Endogenous endothelin attenuates the pressor response to acute environmental stress via the \( \text{ET}_A \) receptor

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D’Angelo, Gerard, Jennifer S. Pollock, and David M. Pollock. Endogenous endothelin attenuates the pressor response to acute environmental stress via the \( \text{ET}_A \) receptor. Am J Physiol Heart Circ Physiol 288: H1829–H1835, 2005. First published November 24, 2004; doi:10.1152/ajpheart.00844.2004.—Clinical studies have documented an abrupt rise in plasma endothelin-1 (ET-1) coincident with an increase in mean arterial pressure (MAP) during the response to acute stress. We therefore examined the \( \text{ET}_A \) and \( \text{ET}_B \) receptor-dependent effects of ET-1 on the pressor response to acute environmental stress in ET-1-dependent hypertension. Stress was induced by administration of air jet pulses (3 min) in \( \text{ET}_B \) receptor-deficient (\( \text{ET}_B \) sl/sl) rats fed normal salt (NS; 0.8% NaCl), high salt (HS; 8% NaCl), and HS plus the \( \text{ET}_A \) receptor antagonist ABT-627 (5 mg·kg\(^{-1}·\text{day}^{-1} \)). Blockade of \( \text{ET}_B \) receptors in Sprague-Dawley rats caused an increase in basal MAP that was enhanced by HS and lowered by mixed \( \text{ET}_A/\text{ET}_B \) receptor antagonism; none of these treatments, however, had any effect on the pressor response. These data demonstrate that increasing endogenous ET-1 suppresses the pressor response to acute stress through \( \text{ET}_A \) receptor activation in a genetic model of ET-1-dependent hypertension. These results are consistent with reports that ET-1 can attenuate sympathetically mediated responses.

HYPERTENSION remains a leading risk factor in the onset of acute cardiovascular incidents as well as the development of chronic cardiovascular disease. Sudden spikes in pressure, such as those caused by acute environmental stress, are believed to play a role in these processes, albeit by mechanisms that are not clearly defined. Numerous clinical studies have demonstrated larger pressor responses to acute stressors in normotensive African American vs. Caucasian adolescents (7, 17, 25, 26, 35–37). A causal relationship between endothelin-1 (ET-1) and the pressor response to stress is suggested by the finding that the elevated pressor response in African American subjects is accompanied by a rapid increase in plasma ET-1 (36).

The responses to ET-1 are subserved by two receptor subtypes, endothelin A (\( \text{ET}_A \)) and B (\( \text{ET}_B \)) (1, 30). Located primarily on vascular smooth muscle, the \( \text{ET}_A \) receptor mediates a sustained vasoconstriction and resultant increase in mean arterial pressure (MAP). Conversely, the \( \text{ET}_B \) receptor has been identified in the vascular endothelium (31, 34) and in the renal medullary epithelium (15), where its activation has been shown to mediate transient vasodilation (3, 14, 18) and natriuretic and diuretic effects (8, 13, 41) in response to the application of exogenous ET-1. Since its discovery, ET-1 has been described as one of the most potent vasoconstrictors known to date. Despite this, the consensus is that ET-1 is involved in elevating MAP principally in experimental models of salt-sensitive hypertension, because selective \( \text{ET}_A \) or mixed \( \text{ET}_A/\text{ET}_B \) receptor antagonists either attenuate the rise in MAP or cause a partial reversal of MAP after the induction of hypertension by a high-salt diet (2, 5, 6, 16).

Epidemiological studies indicate that salt-sensitive hypertension is more prevalent in African Americans and implicate ET-1 as a mediator (reviewed in Ref. 9). In light of findings demonstrating increased pressor responses to acute stress in normotensive black subjects accompanied by an increase in plasma ET-1, the aim of this study was to discern the respective roles of the \( \text{ET}_A \) and \( \text{ET}_B \) receptors during the pressor response to acute environmental stress. The working hypothesis states that \( \text{ET}_A \) and \( \text{ET}_B \) receptor antagonism would blunt and augment, respectively, the pressor response to acute stress. Environmental stress was applied by subjecting animals to acute air jet stress, a well-established method to evoke a rapid increase in sympathetic nerve activity (4, 19, 20). Cardiovascular responses to air jet stress were examined in animals that were continuously monitored using telemetry. To test our hypothesis, we utilized three models of ET-1-dependent hypertension. Experiments were first carried out by using a variant of the spotting lethal (sl) rat that exhibits tissue-specific expression of the \( \text{ET}_B \) receptor (12) and thus is a genetic model of ET-1-dependent hypertension. The extent to which the stress response is mediated by \( \text{ET}_A \) receptors was determined by monitoring the pressor response in animals treated with the highly selective \( \text{ET}_A \) receptor antagonist ABT-627 (27). To determine whether our results are specific to the genetic model, we also assessed relative \( \text{ET}_A \) and \( \text{ET}_B \) receptor activity in Sprague-Dawley rats before and after pharmacological induction of ET-1-dependent hypertension. Finally, experiments were performed with Dahl salt-sensitive rats, a strain that also exhibits ET-1-dependent, salt-sensitive hypertension.

METHODS

Animal model. Three series of experiments were conducted. In the first series, male rats (200–250 g) that were either wild-type (WT; \( \text{ET}_B +/+ \)) or homozygous (sl/sl) for a deficiency of the \( \text{ET}_B \) receptor were used. The costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.
were obtained from our local breeding colony. To prevent death from intestinal aganglionosis, the sl/sl animals express a transgene containing the dopamine β-hydroxylase promoter upstream of the ETβ receptor gene; WT animals also express the transgene (12). In the second series, male Sprague-Dawley rats (200–250 g; Harlan Laboratories, Indianapolis, IN) were used. In the third series, we used male Dahl salt-resistant (DR) and salt-sensitive (DS) rats (200–250 g; Harlan Laboratories). All animals were fed standard rat chow containing normal salt (NS; 0.8% NaCl) and tap water ad libitum up to the point when they were switched to a diet containing high salt (HS; 8% NaCl). Rats were housed in the animal care facility at the Medical College of Georgia, as approved by the Association for the Assessment and Accreditation of Laboratory Animal Care. All protocols were approved by the Institutional Animal Care and Use Committee.

Telemetry. Telemetry transmitters (Transoma, Minneapolis, MN) were implanted according to the manufacturer’s specifications. Rats were anesthetized with ketamine-xylazine (50 mg/kg-10 mg/kg ip). The abdominal aorta was then exposed by a midline incision and briefly occluded. The transmitter catheter was inserted into a hole made by a 21-gauge needle just proximal to the iliac bifurcation and was secured in place with tissue glue (Vetbond). The transmitter body was attached to the abdominal wall along the incision line with 4-0 proline suture as the incision was closed. The skin was closed with staples that were removed 7 days after the incision had healed. Rats were allowed 8–10 days to recover from surgery and were returned to individual housing for data collection before being placed on dietary protocols and subjected to stress. Animals were housed in a room separate from that used for studying the stress response. Individual rat cages were placed on top of the telemetry receivers, and MAP and heart rate (HR) were continuously recorded throughout the study using the Dataquest A.R.T. Acquisition program.

Air jet stress. WT and ETβ receptor-deficient (ETβ sl/sl) rats (n = 6 for each strain) were subjected to acute stress once per week over 3 successive weeks, during which time the animals were maintained on diets containing normal salt (NS), high salt (HS), and HS plus ABT-627 (5 mg·kg⁻¹·day⁻¹). Previous experiments have determined that this dose of ABT-627 yields maximal inhibition of ET-1-mediated responses in the rat (27, 39). Sprague-Dawley rats (n = 5) were subjected to acute stress once per week over 5 successive weeks. During this time, animals were maintained on diets containing NS, NS plus the selective ETβ receptor antagonist ABT-192621 (10 mg·kg⁻¹·day⁻¹) (39), HS, HS plus ABT-192621 (10 mg·kg⁻¹·day⁻¹), and HS plus the mixed ETA/ETβ receptor antagonist SB-209670 (2.5 mg·kg ip, bid). ABT-192621 was given in the food for 2 days before the stress session; SB-209670 was administered for 3 days before the animals were subjected to stress. Preliminary studies have confirmed that administration of the dose of ABT-192621 completely inhibits ETβ receptor-dependent vasoactive responses (unpublished observations).

DR and DS rats (n = 6 for each strain) were subjected to air jet stress over 3 successive weeks. Pressor response was first examined in rats given a NS diet. Animals were subsequently fed a HS diet for 2 wk and subjected to acute stress once per week. On the day they were subjected to air jet stress, rats were quietly brought to a sound-proofed room. Immediately after the telemetry recording software was started, the room was vacated. The animals were then allowed to acclimate to their surroundings for 15–30 min in their cages, until such time as they ceased exploring the new environment and their pressures stabilized. Animals were then placed in tubular Plexiglas restrainers with sufficient aeration, and MAP and HR were continuously monitored using telemetry for at least 15 min before air jet stress was initiated. When necessary, animals were monitored for up to an additional 10 min to allow the animals to adapt to being restrained such that 3–5 min of stable MAP and HR recordings were obtained before exposure to air jet stress. Air jet stress consisted of pulses (2-s duration delivered every 10 s for 3 min) of compressed air (15 lb./in.²) aimed at the forehead from a ½-in. opening at the front of the tube. After the 3-min stress period, MAP and HR were monitored for 20 additional minutes while the animals were still in the restrainer. At the end of this poststress period, animals were returned to their cages and brought back to their holding room.

Statistical analysis. Data are expressed as means ± SD. All baseline MAP and HR values are reported as 24-h means. Total pressor response refers to the change in MAP during the 3 min of air jet stress and was determined using the equation Δ(P − Pbase) × 0.067, where P refers to each MAP data point recorded during the delivery of air jet stress, Pbase is the average pressure during the 3 min just before the onset of the air pulses, and 0.067 is the 4-s data collection interval. Data are expressed as area under the curve (AUC; mmHg · min). Statistical analysis was made by one-way (Sprague-Dawley) or two-way (WT vs. ETβ sl/sl, DR vs. DS) analysis of variance, followed by the Newman-Keuls test for multiple comparisons. Differences are considered significant at P < 0.05.

RESULTS

WT vs. ETβ sl/sl rats. Two approaches were taken to assess the relative roles of the ETα and ETβ receptors in the pressor response to acute stress. The first series involved genetic ETβ receptor deficiency, utilizing a variant of the spotting lethal (ETβ sl/sl) rat, which expresses the ETβ receptor only in neuronal tissue, and its WT control. Baseline (24 h) MAP was significantly greater in ETβ sl/sl vs. WT rats (P < 0.05), and this difference was exaggerated by placing the animals on a diet containing HS (Fig. 1A). Treatment of rats with ABT-627 while still on the HS diet completely normalized MAP in both strains. HR did not change in WT animals during any of the treatments. Conversely, HR was significantly lower in ETβ sl/sl rats placed on the HS diet (P < 0.05 vs. NS; Fig. 1B); ABT-627 fully restored HR to the level observed in animals kept on NS.

Fig. 1. Baseline mean arterial pressure (MAP) (A) and heart rate (HR) (B) in wild-type and ETβ receptor-deficient (ETβ sl/sl) rats. On successive weeks, animals were maintained on diets containing normal salt (0.8% NaCl), high salt (8% NaCl), and high salt plus the ETα receptor antagonist ABT-627 (5 mg·kg⁻¹·day⁻¹). Data represent average values over the 24-h period before animals were subjected to acute air jet stress. *P < 0.05.
Animals were subjected to acute air jet stress after having been placed on diets containing NS, HS, and HS plus ABT-627 on successive weeks. Both movement of animals to a new room and restraint of animals caused transient increases in MAP, which had stabilized during the latter portion of the in-cage acclimation and pre-air jet restraint periods. Similar to the change in MAP caused by the arousal, the average MAP of the restrained animals just before the onset of air jet stress was significantly greater in ET<sub>B</sub> sl/sl vs. WT rats kept on NS and HS diets [NS: 136 mmHg (SD 8) vs. 111 mmHg (SD 6), ET<sub>B</sub> sl/sl vs. WT, P < 0.05; HS: 141 mmHg (SD 15) vs. 119 mmHg (SD 7), ET<sub>B</sub> sl/sl vs. WT, P < 0.05]; ABT-627 lowered the average MAP immediately before air jet stress in both strains and abolished the differences [106 mmHg (SD 6) vs. 107 mmHg (SD 7), ET<sub>B</sub> sl/sl vs. WT, P < 0.05 vs. HS alone for each strain]. Under all conditions, HR remained significantly elevated after restraint compared with immediately before restraint. There was a progressive drop in average HR just before air jet stress such that HR of both ET<sub>B</sub> sl/sl and WT rats was significantly less when fed HS plus ABT-627 compared with NS [ET<sub>B</sub> sl/sl: 489 beats/min (SD 41), NS vs. HS + ABT-627, P < 0.05; WT: 471 beats/min (SD 17) vs. 407 beats/min (SD 22), NS vs. HS + ABT-627, P < 0.05]. For a given diet and drug treatment, however, absolute HR was not different between ET<sub>B</sub> sl/sl and WT rats.

Typical cardiovascular responses to acute air jet stress in ET<sub>B</sub> sl/sl and WT rats maintained on NS are shown in Fig. 2. The initial burst of air caused a rapid rise in MAP, followed by oscillatory changes that were coincident with the delivery of subsequent air pulses (Fig. 3). The initial response was significantly larger in ET<sub>B</sub> sl/sl vs. WT rats maintained on NS and HS diets [NS: 41 mmHg (SD 16) vs. 28 mmHg (SD 8), ET<sub>B</sub> sl/sl vs. WT, P < 0.05; HS: 46 mmHg (SD 10) vs. 32 mmHg (SD 15), ET<sub>B</sub> sl/sl vs. WT, P < 0.05] but not on HS plus ABT-627. The average increase in MAP during the last minute of air jet stress was similar in ET<sub>B</sub> sl/sl vs. WT rats on a NS diet; conversely, the change in MAP was markedly attenuated in ET<sub>B</sub> sl/sl rats on the HS diet compared with both those kept on the NS diet [-4 mmHg (SD 5) vs. 8 mmHg (SD 4), HS vs. NS, P < 0.05] and their WT counterparts fed the HS diet [5 mmHg (SD 7), HS vs. ET<sub>B</sub> sl/sl, P < 0.05]. ABT-627 reversed this effect such that the stress-induced increase in MAP was significantly higher in both strains [14 mmHg (SD 4) vs. 12 mmHg (SD 10), ET<sub>B</sub> sl/sl vs. WT, P < 0.05 vs. respective NS]. Consequently, the total pressor response was significantly reduced in ET<sub>B</sub> sl/sl rats fed HS yet augmented by ABT-627 in both strains compared with their respective responses on NS (Fig. 3). In time control experiments, WT and ET<sub>B</sub> sl/sl rats were maintained on a NS diet and subjected to the air jet stress protocol over 3 successive weeks; no difference in the total pressor response was observed in either strain on each successive week (data not shown).

We also tested whether ET<sub>B</sub> receptor blockade could elicit similar results. Male Sprague-Dawley rats were fed on successive weeks diets containing NS, NS plus the selective ET<sub>B</sub> receptor antagonist ABT-192621, HS, HS plus ABT-192621, and HS plus the mixed ETA/ET<sub>B</sub> receptor antagonist SB-209670. Thus these treatments were designed to pharmacologically mimic the conditions to which the ET<sub>B</sub> sl/sl rats were exposed. The effects of these treatments on baseline MAP and HR are summarized in Fig. 4. ABT-192621 caused a modest but not statistically significant increase in baseline MAP (P = 0.10), whereas HS had no effect (Fig. 4A). The combination of HS and ABT-192621 did elicit a rise in baseline MAP greater than that produced by ABT-192621 or HS alone (P < 0.05 vs. NS). A significant drop in basal HR accompanied this increase in MAP (P < 0.05 vs. NS; Fig. 4B). SB-209670 prevented the rise in baseline MAP. Before air jet stress was initiated, MAP of restrained animals was comparable to that observed imme-
normal salt plus the ETB receptor antagonist ABT-162621 (10 mg/kg, bid). Values were calculated as the sum of MAP data points during air jet stress minus the average MAP obtained over the 3 min before the initiation of air jet stress. *P < 0.05. The total pressor response to air jet stress for DR rats to the extent that the AUC was significantly greater in DR vs. DS rats (P < 0.05) by the end of the second week on the HS diet (Fig. 7). However, the HS diet had minimal effect on the total pressor response in DS animals.

**DISCUSSION**

Clinical studies have noted an increased pressor response to acute stressors in normotensive black vs. white adolescents (7, 17, 25, 26, 35–37) that is accompanied by an increase in plasma ET-1 (36). Because epidemiological studies have documented an increased prevalence of salt-sensitive hypertension among blacks, we utilized an experimental model of salt-sensitive hypertension to discern the roles of ETA and ETB receptors during the pressor response to acute stress. Activation of ETA receptors on vascular smooth muscle mediates vasoconstriction, whereas stimulation of endothelial ETB receptors causes vasodilation; thus we hypothesized that ETA and ETB receptor antagonism would reduce and augment, respectively, the pressor response to acute stress. Experiments were carried out using the ETB receptor-deficient rat, whose effects of ET-1 in the vasculature are mediated exclusively through the ETA receptor. Moreover, it is well established that a HS diet increases the ET-1 content in the vascular tissue of animal models of salt-sensitive hypertension (2, 22, 24). Thus comparison of responses of animals fed a NS vs. HS diet enabled us to determine the extent to which a diet-induced alteration in the level of endogenous ET-1 affected the pressor response.

One major finding of this study is that the pressor response was markedly inhibited in ETB−/−sl/sl rats fed a HS diet. Given the documented effect of HS on vascular ET-1 content in salt-sensitive hypertension (2, 22, 24), our data suggest that DR rats [115 mmHg (SD 11) and 118 mmHg (SD 8), week 1 and 2, respectively], but increased steadily in DS animals [137 mmHg (SD 14) and 160 mmHg (SD 21), week 1 and 2, respectively]. Conversely, there was a progressive increase in the total pressor response to air jet stress for DR rats to the extent that the AUC was significantly greater in DR vs. DS rats (P < 0.05) by the end of the second week on the HS diet (Fig. 7). However, the HS diet had minimal effect on the total pressor response in DS animals.

**Dahl salt-resistant vs. salt-sensitive rats.** In DS rats, the dependence of salt-induced hypertension on ET-1 is well established (2, 5, 6, 16). Thus, to confirm whether modulation of the pressor response by ET-1 is specific to genetic models of ET-1-dependent hypertension, we also examined the pressor in DS and DR rats fed NS and HS diets. Animals were subjected to acute air jet stress on 3 successive weeks after being fed NS and were then switched to HS for the remaining 2 wk. Baseline (24 h) MAP was slightly higher in DS vs. DR rats fed NS, but this difference was not significant (Fig. 6A). Both DS and DR animals exhibited salt sensitivity after being fed HS for 2 wk, although this was much more pronounced with DS rats (P < 0.05). HR was not different between strains, and there was no significant effect of diet (Fig. 6B).

On NS, MAP of restrained animals before initiation of air jet stress tended to be higher in DS vs. DR rats [128 mmHg (SD 14) vs. 115 mmHg (SD 9), DS vs. DR, P = 0.08]. The total pressor response was significantly higher in DS vs. DR rats (P < 0.05; Fig. 7). On HS, pre-air jet MAP did not change in
endogenous ET-1 may actually suppress the response to acute stress. Furthermore, the ET<sub>A</sub> receptor antagonist ABT-627 significantly enhanced this response, suggesting that contrary to our original hypothesis, the inhibitory effect is ET<sub>A</sub> receptor dependent. Interestingly, ABT-627 also potentiated the pressor response in WT rats fed HS, suggesting a more generalized suppression by ET<sub>A</sub> receptor activation. On the other hand, a functional ET<sub>B</sub> receptor may ameliorate the initial response, because this was significantly lower in WT vs. ET<sub>B</sub> s/sl rats fed either a NS or HS diet. Finally, we observed that the total pressor responses in untreated ET<sub>B</sub> s/sl rats fed a NS diet were nearly twice that of their WT counterparts, although this difference did not reach significance.

In the present study, we found that augmentation of the pressor response by ET<sub>A</sub> receptor blockade occurred in ET<sub>B</sub> s/sl rats, a genetic model of ET-1-dependent hypertension, and, curiously, their WT controls. This response in WT animals may be explained by our finding that these animals exhibited a modest but significant increase in 24-h MAP that was abrogated by ET<sub>A</sub> receptor blockade, reflecting a dependence of elevated blood pressure on ET-1. Nevertheless, our results suggest that pharmacological manipulation alone is not sufficient to elicit this potentiating effect but may be reflective of genetic modifications inherent to salt-sensitive strains and their respective genetic controls. However, potential mechanisms for this remain unclear. In addition, our results suggest that attenuation of the pressor response occurs independently of alterations in baseline MAP. Although the changes in baseline MAP in Sprague-Dawley rats produced by the different diets and drug treatments were nearly equal to those changes observed in ET<sub>B</sub> s/sl rats under similar conditions, the pressor response in Sprague-Dawley animals was not affected by any of these manipulations. Alternatively, it remains possible that the duration of heightened ET<sub>A</sub> receptor activation may contribute to the different effects caused by ET<sub>A</sub> receptor blockade in ET<sub>B</sub> s/sl vs. Sprague-Dawley rats. Among its effects, ET<sub>A</sub> receptor activation leads to the induction of numerous genes. Thus the relatively short time frame during which ET<sub>B</sub> receptors were blocked in Sprague-Dawley rats, thereby directing all of the effects of ET-1 through the ET<sub>A</sub> receptor, may not be sufficient to elicit comparable changes in gene transcription that may in turn alter the pressor response to stress.

The experimental paradigm used in the present study involved two stressors, restraint followed by air jet stress. In most experiments, the MAP of restrained ET<sub>B</sub> s/sl and WT rats returned to that level noted immediately before restraint. Conversely, HR was always significantly higher, offering the possibility that sympathetic activation caused by restraint may differentially impact the subsequent pressor responses to air jet stress in these animals. However, our results do not consistently support this notion. We found that there was a larger increase in HR caused by restraint relative to room switch in WT [136 beats/min (SD 22)] vs. ET<sub>B</sub> s/sl rats [95 beats/min (SD 12)] fed NS. If HR were to be used as an index of sympathetic activity, these data would therefore suggest a greater increase in sympathetic activity in WT rats under this condition and perhaps less susceptibility to further increases in MAP upon additional stress. Conversely, in animals fed HS and HS + ABT-627, the increases in HR caused by restraint were comparable between ET<sub>B</sub> s/sl and WT rats. Despite the equivalent changes in HR, ET<sub>B</sub> s/sl rats fed HS consistently demonstrated a blunted pressor response to air jet stress, whereas WT animals exhibited no change. In comparing absolute HR before the administration of air jet stress, we found no difference between ET<sub>B</sub> s/sl and WT rats within each diet and drug treatment. Thus it can be argued that ET<sub>B</sub> s/sl and WT animals are in a similarly stressed state for a given treatment and that this level of stress does not uniformly affect the pressor response to air jet stress.

Fig. 6. Baseline MAP (A) and HR (B) in Dahl salt-resistant and salt-sensitive rats fed a diet containing normal salt (0.8% NaCl) or high salt (8% NaCl) for 2 wk. Data represent average values over the 24-h period before animals were subjected to acute air jet stress. *P < 0.05.

Fig. 7. Summary of total pressor response (AUC) to acute air jet stress in Dahl salt-resistant and salt-sensitive rats. Animals were subjected to acute air jet stress on 3 successive weeks after being fed a diet containing normal salt (0.8% NaCl) for the first week and then high salt (8% NaCl) for the next 2 wk. Values were calculated as the sum of MAP data points during air jet stress minus the average MAP obtained over the 3 min before the initiation of air jet stress. *P < 0.05.
To determine whether diet-induced changes were unique to the ETB sl/sl strain, we tested this response in DR and DS maintained on NS and HS diets. On a NS diet, the total pressor response was elevated in DS compared with DR rats. Because the DS rat is a well-established model for the salt-sensitive, low-renin hypertension typically found in African Americans, our results are consistent with the previous reports of heightened responses to environmental stressors in normotensive black compared with white adolescents (7, 17, 25, 26, 35–37). On the other hand, a HS diet had minimal effects on the pressor response in DS rats yet caused a progressive enhancement in DR animals such that after 2 wk of HS feeding, the AUC was significantly greater in DR vs. DS rats. Previous studies have demonstrated that HS causes an increase in the vascular ET-1 content of DS but not DR rats (2, 28, 29). Our data therefore support the notion that elevated vascular ET-1 may prevent the augmentation of the pressor response in DS rats maintained on HS.

Unlike our results with ETB sl/sl rats, HS did not lower the total pressor response in DS animals. One possible reason for this may be the presence of a functional ETB receptor in DS rats. If, as our data suggest, the inhibitory effect of ET-1 is ETA receptor dependent, then one would expect to see greater inhibition if all of the actions of ET-1 are mediated through the ETA receptor, as occurs in ETB sl/sl rats. Because ET-1 binding can be distributed to both ETA and ETB receptor subtypes in DS rats, it follows that the negative influence exerted by endogenous ET-1 on the pressor response may be reduced. Alternatively, ETB sl/sl rats may have higher ET-1 content in the vascular wall compared with DS rats because of the lack of ETB receptor-dependent clearance of ET-1.

It is well established that the cardiovascular responses to acute stress are initiated by the sympathetic nervous system. Previously, Koepeke et al. (20) demonstrated an abrupt increase in renal sympathetic nerve activity in restrained DR and DS rats subjected to air jet stress, yet, in contrast to our results, they reported no increase in MAP. One explanation for this difference may be related to the temporal characteristics of the stimulus and mode of analysis. In the study by Koepeke et al., the authors applied a continuous air jet stimulus for 10 min and calculated the average MAP value over that period. In a pilot study, we found that 3 min of continuous, rather than pulsatile, air jet stress elicited a transient increase in MAP, whereby MAP actually returned to a level below the pre-air jet baseline. Under this condition, the average MAP was not significantly higher than the pre-air jet baseline (unpublished observation). On the other hand, applying pulses over that same interval yielded repetitive spikes in MAP. Thus it appears as though the animals can rapidly habituate to a continuous stimulus and that this may be circumvented by applying air pulses.

Given that elevated sympathetic nerve activity has been proposed to contribute to the development of essential hypertension (see Refs. 10 and 23 for reviews), it therefore stands to reason that mechanisms that can regulate adrenergic activity either pre- or postsynaptically may participate in the disease process. The results from several studies suggest that ET-1 may exert an inhibitory effect prejunctionally. Specifically, pretreatment of arterial preparations in vitro with ET-1 has been shown to reduce the fractional release of [3H]norepinephrine (NE) and thereby attenuate the contractile response caused by transmural stimulation (32, 33, 40).

Because of the extensive overlap of the signaling pathways downstream of ET-1 and α1-adrenergic receptors, it is also possible that ET-1 can exert an effect postsynaptically to downregulate α1-adrenergic receptor activation by heterologous desensitization. In cell cultures, ET-1 markedly increased basal phosphorylation of both α1b- and α1D-adrenergic receptors and attenuated the NE-induced rise in cytosolic calcium (11, 38). Moreover, Kong et al. (21) found that the sensitivity to NE of perfused mesenteric arteries from DS rats is negatively correlated with systolic arterial pressure with prolonged hypertension. Together, these findings support the notion that ET-1 activity may dampen α-adrenergic-mediated whole animal pressor responses, especially in experimental models of salt-sensitive hypertension, where the elevation in blood pressure is more dependent on heightened ET-1 activity, either through inhibition of NE release or by heterologous desensitization.

In summary, we found that, contrary to our original hypothesis, conditions reflective of elevated tissue ET-1 attenuated the pressor response to air jet stress, whereas ETA receptor blockade significantly enhanced this response. We propose that endogenous ET-1 modulates sympathetically mediated responses such that inhibition of ET-1-mediated effects reveals an increased responsiveness to adrenergic stimulation. This modulation may serve as an important protective mechanism to dampen deleterious effects caused by sudden increases in pressure, particularly in models of salt-sensitive hypertension.

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REFERENCES

ET(A)-DEPENDENT SUPPRESSION OF STRESS-INDUCED PRESSOR RESPONSE


