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

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Ferrario found that ablation of the AP attenuated one-kidney, one-clip hypertension in the dog. In the rat, there are few studies of the role of the AP in hypertension. Haywood et al found that ablation of the AP did not alter Grollman renal hypertension, and did not reduce pressor responses to short-term infusion of Ang II, but Fink et al have found that chronic Ang II-induced hypertension was prevented in rats with AP lesions. In addition, these authors found that ablation of the AP completely blocked the development of deoxycorticosterone acetate (DOCA)-salt hypertension. Thus, ablation of the AP prevented hypertension in a model in which plasma renin activity is known not to be elevated (DOCA-salt), as well as in the chronic Ang II-infusion model. In the present study, we exam-
Determined 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 stereotaxic instrument (David Kopf). The atlanto-occipital membrane 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-
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Systolic Pressure (mm Hg)

FIGURE 1. Effect of area postrema (AP) or sham lesion on weekly values of systolic pressure in the four lesion groups. Lesions of the AP or sham lesions were made at age 4 weeks. The development of hypertension was markedly attenuated in the SHR-LESION group. Values are mean±SEM. *p<0.001. *p<0.01.

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 μm 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=10). 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 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

| Table 1. Cardiovascular Effects of Area Postrema Ablation in SHR and WKY |
|-----------------|-----------------|-----------------|-----------------|
|                 | SHR             | WKY             |
|                 | Sham            | Lesion          | Sham            | Lesion          |
| MAP* (mm Hg)    | 164±7.8         | 131±4.7††       | 113±2.8         | 109±3.1         |
| Heart rate (bpm)| 342±10          | 314±7§          | 329±13          | 330±12          |
| SD (mm Hg)      | 7.5±0.7         | 5.4±0.7||        | 6.2±0.9         | 6.8±1.1         |
| Phenylephrine1 baroreflex slope (Pi/mm Hg) | 0.87±0.1 | 0.74±0.02 | 1.8±0.3 | 1.5±0.2 |
| Ang II1 baroreflex slope (Pi/mm Hg) | 0.83±0.1 | 0.65±0.1 | 1.5±0.2 | 2.2±0.3§ |

SHR, spontaneously hypertensive rats; WKY, Wistar-Kyoto rats; MAP, mean arterial pressure; SD, standard deviation of 1,370 samples of MAP; and Pi, pulse interval (1/heart rate).

All values are mean±SEM.

*Difference between genetic strains (significance level in "Results").
†$p<0.005$ vs. SHR sham.
‡$p<0.01$ vs. WKY sham.
§$p<0.025$ vs. SHR sham.
||$p<0.05$ vs. SHR sham.
¶$p<0.05$ vs. WKY sham.
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 ambiguous 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.
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.

Acknowledgments

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