla in three cats did not affect the immediate dilator response to DRN stimulation but significantly reduced the poststimulus constrictor response ($F_{0.05;1,18} = 5.12$).

Supracollicular decerebration in four cats (which included removal of the hypothalamus) left both the vasodilator and vasoconstrictor responses to DRN stimulation intact, the mean response at $50 \cdot s^{-1}$ after decerebration being a 12.2 ± 6.0% decrease in resistance and a 104.0 ± 17.9% poststimulus increase in resistance.

Section of the spinal cord at the C_1-C_2 level completely blocked the delayed constrictor response, leaving only the dilator response intact ($F_{0.05;1,108} = 2.03$; Fig. 6). The mean response after section was a decrease of 12.8 ± 1.6% at $50 \cdot s^{-1}$. Section of the facial nerve intracranially contralateral to the side of carotid resistance measured lead to a significant change in that dilator response, and bilateral facial nerve section blocked the response completely.

Pharmacological intervention. Administration of the nicotinic autonomic ganglion blocking agent hexamethonium (20 $mg \cdot kg^{-1}$ iv) to seven cats caused an immediate decrease in blood pressure which lasted during the time the observations were made. Both the immediate dilator ($F_{0.05;1,9} = 1.73$) and delayed constrictor responses to DRN

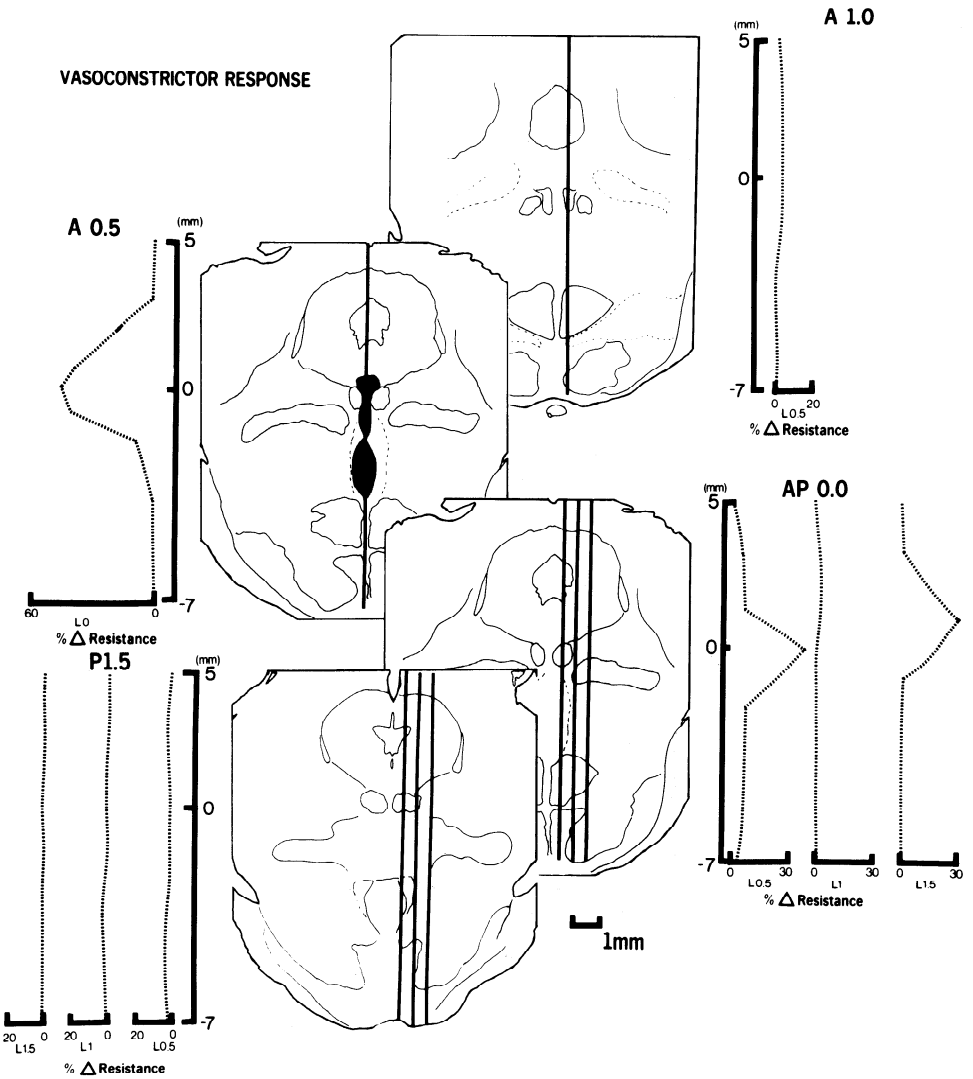

FIG. 3. Cross section of brain stem of cat showing localization of poststimulus constrictor response to electrical stimulation for 15 s at $50 \cdot s^{-1}$. Each point represents mean for 4–12 cats. See Fig. 4 for more anatomic details and Fig. 2 for abbreviations.

stimulation were abolished.

Intravenous administration of the serotonin antagonist methysergide ($5\ mg \cdot kg^{-1}$) had no effect on either the immediate dilator or delayed constrictor responses to DRN stimulation ($F_{0.05;1,9} = 2.22$).

Pretreatment of four cats with PCPA preceded by desmethylimipramine significantly reduced the immediate dilator and secondary constrictor responses to DRN stimulation to a mean maximum of 5.7 ± 2.9 and 9.9 ± 3.3% at $100 \cdot s^{-1}$, respectively. As a control measure, stimulation of the locus coeruleus during the same experiment produced the pressor and carotid vasodilator responses previously recorded ($t_6 = 0.76$) (4).

Intravenous administration of the α-receptor blocking agent phentolamine ($5\ mg \cdot kg^{-1}$) in four cats caused immediate hypotension and abolished the poststimulus constrictor response but not the immediate dilator response. Although the mean dilator response at a frequency of stimulation of $50 \cdot s^{-1}$ was increased to 32.6 ± 3.3%, this change was not significant ($t_{11} = 1.15$).

DISCUSSION

We have used the cat to extend our observations of the craniovascular responses to DRN stimulation because intracranial surgical procedures are easier and the animals withstand spinal cord section and other surgical maneuvers better.

The response in the cat to DRN stimulation was biphasic, an immediate dilatation followed by poststimulation constriction of the common carotid vascular bed. By analogy with the monkey, dilatation in the common carotid vasculature probably reflects summation of the cerebral and extracerebral effects seen in the monkey. The fact that the overall response in the cat was smaller than would be predicted by addition of the cerebral and extracerebral components of the monkey response is probably related to the early constrictor response in the cerebral vasculature of the monkey seen at high frequencies of stimulation and perhaps to quantitative differences in the response of the cat and monkey circulations. Similarly the poststimulus vasoconstrictor response is likely to be the same phenomenon as that seen in the external carotid vasculature of the monkey (5), somewhat dampened by the slow resolution of the cerebral component of the dilator response.

Localization. The localization to the DRN of electrical stimulation producing the dilator response was not as

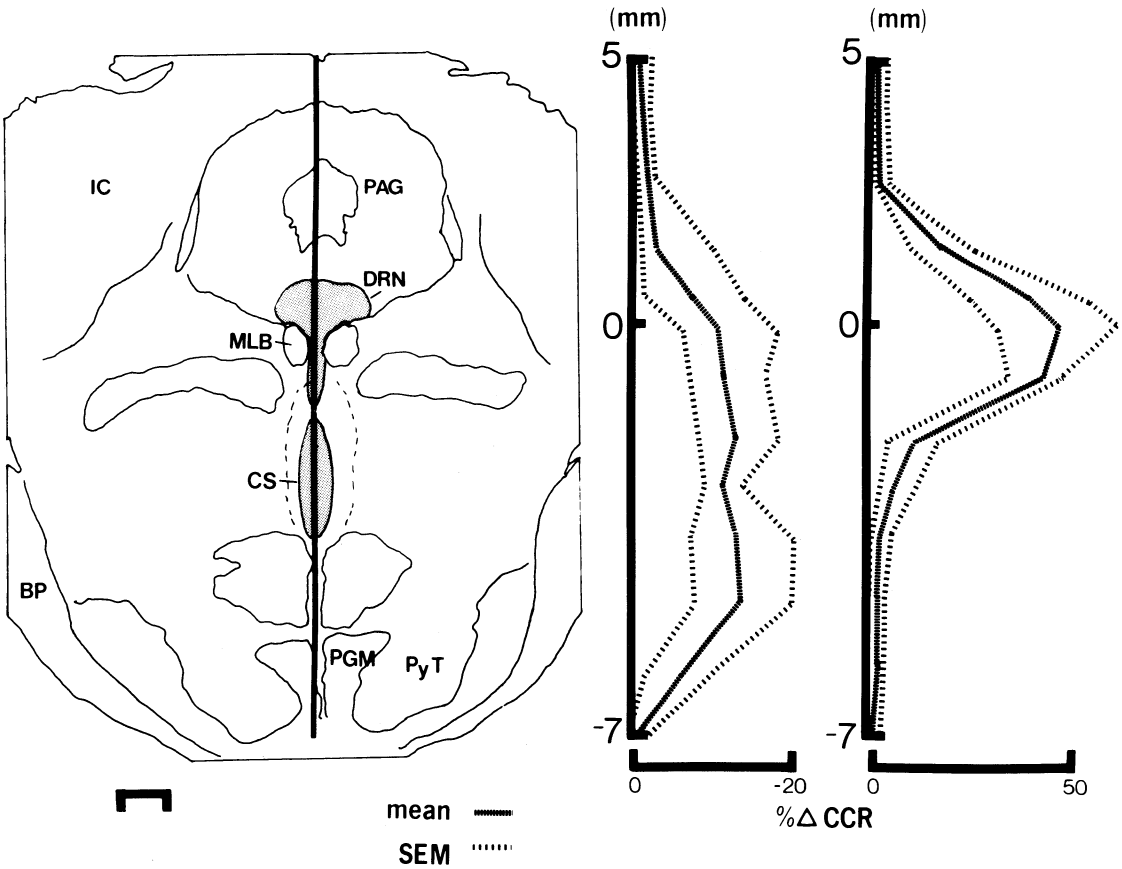

FIG. 4. A more detailed representation of the changes in common carotid resistance (CCR) (dilatation and constriction) resulting from electrical stimulation (15 s at $50 \cdot s^{-1}$) of midbrain raphe nuclei. Mean and SE ($n = 10$) for each point in brain stem are shown plotted next to their site of origin. PAG, periaqueductal grey matter; IC, inferior colliculus; DRN, dorsal raphe nucleus; MLB, medial longitudinal bundle; CS, raphe centralis superior; BP, brachium pontis; PGM, pontine grey matter; Py T, pyramidal tract.

well marked as that for the poststimulus constrictor response. This is probably due to the excitation of fibers passing through the dorsal midbrain, because both the dilator and poststimulus constrictor responses were found to be well localized to the DRN by chemical stimulation. The elicitation of both responses by chemical stimulation using DL-H supports the notion that the responses we have reported are due to activation of cell bodies and not merely to stimulation of fibers of passage.

Interestingly, L-glutamate has very little effect when injected into the DRN. Krnjevic (11) listed DL-H as being in general a stronger excitatory amino acid than L-glutamate. Hösli and Tebecis (7) have also shown a strong excitation of reticular-activating neurons by DL-H in the absence of any effect of L-glutamate. The reason for this difference may reside in the differences in uptake mechanisms for each substance. L-glutamate is actively taken up by glial cells (12) and neurons (2), whereas no specific mechanism for DL-H uptake has been described. Because the DRN is relatively diffuse, it is conceivable that L-glutamate is taken up before it can activate the whole nucleus, whereas DL-H can spread throughout the whole nucleus, thus reproducing the changes seen with electrical stimulation more closely. The responses to DL-H have a longer latency than those to electrical stimulation, supporting the concept that DL-H has to diffuse through the nucleus to exert its effect.

Surgical maneuvers. To eliminate peripheral effects of DRN stimulation, animals were spinalized at the C_1-C_2 level. Although monkeys consistently suffered a drop in blood pressure to ≤10 mmHg and died after this procedure, cats remained for hours at blood pressures of ≥100 mmHg with only the encouragement of a normal saline drip. The poststimulus constrictor response was blocked by spinal cord section, consistent with the view that it is peripherally mediated, but the cranial vasodilator response was not. Bilateral section of the facial nerve blocked the dilator response entirely in cats, confirming that its pathway was the same as in monkey (5).

The reduction of the poststimulation constrictor response after bilateral clamping of the hila of the adrenal medulla showed that it depended substantially on the release of catecholamines. The dilator response was predictably unaffected by this procedure.

Supracollicular decerebration, which included removal of the hypothalamus, was conducted to check whether the dilator or constrictor responses were mediated by rostral projections of the DRN. Because both dilator and constrictor responses were intact after decerebration both responses are obviously mediated by brain stem mechanisms and connections. It is especially interesting from the viewpoint of integrative physiology to note the lack of participation of the hypothalamus in any of the responses reported here.

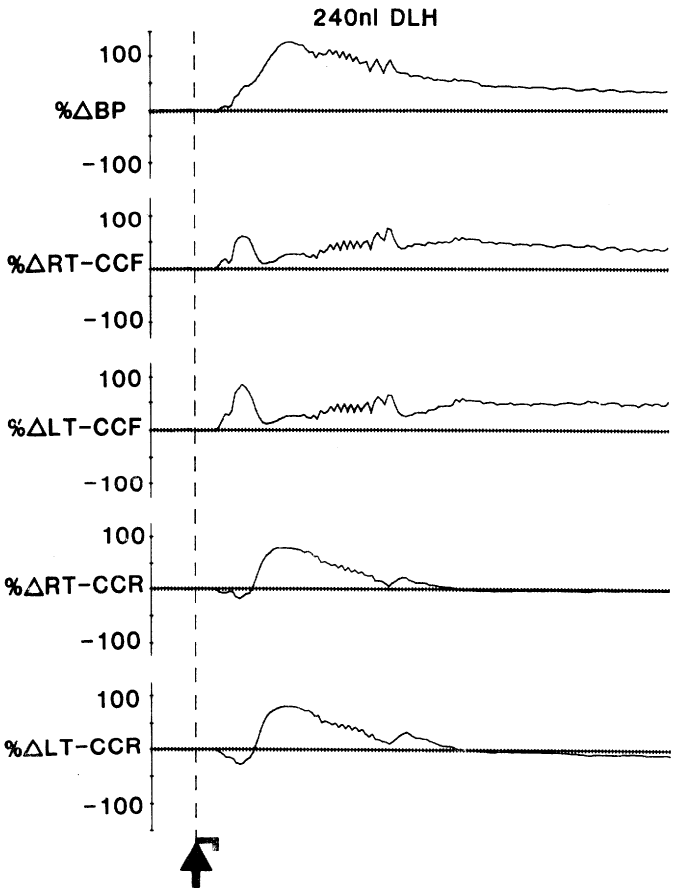

FIG. 5. Responses to stimulation of dorsal raphe nucleus with DL-homocysteic acid (DLH) are shown here to be similar to those obtained with electrical stimulation. *Arrow*, point of injection, and 15-s time *bar* follows. See Fig. 1 for abbreviations.

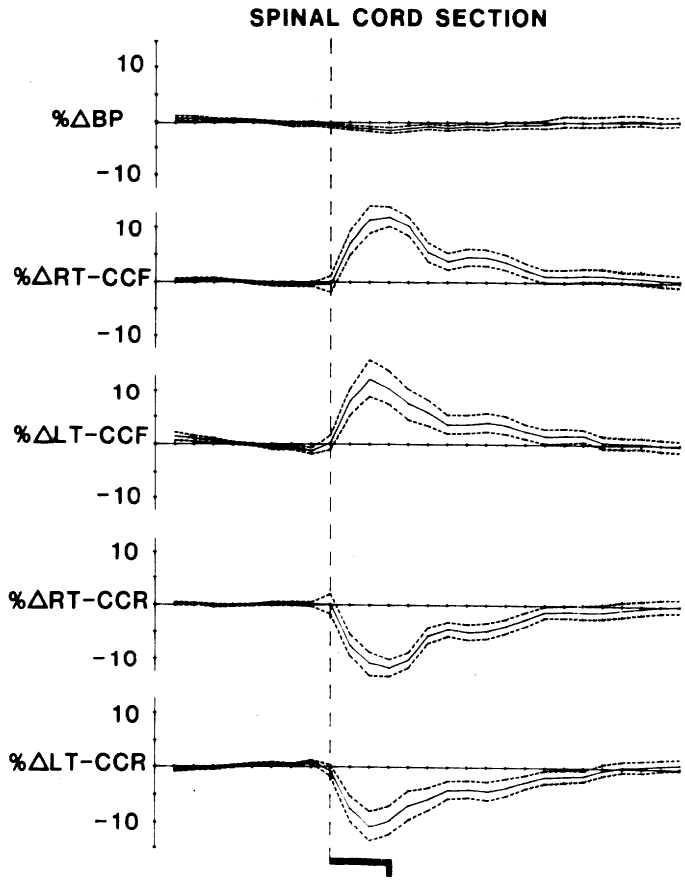

FIG. 6. Response to stimulation (15 s at 50·s^{-1}) of dorsal raphe nucleus after spinal cord section ($n = 9$); here only the cranial vascular changes remain intact. See Fig. 1 for abbreviations.

Pharmacological intervention. The lack of effect of methysergide on either the dilator or constrictor responses is consistent with the results in the monkey and leaves a very important question unanswered. Are the craniovascular responses to DRN stimulation due to activation of serotonin or nonserotonin-containing cell bodies in the DRN? The DRN is not exclusively serotonergic because ≥30% of the cell bodies contain some other transmitter (17). Cholecystokinin and substance P have been demonstrated even within serotonin-containing cell bodies in the DRN (15). For this reason cats were pretreated with PCPA and desmethylimipramine to deplete central serotonin stores without affecting central catecholamine nuclei. Stimulation of the DRN 3 days after pretreatment demonstrated a marked attenuation of both the immediate dilator and poststimulus constrictor responses, demonstrating that a serotonergic synapse is involved in the expression of these responses. As a control measure to determine whether other aminergic (especially noradrenergic) systems were intact, locus coeruleus was stimulated in PCPA-treated cats with a normal response both in the periphery and in the cranial vasculature as previously reported (4).

The original impetus to these experiments was the description by Reinhard et al. (13) of projections from the midbrain raphe to the cerebral parenchymal vessels. No evidence for any direct effect of the midbrain raphe on cerebral blood flow has been detected in either of these investigations (5). This may simply be that no effect exists, or it may be due to selectivity in the regions affected by the midbrain raphe nuclei so that changes in the microcirculation cannot be measured by the bulk flow techniques employed in this study. The ultimate solution to this question can probably be determined by measurement of regional cerebral blood flow with such methods as autoradiography or radiolabeled microspheres.

The cerebral dilator effect of the DRN is in marked contrast to the constrictor effect of the locus coeruleus, the major noradrenergic nucleus in the central nervous system, which when stimulated leads to a frequency-dependent increase in internal carotid vascular resistance (3). The effect of the DRN on internal carotid resistance is mediated by an intermediate pathway, the greater superficial petrosal nerve dilator pathway, whereas the locus coeruleus projects directly to the intraparenchymal vasculature. The existence of these two pathways suggests that cerebral blood flow may be reflexly controlled through brain stem aminergic nuclei. It is conceivable that an imbalance in these two systems may result in pathologically abnormal states of the cranial vasculature, such as migraine headache, where aminergic mechanisms have long been suspected.

The authors thank J. W. Duckworth for help in the preparing and executing these experiments. The authors also thank the Dept. of

Medical Illustration, Univ. of New South Wales, for photographing the illustrations in this paper.

This program has been supported by the National Health and Medical Research Council of Australia and by grants from the J. A. Perini Family Trust, the Basser Trust, and the Australian Brain Foundation.

Received 9 January 1984; accepted in final form 20 September 1984.

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