Role of ET\textsubscript{A} receptors in experimental ANG II-induced hypertension in rats

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Ballew, Jennifer R., and Gregory D. Fink. Role of ET\textsubscript{A} receptors in experimental ANG II-induced hypertension in rats. Am J Physiol Regulatory Integrative Comp Physiol 281: R150–R154, 2001.—The objectives were to determine if ANG II-induced hypertension is maintained by activation of endothelin type A (ET\textsubscript{A}) receptors by endogenous ET-1 and if this effect is influenced by salt intake. Male rats were maintained on high sodium intake (HS; 6 meq/day) or on normal sodium intake (NS; 2 meq/day). Hypertension was produced by intravenous infusion of ANG II (5 ng/min) for 15 days. Five-day oral dosing with the selective ET\textsubscript{A}-receptor antagonist ABT-627 (−2 mg·kg\textsuperscript{-1}·day\textsuperscript{-1}) reduced mean arterial pressure (MAP) to baseline levels in rats on HS receiving ANG II infusion, but it did not affect MAP in normotensive HS controls. In rats on NS, ABT-627 only transiently decreased MAP in rats receiving ANG II and slightly reduced MAP in normotensive controls. ABT-627 produced mild retention of sodium and water in HS rats receiving ANG II, but not in any other group. These results indicate that ET-1 plays a role in ANG II-induced hypertension via activation of ET\textsubscript{A} receptors and that this role is more prominent in rats on HS.

endothelin; blood pressure; salt

THE RENIN-ANGIOTENSIN SYSTEM (RAS) is an important factor in human hypertension, even though most hypertensives do not exhibit elevated renin or ANG II levels. Changes in sensitivity to the pressor actions of ANG II may explain this anomaly. Animals receiving continuous small-dose infusions of ANG II develop progressive hypertension (ANG II-induced hypertension) after a period of normotension (16). We and others (4, 17) have proposed that changes in sensitivity to this slow pressor effect of ANG II could explain apparent “renin-dependent” forms of hypertension not associated with marked RAS activation. One condition known to increase sensitivity to ANG II-induced hypertension is high salt intake (21, 24). But the relative contribution of excess salt retention, vs. neural and/or vascular factors, to this effect is not yet completely resolved.

We reported that hypertension in animals receiving continuous low-dose infusions of endothelin-1 (ET-1) is also salt sensitive (20). Furthermore, there are several known relationships between ANG II and ET-1. ANG II stimulates the ET-1 promotor to cause the upregulation of ET-1 synthesis (6, 9, 12), ET-1 acts as an amplifier of the pressor effects of ANG II (6), and ET-1 partially mediates ANG II-induced vascular hypertrophy (8). Most pressor actions of ET-1 are caused by activation of the ET\textsubscript{A} subtype of ET-1 receptors (10). Therefore, in the present study, we assessed the hypothesis that ANG II-induced hypertension is enhanced during high salt intake through stimulation of ET\textsubscript{A} receptors by endogenous ET-1.

METHODS

Animals. Male Sprague-Dawley rats (Charles River Laboratories, Portage, MI) weighing 350–400 g were used in these experiments. On arrival at our facility, rats were maintained according to standards approved by the Michigan State University All-University Committee on Animal Use and Care. All experimental procedures were carried out in accordance with the Guide for the Care and Use of Laboratory Animals of the American Physiological Society. Rats were acclimatized for at least 2 days before surgical procedures in clear plastic boxes and were allowed access to standard rat chow (Teklad 22/5 Rodent Diet W 8640, Madison, WI) and tap water ad libitum.

Surgical procedures. All surgical procedures were performed after administration of pentobarbital sodium (50 mg/kg ip; Nembutal, Abbott Laboratories, Chicago, IL) and atropine sulfate (0.2 mg ip; Sigma, St. Louis, MO). If necessary, anesthesia was supplemented using methohexital sodium (5–10 mg/kg iv; Brevital, Eli Lilly, Indianapolis, IN). At least 2 days before initiation of the experimental protocol, rats were catheterized via the femoral artery and vein as previously described (17).

Chronic rat maintenance and measurements. The stainless steel metabolism cages housing the rats during the experiments were located in a climate-controlled room with a 12:12-h light-dark cycle. The rats were allowed access to distilled water and sodium-deficient rat chow (Teklad 170950) ad libitum, depending on the experimental protocol. One-half of the rats was maintained on a daily sodium intake of 2 meq/day (normal salt intake), and the remaining one-half was maintained on 6 meq/day (high salt intake). Because the rat chow contained negligible amounts of sodium, all sodium was delivered by continuous intravenous infusion of a sodium chloride solution at a volume of 5 ml/day. Catheters were...
flushed daily to maintain patency, and the arterial catheters were filled with a heparinized sucrose solution and occluded when not in use.

Mean arterial pressure (MAP) and heart rate (HR) were recorded via the femoral arterial catheter each morning of the protocol between 8 and 11 AM. The arterial catheters were connected to external pressure transducers (TXD-300, Micro-Med, Louisville, KY) that were first zeroed at the level of the rat's heart. The transducers were connected to digital pressure monitors (BPA-200 Blood Pressure Analyzer, Micro-Med) that provided input to a computerized digital pressure-monitoring system. Data were collected once every second for 15–30 min. The daily value was determined by the average of the 1-s recordings taken over the last 5 min of the recording session.

Electrolyte and metabolite concentrations were measured in blood and urine using ion-selective electrodes (Nova Electrolyte 16+ Analyzer, Nova Biomedical). Blood was collected in 0.5- to 1.0-ml volumes into heparinized syringes. Plasma volumes were determined on control day 2, and ANG II infusion days 5, 10, and 14 by Evans blue dye dilution (total blood withdrawal = 1.5 ml). Blood volume was calculated according to the standard formula based on arterial hematocrit. Daily electrolyte balance, water balance, and other variables were calculated as previously described (17). Briefly, water balance was calculated as the difference between daily urine volume and the combination of voluntary water intake and the 5-ml/day intravenous infusion volume. Sodium balance was calculated by subtracting daily urinary sodium excretion from the fixed daily sodium intake produced by intravenous infusion of sodium chloride. Our metabolic cage collection system allows 90–95% recovery of daily urinary sodium and water excretion.

**Experimental protocol.** After blood pressure (BP), HR, and other variables were recorded for 2 control days, ANG II (Ile-5 ANG II acetate, Sigma) was continuously infused intravenously at a rate of 5 ng/min for 15 days. On day 5, the rats received a one-time bolus injection of the selective ETA-receptor antagonist ABT-627 (2 mg/kg iv; Abbott Laboratories, Abbott Park, IL), followed by ABT-627 in their drinking water at a concentration (20 mg/l) to deliver an approximate dose of 2 mg·kg⁻¹·day⁻¹ for days 5-10. The dose of ABT-627 was chosen based on preliminary studies in which the antagonist was tested against chronic pressor actions of ET-1 and ANG II (data not shown). BP and HR recordings were taken continuously for 1 h immediately following the bolus injection of ABT-627 and then once each subsequent day of the protocol. Urine samples were collected daily in calibrated containers, blood samples were obtained on control day 2, and ANG II infusion days 5, 7, 10, and 14.

In a separate set of experiments, normal conscious male Sprague-Dawley rats were catheterized and housed as described above. After having BP and HR recorded for 2 control days, each rat received injections (over a time frame of a few seconds) of increasingly large bolus doses of ANG II. Each dose was separated by 10–15 min. After generation of this acute ANG II dose-response curve, the rats received ABT-627 in their drinking water (−2 mg·kg⁻¹·day⁻¹) for 72 h. At this time, each rat again received ANG II to produce a second acute dose-response curve.

**Statistical analysis.** Results are expressed as means ± SE. For all data, within- and between-group differences were analyzed using mixed-design ANOVA. Post hoc comparisons were performed by testing for simple main effects (compare group means within only 1 level of a factor). Analyses were performed with Crunch version 4.0 software (Crunch Software, Oakland, CA). Criterion for statistical significance was a probability level of less than 0.05.

**RESULTS**

**MAP and HR.** As shown in Fig. 1, continuous intravenous infusion of ANG II (5 ng/min) induced a significant increase in MAP compared with control period values. No significant change in MAP was observed in rats that did not receive ANG II. The effect of ANG II was larger and occurred more rapidly in rats on high salt intake (5-day average = 18 ± 3.1 mmHg) compared with rats on normal salt intake (5-day average = 11 ± 3.2 mmHg), but the difference between the two groups was not statistically significant (P = 0.13). With chronic oral dosing in rats on high salt intake and normal salt control group.
receiving ANG II, ABT-627 (2 mg·kg⁻¹·day⁻¹) produced a sustained reduction in MAP to levels not significantly different from that of normotensive control rats within 24 h. ABT-627 did not affect MAP in normotensive control rats on high salt intake.

In rats on normal salt intake, ABT-627 also decreased MAP in rats receiving ANG II, but the effect was significantly less (5-day average 52±15 mmHg) than in rats on high salt intake (5-day average 52±22 mmHg; P=0.0391). Furthermore, in normal salt rats receiving ANG II, ABT-627 did not reduce MAP to the level of normotensive control rats except on the first day of treatment. Also, by day 5 of ABT-627 treatment, MAP in ANG II-infused rats on normal salt intake was no longer significantly less than pretreatment values. ABT-627 did not reduce MAP in normotensive control rats on normal salt intake. ABT-627 did not produce a significant change in HR in any of the four groups studied (data not shown).

On day 5 of ANG II infusion, acute intravenous injection of ABT-627 gradually and significantly lowered MAP over a 1-h time frame in hypertensive ANG II-infused rats, but it had no significant effect in normotensive controls (Fig. 2).

Pressor responses to acute bolus doses of ANG II were identical before and after administration of ABT-627 (given in the drinking water ~2 mg·kg⁻¹·day⁻¹ for 3 days) (Fig. 3).

**Metabolic parameters.** ABT-627 produced a slight retention of water in rats with ANG II-induced hypertension and on normal salt intake, but it did not significantly affect water balance in any other group (Fig. 4). Likewise, the ETA-receptor antagonist produced slight retention of sodium in rats with ANG II-induced hypertension and on normal salt intake (5-day cumulative sodium retention of 1.56±0.66 meq), but it did not significantly affect sodium balance in any other group (Fig. 5).

In rats on high salt intake and with ANG II-induced hypertension, a modest progressive decrease in blood volume was observed across the duration of the protocol. No changes in blood volume were seen in the other three groups (data not shown). No significant changes in routine blood chemistries (e.g., plasma sodium, potassium, glucose, osmolality, blood urea nitrogen, or creatinine concentrations) were observed in any group throughout the protocol (data not shown).

**DISCUSSION**

Previous experiments show that ET-1-receptor blockade begun before the start of ANG II infusion attenuates or eliminates ANG II-induced hypertension (7, 11, 22). These results strongly support involvement of ET-1 in ANG II-induced hypertension. Earlier studies differ from the current one, however, in that they all used higher rates of ANG II infusion (200 ng·kg⁻¹·min⁻¹ sc), used only rats on normal salt intake, and produced a more severe level of hypertension (~35–85 mmHg over control levels) than observed here (~11–18 mmHg over control levels). Furthermore, no published studies addressed potential renal effects of ET-1-receptor blockade in this model of hypertension.

The new findings in this study are as follows: the selective ETA-receptor antagonist ABT-627 entirely normalized BP in rats with preexisting ANG II-induced hypertension; the reduction in MAP was larger...
and longer lasting in rats on high salt intake compared with rats on normal salt intake; and changes in sodium and water excretion were not consistent with the kidney being the primary target for the initial antihypertensive response to ABT-627. We also showed that acute pressor responses to ANG II were unaffected by ABT-627 treatment, indicating that this drug does not block ANG II receptors. Our results confirm that ET-1 acting at ETA receptors plays an important role in ANG II-induced hypertension and further indicate that its role is magnified in rats on high salt intake.

ET-1 is an accepted etiologic factor in several common models of hypertension, most of which are salt sensitive (23). Although the severity of the hypertension caused by ANG II infusion in this particular study was not statistically different between the high and normal salt intake groups, ANG II infusion in the rat is well-known to be a salt-dependent model of hypertension (1, 5, 15, 21, 24). Elevated salt intake may increase the synthesis of ET-1, amplify the pressor actions of ET-1, or do both. Results of published studies suggest no change in vascular ET-1 formation in rats or humans receiving high salt intakes (11, 22). We have previously shown, however, that hypertension produced by exogenous infusion of a low dose of ET-1 is markedly enhanced by high salt intake (20). Thus salt most likely amplifies the pressor actions of ET-1 rather than increasing synthesis and release of the peptide in vascular endothelial cells.

Other investigators have reported that chronic ANG II infusion in rats increased the formation of ET-1 in arteries (8, 12, 19), although the effects of salt intake were not examined. So tonic constriction of resistance arteries by endothelium-derived ET-1 is a probable mechanism of elevated BP in ANG II hypertension. In this study, we showed that ABT-627 significantly lowered MAP within 1 h of bolus intravenous injection. This time course is similar to the effects of acute pressor responses to ANG II.

**Fig. 4.** Line plots show water balance responses in ml/day to infusion of ANG II at 5.0 ng/min and administration of ABT-627 at 2 mg·kg⁻¹·day⁻¹ in rats on either high sodium (A) or normal sodium (B) intake. The horizontal bars from protocol days A1 to A15 depict ANG II-infusion period. The horizontal bar from protocol days A5 to A10 depicts ABT-627 administration. *Significant (P < 0.05) difference in water balance from protocol day A5.

**Fig. 5.** Line plots show sodium balance responses in meq/day to infusion of ANG II at 5.0 ng/min and administration of ABT-627 at 2 mg·kg⁻¹·day⁻¹ in rats on either high sodium (A) or normal sodium (B) intake. The horizontal bars from protocol days A1 to A15 depict ANG II-infusion period. The horizontal bar from protocol days A5 to A10 depicts ABT-627 administration. *Significant (P < 0.05) difference in sodium balance from protocol day A5.
consistent with ANG II-induced hypertension being dependent on ETA receptor-mediated arterial constriction. In addition to enhancing ET-1 release from endothelial cells, ANG II could increase vascular reactivity to endogenous levels of ET-1, although existing data do not support this hypothesis (11, 22).

Reduction of sodium excretion by the kidney is a known cause of hypertension development, and ET-1 has been shown to be an antinatriuretic peptide under some circumstances (14). A recent report also demonstrated that chronic ANG II infusion in rats increases ET-1 gene expression in the kidney (2, 3). The current study is consistent with an antinatriuretic role for ET-1 in ANG II-induced hypertension, because during ABT-627 treatment, sodium excretion was maintained constant, whereas arterial pressure was lowered (i.e., a shift in the renal pressure-natriuresis relationship). The hypotensive response to ABT-627, however, occurred rapidly and was not preceded by any measurable increases in renal sodium or water excretion, or changes in plasma electrolytes, or blood volume. In fact, in rats on normal salt intake, the hypotensive effect of ABT-627 was accompanied by significant retention of sodium and water. This probably was an important factor in the rapid return of BP to hypertensive levels in these rats during continued ETA-receptor blockade. Recent data from our laboratory showed that a thiazide diuretic was able to significantly reduce MAP in rats on high salt intake in the same protocol as described here (J. R. Ballew and G. D. Fink, unpublished data). But the antihypertensive response was delayed (1–2 days) and associated with negative salt and water balances. Thus the renal actions of ABT-627 probably did not contribute to the initial decrease in arterial pressure observed in hypertensive rats, but they were an important part of the sustained anti hypertensive response in rats on high salt intake via a shift in the pressure-natriuresis relationship.

In summary, our data suggest that ANG II-induced hypertension depends on both increased arterial constriction and a shift in renal sodium excretion due to actions of ET-1 on ETA receptors. These effects are greater under conditions of elevated salt intake. It is, therefore, conceivable that ETA-selective receptor antagonists will be effective antihypertensive agents in patients with salt-sensitive forms of hypertension.

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REFERENCES


