ANG II-induced hypertension and the role of the area postrema during normal and increased dietary salt

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Nahey DB, Collister JP. ANG II-induced hypertension and the role of the area postrema during normal and increased dietary salt. Am J Physiol Heart Circ Physiol 292: H694–H700, 2007. First published September 15, 2006; doi:10.1152/ajpheart.00998.2005.—It has been shown that the area postrema (AP) plays a role in the development of certain types of chronic angiotensin II (ANG II)-induced hypertension in the rat but is not of great importance in the salt sensitivity of arterial pressure. It has recently been proposed, however, that elevated sodium levels may exacerbate the hypertensive effects of ANG II, which by itself dramatically affects salt sensitivity, by acting at sodium-sensing neurons in certain circumventricular organs of the brain. Thus the interactions of ANG II, sodium, and the central nervous system remain to be fully understood. The purpose of this study was to examine the role of the AP in ANG II-induced hypertension during periods of normal and elevated dietary salt. We hypothesized that an intact AP was necessary for the full development of ANG II-induced hypertension and that its role would be pronounced during periods of increased dietary sodium. To test this, male Sprague-Dawley rats underwent ablation of the area postrema (APx, n = 6) or sham operation (sham, n = 6). After 3 wk of recovery, rats were instrumented with radiotelemetry transducers for constant blood pressure and heart rate monitoring and venous catheters for vehicle infusion. After a 3-day control period of 0.9% saline infusion and a 10-day period of 0.4% dietary sodium, a 10-day period of ANG II infusion (7 ml/day) and 0.4% dietary sodium, a 10-day period of ANG II infusion (10 ng \cdot kg^{-1} \cdot min^{-1}) was begun, immediately followed by a second 10-day period during which rats were fed a 4.0% sodium diet. By day 6 of ANG II infusion, mean arterial pressure (MAP) in APx rats had increased to 139 ± 4 mmHg, whereas MAP in sham rats had increased to 126 ± 3 mmHg. This difference was found to be significant and continued through day 1 of the high-salt period, after which MAP of the two groups had risen to similar levels. On day 9 of high salt, MAP was again observed to be significantly higher (162 ± 1 mmHg) in APx rats when compared with sham rats (147 ± 4 mmHg). These results do not support the hypothesis that the AP is necessary for the full development of ANG II-induced hypertension at normal or elevated levels of dietary sodium.

angiotensin; circumventricular organs; blood pressure; sodium and water balance

THE AREA POSTREMA (AP), located on the dorsal surface of the medulla near the fourth ventricle, has been implicated in the control of cardiovascular function for some time (26, 38). One of the ways in which the AP is presumed to participate in cardiovascular control is in direct response to circulating hormones. As one of the circumventricular organs (CVO), the AP lacks a normal blood-brain barrier (25), rendering it susceptible to larger circulating substances such as peptide hormones. The AP has been shown to have a high number of receptors for such cardiovascular regulating hormones as vasopressin and, most notably, angiotensin II (ANG II) (35), which makes it an attractive candidate for participation in the central control of cardiovascular function. ANG II has been shown to have numerous effects on cardiovascular function, ranging from acute effects on vascular tone to long-term effects on sympathetic output (10, 32). Furthermore, ANG II-induced hypertension is a widely accepted and examined model of hypertension.

The AP is also widely acknowledged as a functioning part of a complex neural pathway regulating cardiovascular function. Centrally, the AP sends projections to and receives input from several areas in the brain that have been shown to play important roles in the neural control of blood pressure (BP) and heart rate (HR), including the paraventricular nucleus (25), dorsal motor nucleus of the vagus (33, 36), rostral ventral lateral medulla (17, 25), and nucleus tractus solitarius (13, 17). Because of its interrelationship with these areas and other structures that participate in cardiovascular regulation, such as osmoreceptors (3, 32), it is expected that the AP plays an integral role in the central pathway that helps regulate cardiovascular function and homeostasis.

Early studies of the AP showed no role in cardiovascular control in response to intra-aortic or intravertebral administration of angiotensin in the rat (22). Subsequent studies, however, demonstrated a profound hypertensive response to acute injections of ANG II directly into the AP of rats (4, 28). Chronic studies have since demonstrated a role of the AP in more long-term regulation of BP and HR as well. Specifically, a high-salt model of ANG II-induced hypertension (19) and a chronic model of hypotension induced by ANG II type 1 (AT1) receptor blockade (7) have provided valuable information to explain the role(s) of AP in cardiovascular control with regard to ANG II.

Chronic ANG II hypertension has been shown to be exacerbated by increased sodium intake (14, 31). It has been proposed that elevated plasma sodium levels may be contributing to this effect by activating sodium or osmoreceptors at CVO such as the AP (3). Although the role of the AP in ANG II-induced hypertension in saline loaded rats has been described (19), its role in the chronic hypertensive effects of ANG II during normal sodium intake has not. Is the AP involved solely in the chronic effects of ANG II or only in the combined effects of ANG II and elevated salt? The present study was conducted to determine the role of the AP in the chronic hypertensive effects of ANG II during normal dietary salt as well as in the salt-sensitive effects of ANG II.
METHODS

Adult male Sprague-Dawley rats (250–275 g; Charles River, Wilmington, MA) were used in all experiments. All rats were housed in Nalgene metabolic cages (Harvard Apparatus, Holliston, MA) and maintained in a controlled environment with a 12-h:12-h light-dark cycle. All procedures were conducted in accordance with institutional and National Institutes of Health (NIH) guidelines, complied with the Guide for Care and Use of Laboratory Animals (NIH Publication No. 85-22, Revised 1996), and were approved by the Institutional Animal Care and Use Committee of the University of Minnesota (St. Paul, MN).

Surgical procedures. Rats were randomly selected for either AP lesion (APx, n = 6) or sham (n = 6) operation. Rats were preanesthetized with pentobarbital sodium (32.5 mg/kg ip) and atropine (0.2 mg/kg/ip). Surgical anesthesia was achieved with a second intramuscular injection containing a combination of anesthetic agents (0.2 mg/kg acetylpromazine, 0.2 mg/kg butorphanol tartrate, and 25 mg/kg ketamine). Rats were then placed in a stereotaxic apparatus with the neck flexed. The surgical technique utilized for APx and sham operation was the same as previously described (6). Briefly, a dorsal midline incision was made from the occipital crest of the skull to the spine of the first vertebrae, and the underlying muscle was separated to expose the atlanto-occipital membrane. The membrane was punctured, and a small portion of the skull was removed with rongeurs to allow visualization of the AP. The AP was removed by suction using a blunt 26-gauge needle attached to a vacuum line. Except for the attached vacuum line, sham operations were identical. The muscular layer was closed with 3-0 chronic gut suture, and the skin layer was closed with 3-0 silk sutures. Rats were given an intramuscular antibiotic injection of 2.5 mg gentamycin and a subcutaneous injection of 0.075 mg butorphanol tartrate for analgesic purposes postoperatively. Because APx rats exhibit a transient decrease in food intake after AP ablation, rats were allowed 3 wk of recovery before undergoing further surgical procedures. Standard rat chow and distilled water were provided ad libitum throughout the recovery period. Implantation of radiotelemetry transducers and venous catheters. After 3 wk of recovery from APx or sham operation, rats were instrumented with radiotelemetric pressure transducers (model TA11PA-C40, Data Sciences International, St. Paul, MN), for continuous sampling of mean arterial pressure (MAP) and HR, and venous catheters for delivery of ANG II. Surgical anesthesia was achieved as described above. The telemetry device was implanted as previously described (8) and secured to the abdominal wall with 3-0 silk sutures during closure of the abdominal cavity. The skin was closed with surgical staples. For implantation of the venous catheter, a ventral incision was made in the left leg to expose the femoral vein. Flexible tubing (Helix Medical silicone tubing, Fisher Scientific, Pittsburgh, PA) was advanced through the vein cranially ~9 cm and anchored using 3-0 silk suture. The catheter was passed subcutaneously to exit between the scapulae and passed through a flexible spring connected to a single-channel hydraulic swivel. Each rat received a postsurgical subcutaneous injection of 0.075 mg butorphanol tartrate for analgesic purposes and received intravenous antibiotics consisting of 15 mg ampicillin for 3 days following surgery. Rats were given a 7-day recovery period postsurgery, during which standard rat chow and distilled water were provided ad libitum. After this recovery period, rats were placed on a 0.4% NaCl diet (Research Diets, New Brunswick, NJ) and given continuous infusions of 0.9% sterile saline (7 ml/day iv) from 3–4 days before the start of the 3-day control period to ensure that sodium and water balance had been reestablished.

Experimental protocol. To begin the protocol, baseline control levels were recorded for 3 days during which rats were fed ad 0.4% (normal) NaCl diet and infused with 0.9% sterile saline (7 ml/day). This combination results in a sodium intake of ~2 meq/day, which is equivalent to normal sodium intake on standard (1.0% NaCl) rat chow. Rats were weighed to calculate infusion concentrations of ANG II the day before ANG II infusion was begun. The average weights on day 1 of infusion of APx and sham rats were 320 ± 16 and 376 ± 7 g, respectively. On days 1–10 of the experimental phase, ANG II was added to the saline and infused at a rate of 10 ng·kg\(^{-1}\)·min\(^{-1}\) for all rats; their diet was unchanged. On days 11–20 of the experimental phase, all rats were given a 4.0% (high) NaCl diet while remaining on the same dose of ANG II. Infusion of ANG II was terminated while all other variables remained unchanged for a 6-day recovery period to complete the protocol. During the protocol, MAP and HR were monitored 24 h per day, and data points reported are 24-h averages.

Food, water, urine, and sodium measurements. Ad libitum food and water intake as well as urine output were gravimetrically measured daily at approximately hour 6 of the daily light cycle. Total water intake was calculated as the sum of voluntary drinking and the 7 ml of infused volume. Total sodium intake was calculated as the sum of sodium received in the daily infusion and the product of food intake and sodium content of the food. Urine sodium concentration was analyzed using the Nova 1 Analyzer (Nova Biomedical, Waltham, MA), and urine sodium excretion was calculated as the product of urine sodium concentration and daily excreted urinary volume. Water balance was calculated as the difference between total water intake (ingested and infused) and urine output. Sodium balance was calculated as the difference between total salt intake (which included both dietary and infused sodium) and sodium excretion.

Histological verification. After conclusion of the experimental protocol, rats were anesthetized (as described above) and perfused intracardially with 4% paraformaldehyde. Whole brains were removed and kept refrigerated in 4% paraformaldehyde for 2 days, then transferred to a 30% sucrose solution for 3 days. Coronal serial sections (50 µm) were sliced using a freezing microtome and mounted on gelatin-treated slides. Sections were stained with cresyl violet and examined using light microscopy for confirmation of an intact (sham) or lesioned AP (APx). All APx rats included in the study were confirmed to have undergone complete AP ablation with minimal damage to the surrounding tissue (Fig. 1).

Statistical analysis. All values are reported as means ± SE. Statistical comparison within and between experimental groups was performed by a two-way ANOVA with repeated measures, the factors being lesion and diet/ANG II condition. If interaction was reported between factors, post hoc analysis was performed using the Bonferroni multiple comparison test. The Geisser-Greenhouse adjusted P value was used to account for violations of the assumption of compound symmetry that accompany this experimental design (21). A value of P < 0.05 was considered statistically significant for all tests.

RESULTS

Cardiovascular effects of ANG II. The effects of infused angiotensin II on MAP during both levels of dietary sodium are shown in Fig. 2. During the control period, the average MAP for APx rats was 95 ± 1 mmHg and 98 ± 2 mmHg for sham rats. There was no significant difference observed between the two groups during this time. By day 6 of ANG II infusion during normal dietary sodium intake, APx MAP had increased to 139 ± 4 mmHg while sham MAP had increased to 126 ± 3 mmHg. This difference was found to be significant and continued throughout the period of normal dietary salt intake and into day 1 of high salt, at which point MAP for APx and sham groups reached 157 ± 7 mmHg and 143 ± 5 mmHg, respectively. From day 2 through day 8 of high salt, no significant difference was observed in MAP between the two groups. On day 9 of high salt, APx MAP levels once again were observed to be significantly higher. MAP in APx rats was also observed to be significantly higher on days 2 and 3 of recovery after termination of ANG II infusion, because sham rats returned more quickly to baseline. Both groups of rats showed signifi-
significantly increased MAP from day 2 of ANG II infusion through day 1 of recovery when compared with their respective control levels. This significance continued through day 4 of the recovery period for APx rats.

The effects of ANG II on HR are also shown in Fig. 2. Although HR tended to be lower in APx rats throughout the protocol and ANOVA revealed a significant effect of lesion and of diet/ANG II treatment, there was no interaction between these factors. Therefore, no specific differences in HR between groups are reported.

Food intake and water and sodium balance responses. Food intake data are shown in Fig. 3. Although food intake tended to decrease slightly in both groups over the control period, it was not different between groups (APx, 24 ± 1 g/24 h; sham, 26 ± 2 g/24 h) during this time and had no significant impact on sodium balance (see Fig. 5). Upon beginning ANG II treatment, APx rats tended to eat less for the remainder of the protocol, but this difference was never statistically significant.

Water balance data are shown in Fig. 4. During the control period, water intake and urine output averages for APx rats were 30 ± 3 and 22 ± 3 ml/24 h, respectively. Water intake and urine output averages for sham rats during the same period were 23 ± 3 and 15 ± 3 ml/24 h, respectively. Although there was some instability in water intake in both groups during the control period, there was no significant difference observed between groups on any day during this time, and water balance was not affected in either group. Similarly, even though APx rats tended to have lower (though not significant) water intake and urine output than sham rats when placed on the high-salt diet, there was no significant difference observed in water balance between the two groups over the protocol. Water intake in APx rats was significantly increased from baseline on days 3 and 4 of high salt, and urine output was significantly increased on days 2–4 and 7 of the same period. The water
intake and urine output of sham rats increased to significantly higher levels on all days of elevated sodium.

Sodium balance levels are shown in Fig. 5. During the control period, sodium intake and excretion averages for APx rats were $3 \pm 0.1$ and $2 \pm 0.2$ meq/24 h, respectively. Levels for sham rats were $3 \pm 0.1$ and $2 \pm 0.2$ meq/24 h, respectively, over the same period. Sodium intake was significantly lower in APx rats compared with sham rats on days 1, 2, 6, and 9 of high salt and days 2, 4, and 5 of recovery. Sodium excretion was significantly lower in APx rats compared with sham rats every day but day 7 of high salt. Consequently, no differences were observed in sodium balance between groups throughout the protocol. Both sodium intake and excretion were significantly elevated from baseline in APx and sham rats throughout the period of high salt.

**DISCUSSION**

The current study aimed to further explain the contribution of the AP in the neural control of cardiovascular function in ANG II-induced hypertension and its relationship to salt sensitivity by chronically infusing AP-lesioned and sham rats with ANG II during periods of normal and elevated dietary salt. HR was generally lower in APx rats than in sham rats throughout the protocol, a phenomenon consistent with previous APx studies (6, 12, 34), although statistical significance was not reported. With regard to MAP, though we predicted that ANG II-induced hypertension requires an intact AP to achieve maximal pressor levels, the present study does not support this hypothesis. In fact, rats that received chronic infusion of ANG II showed an exaggerated increase in BP when lacking the AP compared with those with an intact AP, a trend that was observed on all but the first day of the experimental protocol. Our data showed a significant increase in MAP in APx rats when compared with sham rats over the last 5 days of normal and 1 out of the last 2 days of elevated dietary sodium during ANG II infusion. Specifically, APx MAP increased from 95 mmHg during the control period to maximums of 151 mmHg during normal salt and 162 mmHg during high salt, whereas sham MAP increased from 98 mmHg during the control period to maximums of 135 and 152 mmHg during normal and high salt, respectively. Clearly, lesion of the AP did not prevent the chronic hypertensive effects of ANG II in this model.
dose of ANG II, however, it was observed that MAP in APx rats increased to significantly higher levels than MAP in sham rats only after 5 days of ANG II infusion. It could thus be hypothesized that the role of the AP may be of negligible influence in the neurogenic control of MAP over the initial few days of this model of ANG II-induced hypertension. This trend is consistent with previous observations from our lab that showed a significant difference in the MAP response of APx rats compared with sham rats to the AT1 receptor blocker losartan only after 7 days of chronic infusion (32). Similarly, despite conflicting results, Fink et al. demonstrated a difference between MAP of APx and sham rats only after 4 days of ANG II infusion (19). Further investigation of this timeline remains to be done.

This temporal trend has also been observed in studies of the subfornical organ (SFO), another CVO that plays a major role in the central control of cardiovascular function (5, 29). Using the same model of hypertension as the present study, we recently reported that SFO lesion attenuates the development of ANG II-induced hypertension (23). In that study, as in the current AP study, MAP differences between SFO-lesioned rats and sham rats did not achieve significance until day 5 of ANG II infusion. This is of relevance in that our lab has hypothesized that the SFO and the AP may have overlapping capabilities, implying that, on removal of one of these brain structures, the other, in time, can compensate for the removed area. It also is possible that these two structures work in opposition to each other during certain physiological conditions. Indeed, the results observed here imply that the AP may be exerting a negative control or inhibitory mechanism to buffer or counteract the pressor response mediated through the SFO (23) during ANG II infusion. This idea is supported by the observation that the AP contains two separate populations of neurons that behave in contrast to one another, one serving to increase BP and one decreasing BP when activated (16). It is possible that these distinct populations of neurons within the AP may respond uniquely to different levels of sodium and/or ANG II. For instance, the neuronal population in the AP that serves to decrease BP may be activated by an increase in ANG II levels while the subpopulation that normally drives an increase in BP could be inhibited. Complete lesion of the AP would, therefore, remove brain areas that normally may buffer ANG II-induced hypertension. This possibility remains to be examined.

As in all lesion studies, it must be considered that, upon AP ablation, pathways involving other cardiovascular control centers are severed as well. Although little damage to areas around the AP was noted in the current study, it is possible that connections between brain sites involved in cardiovascular regulation merely passing through the AP were destroyed. Additionally, it has been shown that the AP receives input from the SFO (20, 33) and other major autonomic control centers (17, 24, 36), so projections directly synapsing at the AP would be unable to induce downstream effects as well. This type of disconnection could partially explain the different results observed by Hendel and Collister (23) when investigating the role of the SFO during the same model of ANG II hypertension. If, indeed, projections from the SFO relay signals to the AP that act to support BP, their purpose may be to suppress hypotensive effects originating at the AP. Ablation of the SFO would allow these hypotensive signals to be fully expressed. Similarly, as in the present study, removal of the AP would sever pathways originating at the SFO and ultimately communicating downstream with other influential areas such as the paraventricular nucleus (37). Unchecked by communication with the AP, these pathways would be allowed to exert their full pressor effects in response to elevated ANG II.

The second phase of the current study investigated the role of the AP in the hypertensive effects of ANG II during elevated dietary salt. During the 10 days of high dietary sodium and ANG II infusion, MAP of APx rats was observed to be significantly higher than sham MAP only on days 1 and 9.
reaching a maximal level of 162 vs. 152 mmHg for sham rats. Interestingly, the difference observed on day 9 appears to be more attributable to a slight decrease in MAP in sham rats over the last few days of ANG II than to a substantial increase in APx MAP over that period. Overall, in fact, it appears that BP and HR in lesioned animals were not markedly influenced by increased sodium levels during elevated ANG II, because sham rats experienced a modest rise in MAP during the period of high salt that was absent in APx rats. Taking into consideration that it has previously been demonstrated that lesioning the AP has no effect on MAP during elevated sodium intake (8), it is questionable whether changes in dietary salt have any effects directly at the AP. However, even though these data do not appear to support the idea of a relationship among salt, the AP, and MAP, the possibility must also be considered that, by the time the high-salt diet was begun, MAP in APx rats had already reached near-maximal levels achievable with this model of hypertension.

Recently, Brooks et al. (3) hypothesized that even a slight increase in osmolality from high dietary salt induces excitation of the sympathetic nervous system via central osmoreceptor activation, ultimately increasing BP, and that ANG II may be acting on sodium-sensing neurons at these osmoreceptor sites to amplify the sympathoexcitatory effects of small increases in osmolality. Although sodium-sensing neurons have been identified in the AP (1), it has been proposed that input from visceral osmoreceptor sites to the AP may be more influential than osmoreceptors intrinsic to the AP (20). Rather, the organum vasculosum of the lamina terminalis (OVLT), another CVO of the third ventricle, and the SFO are thought to be the primary central sites for osmoreception (2, 3). The actions of ANG II at osmoreceptor sites in these two CVO as proposed by Brooks et al. (3) could partially explain the rise in BP that was observed in sham rats but appeared to be attenuated in APx rats during high salt. That is, the effects of ANG II and increased sodium acting at the OVLT and SFO could be overriding the antihypertensive influence of the AP. Further investigation of this mechanism remains to be done.

In agreement with previous data (8), no difference in food intake was observed between APx and sham rats by completion of the 3 wk of postoperative recovery, although body weights were slightly lower in APx versus sham rats at this time (308 ± 11 and 373 ± 20 g, respectively). We do not believe that the difference in body weight between groups is contributing to the present results, because our lab has not observed any variations in responses within groups, despite a naturally wide range of body weights, in other experiments employing this model of hypertension. With specific regard to food intake, it must be considered that the decreased food intake consistently observed in rats during the 2- to 3-wk period after AP lesion could influence other factors contributing to cardiovascular regulation, such as baseline plasma renin activity. However, we have previously shown that sham rats with food intake restricted to approximately that of postoperative APx rats experienced a decrease in BP to the same level as sham rats allowed to feed ad libitum when treated with chronic losartan (6) and that plasma renin activity in APx and both food-restricted and non-food-restricted shams were similar after postoperative recovery periods (7).

With regard to sodium and water balance, there was no difference between groups throughout the protocol, although differences in sodium intake and excretion were detected between groups during the period of high salt. While others have reported changes in osmoregulation (11) and sodium appetite (9, 15) after lesion of the AP, neither differences in sodium balance nor increases in ad libitum sodium intake were observed in the present study in APx rats. Rather, sodium intake and excretion differences are most easily explained by the observation that APx rats tended to eat less upon introduction of ANG II. This translated to a decreased sodium intake and excretion in APx rats relative to shams that became exaggerated and significant during the period of high salt.

The results of this experiment are not consistent with those previously observed by Fink et al. (19). One possibility for this discrepancy is that MAP and HR data were collected in the present study using telemetry, allowing continuous 24-h sampling of all subjects. This technique is more accurate and representative of true chronic effects and buffers any extraneous and transient factors that could influence MAP and HR using intermittent data-collecting techniques. Another possibility is that the earlier study was conducted using an ANG II infusion rate of 10 ng/min, whereas the present study employed a rate of 10 ng·kg⁻¹·min⁻¹. With rats weighing ~350 g at the start of the current experiment, this factor resulted in an approximate threefold decrease in administered ANG II compared with the previous study. This is important because higher doses of ANG II are capable of producing acute elevations in BP. It is quite possible that areas in the brain acting to regulate BP in response to ANG II respond differently, and perhaps at different time points of the developing hypertension, to varying levels of the hormone. Also, Fink et al. (19) supplied their rats with a low-sodium diet (0.002 meq) while infusing 0.9% NaCl at a relatively high rate of 40 ml/day to fix sodium intake, whereas, in the present study, rats were infused with a total volume of 7 ml/day isotonic saline throughout the protocol. Clearly, in this high volume-loaded model, the AP could be playing a different role in modulating the chronic effects of ANG II. Finally, APx lesions in the present study were performed using suction rather than the previously reported method of electrolytic lesioning.

In summary, our results suggest that the AP is not necessary for the full development of ANG II-induced hypertension in this model. In fact, ablation of the AP resulted in a pronounced hypertension in rats chronically infused with ANG II during levels of normal as well as elevated dietary salt. Further studies are necessary to determine the exact role of the AP in this effect, how its contribution may be influenced by salt and differing levels of ANG II, and whether and how the neural components and pathways that oppositely affect chronic BP regulation are playing a part. Is it also possible that some or all of the CVO have redundant, compensatory, or opposing roles in response to different physiological conditions? These interrelationships and the possible multiple mechanisms by which the AP acts to influence BP remain to be fully understood.

(18, 30)

GRANTS

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