Influence of the Anteroventral Third Ventricle Region and Sinoaortic Denervation on the Pressor Response to Carotid Occlusion

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SUMMARY The effect of anteroventral third ventricle (AV3V) lesion on the pressor response to occlusion of the common carotid artery was studied in freely moving rats with cuffs implanted 1 day before the tests. Short-term (6 hours) and long-term (2, 14, and 30 days) lesions greatly depressed the pressor responses to 60 seconds of common carotid occlusion. The initial peak, which depends on carotid innervation, was reduced by 55% (from 42 ± 2 to 20 ± 2 mm Hg), and the maintained response, which is of central origin (probably ischemic), was reduced by 32% (from 31 ± 2 to 21 ± 2 mm Hg). The effect of carotid or aortic denervation (or both) was also studied on control and lesioned rats. Carotid denervation produced similar extent of depression of the normal and reduced responses of the AV3V-lesioned rats 35% and 37%, respectively. Aortic denervation produced similar relative potentiation of the responses to common carotid occlusion of control and lesioned rats (72% and 66%, respectively). These data indicate the following: 1) Both short-term and long-term lesions greatly reduce the reflex and central (ischemic) components of the pressor responses to common carotid occlusion in freely moving rats; and 2) the importance of carotid innervation for development of the initial peak and the marked inhibitory effect of the aortic baroreceptor on both components are unchanged after AV3V lesion, when the depressed responses are evaluated as percent changes of the control values rather than as absolute changes. (Hypertension 11 [Suppl I]: 1-178—1-181, 1988)

KEY WORDS  • carotid artery occlusion  • baroreceptor reflex  • anteroventral third ventricle lesion  • cerebral ischemia  • central integration

We have shown that the pressor response to 60-second bilateral occlusion of the common carotid artery produced by implanted cuffs in freely moving rats has two components. The initial peak of the pressor response is of carotid reflex origin, whereas the maintained response, that is, a plateau of lower intensity observed during the last 30 seconds of occlusion, does not depend on carotid innervation and is probably of central (ischemic) origin. In dogs, cats, and rabbits the pressor response to common carotid occlusion (CCO) is considered to be only of reflex origin (baroreceptor and chemoreceptor), since cerebral ischemia is believed to be absent because the relative flow contribution of the vertebral arteries is sufficient in these animals to support cerebral irrigation (see Reference 2). In rats, bilateral common carotid ligation reduces the cerebral flow by 50%, and irrigation by the vertebral arteries is believed to be insufficient to prevent cerebral ischemia, which occurs mainly in the supramedullary areas. The importance of the suprabulbar areas for the full development of the pressor responses to CCO has been demonstrated in the rat. Hypothalamic disconnection at the level of the hypothalamic arcuate nucleus and bilateral lesion of the medial forebrain bundle reduce the pressor response in anesthetized rats. In conscious rats we demonstrated that the integrity of the medial forebrain bundle is important for the development of the responses to CCO, more so for the maintained response than for the initial peak.

Great importance has been attributed to the role of the anteroventral third ventricle (AV3V) region in the cardiovascular regulation of the rat. Because the supramedullary region participates in the CCO response and since the AV3V area in the rat greatly affects cardiovascular regulation, the objective of the present work was to study the effect of AV3V lesion on the pressor responses to CCO of freely moving rats. The
relative importance of aortic and carotid denervation for the development of the pressor response to CCO of the AV3V-lesioned rats was also evaluated.

Materials and Methods
Male Wistar rats weighing 240 to 270 g were used. The common carotid arteries were occluded in conscious, freely moving rats by means of pneumatic cuffs implanted 1 day earlier under ether anesthesia, as detailed previously. Carotid and aortic denervations were performed under ether anesthesia as described elsewhere. Under ether anesthesia, electrolytic lesions were made in the periventricular tissue of the AV3V region according to the technique of Buggy et al. A monopolar, stainless steel electrode 0.4 mm in diameter, its wire bared at the tip, was positioned in a stereotaxic instrument (David Kopf, Tujunga, CA, USA), 0.0 to 0.3 mm posterior to the bregma in the midline to a depth of 7.0 mm from the dura mater. The anodal lesion was made with a 2-mA current for 15 seconds. A clip attached to the tail was used as the indifferent electrode. Rats with bilateral damage of the AV3V region exhibited an initial period of adipsia. The electrode was placed at the same coordinates in the sham-lesioned rats except that the depth was 5.5 mm from the dura mater and no electrical current was passed.

The position of the lesions was confirmed by histological examination of the brain tissue. At the end of the experiments, under ether anesthesia, the hearts were exposed for intracardiac perfusion with saline followed by 10% formalin. The brains were removed and stored in 10% formalin for at least 2 weeks. The tissue was then frozen and transverse sections (20–30 μm) were stained with hematoxylin and eosin for examination by light microscopy.

Only the results obtained for rats with a typical AV3V lesion were used. The typical lesioned area was located below the level of the anterior commissure, with bilateral destruction of the periventricular tissue surrounding the optic recess of the lamina terminalis through the preoptic and anterior hypothalamus, never extending caudally to the arcuate nucleus–median eminence region. The structures destroyed by the AV3V lesion included the preoptic and the anterior hypothalamic periventricular nuclei, the median preoptic nucleus, and the anterior wall of the third ventricle with the associated organum vasculosum of the lamina terminalis.

The effects of short-term (6 hours) AV3V lesion were studied in sham-lesioned (n = 11) or AV3V-lesioned rats (n = 10). The effects of long-term lesion were evaluated 2 (n = 10), 14 (n = 8), and 30 days (n = 9) after sham lesion, or 2 (n = 11), 14 (n = 10), and 30 days (n = 10) after AV3V lesion.

Responses to CCO after aortic denervation (6 days) followed by carotid denervation (6 hours) were studied in six rats. Carotid denervation (6 days) followed by aortic denervation (6 hours) was studied in seven rats. Sinoaortic denervation (6 days) performed in a single operation was studied in six rats. The influence of AV3V lesion was studied in rats with carotid denervation (sham, n = 10; lesioned, n = 11), aortic denervation (sham, n = 12; lesioned, n = 11), or sinoaortic denervation (sham, n = 10; lesioned, n = 10) performed 7 days before the CCO test.

Blood pressure was recorded by means of a strain-gauge transducer (Model P23Db, Statham Instruments, Hato Rey, Puerto Rico) connected to a recorder (Model 7754A, Hewlett-Packard, San Diego, CA, USA) from an indwelling catheter implanted 1 day earlier into the femoral artery and exteriorized through the back of the rat.

Data are reported as means ± SEM. Statistical significance was calculated by means of paired and unpaired Student's t tests. Differences were considered to be significant at a p below 0.05.

Results
Effect of AV3V Lesion on Pressor Responses
Six hours after AV3V lesion (mean arterial pressure, 111 ± 6 mm Hg) the pressor responses to CCO were reduced (G1 in Figure 1). The initial peak decreased 55% (from 44 ± 2 to 20 ± 2 mm Hg) and the maintained response decreased 52% (from 31 ± 2 to 21 ± 2 mm Hg), thus suppressing the difference between the initial peak and maintained response. No changes in responses were observed 6 hours after sham lesion (data not included in Figure 1). Similar reductions of the two components of pressor responses were observed 2, 14, and 30 days after AV3V lesion (see G2, G3, and G4 in Figure 1). Thirty days after lesion (mean arterial pressure, 112 ± 8 mm Hg), the initial peak compared to that of sham animals was reduced from 44 ± 1 to 22 ± 2 mm Hg, and the maintained response was reduced from 33 ± 1 to 24 ± 2 mm Hg.

Effect of Aortic and Carotid Denervations
It is known that aortic denervation potentiates the pressor responses to CCO, thereby reducing the difference between the initial peak and maintained response, and that sinus (carotid) denervation almost abolishes the initial peak. Figure 2 shows the effect of
sinoaortic denervation performed in one or two stages. Six hours after sinus denervation the increased response observed 6 days after aortic denervation was reduced substantially, but the potentiation was not completely abolished (see G2 in Figure 2). Similar responses were obtained 6 hours after aortic denervation on rats subjected to sinus denervation 6 days before (see G3 in Figure 2). When the CCO tests were carried out 6 days after sinoaortic denervation was performed in a single operation (see G4 in Figure 2), the pressor responses were the same as when aortic and carotid denervations were performed separately, thus showing that aortic denervation potentiates the response even after carotid innervation is eliminated. Pressor responses with double peak reappeared after elimination of both aortic and carotid innervation (the differences between the initial peak and maintained response, which were statistically significant).

**FIGURE 2.** Combined effect of sinus (SD) and aortic (AD) denervation on the pressor response produced by common carotid occlusion (CCO). The time after denervation is indicated in parentheses below the bars, and the number of the rats is at the top of the figure. Asterisks indicate significant difference (p<0.05) compared with values of 6 days AD, open circles indicate significant difference (p<0.05) compared with values of 6 days SD, closed circles indicate significant difference (p<0.05) between group with sinoaortic denervation (SAD) and control group.

**FIGURE 3.** Combined effect of AV3V lesion and sinoaortic denervation on the pressor responses produced by common carotid occlusion (CCO). Sinus (SD), aortic (AD), and sinoaortic denervations (SAD) were performed 7 days before CCO. AV3V lesion (L) or sham lesion (S) was performed 2 days before CCO. The number of rats is given in parentheses. Asterisks indicate significant difference (p<0.05) compared with values of sham-lesioned group.

**Combined Effect of AV3V Lesion and Sinoaortic Denervation**

Seven days after sinus denervation the response to CCO was reduced, with no difference between the initial peak and maintained response (G2 in Figure 3). Two days after AV3V lesion an additional reduction of 47% was observed (initial peak decreased from 28 ± 2 to 15 ± 2 mm Hg, and maintained response from 26 ± 3 to 14 ± 2 mm Hg). Two days after AV3V lesion the increased response observed after 7 days of aortic denervation was reduced to approximately 45% (see G3 in Figure 3). No significant difference was noted between the initial peak and maintained response, which were similar to the initial peak of the control group. When the AV3V lesion was performed 7 days after sinoaortic denervation (see G4 in Figure 3), the response was quite similar to that observed in animals submitted only to aortic denervation (see G3 in Figure 3).

**Discussion**

The present study clearly shows that the integrity of the AV3V area is important for the full development of the pressor responses to CCO in freely moving rats. Both components of the pressor response were markedly depressed by short-term and long-term AV3V lesion: the initial peak decreased by 53% and the maintained response by 32%, thus suppressing the difference between them. Therefore the AV3V area not only participates in the development of the initial peak, which depends on carotid innervation (baroreceptors and chemoreceptors), but also on the maintained response, which is probably activated by cerebral ischemia. A very interesting finding is that the depressed responses in the lesioned rats were influenced by carotid or aortic denervation (or both) to the same extent as in intact rats. Calculated data from Figures 2 and 3 showed that carotid denervation reduced the response of control (43 ± 3 mm Hg) and lesioned rats (24 ± 1 mm Hg) by 35% and 37%, whereas aortic denervation increased the responses by 72% and 66%, respectively. Aortic denervation potentiated the reduced responses seen after carotid denervation in the control (28 ± 2 mm Hg) and lesioned (15 ± 5 mm Hg) rats to a similar extent — 89% and 87%, respectively. Therefore, the AV3V lesion produces an overall depression of the development of the pressor response to CCO but maintains the inhibitory action of the aortic baroreceptors on the responses almost unchanged, when the potentiation is calculated as a percentage of the control response. Furthermore, the relative importance of carotid innervation for the development of the initial peak was unchanged in the rats with AV3V lesion. An original finding of the present study is that, in addition to their well-known inhibitory effect on the reflex component of CCO, the aortic baroreceptors produce marked influences on the central component (ischemia), as seen in the experiments with carotid-denervated rats.

We demonstrated previously that the lesion of the medial forebrain bundle depresses the pressor response obtained in freely moving rats, more intensely during
the maintained response. We have now shown in conscious rats that AV3V lesion depresses both components of the pressor response, especially the initial peak. These data extend and confirm the original observation of Manning that the supramedullary structures are important for the complete development of the pressor response to carotid occlusion. More recently, several investigators (see Reference 15) demonstrated the important role of the anterior hypothalamus and preoptic area in the normal development of baroreceptor function. Functional projection of the baroreceptors to the AV3V region and preoptic area has been described in the rat. This species also shows anatomical evidence of direct projection from the nucleus tractus solitarii to the hypothalamic and preoptic areas. Furthermore, the AV3V region has an important role in cardiovascular and fluid volume regulation (see Reference 11), with vasomotor projections through the mesencephalic central gray matter and brainstem. Further studies are necessary to determine in detail how the AV3V region influences the central (ischemic) and reflex components of the response elicited by CCO in conscious rats.

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References

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