Profound Hypotension and Abolition of the Vasomotor Component of the Cerebral Ischemic Response Produced by Restricted Lesions of Medulla Oblongata in Rabbit

Relationship to the So-Called Tonic Vasomotor Center

MAMORU KUMADA, ROGER A.L. DAMPNEY, AND DONALD J. REIS

SUMMARY We studied the effect of bilateral electrolytic lesions in the medulla oblongata on the cerebral ischemic reflex in 24 anesthetized rabbits. In 15 animals lesions were placed in areas from which the differentiated vasomotor component of the response was elicited by electrical stimulation. In four rabbits (group A) the lesions entirely abolished the vasomotor, but not cardiac responses to cerebral ischemia, and resulted in an irreversible fall, to 30-40 mm Hg, of the arterial pressure (AP). These lesions destroyed large portions of the nucleus reticularis parvocellularis, the dorsal part of the nucleus reticularis gigantocellularis, and the ventromedial portion of the medial vestibular nuclei at the level of the inferior olive (3 mm rostral to the obex). In four other rabbits (group B) the pressor response was reduced to 25-87% of control with a fall of AP not as marked as that in group A. These lesions were within the same areas or very close to those of group A but smaller. In the remaining seven rabbits (group C) the lesions did not alter the ischemic response or AP; they were either restricted to the nucleus reticularis gigantocellularis alone, were smaller than in group B, or if large, were located several millimeters more rostral to those of group A. In nine control rabbits lesions placed elsewhere in the medulla failed to alter the ischemic response or resting AP. We conclude that the vasomotor, but not the cardiac or respiratory, components of the cerebral ischemic response depend upon a restricted portion of the bulbar reticular formation. Moreover, the integrity of this region is essential for maintenance of normal resting levels of AP and, hence, appears to function as the so-called tonic vasomotor center of the brainstem.

THE CEREBRAL ischemic response is a stereotyped autonomic reflex consisting of an elevation of arterial pressure, bradycardia, and apnea elicited by rendering the brainstem ischemic (Guyton, 1948; Sagawa et al., 1961; Downing et al., 1963; Miyakawa, 1966; Dampney et al., 1979). In our previous study in anesthetized rabbits (Dampney et al., 1979), we characterized the primary cardiodynamic features of the response (i.e., those due to direct effects of ischemia on the brain). We demonstrated: (1) that the response is entirely elicited by ischemic stimulation of neurons in the medulla oblongata, and (2) that an elevation of arterial pressure with a redistribution of regional blood flow and conductance comparable in pattern and magnitude to that produced by ischemia could be elicited by electrical stimulation of specific regions within the medulla. The active sites for eliciting for the vasomotor response (but not the brachycardia or apnea) were not restricted to a single nucleus. Rather, they were localized to a zone which partially overlapped portions of several nuclear subgroups of the dorsal medullary reticular formation, including the nucleus reticularis parvocellularis and nucleus gigantocellularis. On this basis we proposed that this localized region of the dorsal medullary tegmentum mediated the vasomotor component of the ischemic response.

In the present study we have examined the effects on the cerebral ischemic response of placing bilateral lesions within those same medullary areas. We shall demonstrate that lesions restricted to caudal portions of this zone abolish the vasomotor response to ischemia. Moreover, lesions restricted to only this area result in a fall of arterial pressure to levels equivalent to those produced by spinal cord transection. These observations suggest that the region mediating the vasomotor component of the cerebral ischemic response and the so-called tonic vasomotor center of the brainstem (Alexander, 1946) may be one and the same.

Methods

The detailed methods for: (1) preparation and maintenance of the animals, (2) elicitation of the

From the Laboratory of Neurobiology, Department of Neurology, Cornell University Medical College, New York, New York.

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Address for reprints: Donald J. Reis, M.D., Laboratory of Neurobiology, Department of Neurology, Cornell University Medical College, 1300 York Avenue, New York, New York 10021.

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cerebral ischemic response, (3) recording of cardiovascular activity, (4) electrical stimulation of the brain, and (5) histological reconstruction of lesions, are described in detail in the preceding paper (Dampney et al., 1979) and will only be summarized:

The studies were performed in 24 rabbits of both sexes, anesthetized with urethane (1.25 g/kg, iv) and, except when spontaneous respiration was to be observed, paralyzed with gallamine triethiodide (0.7 mg/kg, im) and artificially ventilated. After anesthesia and placement of tracheal, arterial, and venous cannulas, the vertebral arteries, at the level of the 3rd cervical vertebra, and one carotid artery were occluded. The rabbit then was placed in a stereotaxic frame with the head flexed at 45°. Body temperature was maintained in the range of 37-38°C by a thermostatically regulated heating pad and ventilation continued on a mixture of 50% O₂ and 50% N₂. The end-expired CO₂ was maintained at 2-3%.

After control values were established for the cerebral ischemic response as elicited by occlusion of the patent carotid artery for 20 seconds (Dampney et al., 1979), lesions were placed bilaterally in the brainstem. The electrodes for placement of lesions were similar to those used for electrically stimulating the brain and consisted of Teflon-coated stainless steel wires bared at the tip for 0.5 mm. Lesions were made by passing a DC current of 2-5 mA from a constant current source for 5-15 seconds. The cathode was a disc placed between two layers of neck muscle. At the end of each experiment the rabbit was perfused with 10% formalin, and the brain was removed and cut frozen through the area of the lesion for subsequent histological examination. The localization and distribution of the lesions were made according to the atlas of Meesen and Olszewski (1949). The area of the lesion was plotted on graph paper and, in all illustrations, the areas of its largest dimensions were described.

The brainstem was exposed by an occipital craniotomy. All cut edges were sealed with bone wax. Electrodes were inserted stereotaxically into the brainstem with lateral and rostrocaudal coordinates referred to the obex and the dorsolateral coordinates to the dorsal margin of the brainstem.

In 15 of the 24 rabbits (experimental groups A-C, Table 1), lesions were placed within the area from which a simulated cerebral ischemic response, i.e., elevation of arterial pressure and bradycardia (Dampney et al., 1979), was produced by electrical stimulation. To do this the electrodes were first connected to a stimulator and, as the electrode was lowered to the brainstem in 0.5-mm steps, a constant current stimulus (12-sec train, 50 Hz, 100 µA) was delivered at each point. At the point along the electrode track from which the maximal response was elicited (usually an elevation of arterial pressure of greater than 50 mm Hg), a lesion was made. The same procedure was repeated at a homologous point on the contralateral side. Following the placement of lesions, the cerebral ischemic response was tested approximately once every 10-15 minutes over the following 2-hour period. The area explored ran rostrocaudally from 1 mm to 8 mm anterior to the obex, laterally approximately 1-3 mm from the midline, and in depth to approximately 3 mm below the surface of the brainstem. In several rabbits a stimulating electrode was inserted caudal to the obex in the lateral reticular area. A stimulus train (12 sec, 50 Hz, 100 µA) at this site elicited an elevation of blood pressure. This site was stimulated before and after placement of lesions to determine the viability of the descending sympathetic pathway.

In nine other rabbits, comprising the control group (group D, Table 1), lesions were placed in other regions of the brainstem at rostrocaudal levels comparable to those of the experimental group. Placements were made by use of stereotaxic coordinates and were placed within the nucleus tractus solitarii (rabbits 16, 21, Table 1), the inferior olivary nuclei (rabbit 17), portions of the vestibular nuclear complex (rabbits 18, 19, 22), the trigeminal complex (rabbits 20, 23), and the lateral reticular nucleus (rabbit 24). These sites were chosen since they were known to affect or possibly affect the cardiovascular system. After placement of lesions, a period of 30-60 minutes was allowed to elapse before retesting. In instances (see below) in which the lesions resulted in a fall of arterial pressure to shock levels, pressure was maintained near pre-lesion levels by infusion of norepinephrine, often in association with a plasma expander (Dextran 70, Pharmacia).

**Results**

Bilateral electrolytic lesions of the medulla oblongata, placed in 15 rabbits within regions from which a simulated ischemic response was elicited by electrical stimulation, had variable effects on the reflex elevation of arterial pressure elicited by ischemia (Table 1). In four rabbits designated as group A (rabbits 1-4), the pressor response was almost entirely abolished. In another four animals (designated as group B, rabbits 5-8), it was attenuated by 33-75%. In group C, comprising the remaining seven experimental animals (Table 1) and 9 controls (group D), the cerebral ischemic response was reduced by only 20% or less.

In controls the lesions did not reduce the ischemic pressor response by more than 18% and averaged a reduction of 9% for the group. The groups are described individually below:

**Group A: Almost Total Loss of the Ischemic Pressor Response**

In the four rabbits of group A, as illustrated by a representative tracing in Figure 1, the bilateral
lesions resulted in: (1) virtual disappearance of the rise of arterial pressure reflexly elicited by cerebral ischemia; (2) an immediate fall of mean arterial pressure to 30-40 mm Hg, which was approximately 25-35% of control values (Figs. 1, 2); (3) preservation of the reflex bradycardia elicited by cerebral ischemia. The remaining pressor response, amounting to 3-7% of that before the lesion, could be accounted for by a mechanical effect of occluding the common carotid artery. The change in arterial pressure and reflex responsiveness occurred immediately after placement of the lesions and did not recover despite maintenance of mean arterial pressure at pre-lesion levels by the infusion of norepinephrine and Dextran. The mean arterial pressure was restored to the pre-lesion level to avoid non-specific damage of the brain by sustained subphysiological arterial pressure.

The magnitude of the elevation of arterial pressure elicited by electrical stimulation through an electrode placed caudal to the lesion site in the lateral reticular area was unaltered (Fig. 1). The preservation of the reflex bradycardia and pressor response to electrical stimulation of the lower brainstem demonstrates that the suppression of reflex and tonic vasomotor activity produced by the lesion was not the result of non-specific suppression of bulbar or spinal neurons or vasomotor pathways.

The localization of the lesions that abolished the ischemic response in the four rabbits of group A is illustrated as an overlap for all four animals in Figure 3, and data are presented in Table 1. In all four rabbits the lesions were placed at a level approximately 3 mm rostral to the obex (A3 level). The nucleus parvocellularis reticularis, the dorsal part of the nucleus gigantocellularis reticularis, and the ventromedial portion of the medial vestibular nucleus were almost entirely destroyed by the les-
Figure 1 Effect of bilateral electrolytic lesions of the medulla oblongata on the cerebral ischemic response in anesthetized rabbits (rabbit 2, Table 1). Lower panel: (a) cerebral ischemic response prior to lesion (the aortic nerves had been previously sectioned); (b) pressor response to electrical stimulation (100-μA amplitude, 50 Hz frequency, 0.5-msec pulse duration) of a point within the lateral funiculus of the spinal cord 2 mm caudal to the obex; (c) blood pressure and heart rate 1-2 minutes after placement of the lesions. Norepinephrine (4 μg/ml) was infused slowly so as to restore and maintain blood pressure at control levels after the lesions had been placed; (d) cerebral ischemic response 2 hr 10 min after placement of the lesions; (e) pressor response to electrical stimulation, 2 hours and 15 minutes after placement of the lesions. Note that the cerebral ischemic response is virtually abolished by these lesions, but that the pressor response to stimulation at the point 2 mm caudal to the obex was little affected. The lesions also resulted in an immediate fall in blood pressure to 33 mm Hg from the control level of 110 mm Hg. Upper panel, right = reconstruction of the lesion at point of maximal damage; left = site of electrode from which pressor response was elicited. Abbreviations as in Figure 3.

sions. There was some damage to the ventromedial portion of the nucleus tractus solitarii.

Group B: Partial Impairment of the Ischemic Reflex

In the four rabbits of group B in which only partial impairment of the cerebral ischemic response was obtained, the lesion attenuated the rise of arterial pressure elicited by cerebral ischemia to approximately 45-55% of control values (to about 50-60 mm Hg) (Fig. 2). Lesions in these rabbits also were placed at the A3 level and in an area from which a pressor response was evoked. However, the lesions were smaller than those of group A. Thus, in one rabbit the lesion was restricted only to the nucleus parvocellularis (Rpc, Fig. 4a); in another, only the medial portions of the nucleus gigantocellularis were injured (Fig. 4b). In the remaining two
animals, portions of both the parvocellularis and gigantocellularis nuclei were damaged. In aggregate, however, the size of the lesion was smaller than those in group A.

**Group C: Little or No Effect on the Reflex**

Seven rabbits in the experimental group (Table 1) had lesions which, although placed in active sites from which a pressor response was elicited, failed to impair the cerebral ischemic response by more than 20%, and did not result in changes in resting arterial pressure (Fig. 2). In three rabbits electrodes were placed at the A3 level. These lesions, however, were more ventral than in animals in the A and B groups, and the damage was restricted only to the nucleus reticularis gigantocellularis. Four animals had lesions placed more rostrally, 5–6 mm from the obex (A5–6, Table 1). Of interest was the fact that in two of these (rabbit 11, Fig. 4c, and rabbit 24, Table 1) the lesions were of comparable size to ones which at the A3 level abolished the ischemic response. The observations from group C suggest that there is specificity to the rostrocaudal extent of the lesion site with respect to its ability to abolish the cerebral ischemic response: the critical zone resides in the caudal portions of the electrically excitable zone.

**Controls**

Control lesions (Table 1), including bilateral lesions of NTS (Fig. 4d), did not substantially alter the cerebral ischemic response.

**Discussion**

The present study has demonstrated that the elevation of arterial pressure but not the reflex bradycardia elicited by cerebral ischemia can be abolished by bilateral electrolytic lesions limited to a restricted portion of the medullary reticular formation. The critical area, as illustrated in a summary diagram in Figure 5, lies within the caudal portions of the highly restricted region from which electrical stimulation can reproduce the pattern of changes in peripheral blood flow and conductance elicited by ischemia (Dampney et al., 1979). The effective lesions appear to require damage to three nuclei of the medullary reticular formation: the nucleus parvocellularis, the dorsal part of the nucleus gigantocellularis, and the ventromedial portion of the medial vestibular nucleus. Also, to be effective, the lesion had to damage these nuclei near the caudal portion of the pressor system located at the rostral pole of the inferior olive, about 3 mm rostral to the obex (the A3 level). Comparable lesions which damaged the same nuclei 3 mm rostral to this zone, even though lying within the pressor area, failed to impair reflex elevations of arterial pressure.

**Figure 2** Relationship between the reduction in the magnitude of the vasopressor component of the cerebral ischemic response and the level of mean arterial pressure resulting from the bilateral electrolytic lesions of the brainstem in 24 rabbits, expressed as percent of the corresponding pre-lesion values. Individual animals were segregated into groups A–D, as described in text, with respect to the magnitude of change of the cerebral ischemic response.

**Figure 3** Composite drawing of the extent of bilateral lesions 3 mm rostral to the obex in four rabbits (group A, Table 1) in which the lesions resulted in almost total abolition of the cerebral ischemic response. The broken lines represent the extent of the lesions in individual rabbits; the shaded area represents the zone of overlap. The lesions involved major parts of the nucleus reticularis parvocellularis, the dorsal part of the nucleus reticularis gigantocellularis, part of the medial vestibular nucleus, and the medial portion of the nucleus tractus solitarii. Abbreviations: VII, facial nerve; Dvn, descending vestibular nucleus; Lvn, lateral vestibular nucleus; Mvn, medial vestibular nucleus; N V, nucleus of trigeminal nerve; N VII, nucleus of facial nerve; N XII, nucleus of hypoglossal nerve; Nm X, dorsal motor nucleus of vagus; Nts, nucleus of solitary tract; Oli, nucleus of the inferior olive; Ph, nucleus prepositus hypoglossi; Pyr, pyramidal tract; Rgc, nucleus reticularis gigantocellularis; Rl, nucleus reticularis lateralis; Rpc, nucleus reticularis parvocellularis; Trsp V, spinal tract of trigeminal nerve; TS, tractus solitarius.
Of the three nuclei, the nucleus parvocellularis seems most critical: lesions failing to damage this nucleus never altered the cerebral ischemic response. However, lesions restricted only to the nucleus parvocellularis were not effective in blocking the response. This observation suggests that it was essential to either: (1) damage particular portions of the marginal zone between the parvocellular nucleus and the others or, (2) impair a critical mass in the area of the reticular formation.

It is impossible to determine from these studies whether it is the destruction of neurons within these nuclei, or of the fibers passing through them, which resulted in the blockade of ischemic hypertension. The damaged area lies within the central segmental tract and also, interestingly, within the trajectory of ascending noradrenergic fibers (Ungerstedt, 1971). The rather selective localization to a caudal portion of the zone from which the response is elicited by electrical stimulation suggests it is more likely that damage to neurons within this area is responsible.

The fact that lesions of the responsive area impaired only the reflex vasomotor but not a heart rate component of the ischemic response is of interest. First, the preservation of the reflex bradycardia indicates that the effective lesions had a specific effect in blocking the vasomotor reflex rather than generally suppressing neural activity of the medulla. This contention is also supported by the fact that, after abolition of the reflex vasomotor responses, pressor responses could also be elicited by stimulation of brainstem sites from electrodes placed caudal to the lesion. Second, the selective impairment of only the vasomotor responses suggests that neurons mediating the heart rate (and possibly respiratory) responses to ischemia are located elsewhere in the medulla. This conclusion is supported by our own observations (Dampney et al., 1979) that electrical stimulation within the pressor area failed to excite cardiovagal neurons or to elicit apnea. It would also be in agreement with the conclusions of Borison and Domjan (1970) who observed that the bradycardia elicited by cerebral ischemia was due to direct excitation of cardiovagal neurons within the nucleus ambiguous.

The cardiovascular and respiratory changes elicited by cerebral ischemia are very similar to those produced by distortion of the brainstem in the so-called Cushing response (Cushing, 1902; Hoff and Reis, 1970; Doba and Reis, 1972). Both consist of a large increase in arterial pressure resulting from a profound and differentiated pattern of peripheral vasoconstriction, bradycardia, and apnea (Sagawa et al., 1961; Downing et al., 1963; Miyakawa 1966; Doba and Reis, 1973). Like the ischemic response, the Cushing response is initiated by direct stimulation from regions of the medulla that in cat correspond almost exactly to those from which the ischemic response is elicited (Hoff and Reis, 1970; Doba and Reis, 1972; Dampney et al., 1979). It would consequently appear that the vasomotor components of the cerebral ischemic and Cushing responses are the result of being stimulated, respectively, by distortion or hypoxia of similar if not identical regions of the medulla oblongata. Conceivably, the cerebral ischemic and Cushing reflexes are part of a pattern of oxygen-conserving reflexes, like the diving (Andersen, 1966) or inhalation reflexes (White et al., 1975), which serve to conserve oxygen and redistribute a greatly reduced cardiac output to brain and heart.

Lesions of the pressor zone, which resulted in an abolition of the cerebral ischemic response, always resulted in a profound and irreversible fall of the systemic arterial pressure. The level obtained by such lesions was roughly equivalent to that observed following transection of the spinal cord at Cl. The close relationship between the fall of blood pressure and the abolition of the cerebral ischemic response was clearly demonstrated by the finding that lesions which only partially impaired the ischemic reflex resulted in only a partial reduction of arterial pressure. It seems unlikely that the fall of arterial pressure produced by the lesions was itself responsible for the disappearance of the cerebral ischemic response. First of all, when the blood pressure was maintained by infusion of pressor agents or plasma expanders, the reflex did not reappear. Second, as indicated above, despite the fall of arterial pressure the reflex cardiovagal response to ischemia persisted. More likely the experiments...
BRAIN LESIONS AND THE CEREBRAL ISCHEMIC RESPONSE

**Localization of area mediating the vasomotor component of the cerebral ischemic response in rabbit brainstem. Left = dorsal view of floor of IVth ventral. The distribution of points from which the vasomotor component can be elicited by electrical stimulation of the brainstem is indicated by the elongated cross-hatched area which, although bilateral, is only shown on the right. The area in which lesions abolished the response is represented bilaterally as round dotted area. The lightly shaded strips on both sides of the drawing descending behind the obex represent the distribution of the nucleus tractus solitarii. Right = cross-section of medulla 3 mm rostral to obex, showing critical zone. Abbreviations as in Figure 3.**

Demonstrate that the regions mediating the cerebral ischemic response are also essential for maintaining normal levels of arterial pressure.

Although it has been known for over a century that the integrity of the medulla oblongata is essential for maintenance of normal levels of arterial pressure, the localization of the region, commonly referred to as the tonic vasomotor center (Alexander, 1946), had never been established. Bilateral lesions placed by various investigators in a number of nuclear areas of the medulla in cat, rat, or rabbit have failed to lower the arterial pressure. Such lesions include the nucleus tractus solitarii (Fallert and Bucher, 1966; Miura and Reis, 1970; Miura and Reis, 1971; Doba and Reis, 1973; Nathan and Reis, 1977; Kumada et al., 1978), inferior olivary nuclei, nucleus gigantocellularis, lateral reticular nuclei and paramedian reticular nuclei (Miura and Reis, 1970; Miura and Reis 1971), the spinal trigeminal system (Kumada et al., 1978), and large areas of the medullar dorsal reticular formation, including the vestibular nuclei (Fallert and Bucher, 1966; Chai and Wang, 1968; Manning, 1965). Such investigations led to a view that the representation of tonic vasomotor function in the medulla was diffuse. Indeed, the only study to our knowledge in which a comparable fall of arterial pressure was produced by small bilateral lesions was that of Fallert and Bucher (1966). Their study demonstrated that lesions placed in roughly comparable regions of the brainstem of the rabbit resulted in an immediate profound fall of arterial pressure. Collectively, these observations suggest that the region of the medulla oblongata necessary for maintenance of normal levels of blood pressure is restricted, and moreover corresponds to the area integrating the cerebral ischemic response.

The area of the dorsal medulla which mediates the vasomotor component of the cerebral ischemic and Cushing responses, and is necessary for maintaining normal blood pressure lies within the so-called pressor area of the lower brainstem, i.e., a region from which profound elevations of arterial pressure can be elicited by electrical stimulation (Alexander, 1946). This region is also richly innervated by noradrenergic fibers (Ungerstedt, 1971) and receives projections from afferents in the carotid sinus nerve (Miura and Reis, 1969). Since lesions of this region result in a fall of arterial pressure while stimulation elevates the arterial pressure (Dampney et al., 1979), it is most probable that the region is maintaining a background of normal neural activity, ultimately projected onto spinal vasomotor preganglionic neurons.
Our findings therefore suggest that the cerebral ischemic and possibly Cushing responses are not due to activation of neural areas which normally are quiescent. Rather, they suggest that the responses result from an increase in the background activity of an area of the brain already tonically active and essential for driving the activity of sympathetic vasomotor fibers. Both the cerebral ischemic and Cushing responses, therefore, may represent merely exaggerated, but otherwise normal, inputs to an intrinsic neural network in the lower brainstem which is continuously driving the sympathetic nervous system.

If indeed this area provides normal background drive onto the spinal preganglionic outflow, the question naturally arises as to what makes this area tonically active? One possibility relates to the fact that small distorting pressures within this area, in the range of 10 cm of H2O (Doba and Reis, 1972), can produce substantial elevations of pressure. This is comparable in magnitude to the normal pressure transients measured interestially in brain from reflected arterial and respiratory pulses (Brock et al., 1972; Poll et al., 1972). This fact raises the possibility that neurons in this region may be driven by naturally occurring local distortions and possibly even small variations in O2 tension. Thus, these medullary neurons would maintain tonic background activity and respond to processes inexcitably linked to the life process itself.

References

Borison HL, Domjan D: Persistence of the cardio-inhibitory response to brain stem ischaemia after destruction of the area postrema and the dorsal vagal nuclei. J Physiol (Lond) 211: 263-277, 1970
Doba N, Reis DJ: Localization with the lower brainstem of a receptive area mediating the pressor response to increased intracranial pressure (the Cushing response). Brain Res 47: 487-491, 1972
Miura M, Reis DJ: The paramedian reticular nucleus: A site of inhibitory interaction between projections from fastigial nucleus and carotid sinus nerve acting on blood pressure. J Physiol (Lond) 218: 441-460, 1971
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