Lesions of the Anteroventral Third Ventricle and Development of Stress-Induced Hypertension in the Borderline Hypertensive Rat

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The anteroventral third ventricle (AV3V) region plays a critical role in the pathogenesis of many forms of experimental hypertension. The present study sought to determine whether the integrity of this area was necessary for the development of stress-induced hypertension in the borderline hypertensive rat (BHR). Male BHRs were assigned to three groups at 8 weeks of age: 1) AV3V lesion, 2) sham lesion, and 3) maturation control. BHRs with AV3V and sham lesions were exposed to 12 weeks of conflict stress (2 hr/day, 5 days/wk). At the end of the conflict protocol period, direct measurement of resting mean arterial pressure indicated that BHRs with sham lesions had significantly higher blood pressure (153±2.9 mm Hg) than rats with AV3V lesions (126±5.2 mm Hg) and maturation control rats (133±4.3 mm Hg). Although AV3V lesions prevented stress-induced hypertension in BHRs, these rats were still capable of transiently raising blood pressure. Specifically, the results also indicate that BHRs with AV3V lesions showed greater increases in blood pressure in response to an electric foot-shock paradigm. This study suggests a critical role for this forebrain region in the production of stress-induced hypertension in genetically predisposed animals. (Hypertension 1989;13:817–821)

The relation between environmental stressors and blood pressure regulation has long been appreciated.1-2 Many experiments using dogs,3 rats,4 and nonhuman primates5 have been successful at demonstrating that environmental stressors can transiently alter blood pressure and heart rate. However, the demonstration that repeated exposure to stressful events can be translated into permanent cardiovascular disease in experimental animals has been an elusive goal. The genetic background of the experimental animals studied and the nature of the behavioral stressors used have been suggested to be responsible, at least in part, for the difficulty in producing environmentally induced hypertension in these animals.

Several recent studies have focused on the first generation offspring of the spontaneously hypertensive rat (SHR) × the Wistar-Kyoto (WKY) rat. These rats have genetic components of both a hypertensive and normotensive parent, exhibit resting systolic blood pressures between 140 and 160 mm Hg, and do not display the characteristic age-related increase in pressure observed in their SHR parent. Therefore, this rat has been termed the borderline hypertensive rat (BHR). It appears that the genetic background of the BHR makes it susceptible to the hypertensive effects of at least two environmental challenges. It has been demonstrated that the BHR becomes hypertensive when exposed to a shock-shock conflict paradigm. This hypertension persists long after the termination of the behavioral stress and is attended by significant cardiac pathology.6 In addition, increased dietary salt also has been shown to produce hypertension in this animal model.7 The importance of these findings is highlighted by the fact that stress and increased salt intake are widely considered to be contributory factors in the pathogenesis of human essential hypertension.8,9

Although the development of the BHR has significant implications for the study of the interaction of environmental stressors and blood pressure, little is known about the central mechanisms that may be involved in the elaboration of hypertension in the BHR. An area of the central nervous system that has been implicated in several forms of experimental hypertension is the anteroventral portion of the...
third ventricle (AV3V). Electrolytic ablation of the AV3V region has been demonstrated to prevent or attenuate Grollman,¹⁰ DOCA-salt,¹¹ and Goldblatt¹² models of hypertension as well as neurogenically mediated hypertension.¹³

Although the effects of AV3V lesions have been studied in many models of experimental hypertension, this treatment has not been examined with respect to the development of stress-induced hypertension. This has been due in part to the fact that most animals do not show large, sustained increases in arterial pressure when stressed.¹,² and the SHR is destined to become markedly hypertensive, making it difficult to study the relatively small effects of environmental stressors on the hypertensive process. Since the BHR will not develop severe hypertension unless exposed to exogenous challenges (e.g., stress or increased dietary salt), this model affords the opportunity to test the hypothesis that the AV3V is involved in the production of hypertension that is provoked by exposure to a psychological stressor. The current study was designed specifically to determine whether the integrity of the AV3V region is a necessary prerequisite for the elaboration of hypertension produced by a behavioral stress paradigm in the BHR. An ancillary goal of the experiment was to explore the responses of these rats to an acute stressor.

Materials and Methods

Animals

Male BHRs were used in this study. All rats were bred in the animal facility in the Department of Psychology at the University of Iowa from female SHRs and male WKY rats purchased from Taconic Farms (Germantown, New York). All rats were weaned at 3 weeks of age and individually housed at 7 weeks of age. Standard laboratory rat chow (Purina) and tap water were available ad libitum throughout the experiment.

Experimental Protocol

At 8 weeks of age, all rats were adapted to the tail-cuff plethysmograph for 5 days. The following week, indirect systolic blood pressure measurements (IITC Inc., Woodland Hills, California) were taken, and the rats were then randomly assigned to one of two groups. There were no differences in systolic blood pressures between groups at this point (lesion group, 154±5.5 mm Hg vs. sham-lesion group, 150±4.3 mm Hg; p>0.634). Rats in one group received an electrolytic lesion directed to the periventricular tissue of the AV3V region. The rats were anesthetized with ketamine and acepromazine (50 mg/ml i.p.) and positioned in a Kopf (Tujunga, California) stereotaxic instrument. Bregma and lambda were exposed, the head was leveled, and a midline opening was made in the skull 0.3 mm caudal to bregma. A lesioning electrode (24-gauge nichrome wire insulated except at the tip) was lowered on the midline 0.3 mm caudal to bregma to a depth of 7.5 mm from dura. Direct anodal current (2-3 mA) was passed for 25-30 seconds (rectal cathode). Sham-lesion rats were treated similarly except that the electrode was lowered to 4 mm below dura, and no current was passed. A separate group of male BHRs served as nonstressed controls. Indirect blood pressure measurements taken before induction of the stress protocol revealed no difference between groups (lesion group, 150.8±1.6 vs. sham-lesion group, 146.8±1.9; p=0.19). Since there was no difference between these two groups, a lesioned nonstressed group was not included in the current study.

At 11 weeks of age, lesioned and sham rats began a 3-week training period of signaled avoidance. In this paradigm, rats were placed in Plexiglas conditioning cages (28x8.5x7 cm) for 2 hr/day, 5 days/wk and trained to turn a small wheel (7.6 cm) one quarter turn during the presentation of a 1 KHz tone to delay by 10 seconds five cutaneous tail shocks (0.2-0.4 mA, 1 second in duration) delivered via two cutaneous tail electrodes. The Plexiglas cages were placed in sound-attenuating chambers equipped with a ventilation fan, outlets for tail electrodes, and a buzzer (Tandy Corp., Ft. Worth, Texas). The onset and offset of tones and shocks were controlled by Apple IIe computers using software developed in this laboratory. After this training period, all rats were exposed to an avoidance-avoidance conflict paradigm (2 hr/day, 5 days/wk for 12 weeks). Conflict differs from signaled avoidance; in the conflict paradigm, it is still necessary to turn the wheel to avoid five shocks, but every time the wheel is turned, one tail shock is delivered. This paradigm and the hypertensive results it produces have been discussed in detail elsewhere.⁶ Parent strains are not typically used with this paradigm. Since the SHR is genetically destined to become hypertensive, it complicates the analysis of the effects of exogenous factors on this process, and WKY rats have been shown to be resistant to the hypertensive effects of conflict stress.¹⁴

At the end of the conflict protocol, all rats were anesthetized as described above and instrumented with femoral arterial catheters (PE-50) for the direct measurement of mean arterial pressure (MAP) and heart rate (HR). The catheter was tunneled up the back, exited at the nape of the neck, filled with heparinized saline (100 units/ml), and plugged.

After a 48-hour recovery period, baseline cardiovascular measures were obtained in all rats while in their home cages. The catheter was attached to a pressure transducer (Ailetch 200, City of Industry, California), and the blood pressure signal was recorded on a Beckman Dynograph (model 711, Beckman Instrs., Inc., Fullerton, California). After each rat had been in a quiet state for at least 1 hour, baseline MAP and HR were measured and then the rats were transferred to a Plexiglas foot-shock chamber (25x30x30 cm). After the rat was placed in the
chamber, electric shock pulses were administered through the stainless steel grid floor for a 5-minute period (1.0 mA, 0.5 seconds in duration, every 5 seconds). Cardiovascular data were measured after transfer and immediately and 5 minutes after cessation of foot shock.

**Histology**

At the end of the experiment, all rats were anesthetized as described above and perfused transcardially with 0.9% saline followed by 10% formalin. The brains were removed and stored in the fixative until frozen sections were taken and stained for Nissl substance with cresyl violet.

**Statistical Analysis**

The data collected in this study were analyzed with analyses of variance. Analysis of variance with repeated measures (referred to as time) was used to examine the transfer and postfoot-shock data. Significant effects were further analyzed using Tukey's honestly significant difference test. A p<0.05 level was chosen for statistical significance. Data are presented as mean±SEM.

**Results**

Verification of lesion placement was carried out as previously described. 10-12 This analysis revealed that all rats that failed to develop conflict stress-induced hypertension shared a common area of damage to the periventricular tissue of the optic recess at the level of the anterior commissure. The lesion consistently damaged the preoptic-anterior hypothalamic periventricular tissue, median preoptic nucleus, and organum vasculosum of the lamina terminalis. Damage was infrequently observed in surrounding structures such as the suprachiasmatic, anterior hypothalamic, and paraventricular nuclei.

Figure 1 depicts the baseline MAP for all groups. Analysis of variance performed on these data revealed a significant main effect of groups (p<0.003). Follow-up analyses indicated that the MAP of the AV3V-lesion group was significantly lower than the sham-lesion group (126.0±5.2 vs. 152.8±2.9 mm Hg). Furthermore, AV3V lesions kept MAP in stressed rats at levels not significantly different from nonstressed control BHRs (126.0±5.2 vs. 133.0±4.3 mm Hg). As can be seen in Figure 2, there were no significant differences between groups with respect to baseline HR (p>0.328).

Figure 3 shows the change in MAP to transfer and foot shock in all groups. Analysis of these data revealed a significant groups-by-time interaction effect (p=0.003). Subsequent analyses indicated that BHRs with AV3V lesions displayed a significantly greater increase in MAP to transfer than sham-lesion BHRs and nonstressed control rats (19.2±3.7 vs. 2.7±1.2 mm Hg; 19.2±3.7 vs. 6.6±0.7 mm Hg, respectively). Also, AV3V-lesion rats showed a significantly greater increase in MAP immediately after foot shock compared with sham-lesion rats (17.0±5.3 vs. 11.7±5.8 mm Hg) and...
nonstressed control rats (17.0±5.3 vs. 5.8±2.1 mm Hg). At 5 minutes after foot shock, there were no significant differences between the three groups.

Figure 4 illustrates the HR response to transfer and foot shock in all three groups of rats. Analysis of variance performed on these data revealed a marginally significant groups-by-time interaction (p=0.055). Inspection of Figure 4 suggests that the marginally significant interaction may be partially accounted for by the relatively large HR responses of nonstressed control BHRs.

Discussion

The purpose of the present study was to examine the effect of AV3V lesions on the development of hypertension produced by exposure to an exogenous stressor in the BHR. Previous experiments have investigated the mechanisms involved in the production of hypertension in the BHR and may provide insight into the results reported here. Specifically, since alterations in both autonomic and renal functioning may be involved in the production of environmentally induced hypertension in the BHR, and these systems are disrupted by AV3V lesions, the rat’s capacity to effect volume expansion. Two observations in the BHR suggest that stress-induced hypertension may have a volume expansion component. First, bilateral surgical renal denervation inhibits the elaboration of hypertension in this model. Also, acute bouts of stress and a high salt diet appear to interact to produce a slight decrease in renal plasma flow and urinary sodium excretion in BHRs. BHRs with AV3V lesions may be protected from hypertension partially because of an inability at the central level to orchestrate alterations in renal functioning (e.g., enhanced retention of sodium/water) that contribute to the disease process.

From another perspective, it was somewhat surprising that this manipulation was successful at pared with WKY rats after an episode of acute stress. These observations are consistent with the hypothesis put forth by Folkow that repeated activation of the cardiovascular system may be responsible for producing essential hypertension in genetically predisposed individuals. It may be the case that BHRs with AV3V lesions do not develop hypertension because they exhibit reduced sympathetic drive compared with sham-operated BHRs that become hypertensive.

In the present study, however, rats with AV3V lesions displayed robust increases in MAP as compared with neurologically intact rats after an episode of acute stress. The mechanisms by which BHRs with AV3V lesions raise pressure in this situation are unclear. Since the acute stress session was 5 minutes in duration, there was adequate time for the rat to invoke a variety of endogenous pressor mechanisms that may remain intact. In addition, the rats with lesions may be more responsive to circulating vasoactive agents (e.g., norepinephrine, vasopressin, and angiotensin) during stress than their sham-lesion counterparts that are hypertensive. Furthermore, it is possible that the sham-operated hypertensive BHRs are incapable of further elevating pressure to an acute stressor; this has been previously observed in BHRs with environmentally induced hypertension. Finally, it must be pointed out that the acute shock paradigm employed in this study represents a novel stressor for all subjects. Therefore, it is difficult to determine whether cardiovascular responses recorded during a typical 2-hour conflict stress session would be similar. That is, it is possible that during the 12-week conflict protocol the magnitude and duration of these responses may have been altered in AV3V-lesioned BHRs. It clearly remains to be determined which physiological strategies these rats use to transiently raise pressure under these circumstances.

Ablation of the AV3V may also interfere with the hypertensive process by disrupting the organism’s control of body fluid balance. Since this lesion impairs the release of vasopressin, it is possible that one mechanism by which this maneuver exerts its antihypertensive effect is through compromising the rat’s capacity to effect volume expansion. Two observations in the BHR suggest that stress-induced hypertension may have a volume expansion component. First, bilateral surgical renal denervation inhibits the elaboration of hypertension in this model. Also, acute bouts of stress and a high salt diet appear to interact to produce a slight decrease in renal plasma flow and urinary sodium excretion in BHRs. BHRs with AV3V lesions may be protected from hypertension partially because of an inability at the central level to orchestrate alterations in renal functioning (e.g., enhanced retention of sodium/water) that contribute to the disease process.

From another perspective, it was somewhat surprising that this manipulation was successful at
completely blocking stress-induced hypertension in the BHR since lesions of the AV3V have been found to be ineffective at preventing or attenuating the development of hypertension in the SHR.18,19 Although the hypertensive parent strain was not studied in the present experiment, the previous observations that the lesion does not protect the SHR but does prevent the development of several other models of hypertension10-13 and the current finding that the lesion has a prophylactic effect in the hybrid F1 are intriguing. These earlier findings, coupled with the present investigation, are consistent with the suggestion that the efficacy of AV3V lesions in preventing hypertension is through alterations in mechanisms involved in the pathogenesis of the disorder rather than due to a more global or nonspecific blood pressure–lowering effect. The differing responses between the SHR and BHR suggest that the mechanisms mediating stress-induced hypertension in the BHR are, at least to a degree, different from those responsible for SHR hypertension. AV3V lesions may protect the BHR from developing stress-induced hypertension by disrupting the organism’s control of physiological systems (such as those discussed earlier) involved in the pathogenesis of the disorder that are either inoperative or resistant to the effects of the lesion in the SHR. Future studies will be necessary to determine precise commonalities and differences in the hypertensive processes between these two strains.

In summary, the current study has demonstrated that the integrity of the AV3V is necessary for the development of environmentally induced hypertension in the BHR. Since the pathogenesis of hypertension in this model appears to have both an autonomic and renal component, it is likely that the prophylactic effect of this lesion, in part, involves disruption in one or both of these systems. The results of the present study provide the rationale for the development of experimental strategies designed to better understand the role of this forebrain area in the translation of environmental stressors to hypertension.

References

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