Lesion of the Area Postrema Region Attenuates Hypertension in Spontaneously Hypertensive Rats

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To determine whether the area postrema contributes to the development of hypertension in spontaneously hypertensive rats (SHR), sham or electrolytic lesions of the area postrema (AP) were made in 4-week-old SHR and Wistar-Kyoto (WKY) controls. From weeks 5 through 16, systolic pressure was measured via tail plethysmography. While blood pressure rose markedly in sham-operated SHR, increases in pressure were small in AP-ablated SHR and similar to those seen in all WKY. Subsequent direct measurements of mean arterial pressure in the same rats showed a significant correlation (r=0.87, p<0.01) with the pressure data acquired via weekly tail-cuff measurement, thereby confirming that hypertension in AP-ablated SHR had indeed been attenuated. Analysis of several hundred computer-acquired measurements of mean arterial pressure from each rat showed that AP ablation shifted the distribution of mean arterial pressure to a lower range in SHR but not WKY. Ablation of the AP also decreased resting heart rate in SHR but not WKY. Suppression of heart rate in response to intravenous phenylephrine was equivalent in sham-operated and AP-ablated rats, suggesting that baroreflex-mediated slowing of heart rate was not impaired. In response to intravenous angiotensin II, suppression of heart rate was similar in sham and AP-ablated SHR, and actually was enhanced in AP-ablated WKY. Histological evaluation of the lesions indicated that visible damage to the adjacent nuclei of the solitary tracts was confined to a small portion of the commissural nucleus. Although we cannot rule out the possibility of damage to the remaining nuclei of the solitary tracts, gross functional damage (which would be revealed by increased lability of arterial pressure and/or decreased baroreflex sensitivity) was undetectable in the baroreflex and lability data. We conclude that ablation of the area postrema region markedly attenuates the development of hypertension in the SHR model. (Circulation Research 1989;64;129–135)
ined the effect of the AP lesion on blood pressure in the spontaneously hypertensive rat (SHR), another model in which high renin activity levels are not usually present. We found that in SHR, the AP lesion markedly attenuated the development of hypertension.

Materials and Methods

Animals

Seventeen weanling male SHR and fourteen Wistar-Kyoto controls were obtained from Charles River Laboratories (Wilmington, Massachusetts) at 4 weeks of age. Animals were housed in group cages until 16 weeks of age and single-caged thereafter. Purina Lab Chow pellets and tap water were available ad libitum. Lighting was maintained on a 12 hour light-dark cycle.

Experimental Groups

Four groups of rats were studied. At 4 weeks of age, seven SHR and seven WKY received sham lesions, while 10 SHR and seven WKY received complete AP lesions. Only animals with complete AP lesions (defined as greater than 90% destruction of the AP) were included in the two AP lesion groups. Completeness of the lesion was determined by histological analysis of the brains of these rats subsequent to the experiments (see below).

Area Postrema Lesions

Immediately before the lesion procedure, all rats were anesthetized with a pentobarbital-chloral hydrate mixture and then immobilized in a stereotoxic instrument (David Kopf). The atlanto-occipital membrane then was exposed via a dorsal midline incision. An incision was made in this membrane that allowed access to the dorsal medulla in the region of the AP. With the help of a dissecting microscope, the AP was clearly visualized on the surface of the medulla. A tungsten microelectrode was then positioned on the surface of the AP. The AP was ablated by passing a 700 μA current through the electrode for 7-10 seconds (4.9-7.0 mC). Visualization of the AP allowed us to pass only the minimum current necessary to destroy the AP, guaranteed small and specific lesions and ensured that a complete lesion was produced in every rat. Sham-lesion rats were subjected to the same surgery, except that no current was passed through the electrode. After surgery, all rats were given 100,000 IU of procaine penicillin i.m. and returned to their cages.

Determination of Blood Pressure

Indirect measurement. Beginning at 5 weeks of age (1 week after lesion), we measured systolic pressure in all rats twice weekly up to and including the 12th week of age. In addition, systolic pressure was assessed twice weekly at 14 and 16 weeks of age. The measurements were accomplished via pho-
toelectric sensing of tail blood flow during deflation of an occluding cuff. We used the IITC (Landing, New Jersey) nonheating tail-cuff system. During these measurements, rats were placed in a noise-attenuating chamber in which temperature was maintained at 27°C. For statistical analysis, a single weekly pressure value was obtained for each rat by averaging the results of two weekly measurement sessions. In each session, five separate determinations of systolic pressure were made. Thus, each indirect blood pressure data point was the average of 10 measurements.

Direct measurement. After the completion of indirect measurements at 16 weeks of age (and before direct measurement of pressure), all rats were housed in 8-inch diameter cylindrical Plexiglas cages in the laboratory. A minimum of three days was allowed for the rats to adapt to laboratory conditions. After adaptation, all rats were anesthetized as above and prepared with ethyl vinyl acetate arterial and venous catheters via the femoral vessels. The catheters were led subcutaneously to an exit at the scapulae. After surgery, all rats were given 100,000 IU of procaine penicillin i.m. and were returned to their Plexiglas cages in the laboratory. After a recovery period of at least 48 hours, resting mean arterial pressure (MAP) was measured in each rat while in its home cage. For this purpose, the arterial catheter was connected to a low-volume pressure transducer (model CP-02, Century Technology). All rats were then allowed at least one-half hour to adapt to the test situation. After this period, pressure was recorded continuously for 1 hour and displayed on a Beckman R611 Dynograph. For subsequent computer analysis, MAP data were collected from the Dynograph via an IBM Personal Computer and stored on magnetic media. MAP was sampled at 0.38 Hz by an analog-to-digital converter (Data Translation 2805), yielding 1,370 samples of MAP for each rat. Finally, heart rate was obtained by averaging the results of direct counting of pulsatile arterial pressure signals over four different 30 second intervals.

Baroreflex Testing

Baroreflex tests were conducted at 17 weeks of age by assessing the reduction in heart rate which occurred in response to a 3-4 minute intravenous infusion of either phenylephrine or Ang II. Three doses of each drug were administered to each rat: 10, 20, and 40 μg/kg/min of phenylephrine; 40, 80 and 160 ng/kg/min of Ang II. Heart rate was measured during a 10-second interval just before the infusion and once again between 3 and 4 minutes after infusion was started, at which time the pressor response to the infusion was maximum and constant.

Histology

At the end of the experimental protocol, each rat was anesthetized and perfused intracardially with
saline followed by buffered formalin. Its brain was then removed and coded for a blind histological analysis of lesion size and location. Serial frozen sections (30 \( \mu \)m thick) were cut through the region of the obex, slide-mounted, and then stained for Nissl substance (cresyl violet stain). The sections were then carefully examined via light microscopy to determine the completeness and location of the lesion. The examiner had no knowledge of the data for any particular animal. For statistical analysis, rats whose brains had no discernible damage were assigned to the two sham groups: WKY-SHAM (n=7), and SHR-SHAM (n=7). Rats whose brains showed complete destruction of the AP were assigned to the two lesion groups: WKY-LESION (n=7), and SHR-LESION (n=10). No incidence of partial destruction of the AP was observed.

Statistical Analysis
Differences between groups over time were analyzed with a three-way mixed design analysis of variance (ANOVA). Direct measurement data were analyzed via two-way, factorial design ANOVA. Comparisons within experimental groups over time were analyzed using Duncan's multiple range tests. Where appropriate, planned comparisons were analyzed with the \( t \) test.

Results
Systolic Pressure
Systolic pressures rose significantly in all four groups between weeks 5 and 16, but a pronounced blood pressure elevation occurred only in sham-operated and not in AP-ablated SHR (Figure 1). In the SHR-SHAM group, systolic pressure averaged 102±3 mm Hg at 5 weeks of age and increased to 200±6 mm Hg by week 16, an increase of 98±5 mm Hg. In contrast, pressure increased from 114±4 mm Hg to 152±3 mm Hg in the SHR-LESION group, an increase of only 38±4 mm Hg. Analysis of variance showed that there was a significant difference in pressure between the SHR-SHAM and SHR-LESION groups (\( p<0.001 \)). Over the same time period, the WKY-SHAM and WKY-LESION groups showed pressure increases of 41±8 and 32±4 mm Hg, respectively. Thus, the change in pressure shown by the SHR-LESION group was similar to those shown by the WKY groups.

The effects of the AP lesion on systolic pressure were confirmed by direct assessment of MAP at 17 weeks of age. The effects of the AP lesion on the distribution of mean arterial pressures sampled for 1 hour at 17 weeks of age are illustrated in Figure 2. It is evident that the AP lesion 1) decreased the range of pressures in the SHR and 2) shifted the frequency distribution to the left (and thus to lower pressures). In contrast, neither the range nor the distribution of arterial pressures was significantly changed in the WKY.

Mean Arterial Pressure
The MAPs for the four groups, along with other data collected at 17 weeks of age, are shown in Table 1. In confirmation of the indirect measures, MAP was significantly lower (\( p<0.005 \)) in the SHR-LESION group compared with the SHR-SHAM group. The MAP of the SHR-LESION group was nonetheless significantly higher (\( p<0.01 \)) than that of either WKY group. There was no significant difference between the pressures of the WKY-LESION and WKY-SHAM groups. A significant correlation was found to exist between the indirectly and directly measured pressures (\( r=0.87, p<0.01 \)).

Lability of Arterial Pressure
To quantify the lability of arterial pressure, we calculated for each rat the standard deviation (SD) of 1,370 MAP samples collected over a 1 hour period at 17 weeks of age. These values are shown in Table 1. There was no significant difference between rat strains in terms of the SD; however, the AP lesion reduced the SD in SHR. This result suggests that the lesion reduced lability.
Heart Rate

The AP lesion also caused significant bradycardia in SHR as shown by measurements taken at 17 weeks of age (see Table 1). Heart rate was significantly reduced (p<0.025) in the SHR-LESION group when compared with the SHR-SHAM group. No significant difference was observed between the heart rates of the WKY-LESION and WKY-SHAM groups.

Baroreflex Response

Baroreflex-mediated slowing of heart rate was not impaired by AP ablation. For each rat, baroreflex slopes were obtained by linear regression, plotting pulse interval versus MAP. For each group, the individual slopes were averaged to yield mean slopes. As Table 1 shows, the slope of the baroreflex produced by phenylephrine injection was not different in sham-operated and AP-ablated rats. In response to Ang II injection, baroreflex slopes generated in sham-operated and AP-ablated SHR were similar; however, in WKY, AP ablation increased the slope of the baroreflex, indicating greater baroreflex sensitivity.

Histology

Photomicrographs of the caudal medulla showing representative sham and complete AP lesions are shown in Figure 3. The AP was completely destroyed in all animals in the SHR-LESION and WKY-LESION groups; in seven of 10 SHR-LESION rats and four of seven WKY-LESION rats, significant damage occurred in the immediately subjacent commissural nucleus of the tractus solitarius (NTS). Since only a small portion of the commissural nucleus is subjacent to the AP, this amounted to destruction of about 10% of the total of the commissural nucleus. In all cases, the solitary tracts and...
Figure 3. Unretouched photomicrographs of coronal sections of rat brain at the level of the obex in the dorsal medulla. Each photomicrograph is from a different rat. Top left: Intact area postrema (AP) from rat in SHR-SHAM group. Area postrema is the dark-staining, diamond-shaped structure on the surface of the medulla. Section transects AP at its midpoint. Top right: AP lesion in rat from SHR-LESION group. Section transects lesion at the caudal extent of the AP. Bottom left: AP lesion in rat from SHR-LESION group. Section transects lesion at the midpoint of the AP. Bottom right: AP lesion in rat from SHR-LESION group. Section transects lesion at the most rostral extent of the AP. These lesions destroyed the AP and significantly damaged, in most cases, the area subpostrema. Note that nuclei of the solitary tract are largely intact; in addition, the dorsal motor nucleus of the vagus is undamaged throughout the extent of the lesions. Calibration bar at top left is 400 μm in length.

dorsal motor nuclei of the vagi, as well as the medial, dorsolateral, intermediate, interstitial, ventrolateral, and ventral nuclei of the NTS (terminology of Kalia and Sullivan) were undamaged. Thus, the actual damage to the totality of the NTS was minor, and the only common area of destruction in all rats appeared to be the AP. Finally, there was no discernible difference in the attenuation of hypertension or heart rate in rats with and without damage to the commissural nucleus.

Discussion

Although many explanations have been proposed to account for the occurrence of hypertension in SHR, much evidence indicates that increased sympathetic nerve traffic is a major factor. This, in turn, suggests that the central neural networks controlling sympathetic outflow may be overactive. Okamoto et al. showed that the central nervous origin of the hypertension in SHR was located in the medulla. In agreement with the findings of Okamoto et al., the main finding of the present study is that ablation of a medullary structure, the AP, at 4 weeks of age greatly attenuated the development of hypertension in SHR. Because of the nature of neural networks and of limitations on the interpretation of lesion studies, our data do not necessarily suggest that the AP is the origin of the enhanced sympathetic drive seen in SHR. Nonetheless, the efferent flow of fibers from the AP certainly suggests that it could modulate sympathetic activity. These connections include pathways from the AP to the NTS (which influence the baroreflex), the dorsal motor nuclei of the vagi and the ambiguus nuclei (which control heart rate), and the ventrolateral medulla (thought by many to be a major controller of resting sympathetic tone). The hypothesis that efferents of the AP function in control of sympathetic outflow is buttressed by the findings that electrical stimulation of the rat or dog AP increases arterial pressure by a neurogenic mechanism. On a neurobiological level, we have attributed the attenuation of hypertension to ablation of the AP; however, underlying the AP is the NTS. Because of the nature of the electrolytic lesion technique, it is not possible to conclusively exclude the possibility
that damage to the NTS may have occurred, or that such damage might be the cause of the attenuation of hypertension. We went to great lengths to try to determine whether NTS damage occurred and if so, what effects it had. First, histological analysis revealed that only a small part of the commissural nucleus of the NTS was visibly damaged and not in all animals. The remainder of the NTS appeared undamaged. Second, in order to assess the possibility of unseen functional damage, we conducted an assessment of the baroreflex slowing of heart rate in all rats; this revealed that ablation of the AP did not impair the baroreflex response in either SHR or WKY. Consistent with these findings, we have found no impairment of the baroreflex response to phenylephrine in AP-ablated Sprague-Dawley rats, and Undesser et al have obtained similar findings in the AP-ablated rabbit. Finally, no increase in lability of arterial pressure could be detected in AP-ablated rats. Thus, visible damage to the NTS was small, and gross functional damage to the NTS (which is usually demonstrated by decreased baroreflex sensitivity and increased pressure lability) was undetectable. In view of these findings, it is likely that the attenuation of hypertension was due to ablation of the AP; however, we cannot rule out the possibility that damage to adjacent areas of the NTS contributed to this effect.

In WKY, the baroreflex response to Ang II was significantly enhanced by AP ablation. This result is consistent with hypotheses suggesting that Ang II inhibits the baroreflex and does so via an action at the AP. In contrast, the baroreflex response to Ang II was not enhanced by the lesion SHR; had the reflex been enhanced, such a result would have provided a possible mechanism for the antihypertensive effect of the lesion. We have no explanation for this difference between the two strains.

When direct measurements of MAP were made at week 17, the differences between groups were somewhat smaller than those seen with the tail-cuff technique at week 16. While it could be postulated that the difference in pressures grew smaller in the 1 week interval, another explanation seems more likely. The difference may be accounted for by the tendency of SHR to have larger pulse pressures than WKY. Thus, SHR have greater mean, systolic, and pulse pressures than WKY. When the usual formula to calculate mean pressure is used, it is inevitable that if one rat has a larger mean, systolic, and pulse pressure than another, the difference in mean pressures between the two rats will be smaller than the difference in systolic pressures.

Heart rate was also significantly reduced by AP ablation in SHR but not in WKY. It may be that reduced heart rate, which reflects reduced sympathetic or increased parasympathetic drive to the heart, is part of the mechanism by which the lesion reduces pressure in SHR. As mentioned above, the AP has efferents to both the nuclei ambiguous and the dorsal motor nuclei of the vagi, suggesting that it may modulate heart rate. If AP input to these structures were inhibitory, the bradycardia could have resulted because of reduced AP input to them. We have also observed bradycardia after AP ablation in Sprague-Dawley rats. Why the WKY is an exception is unknown.

Examinations of the cardiovascular function of the AP have been stimulated for nearly 20 years by the idea that it is a target of circulating Ang II. The present study, in conjunction with the findings that AP ablation prevents both Ang II and DOCA-salt hypertension, suggests that ablation of the rat AP does more than simply remove a target of circulating Ang II. DOCA-salt hypertension and spontaneous hypertension are not believed to be caused by increased plasma Ang II and are known to be associated with normal or suppressed plasma renin activity. Thus, removal of AP Ang II receptors would not appear to account for the prevention of hypertension in these models.

Another possibility is that the lesion is antihypertensive via interference with hydromineral balance; one report indicates that in rats ablation of the AP causes significantly increased urinary sodium losses for as long as 23 weeks postlesion, while another indicates that AP ablation impairs sodium balance. Nonetheless, we have found that when sodium balance is maintained and equivalent in sham-operated and AP-ablated rats, both Ang II and DOCA-salt hypertension are still prevented in the ablated animals.

Our findings suggest that the AP is a significant component of the central network controlling the autonomic nervous system. What remains unclear is the functional relationship of the AP to other members of this network which may be involved in the generation of increased central neurogenic drive in hypertensive models. For example, the AP might be a relay passing information to the brainstem from hypothalamic regions involved in hypertension. One such region borders the anterior ventral third ventricle (AV3V). Many studies have shown that ablation of the AV3V region in rats blocks or attenuates most forms of experimental hypertension. The AV3V region has projections to the paraventricular nuclei, which project to the AP. Nonetheless, spontaneous hypertension is not affected by ablation of the AV3V region. Clearly, then, the effects of the AP lesion on SHR hypertension do not depend on the interruption of information coming from the AV3V region. In addition, Grollman hypertension is blocked by the AV3V lesion but not by the AP lesion. If ablation of the AP were antihypertensive via disruption of the function of the AV3V, one would expect that Grollman hypertension should have been blocked by AP ablation. Thus, the results of the SHR and Grollman experiments both argue that the AP lesion does not create the functional equivalent of an AV3V lesion.

In conclusion, we found in the present study that ablation of the AP markedly attenuates hyperten-
sion in the SHR model. This finding suggests that the AP may participate in the generation of the increased neurogenic drive to arterial pressure that characterizes this model of hypertension.

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