Area postrema lesion attenuates the long-term hypotensive effects of losartan in salt-replete rats

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Collister, John P., and John W. Osborn. Area postrema lesion attenuates the long-term hypotensive effects of losartan in salt-replete rats. Am. J. Physiol. 274 (Regulatory Integrative Comp. Physiol. 43): R357–R366, 1998.—We reported that the AT1 receptor antagonist losartan decreases arterial pressure in sodium-replete rats and that this response is attenuated in area postrema-lesioned (APx) rats (J. P. Collister, B. J. Hornfeldt, and J. W. Osborn. Hypertension 27: 598–606, 1996). In that study, food intake for the 3-wk period after sham lesion was restricted to that observed in APx rats. Food-restricted sham rats had lower arterial pressures and attenuated responses to losartan compared with control rats fed ad libitum. The present study examined whether these differences persisted months, rather than weeks after APx or sham lesions. Losartan was administered for 10 days to APx and two groups of sham rats 3 mo after APx or sham surgery. The first sham group was food restricted (SFR) for 3 wk after surgery, whereas the second sham group was allowed ad libitum (SAL) access to food. By day 8 of losartan administration, both sham groups demonstrated a marked hypotension (SFR: −38 ± 4; SAL: −33 ± 4 mmHg). This response was attenuated (P < 0.05) on the same day in APx rats (−17 ± 3 mmHg). This trend continued throughout days 9 and 10. Because both sham groups responded similarly to losartan (yet significantly different from APx rats), these results demonstrate that transient decreases in food intake do not affect the response to losartan if rats are allowed an adequate recovery period. We conclude that the area postrema mediates part of the long-term hypotensive effects of AT1 receptor blockade in the conscious rat.

circumventricular organ; angiotensin II; food restriction; chronic arterial pressure regulation

THE AREA POSTREMA (AP) is a circumventricular organ located in the dorsomedial medulla at the caudal end of the 4th ventricle. As is characteristic of circumventricular organs, the AP has a rich vascular supply that lacks the normal blood-brain barrier (38). This anatomy allows for circulating substances, such as peptides, to gain access to the central nervous system and be sensed by neural components of the AP. As such, the AP has been the topic of study in many areas of integrative physiology. It has been implicated as an important regulatory center for such functions including satiety (17, 63), vomiting (5), body fluid homeostasis (1, 36), respiration (45), and cardiovascular control (2, 4, 18, 19, 21, 65, 67).

In terms of cardiovascular regulation, many studies have addressed the role of the AP in blood pressure regulation and hypertension. In particular, there is much evidence to support a role for the AP in the actions of angiotensin II (ANG II). For example, infusions of ANG II into the vertebral artery in the dog cause a greater increase in arterial pressure compared with systemically infused intravenous ANG II (22, 57), indicating the hindbrain as a potential site of action of this hormone. Furthermore, microinjection of ANG II into the AP in rats causes an elevation in arterial pressure, a response that is blocked with prior intravenous treatment with the AT1 receptor antagonist losartan (9, 44).

Many researchers have used lesioning of the AP (APx) to gain insight into the function of this specialized structure. This approach has been used to show that hormonal resetting of the arterial baroreflex by the peptide hormones ANG II and vasopressin is mediated through actions at the AP (4, 32, 46). Furthermore, AP ablations eliminate exaggerated pressor responses to intravertebral arterial ANG II injection in the dog (39) and attenuate chronic ANG II hypertension in both rabbits (15) and rats (24). APx has also been shown to prevent chronic hypertension in the two-kidney, oneclip (25) and deoxycorticosterone acetate (DOCA)-salt (26) rat models of hypertension.

Clearly, APx studies have been helpful in gaining insight into the role of this structure in long-term cardiovascular regulation. Because the AP is an integrated control structure for many systems, the effect of APx on numerous other physiological entities must not be overlooked when observing for a narrow result within the confines of a single physiological variable, such as arterial pressure. Such results could be mistaken as primary, without the understanding that the observations could be the result of an indirect disruption of a seemingly less-related system. For example, the AP is a primary regulator of autonomic function, as well as sodium, water, and overall food intake. Therefore, it is difficult to assess AP control over blood pressure after lesion of this structure, because disruption of these other variables may indirectly affect blood pressure as well. Only after addressing such interrelated complications of AP lesioning can experimental conclusions be made with any certainty.

In addition, another confounding factor in the literature concerning APx is the length of recovery after surgery before the experiments were conducted. Experiments carried out days, weeks, or months after lesion have been reported. This discrepancy could contribute to some of the contrasting results in the literature. Initial reports stated that APx had no effect on basal arterial pressure in the rat (69). Subsequently, Skoog and Mangiapane (59) reported both lower arterial pressures and heart rates in APx rats up to 1 wk after APx (59). APx rats have also been reported to have other postsurgical transient changes in water, sodium, and food intake. An increased water intake immediately after APx has been substantiated (35, 38). Like-
wise, APx rats appear to have an increased sodium appetite (12, 19, 31, 54), which has been described as “need free” (16). Furthermore, it is well established that APx rats have a transient period of decreased food intake after lesion (4, 18, 34, 37), which returns to normal by 60 days postsurgery (34). Even after food intake returned to normal, APX animals do not have parallel body weight gains compared with sham-operated rats (34). Again, these metabolic and fluid homeostatic alterations in APX rats need to be considered, as they most likely have an indirect, rather than primary, effect on autonomic cardiovascular function.

We have recently reported that the hypotensive effects of the AT₁ receptor antagonist losartan in normotensive, salt-replete rats is attenuated during the first 4 days of administration, but not the steady state in rats with lesions of the AP (11). In that study, we determined that APx rats regained near normal food intakes 3 wk after surgery. To control for this anorexia, sham-operated rats were food restricted (SFR) to the level consumed by APx rats for 3 wk after surgery. Although this approach has been previously used (34), it has since been shown that food restriction alone decreases arterial pressure in both the aortic coarctation and spontaneously hypertensive rat models of hypertension (53, 64). Indeed, in our study, both food-restricted and APX animals maintained significantly lower basal arterial pressures than non-food-restricted control rats. We suspect subtle differences in the magnitude of the hypotensive response to losartan between APX and food-restricted sham groups were masked due to the overall lower starting level of arterial pressure caused by reduced food intake.

Therefore, the aim of the present study was to determine the hypotensive response to losartan in APX and sham-operated rats after a prolonged period of recovery. We reasoned that after a 3-mo recovery period, the changes in food and water intake, as well as compensatory autonomic changes, would be eliminated or at least maintained at a chronic, steady-state level. Indeed, contrary to our earlier report in which APX attenuated the initial, but not the steady-state, effects of losartan, we found that after prolonged recovery APX rats had normal arterial pressures and an attenuated chronic hypotensive response to losartan.

## METHODS

**Adult male Sprague-Dawley rats (325–375 g, Harlan Sprague Dawley, Indianapolis, IN) were used in all experiments. All procedures were conducted in accordance with institutional and National Institutes of Health guidelines.**

**Surgical Procedures**

**APX.** Rats were randomly selected for APX or one of two sham-operated groups 12–13 wk before catheter implantation. Rats were preanesthetized with pentobarbital sodium (32.5 mgkg ip). Surgical anesthesia was achieved with a second intramuscular injection containing a combination of anesthetic agents (acepromazine 0.2 mg/kg, butorphanol tartrate 0.2 mg/kg, ketamine 25 mg/kg). Rats were placed in a stereotaxic apparatus with the neck flexed. A dorsal midline incision was made through the skin and epaxial musculature. With the aid of a dissecting microscope, the atlantooccipital membrane was visualized and punctured, and a portion of it was removed. To better visualize the brain stem, a small portion of the base of the skull was removed with rongeurs. The AP was visualized on the dorsal surface of the medulla at the caudal extent of the fourth ventricle and removed by suction with a blunt 26-gauge needle attached to a vacuum line as described by Edwards et al. (16). With the exception of the attached vacuum line, sham operations were identical to those described for APX rats. The muscular layer was closed with 3–0 chronic catgut suture. Silk sutures (3–0) were placed in the skin for closure. All rats were given a subcutaneous injection of 0.075 mg butorphanol tartrate for analgesic purposes and allowed 12–13 wk for postoperative recovery. Specifically, the food intake of one group of sham-operated rats (SFR) was restricted to a level similar to that of APX rats during the first 3 wk of recovery. Food intake in this group was restricted to −50, 60, and 80% of normal first, second, and third week after sham surgery, respectively. After this initial period of food restriction, SFR rats were allowed ad libitum access to food for the remainder of the recovery period. We have previously reported that APX rats regain a near normal food intake and growth rate 3 wk after the lesion (11). The APX rats and the other sham-operated group of rats (sham, ad libitum; SAL) were allowed ad libitum access to food for the entire 12–13 wk of recovery.

**Catheter Implantation.** After a period of 12–13 wk of recovery from APX or sham operation, rats were instrumented with arterial and venous catheters. Surgical anesthesia was achieved as described above. Rats were then instrumented with arterial and venous catheters via the femoral vessels. The catheters were exited through the skin on the dorsal surface of the skull and were passed through a flexible spring connected to a single-channel hydraulic swivel to which the venous catheter was attached. At the end of surgery, each rat received a subcutaneous injection of 0.075 mg butorphanol tartrate for analgesic purposes. After recovery from anesthesia, rats were housed individually in metabolic cages with the swivels mounted above. Rats were allowed 3 days to recover from surgery before the experimental protocol began. During this time, each rat received daily prophylactic intravenous antibiotics consisting of 15 mg ampicillin and 1 mg tobramycin. Each rat was also started on a continuous intravenous infusion of sterile 0.9% saline (7 ml/24 h). A 0.4% NaCl diet (Research Diets) and distilled water were provided ad libitum throughout this recovery period.

**Experimental Protocol**

The experimental protocol was begun 3 days after catheter implantation. The first 3 days of the protocol served as a control period, during which a continuous intravenous infusion of 0.9% sterile saline (7 ml/24 h) was maintained. This was followed by a 10-day infusion period of the AT₁ receptor antagonist losartan (10 mg·kg⁻¹·24 h⁻¹). Losartan was dissolved in 0.9% sterile saline and infused at a rate of 7 ml/24 h iv. Finally, a 3- to 4-day recovery period identical to the control period completed the protocol. All infusions were given through a 0.2-μm syringe filter. Throughout the protocol, mean arterial pressure (MAP), heart rate, food intake, water intake, and urine output were measured daily in conscious, unrestrained rats in their home cages. MAP was measured directly by connecting the arterial catheter to a pressure transducer coupled to a polygraph (Grass Instrument). MAP was monitored daily for 15 min by computer at a sampling rate of 1 Hz, as previously described (51). The resulting 900 data points were used to calculate the
average MAP as well as the standard deviation of MAP (SD-MAP) during the recording period. SD-MAP was used as a quantitative index of baroreceptor reflex function, as previously described (51). Heart rate was measured by increasing the chart speed and counting peaks on the pulsatile pressure tracing. Twenty-four-hour food and water intake as well as urinary output were measured gravimetrically. Sodium intake was calculated as the sum of sodium received in the daily infusion (1 mmol/day iv) plus the product of food intake and the sodium content of the food, which had previously been determined (0.4% NaCl, 0.07 mmol/g). Urinary sodium content was measured with an ion-specific electrode (Nova Biomedical). Urinary sodium excretion was calculated as the product of urine flow rate and urinary sodium concentration.

The protocol was carried out in three experimental groups: 1) APx (n = 9), 2) SFR (n = 8), and 3) SAL rats (n = 7).

Measurement of Baseline Plasma Renin Activity and Tests of AT1 Receptor Blockade

Plasma renin activity (PRA) was measured in all rats on the second control day. Blood (500 μl) was obtained via the arterial catheter and placed into a chilled 1-ml syringe containing 1 mg EDTA in 20 μl. Whole blood was centrifuged, and plasma was collected and stored at −70°C for later radioimmunoassay, as previously described (52).

To test the efficacy of AT1 receptor blockade, acute pressor responses to bolus injections of ANG II (30 ng iv) were measured on day 3 of the control period and day 7 of losartan infusion. Responses were measured as the peak increase of arterial pressure.

Histological Verification of APx

On completion of the protocol, all rats were anesthetized as described above and perfused intracardially with 4% paraformaldehyde. Whole brains were dissected and soaked in 8% paraformaldehyde for 2 days. The brains were then transferred to a 30% sucrose solution and allowed to soak for a minimum of 2 days. Frozen serial coronal sections (40 μm) were made at the level of the obex and mounted on slides. The slides were then stained for Nissl substance (cresyl violet stain). Confirmation of complete AP lesioning or intact AP (sham-operated rats) was made under light microscopy. All APx rats included in the final analysis of the data were confirmed to have complete lesions of the AP.

Statistical Analysis

Statistical comparison within and between experimental groups was performed by a two-way analysis of variance (ANOVA) with a commercially available statistical package (Abacus Concepts). Comparisons of specific experimental days (within and between groups) were performed by linear contrast analysis (50). For clarity in data presentation, only between-group differences are shown in Figs. 1–6. One-way ANOVA was used for between-group comparison of baseline control values. In addition, one-way ANOVA was used for between-group comparisons of body weight, PRA, and pressor responses to ANG II. A value of P < 0.05 was considered statistically significant for all tests. All values are reported as means ± SE.

RESULTS

Body Weight and Food Intake

On the day of APx or sham operation, the body weights of all three groups were not significantly different (APx: 359 ± 8; SFR: 351 ± 8; SAL: 344 ± 4 g). As reported previously (11), there were no differences in body weights between APx and SFR rats after the initial 3-wk period of food restriction in SFR (data not shown). Despite having food intakes similar to sham rats 3 wk postsurgery, body weights of APx rats did not increase to the same level of SFR rats after this prolonged recovery. After 3 mo of recovery, the body weights of the three groups were as follows: APx, 407 ± 12; SFR, 495 ± 11; SAL, 492 ± 11 g. Despite the initial period of food restriction, body weights of SFR rats recovered to the same level of SAL rats after 3 mo of recovery. During the 3-day control period of the experimental protocol, ad libitum food intake was not significantly different between the three groups (APx, 18 ± 2; SFR, 15 ± 3; SAL, 15 ± 3 g/day).

Histological Verification of APx

Histological verification of APx was confirmed in all APx rats. A typical example is shown in Fig. 1. In all rats, there was minimal destruction of the adjacent nucleus of the solitary tract (NTS) at the light microscopic level. Further evidence that lesions of the AP did not impair NTS sites involved in the baroreceptor reflex was that the lability of MAP (SD-MAP), a quantitative index of baroreceptor reflex sensitivity (51), was not significantly different between APx (4 ± 1 mmHg), SFR (4 ± 1 mmHg), and SAL (4 ± 1 mmHg) groups of rats.

Baseline Data

Basal data are shown in Fig. 2 as the average of the 3-day control period. Control MAP was not different between the groups (APx, 105 ± 3; SFR, 106 ± 3; SAL, 108 ± 3 mmHg). In contrast, despite the prolonged recovery period, APx rats maintained a significantly lower heart rate (340 ± 6 beats/min) compared with either sham group (SFR, 362 ± 10; SAL, 362 ± 7 beats/min). Basal PRA was not different between the three groups (APx, 4.4 ± 1.0; SFR, 4.7 ± 0.6; SAL, 4.6 ± 0.9 ngANG I·ml⁻¹·h⁻¹).

Pressor responses to 30 ng ANG II were measured as described above during the control period and on day 7 of losartan treatment. Control responses were not significantly different between the three groups (APx, 46 ± 2; SFR, 42 ± 2; SAL, 48 ± 3 mmHg).

Cardiovascular Responses to Losartan Infusion

The hypotensive responses to losartan are shown in Fig. 3 as the change in MAP from the average 3-day control pressure. Figure 3A shows the responses in the APx rats and both sham groups. Because no significant differences were observed between both sham groups (SFR and SAL), they have been plotted as one control group in Fig. 3B. By day 1 of losartan treatment, both APx and sham groups of rats demonstrated significant decreases in MAP compared with baseline (APx, −10 ± 1; sham rats, −12 ± 2 mmHg; statistics not shown). Both groups of rats continued to show increased hypotensive responses to losartan until days 8–10, when both groups achieved a steady-state level of MAP. At
this point, statistically significant differences were observed between APx and sham rats. By day 10 of losartan, MAP had dropped $32 \pm 2$ mmHg from control in the sham group. More importantly, the hypotensive response to losartan was attenuated by almost 50% in APx rats ($21765$ mmHg). Throughout the recovery period, both groups demonstrated increasing levels of MAP. By day 4 of recovery, MAP of both APx and sham rats had achieved a level that was not significantly different from control (statistics not shown). No pressor responses were seen in any of the three groups on day 7 of losartan after bolus infusion of 30 ng ANG II.

The heart rate responses to losartan are shown in Fig. 4. Again, because no differences were seen between both sham groups (SFR and SAL) throughout the entire protocol, they are plotted as one group in Fig. 4B. Both APx and sham rats showed significant increases from baseline in heart rate by day 1 of losartan treatment (statistics not shown), which were maintained throughout the entire losartan treatment. Significant differences between APx and sham rats were seen on days 2, 8, and 9 of losartan treatment. In addition, APx rats tended to demonstrate a greater tachycardic response compared with sham groups. This difference was most apparent on day 9 of losartan treatment, on which sham rats showed a heart rate increase of $15 \pm 9$ beats/min compared with an increase of $50 \pm 14$ beats/min in APx rats. It should be noted, however, there were no differences in the absolute levels of heart rate between all three groups throughout the entire losartan treatment. This can be explained by the fact that (as stated above) APx rats began with an overall lower basal heart rate.

Sodium and Water Balance Responses to Losartan Infusion

Three-day average control water intakes in APx, SFR, and SAL rats were $19 \pm 3$, $13 \pm 2$, and $22 \pm 3$ ml/day, respectively. The control water intake for SFR rats was determined to be statistically different from

![Fig. 1. Photomicrographs of 40-µm sections of a typical sham operation (A) and area postrema (AP) lesion (APx; B). NTS, nucleus of the solitary tract; X, dorsal motor nucleus of vagus nerve; XII, hypoglossal nucleus.](image1)

![Fig. 2. Bar graphs show basal mean arterial pressure (MAP; A), heart rate (HR; B), and plasma renin activity (PRA; C) observed throughout the control (3 days saline) period in APx, sham-lesioned food-restricted (SFR), and sham-lesioned ad libitum-fed (SAL) rats. AI, angiotensin I. *Statistical significance between groups (P < 0.05).](image2)
either APx or SAL rats. Baseline sodium intake was not different between the three groups (APx, 2.2 ± 0.0; SFR, 2.1 ± 0.2; SAL, 2.0 ± 0.1 mmol/day). Cumulative water balance data is shown in Fig. 5A. No differences in water balance were seen APx, SFR, and SAL rats during the 3-day control period (Fig. 5A). Furthermore, there was no difference between SFR and SAL rats throughout losartan treatment and recovery. Beginning on day 1 of losartan, APx rats tended to have less cumulative water balance compared with either sham group. This trend continued and became more apparent throughout losartan treatment and recovery. In terms of cumulative sodium balance (Fig. 5B), no differences were seen between the three groups during the 3-day control period. As with the water balance, APx rats again tended to maintain a lower cumulative sodium balance throughout the entire protocol, compared with either sham group.

**DISCUSSION**

There were three major findings in the present study. First, with the assumption of a high selectivity of losartan for the AT1 receptor, the marked hypotensive response to losartan in the normotensive, salt-replete rat suggests a major role of the renin-angiotensin system (RAS) in the regulation of MAP. Second, the long-term steady-state depressor response to losartan was attenuated in APx rats by ~40%, suggesting this brain stem region as an important target site for this
AT1 receptor antagonist. Third, after prolonged recovery from lesion surgery, sham and APx rats had normal basal arterial pressures compared with rats recovered for a lesser period of time (11). This observation taken with our recent report (11) emphasizes how length of recovery from APx can influence both experimental results and their subsequent interpretation. The importance of these findings is discussed in detail below.

Hypotensive Responses to Losartan in Normal Rats

Not surprisingly, both angiotensin-converting enzyme inhibitors and AT1 receptor antagonists have been reported to be effective antihypertensive agents in the treatment of “renin-dependent” forms of hypertension (61). Likewise, AT1 receptor blockade has been shown to decrease arterial pressure acutely during physiological conditions in which the level of activity of the RAS is increased, such as hypovolemia and chronic salt depletion (62).

Blockade of the RAS could result in a reduction in sympathetic nerve outflow (3, 10). In addition, vascular RAS blockade could result in an overall decreased vascular resistance (28, 41). In these examples, tissue RAS blockade would be an effective antihypertensive approach despite normal circulating renin levels. Alternatively, others hypothesize that in some low-renin models of hypertension, sensitivity to circulating ANG II increases in target tissues (48), possibly due to upregulation of AT1 receptors (49). Again, with this explanation, PRA would not adequately reflect antihypertensive effectiveness of RAS blockade.

The importance of the present study, which confirms our earlier findings (11), is that long-term administration of the AT1 receptor antagonist losartan markedly lowers arterial pressure in normotensive, salt-replete rats. The fact that this response occurred despite the presence of other arterial pressure control systems implies a paramount role of the RAS in the long-term maintenance of arterial pressure under normal physiological conditions. The fact that we have shown here such a profound hypotensive response to losartan in the normotensive rat indeed offers further insight into this role for the RAS in control of arterial pressure. We demonstrated a decrease in pressure of nearly 35 mmHg throughout the course of losartan treatment, which resulted in steady-state levels of arterial pressure of ~75 mmHg. We believe this response to be truly mediated through blockade of the RAS, as we have...
previously shown this chronic effect to be absent in rats consuming a high (8.0%)-NaCl diet in which the endogenous RAS was suppressed (11). It should be emphasized that this occurred without any background of hypertension or preactivation of the RAS.

Role of the AP in Mediating the Hypotensive Actions of Losartan

The mechanism by which blockade of the endogenous RAS lowers arterial pressure over long periods of time is difficult to dissect due to the fact that ANG II acts in many different tissues over a wide time scale. Figure 6 illustrates these well-described actions of ANG II, along with a time scale relating the relative onset of actions of these effects. These include direct peripheral vasoconstriction (7), renal retention of sodium and water (14, 30, 40), sympathoexcitation (56), and vascular hypertrophy (28). Each of these effects is thought to be mediated via AT1 receptors. The obvious question is as follows: which of these inhibited actions of ANG II are responsible for the hypotensive effect of AT1 receptor blockade? As there are a myriad of different time-dependent effects of ANG II, it is quite probable that the observed results of AT1 receptor blockade are likewise the sequelae and/or summation of numerous blocking actions.

We have previously shown that a lower dose (1 mg·kg−1·day−1) of losartan that blocks the acute vasoconstrictor actions of ANG II has no effect on arterial pressure chronically (33). This is in agreement with a previous study suggesting that chronic low-dose ANG II-induced hypertension does not involve ANG II vasoconstrictor activity (27), but rather a large neural component (42). Furthermore, the hypotensive responses to losartan were progressive and did not reach a maximal steady-state level until day 7 or 8. These data suggest that the response was not solely due to blockade of the peripheral vasoconstrictor actions of ANG II. On the other hand, losartan could be acting to block vascular AT1 receptors and a basal ANG II-bound effect to maintain structural changes in resistant vessels. This is thought to be a more long-term (wk) trophic process of ANG II (28) and is probably not an important factor in the subacute to chronic (days) effects presented here. Another possibility is that a portion of the observed hypotensive response to losartan was due to blockade of renal AT1 receptors. Indeed, it has been shown that intrarenal infusion of the AT1 receptor antagonist valsartan lowered arterial pressure in spontaneously hypertensive rats at a dose that did not alter blood pressure when administered intravenously (66). Although we did not observe any profound natriuresis or diuresis during the marked depressor response to losartan, the fact that rats were in a steady-state sodium balance despite arterial pressures of nearly 75 mmHg means that the renal function curve was shifted to a lower pressure level (13, 29). However, we cannot determine the extent to which this was a primary or secondary resetting of the renal function curve.

This study was conducted to test the hypothesis that the hypotensive responses to losartan are mediated through blockade of AT1 receptors at the AP. Indeed, APx rats demonstrated a chronic depressor response to losartan, which was attenuated by ~15 mmHg compared with sham rats (APx: −20 mmHg; sham: −35 mmHg). On the basis of this observation, we conclude that a large portion (~40%) of the observed hypotensive response to losartan is mediated through the AP. This is proposed to be due to blockade of the sympathoexcitatory effects of endogenous ANG II at the AP. The AP has previously been suggested as a primary site of the sympathoexcitatory actions of ANG II (4, 6, 20, 23, 39, 55, 58, 68). Specifically, the hypotension induced by chronic ANG II infusion has been shown to be attenuated in APx rats (24).

The mechanism(s) of the hypotensive effects of losartan that remain in APx rats are unclear. As explained above, it is quite possible that the remaining effect is due to blockade of renal effects of ANG II. Because both groups of rats (APx and sham) responded similarly during the first few days of losartan, this initial phase of hypotension could be explained via blockade of renal reabsorption of sodium and water. It is possible that only after 5 or 6 days of losartan treatment is the blockade of sympathoexcitatory effects manifested, as seen then by the divergence in the pressure responses of the two groups. Interestingly, the attenuated hypertensive response to chronic ANG II infusion in APx rats was similarly not seen until approximately day 5 of ANG II treatment (24). It is also possible, as discussed previously, that a portion of the observed effects is due to blockade of tissue RAS. This could especially be true in the brain, where losartan has been shown to be able to cross the blood-brain barrier (43) and thus able to gain access to other control structures containing AT1 receptors, such as the rostral ventrolateral medulla (60). In addition, other AT1 receptor-bearing circumventricular organs, such as the subfornical organ, may play a role in the actions mediated by ANG II and as such the effects of losartan (37, 38).

Critical Issues Concerning APx Studies

In the present experiment, we examined the effects of losartan on APx and two sham control groups 12–13 wk after surgery. In our previous report (11), APx rats and
ATTENUATED HYPOTENSION IN LOSARTAN-TREATED APx RATS

shams were studied only 3 wk postoperatively. In that study, sham rats were food restricted to the level of intake of APx rats for the initial 3 wk after surgery. Because we have observed that APx rats regain normal food intake ~3 wk postlesion, studies were conducted at that time. Consequentially, we observed that both APx and SFR rats had basal pressures of 95 mmHg, ~10–15 mmHg lower than normal rats measured in our laboratory. More importantly, we reported depressed responses to losartan in food-restricted sham animals of ~20 mmHg. These responses were attenuated in APx rats by 50% on days 1-4 of chronic losartan treatment, but the absolute level of arterial pressure at steady state (day 10 of losartan treatment) was not different between sham and APx rats in that study (11). The magnitude of the depressor responses seen in these APx and food-restricted rats were blunted compared with non-food-restricted control rats reported in that study receiving the same dose of losartan (11). However, it is important to note that both sham food-restricted rats and nonoperated control rats achieved the same low level of overall pressure (75 mmHg) during chronic losartan treatment, despite their basal arterial pressures. It seems plausible that these rats could not exhibit a greater fall in pressure because of other control systems present, preventing decreases <75 mmHg. We hypothesized that because APx and sham rats began at a lower level of basal arterial pressure in that study, both the magnitude and pattern of hypotensive response to losartan (in APx and food-restricted sham rats) were attenuated and altered, respectively. We felt that initial decreased food intake after surgery could not only alter basal arterial pressure but could also modify the cardiovascular responses to, or effects from, chronic losartan.

For this reason, the present study was conducted 3 mo, rather than 3 wk, after lesion or sham surgery. In this study we included SFR rats that were food restricted similarly to the previous study, as well as an ad libitum-fed sham (SAL) group to observe any differences between these two control groups after a prolonged recovery. This experimental design resulted in three important observations. First, all three groups of rats had basal arterial pressures of ~105 mmHg or normal for rats measured in our laboratory. As mentioned above, in our previous study, both APx and SFR rats had basal arterial pressures significantly lower at a level of ~95 mmHg. The fact that the pressures of APx and SFR rats were the same at 3 wk recovery and again at 3 mo recovery (although overall both groups pressures were significantly higher at 3 mo recovery), suggests that the initial depressed basal pressures are the result of the decreased food intake and not the lesion itself. Second, there was no difference in basal control values between SFR and SAL rats at 3 mo postsurgery. Furthermore, their responses to losartan were identical. These results suggest that with a prolonged recovery of several months either sham group is an adequate control for the APx rat. Finally, a clear divergence in MAP between APx and sham rats was observed. APx rats demonstrated an attenuated steady-state hypotensive response to losartan. This contrasts with our previous study (11) and clearly defines a role for the AP in the chronic effects of AT1 receptor blockade on arterial pressure regulation.

Perspectives

This report emphasizes the central nervous system actions of endogenous ANG II in the chronic regulation of arterial pressure in normotensive rats. ANG II has been implicated in the pathogenesis of numerous forms of hypertension. We have demonstrated decreases in pressure of 35 mmHg in the normal, salt-replete rat through chronic blockade of AT1 receptors. Clearly, endogenous ANG II plays an important role in supporting arterial pressure in the normal rat. Additionally, the numerous actions of ANG II and their respective target sites have been studied to gain insight into the mechanisms of this hormone’s regulatory actions and the pathophysiology of certain forms of hypertension. As such, we currently report the AP as a critical site for the long-term actions of endogenous ANG II in the normal rat.

In the present study, we used lesioning of the AP to dissect the actions of AT1 receptor blockade. With surgeries of this nature, it is seemingly easy to draw conclusions from the observed effects of a given manipulation of the lesioned animal. It is important not to overlook the multifaceted and interrelated roles of such a structure while pursuing a relatively narrow goal of a given experiment. It is still unclear as to what numerous functions are being removed with lesioning such cell bodies and their given projections within the AP. For example, with APx we observe different time-dependent changes in basal pressure and heart rate. Although we are interested specifically in the role of the AP in chronic arterial pressure control, we must not forget the numerous visceral inputs to the AP that have been removed and could also indirectly affect autonomic control of cardiovascular function. In addition, it is unknown what compensatory responses are being activated by other possible redundant systems or organs and on what time scale some of these lost functions are being replaced. Certainly, as we have seen in our laboratory and discussed here, a rat with lesion of the AP is not the same rat physiologically at 3 wk and at 3 mo postsurgery. As such, in contrast to our previous report in rats studied 3 wk post-APx, we currently report normal basal arterial pressures in APx rats with a prolonged recovery period of 3 mo, as well as an attenuated response to chronic AT1 receptor blockade.

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