Evidence for endothelin involvement in the response to high salt

DAVID M. POLLOCK AND JENNIFER S. POLLOCK
Vascular Biology Center, Departments of Surgery, Physiology, and Pharmacology and Toxicology, Medical College of Georgia, Augusta, Georgia 30912-2500

Received 7 November 2000; accepted in final form 20 February 2001

Pollock, David M., and Jennifer S. Pollock. Evidence for endothelin involvement in the response to high salt. Am J Physiol Renal Physiol 281: F144–F150, 2001.—Recent evidence suggests that endothelin-1 (ET-1), perhaps through the ET\(_B\) receptor, may participate in blood pressure regulation through the control of sodium excretion. Mean arterial pressure (MAP) was continuously measured via telemetry implants in male Sprague-Dawley rats. After 1 wk of baseline measurements, rats were given either high (10%) or low (0.08%) NaCl in chow for the remainder of the experiment (n = 5 in each group). MAP was significantly increased in rats on a high-salt diet (115 ± 2 mmHg) compared with rats on the low-salt diet (103 ± 2 mmHg; P < 0.05). All rats were then treated with the ET\(_B\) receptor antagonist A-192621 mixed with the food and adjusted daily to ensure a dose of 30 mg·kg\(^{-1}\)·day\(^{-1}\). ET\(_B\) blockade produced an increase in MAP within a few hours of treatment and was significantly higher in rats on the high-salt diet over a 1-wk period (170 ± 3 vs. 115 ± 3 mmHg, P < 0.01). To determine whether the increase in MAP during A-192621 treatment was due to increased ET\(_A\) receptor activation, all rats were then given the ET\(_A\)-selective antagonist ABT-627 in the drinking water while a low-salt/high-salt diet and ET\(_B\) blockade were continued. ABT-627 decreased MAP within a few hours in rats on either the high-salt (113 ± 3 mmHg) or low-salt (101 ± 3 mmHg) diet. These results support the hypothesis that endothelin, through the ET\(_B\) receptor, participates in blood pressure regulation in the response to salt loading.

ENDOTHELIN-1 (ET-1) has been described as the most powerful vasoconstrictor yet discovered. Administration of exogenous ET-1 results in a transient vasoconstriction mediated by ET\(_B\) receptors and a sustained vasoconstriction produced primarily through ET\(_A\) receptors (20). Despite the predominance of ET\(_A\) actions in this setting, physiological regulation of ET-1 action may be more dependent upon ET\(_B\) receptor function (18). The ET\(_B\) receptor has several distinct functions that may play a role in arterial pressure regulation (2, 12). First, there is evidence that ET\(_B\) receptors bind and remove ET-1 from the circulation and thus minimize ET\(_A\) receptor activation (4). Second, ET\(_B\) receptors on vascular endothelium release nitric oxide and prostaglan-

METHODS

Telemetry blood pressure measurements. Telemetry transmitters (Data Sciences, St. Paul, MN) were implanted according to manufacturer’s specifications into male Sprague-Dawley rats (200–220 g; Harlan Laboratories, Indianapolis, IN) while under Na pentobarbital anesthesia (65 mg/kg ip; Abbott Laboratories, North Chicago, IL). In brief, a midline incision was used to expose the abdominal aorta that was briefly occluded to allow insertion of the transmitter catheter. The catheter was secured in place with tissue glue. The transmitter body was sutured to the abdominal wall along the incision line as the incision was closed. The skin was closed with staples that were removed 7-10 days later after the incision was healed. Rats were allowed to recover from surgery and returned to individual housing for at least 1 wk prior to data acquisition was initiated. The individual rat cages were placed on top of the telemetry receivers, and arterial pressure waveforms were continuously recorded throughout the study except on days where rats were placed in metabolic cages (see below).

Protocol. All rats were given free access to regular rat chow (0.8% NaCl) during a 1-wk baseline period. Rats were then given chow containing either low (0.08%) or high (10%) NaCl along with tap water ad libitum. After 1 wk on either low or high NaCl, all rats were given the ET\(_B\) receptor antagonist...
A-192621 (19) at a dose of 30 mg·kg⁻¹·day⁻¹ in the food. The concentration of antagonist in the food was adjusted daily to maintain proper dosage. Following 1 wk of treatment with A-192621, all rats were then given the ET₄ receptor antagonist ABT-627 (21) in drinking water at a concentration to deliver a dose of 5 mg·kg⁻¹·day⁻¹. This treatment was continued for 1 wk. On the last day of each week, rats were placed in metabolic cages to monitor food and water intake as well as excretory variables.

Several additional groups of rats were given different treatments. In a third group, rats were treated exactly the same as the initial group with high NaCl, except the dose of A-192621 was reduced to 10 mg·kg⁻¹·day⁻¹. A fourth group of rats received high NaCl and the A-192621 at 30 mg·kg⁻¹·day⁻¹ as before, but during the final week, amiloride was administered in the drinking water at 3 mg·kg⁻¹·day⁻¹. A fifth group of rats served as a time control group and was given the high-NaCl diet that was maintained without any drug treatment for a period of 3 wk. A final group of rats was given high NaCl followed by only 1 wk of treatment with A-192621 at 30 mg·kg⁻¹·day⁻¹ to examine the recovery from ET₄ receptor blockade.

**Assays and chemicals.** Urine concentrations of sodium were determined by ion-selective electrodes (Beckman EL-ISE), and sodium and water balance were calculated as the difference between intake and excretion. Urinary ET-1 concentrations were measured by radioimmunoassay (Amersham Pharmacia Biotech, Arlington Heights, IL). All normal and special NaCl content rat chow was obtained from Harlan Teklad (Madison, WI). A-192621 and ABT-627 were supplied courtesy of Dr. Jerry Wessale of Abbott Laboratories. Amiloride was obtained from Sigma Chemical (St. Louis, MO).

**Statistical analysis.** ANOVA for repeated measures combined with post hoc tests was used for statistical evaluation of mean values each week. Student’s t-test for unpaired data was used for statistical analysis of the urinary ET-1 concentrations. All data are presented as means ± SE.

![Fig. 1. Mean arterial pressure (MAP; top) and heart rate (bottom) in conscious rats given a low- or high-sodium diet followed by endothelin ET₄ receptor and then ET₄ receptor blockade with A-192621 and ABT-627, respectively. bpm, Beats/min. Treatment periods are indicated by the solid horizontal bars. A-192621 was placed in the food at a concentration to deliver 30 mg·kg⁻¹·day⁻¹, and ABT-627 was dissolved in the drinking water to deliver 5 mg·kg⁻¹·day⁻¹. Values are means for 12-h periods. *P < 0.05 vs. the low-Na group.](http://ajprenal.physiology.org/Downloadedfromhttp://ajprenal.physiology.org/2017/06/25)
was used to determine statistical differences between means at any given time period between the first two groups of rats. Values are reported as means ± SE with \( P < 0.05 \) being considered significant; \( n = 5 \) in all groups.

RESULTS

During baseline periods while being given normal rat chow, rats displayed a typical diurnal variation in arterial pressure (Fig. 1). The overall average mean arterial pressure (MAP) for the entire week was 105 ± 2 mmHg in all rats while on normal NaCl intake. Placing rats on a high-NaCl diet significantly increased arterial pressure, whereas the low-NaCl diet had no effect. The weekly average MAP was 103 ± 2 mmHg for rats on low-NaCl and 115 ± 2 mmHg for rats on a high-NaCl diet \( (P < 0.05) \). Heart rate remained unchanged by alterations in NaCl intake.

Treatment with the ET\(_B\)-selective antagonist A-192621 (30 mg·kg\(^{-1}\)·day\(^{-1}\)) significantly increased arterial pressure in all rats, although the increase in rats on a high-NaCl diet was much larger than those on a low-NaCl diet (Fig. 1). ET\(_B\) blockade lowered heart rate and eliminated the normal diurnal variation in rats on a high-NaCl diet but not when on a low-NaCl diet. During the final week of study, rats were given the ET\(_A\)-selective antagonist ABT-627 (5 mg·kg\(^{-1}\)·day\(^{-1}\)), which lowered the arterial pressure to levels similar to that prior to A-192621 treatment.

As expected, food and water consumption were greater in rats on a high-NaCl diet, although this difference was not significant during the week when rats were given A-192621 alone (Fig. 2). Sodium excretion and urine volume were significantly elevated in rats on the high-NaCl diet compared with baseline periods while they were on normal rat chow (Fig. 2). Sodium excretion decreased below detectable levels in rats on the low-NaCl diet. A-192621 had no significant effect on water intake, sodium excretion, or urine volume, regardless of NaCl intake. Similarly, ET\(_A\) receptor blockade produced no further significant changes in these variables.

Figure 3 shows that urinary ET-1 excretion was significantly increased when rats were placed on a
high-Na diet compared with rats given low-Na chow. In contrast to what has been reported for plasma ET-1 (19), treatment with the ETB receptor antagonist had no effect on urinary ET-1 excretion, regardless of Na intake. Furthermore, additional ETA receptor blockade had no effect on urinary ET-1 excretion.

The next series of rats was first given a high-Na diet followed by treatment with A-192621 at the higher dose (30 mg·kg⁻¹·day⁻¹). To determine whether the hypertension could be reversed by inhibition of epithelial Na channels, rats were given the diuretic amiloride (3 mg·kg⁻¹·day⁻¹) during the final week. Amiloride was unable to reduce arterial pressure in rats made hypertensive by chronic ETB receptor blockade (Fig. 4).

An additional series of rats was studied to determine the effect of a lower dose of ETB antagonist in rats on high-NaCl chow (Fig. 5). Similar to results with a higher dose, A-192621 significantly increased arterial pressure and depressed heart rate. Subsequent treatment with the ETA-selective antagonist, ABT-627, reversed these effects.

Another series of rats served as time controls in that they were given a high-NaCl diet from the second week on, but no other treatments. Again, there was a small, but significant increase in arterial pressure in rats on the high-NaCl diet that remained elevated for the duration of the experiment (data not shown). The average MAP for the initial week was 98 ± 2 mmHg, which was significantly increased to 108 ± 3, 109 ± 3, and 110 ± 2 mmHg for the remaining 3 wk while animals were on the high-NaCl diet.

A final series of rats was studied to examine the reversibility of ETB antagonist-induced hypertension. In rats on a high NaCl intake, A-192621 produced a sustained hypertension as in previous groups (Fig. 6). Although it took 3–4 days, arterial pressure did return to baseline after A-192621 treatment was discontinued.

DISCUSSION

Our laboratory has recently provided evidence that the ETB receptor is upregulated in a model of high-salt hypertension, the DOCA-salt-treated rat (13). This model is characterized by elevated endothelin synthesis and ETA-dependent hypertension (1, 9, 10, 16). Furthermore, blockade of the ETB receptor in the DOCA-salt hypertensive rat exacerbated the hypertension (13). The current study extends these initial findings to explore the role of the ET-1 in normotensive rats on either a high- or low-salt diet. We observed that chronic ETB receptor blockade increases arterial pressure in normal rats and that this hypertension was much greater in rats on a high-salt diet compared with a low-salt diet. The increase in arterial pressure during ETB blockade was much larger than that predicted solely from the elevated salt intake such that there was a significant shift in the pressure-natriuresis relationship. These findings provide strong evidence that ET-1...

Fig. 3. Urinary endothelin-1 (ET-1) excretion in rats during a 1-wk baseline period, followed by rats being given either a low- or high-NaCl diet, followed by A-192621 treatment and then ABT-627 treatment. Values are means ± SE for a 24-h urine collection period. *P < 0.05 vs. the low-Na group.

Fig. 4. MAP (top) and heart rate (bottom) in conscious rats given a high-sodium diet followed by ETB receptor blockade with A-192621 in the food (30 mg·kg⁻¹·day⁻¹) and then amiloride in the drinking water (3 mg·kg⁻¹·day⁻¹). Treatment periods are indicated by the solid horizontal bars. Values are means for 12-h periods.
plays a role in regulating arterial pressure during conditions of high salt intake. Further support for this hypothesis is demonstrated by the observation that renal ET-1 production, as assessed by urinary ET-1 excretion, was increased during high salt intake. Similar conclusions are consistent with our recent findings in the DOCA-salt hypertensive rat (13).

The ET<sub>B</sub> receptor appears to function in a variety of ways. ET<sub>B</sub> receptors located on renal tubular epithelium may function to inhibit sodium and water reabsorption (7, 17), whereas those located on vascular endothelium mediate vasodilation, which could also contribute to the natriuretic and diuretic actions attributed to ET-1 (6). The ET<sub>B</sub> receptor also appears to function as a regulator of ET-1 concentrations within the circulation, i.e., as a "clearance" receptor (4). During ET<sub>B</sub> receptor blockade, circulating levels of ET-1 increase (14, 19), which results in a greater probability of ET<sub>A</sub> receptor activation. Therefore, we proposed that ET<sub>A</sub> receptor activation accounts for most, if not all, of the hypertension associated with chronic ET<sub>B</sub> blockade. Chronic ET<sub>A</sub> receptor blockade dramatically reduced the hypertension produced by chronic ET<sub>B</sub> receptor blockade. Since the increase in arterial pressure was greater in rats on a high-salt diet, these findings suggest that a combination of mechanisms account for the hypertension associated with inhibition of the ET<sub>B</sub> receptor, including both reduced ET<sub>B</sub> and increased ET<sub>A</sub> receptor-mediated events.

One of the more interesting findings of the current study is that urinary ET-1 excretion was increased by a high-salt diet and was unaffected by either ET<sub>A</sub> or ET<sub>B</sub> receptor antagonism. These findings suggest that renal ET-1 synthesis is elevated in response to salt loading and are consistent with the hypothesis that ET-1 plays an important role within the kidney in the regulation of sodium excretion (12). Furthermore, urinary ET-1 levels did not change in response to ET<sub>B</sub> blockade, which is in contrast to what has been re-

![Fig. 5. MAP (top) and heart rate (bottom) in conscious rats given a high-sodium diet followed by ET<sub>B</sub> receptor blockade with a lower dose of A-192621 in the food (10 mg·kg<sup>-1</sup>·day<sup>-1</sup>) and then ET<sub>A</sub> receptor blockade with ABT-627 in the drinking water (5 mg·kg<sup>-1</sup>·day<sup>-1</sup>). Treatment periods are indicated by the solid horizontal bars. Values are means for 12-h periods.](image1)

![Fig. 6. MAP (top) and heart rate (bottom) in conscious rats given a high-sodium diet followed by ET<sub>B</sub> receptor blockade with A-192621 in the food (30 mg·kg<sup>-1</sup>·day<sup>-1</sup>) for 1 wk and then followed by a 1-wk recovery period. Treatment periods are indicated by the solid horizontal bars. Values are means for 12-h periods.](image2)
ported for plasma levels of ET-1 (14, 19). Our data suggest that ET<sub>B</sub> receptors within the kidney, most likely the tubular system of the renal medulla, do not play a role in the clearance of ET-1 but most likely respond to high salt intake. Reduction of arterial pressure with ET<sub>A</sub> blockade also had no influence on the response to salt load, indicating that urinary ET-1 excretion is more closely related to salt intake rather than systemic arterial pressure.

Gariepy et al. (5) have recently demonstrated that rats deficient in ET<sub>B</sub> receptor expression display salt-sensitive hypertension. These findings are somewhat similar to our observations with chronic ET<sub>B</sub> receptor blockade. These investigators hypothesized that the ET<sub>B</sub> receptor normally functions as a regulator of renal tubular sodium reabsorption through inhibition of the amiloride-sensitive epithelial sodium channel. They were able to demonstrate that 3 days of amiloride treatment could normalize arterial pressure increases produced by a high-salt diet in the ET<sub>B</sub>-deficient rats. Although provocative, their studies do not directly address the influence of ET<sub>B</sub> receptors on Na channel activity. We conducted similar studies by giving amiloride to rats on a high-salt diet and chronic ET<sub>B</sub> receptor blockade. In contrast to the findings of Gariepy et al. amiloride had no effect on the hypertension produced by chronic ET<sub>B</sub> receptor blockade. The reasons for these differences in results are not clear. There were minor differences in the protocols, but it is not clear that these differences could account for the different findings. Although the daily dose of amiloride was similar, in the current study the drug was administered in the drinking water, whereas Gariepy et al. used once-a-day intraperitoneal injection. In the later study treated animals with amiloride for only 3 days, whereas our rats were treated for a full week. There was also a minor difference in the sodium content of the high-NaCl diet; i.e., our rats were consuming 10% NaCl, whereas the others received 8% NaCl. Clearly, further investigation is needed to contrast the effects of pharmacological vs. genetic “blockade” of ET<sub>B</sub> receptors.

The use of radiotelemetry allowed us to observe typical circadian rhythms in arterial pressure. During the hypertension produced by ET<sub>B</sub> receptor blockade, we observed that the arterial pressure differences between day and night were much greater when the overall MAP was at its highest level. Thus the mechanisms responsible for the normal circadian fluctuations in arterial pressure remain intact and may even be potentiated during ET<sub>B</sub> receptor blockade. In contrast, heart rate was initially decreased by ET<sub>B</sub> blockade, and the typical circadian pattern was abolished. Heart rate slowly increased during the period of ET<sub>B</sub> blockade without any restoration of a circadian pattern. Changes in heart rate may be due to baroreceptor resetting; however, an ET<sub>A</sub> receptor influence on cardiac contractility cannot be ruled out (15).

In conclusion, our results support an important role for ET-1 and the ET<sub>B</sub> receptor in the regulation of arterial pressure through the control of salt balance. High salt intake is a consistent stimulus for renal ET-1 production, which appears to shift the balance between ET<sub>A</sub> and ET<sub>B</sub> receptor activity. The complex nature of ET<sub>B</sub> receptor-mediated actions requires further investigation into the mechanism of how ET<sub>B</sub> receptor activation controls salt handling within the kidney, whether it is via control of renal tubular transport mechanisms, control of intrarenal hemodynamics, or both.

We thank Hiram Ocasio and Deborah Garner for expert technical assistance. Additional thanks go to Dr. Jerry L. Wessale of Abbott Laboratories for supplying the endothelin antagonists.

These studies were supported by an American Heart Association Scientist Development Grant and by National Heart, Lung, and Blood Institute Grants HL-60653 and HL-34776.

REFERENCES


