Prevention of endothelin-1-induced increases in blood pressure: role of endogenous CGRP

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Wang, Youping, and Donna H. Wang. Prevention of endothelin-1-induced increases in blood pressure: role of endogenous CGRP. Am J Physiol Heart Circ Physiol 287: H1868–H1874, 2004.—To determine the role of endothelin-1 (ET-1) and its receptors in the regulation of calcitonin gene-related peptide (CGRP) release, male Wistar rats were divided into six groups and subjected to the following treatments for 1 wk with or without ABT-627 (an ET_{A} receptor antagonist, 5 mg·kg^{-1}·day^{-1} in drinking water) or A-192621 (an ET_{B}-receptor antagonist, 30 mg·kg^{-1}·day^{-1} by oral gavage): control (Con), ET-1 (5 ng·kg^{-1}·min^{-1} iv), Con + ABT-627, Con + A-192621, ET-1 + ABT-627, and ET-1 + A-192621. Baseline mean arterial pressure (MAP, mmHg) was higher ([P < 0.05]) in Con + A-192621 (122 ± 4) and ET-1 + A-192621 (119 ± 4) groups compared with Con (104 ± 6), ET1 (106 ± 3), Con + ABT-627 (104 ± 3), and ET1 + ABT-627 (100 ± 3) groups. Intravenous administration of CGRP 8 –37 (a CGRP receptor antagonist, 1 mg/kg) increased MAP ([P < 0.05]) in ET-1 (13 ± 1), Con + A-192621 (12 ± 1), and ET-1 + A-192621 (15 ± 3) groups compared with Con (4 ± 1), Con-ABT-627 (4 ± 1), and ET-1 + ABT-627 (5 ± 1) groups. Plasma CGRP levels (in pg/ml) were increased ([P < 0.05]) in ET-1 (57 ± 6.1), Con + A-192621 (53.9 ± 3.4), and ET-1 + A-192621 (60.4 ± 3.0) groups compared with Con (40.4 ± 1.6), Con + ABT-627 (40.0 ± 2.9), and ET-1 + ABT-627 (42.6 ± 1.9) groups. Plasma ET-1 levels (in pg/ml) were higher ([P < 0.05]) in ET-1 (28.2 ± 0.2), ET-1 + ABT-627 (32.2 ± 0.4), Con + A-192621 (3.3 ± 0.4), and ET-1 + A-192621 (4.6 ± 0.3) groups compared with Con (1.1 ± 0.2) and Con-ABT-627 (1.3 ± 0.2) groups. Therefore, our data show that ET-1 infusion leads to increased CGRP release via activation of the ET_{A} receptor, which plays a compensatory role in preventing ET-1-induced elevation in blood pressure.

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endothelin receptor; calcitonin gene-related peptide

CALCITONIN GENE-RELATED PEPTIDE (CGRP) is a 37-amino acid vasoactive neuropeptide resulting from tissue-specific alternative splicing of the primary transcript of the calcitonin–CGRP gene (30). Within the sensory nervous system, CGRP immunoreactivity has been detected prominently in dorsal root ganglia (DRG), which contain the cell bodies of sensory afferents (30). Immunohistochemical studies (30) have also found an abundance of CGRP-containing sensory nerve terminals throughout the body, often associated with vascular smooth muscle. Whereas many biological actions, including the regulation of nociception have been attributed to CGRP, it has been generally accepted that CGRP is one of the most potent endogenous vasodilators. Evidence shows that CGRP plays a vital role in regulating peripheral vascular tone and regional blood flow under both physiological and pathophysiological conditions (3, 27, 30).

Endothelin-1 (ET-1) is a potent vasoconstrictor peptide of 21 amino acids that is synthesized and released by endothelial cells (33). ET-1 activates two subtypes of receptors, endothelin-A (ET_{A}) and endothelin-B (ET_{B}) receptors (9). ET_{A} receptors are present in vascular smooth muscle cells and mediate endothelin-induced vasoconstriction (9). ET_{B} receptors are expressed predominately in endothelial cells and mediate vasodilation by generation of prostacyclin and nitric oxide (5, 10). Moreover, there is evidence showing that ET_{B} receptors bind and remove ET-1 from the circulation and thus serve as clearance receptors that minimize ET_{A} receptor activation (9). Whereas ET-1 plays an important role via its autocrine and paracrine actions, it has been shown that its spillover into the blood stream under pathophysiological conditions associated with endothelial dysfunction elevates circulating ET-1 levels by two- to fivefold (4, 14).

Previous studies have demonstrated that in deoxycorticosterone acetate (DOCA)-salt hypertension, CGRP acts as a compensatory depressor to attenuate the elevation of blood pressure (27). The mechanisms responsible for the action appear to be through an increase in neuronal synthesis and subsequent release of CGRP in this model (27). These studies suggest that altered circulating and/or local factors in DOCA-salt hypertension may change the long-term synthesis and release of CGRP from sensory afferents. It has been shown that DOCA-salt hypertension is associated with an upregulation of the endothelin system (9). It is unknown, however, whether ET-1 plays a role in CGRP synthesis and release. The purpose of this study was, therefore, to determine the chronic action of ET-1 on CGRP release through intravenous administration of this peptide at 5 ng·kg^{-1}·min^{-1}. Our preliminary studies indicate that this dose of ET-1 elevates plasma ET-1 levels by twofold, a condition that mimics certain pathophysiological states (4, 14). In addition, the use of specific ET_{A} and ET_{B} receptor antagonists allowed us to evaluate which receptor subtype is primarily responsible for the ET-1 action.

METHODS

Animal groups. All experiments were approved by the Institutional Animal Care and Use Committee. Male Wistar rats weighing 220–270 g (Charles River Laboratories; Wilmington, MA) were housed in the animal facility for 1 wk before the experiment and were allowed free access to regular rat chow throughout the experiment. All rats were divided into six groups and subjected to the following treatments for 1 wk: control (Con), ET-1, control + ABT-627 [2-(4-methoxyphenyl)-4-(1,3-benzodioxol-5-yl)-1-[(N,N-di(n-butyl)amino carbonyl-methyl]-pyrrolidine-3-carboxylic acid] (Con + ABT-627), control + A-192621 [2-(4-propoxyphenyl)-4-(1,3-benzodioxol-5-yl)-1-(2,5-eth-
Administration of CGRP and the measurement of mean arterial pressure (MAP) or intravenous infusions at each session. At the end of the experiment, rats were sacrificed and the catheter was positioned in the external jugular vein. The pressure was routinely obtained in conscious rats by use of a Narco Bio-Systems Electro-Sphygmomanometer (Austin). The pressure was monitored continuously over 3–6 h at 4°C. The arterial pressure, MAP and heart rate (HR) responses to bolus injection of normal saline via osmotic pumps. ABT-627 (an ETA receptor antagonist) was given at a dose of 5 mg/kg-day in drinking water. This dose was calculated based on the preliminary experiments indicating that each rat consumes about 40 ml/day of water. A-192621 (an ETB receptor antagonist, 30 mg/kg-day) was given by oral gavage twice a day (34). These doses of ABT-627 and A-192621 have been shown to be effective in blocking the ETA and ETB receptors in vivo (1, 20). At the end of the experiment, complete ET-1 delivery was confirmed by ensuring that the pump was empty, and the catheter was positioned in the external jugular vein.

Blood pressure measurement. Indirect tail-cuff systolic blood pressures were routinely obtained in conscious rats by use of a Narco Bio-Systems Electro-Sphygmomanometer (Austin). The pressure was measured starting 1 day before the treatment and continuously every 2–3 days after infusion of ET-1 or vehicle. The blood pressure value was used as the average of three separate measurements at each session. At the end of the experiment, rats were anesthetized with ketamine and xylazine (80 and 4 mg/kg ip, respectively), and the left carotid artery and jugular vein were cannulated for the measurement of mean arterial pressure (MAP) or intravenous administration of CGRP and ET-1 (128 and 3 mmHg) groups compared with Con (112 ± 1 mmHg), ET-1 (113 ± 3 mmHg), Con + ABT-627 (111 ± 2 mmHg), and ET-1 + ABT-627 (110 ± 3 mmHg) (P < 0.05) groups. MAP obtained from direct arterial measurement was consistent with the systolic blood pressure (baseline MAP in Table 1 and Fig. 1). Therefore, neither systolic blood pressure nor MAP was elevated by ET-1 infusion. However, daily oral administration of the ETB antagonist A-192621 increased systolic blood pressure and MAP in both control and ET-1-infused rats. In contrast, the ETA antagonist ABT-627 did not alter systolic blood pressure or MAP in either control or ET-1-infused rats (Fig. 1).

To determine whether endogenous CGRP plays a compensatory role in preventing the ET-1-induced increase in blood pressure, MAP and heart rate (HR) responses to bolus injection of CGRP (1 mg/kg in 200–300 μl of normal saline) were examined under the fully awake and unrestrained state of rats. The MAP elevation began ~15–20 s after administration of CGRP and reached the peak 40–60 s after administration. The pressor activity of CGRP lasted for ~120 s. The baseline and maximal changes of MAP and HR and the differences between the baseline and maximal changes are listed in Table 1. CGRP infusion led to a significant increase in MAP in rats receiving ET-1 compared with the control group (Fig. 2 and Table 1).

RESULTS

After 1 wk of treatment, systolic blood pressure was significantly increased in the Con + A-192621 (130 ± 4 mmHg) and ET-1 + A-192621 (128 ± 3 mmHg) groups compared with Con (112 ± 1 mmHg), ET-1 (113 ± 3 mmHg), Con + ABT-627 (111 ± 2 mmHg), and ET-1 + ABT-627 (110 ± 3 mmHg) (P < 0.05) groups. MAP obtained from direct arterial measurement was consistent with the systolic blood pressure (baseline MAP in Table 1 and Fig. 1). Therefore, neither systolic blood pressure nor MAP was elevated by ET-1 infusion. However, daily oral administration of the ETB antagonist A-192621 increased systolic blood pressure and MAP in both control and ET-1-infused rats. In contrast, the ETA antagonist ABT-627 did not alter systolic blood pressure or MAP in either control or ET-1-infused rats (Fig. 1).

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response induced by CGRP_8–37 was observed in rats receiving the ET_B antagonist with or without ET-1 but not in rats treated with the ET_A antagonist (Fig. 2 and Table 1).

Plasma CGRP concentrations in each of the six groups were shown in Fig. 3. Plasma CGRP levels were increased in ET-1 (57.5 ± 6.1 pg/ml), Con + A-192621 (53.9 ± 3.4 pg/ml), and ET-1 + A-192621 (60.4 ± 3.0 pg/ml) groups compared with Con (40.4 ± 1.6 pg/ml), Con + ABT-627 (40.0 ± 2.9 pg/ml), and ET-1 + ABT-627 (42.6 ± 1.9 pg/ml) groups (P < 0.05).

Therefore, circulating CGRP was significantly increased in response to ET-1 infusion. Plasma CGRP concentration was also significantly higher in rats receiving the ET_B antagonist with or without ET-1 infusion. In contrast, no significant difference in DRG CGRP content was observed among groups (Fig. 3).

Plasma ET-1 levels were higher in ET-1 (2.8 ± 0.2 pg/ml), ET-1 + ABT-627 (3.2 ± 0.4 pg/ml), Con + A-192621 (3.3 ± 0.4 pg/ml), and ET-1 + A-192621 (4.6 ± 0.3 pg/ml) groups compared with Con (1.1 ± 0.2 pg/ml) and Con-ABT-627 (1.3 ± 0.2 pg/ml) groups (Fig. 4, P < 0.05). Therefore, plasma ET-1 concentrations were increased in rats receiving the ET_B antagonist alone or in rats infused with ET-1 with or without receiving the ET_A antagonist. Co-administration of ET-1 plus the ET_B antagonist resulted in a further increase in plasma ET-1 concentration compared with rats receiving ET-1 infusion alone.

Double staining of the ET_A receptor and the marker of primary afferent fibers CGRP revealed that ET_A receptors were present in perivascular sensory nerve fibers and colocalized with CGRP (Fig. 5). All CGRP-positive sensory fibers appeared to express ET_A receptors.

As expected, these CGRP-positive sensory fibers consisted of only a subpopulation of the total perivascular nerve fibers, which were visualized by PGP9.5 (a general neuronal marker) (Fig. 5).

Table 1. Effect of intravenous administration of CGRP_8–37 on MAP and HR in conscious rats

<table>
<thead>
<tr>
<th>Group</th>
<th>MAP, mmHg</th>
<th>After CGRP_8–37</th>
<th>Δ</th>
<th>HR, beats/min</th>
<th>After CGRP_8–37</th>
<th>Δ</th>
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<td>Baseline</td>
<td></td>
<td></td>
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<tr>
<td>Con</td>
<td>104 ± 6</td>
<td>108 ± 5</td>
<td>4 ± 1</td>
<td>383 ± 6</td>
<td>380 ± 5</td>
<td>-4 ± 2</td>
</tr>
<tr>
<td>Con + ABT-627</td>
<td>104 ± 3</td>
<td>108 ± 4</td>
<td>4 ± 1</td>
<td>388 ± 13</td>
<td>387 ± 14</td>
<td>-2 ± 3</td>
</tr>
<tr>
<td>Con + A-192621</td>
<td>122 ± 4*</td>
<td>134 ± 5†</td>
<td>12 ± 1*</td>
<td>382 ± 12</td>
<td>371 ± 11†</td>
<td>-11 ± 4</td>
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<td>ET-1</td>
<td>106 ± 3</td>
<td>119 ± 3†</td>
<td>13 ± 1*</td>
<td>385 ± 9</td>
<td>374 ± 10†</td>
<td>-12 ± 1</td>
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<tr>
<td>ET-1 + ABT-627</td>
<td>100 ± 3</td>
<td>105 ± 3</td>
<td>5 ± 1</td>
<td>390 ± 10</td>
<td>387 ± 10</td>
<td>-3 ± 2</td>
</tr>
<tr>
<td>ET-1 + A-192621</td>
<td>119 ± 4*</td>
<td>133 ± 6†</td>
<td>15 ± 3*</td>
<td>378 ± 12</td>
<td>368 ± 11†</td>
<td>-10 ± 4</td>
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Values are means ± SE; n = 7–8 rats. CGRP, calcitonin gene-related peptide; Δ, difference between the peak value and baseline; ET-1, endothelin-1. Mean arterial pressure (MAP) and heart rate (HR) were measured by direct intra-arterial recording on day 7 of drug treatment in rats fully awake and unrestrained. Values for MAP and HR responses to calcitonin gene-related peptide antagonist (CGRP_8–37) represent maximal changes or peak values (after CGRP_8–37). *P < 0.05 vs. the control group (Con), †P < 0.05 vs. baseline.

Fig. 1. Systolic blood pressure (A) and mean arterial pressure (B) in rats infused with either endothelin-1 (ET-1) or vehicle, alone or in combination with ABT-627 or A-192621. Systolic blood pressure was measured by the tail-cuff method, and mean arterial pressure was measured by indwelling catheter in the conscious state on day 7 of the infusion. Values are means ± SE (n = 7–8). *P < 0.05 vs. control group (Con); †P < 0.05 vs. baseline.

Fig. 2. Changes in mean arterial pressure after administration of calcitonin gene-related peptide (CGRP) antagonist [CGRP_8–37] to rats infused with either ET-1 or vehicle, alone or in combination with ABT-627 or A-192621. Values are means ± SE (n = 7–8). *P < 0.05 vs. Con.
The present study provides new findings regarding the role of ET-1 in the regulation of CGRP release in the rat. Our results showed that blockade of the CGRP receptor by CGRP8–37 leads to an increase in blood pressure in chronically ET-1 infused rats, suggesting that ET-1 infusion leads to an increase in CGRP release, which plays a compensatory role in preventing ET-1-induced elevation in blood pressure. Indeed, we found that the plasma CGRP level, determined by radioimmunoassay, is increased during chronic ET-1 infusion. Furthermore, our data demonstrate that acute pressor responses to CGRP8–37 and increased plasma CGRP levels in ET-1-treated rats are abolished by an ET A receptor antagonist but not an ET B receptor antagonist, indicating that ET-1 promotes CGRP release via activation of the ET A receptor. Indeed, Gokin et al. (7) reported that peripheral administration of ET-1 induces nocifensive behavior that is reversible by administration of an ET A receptor antagonist, suggesting that ET A receptors are present in sensory afferents. This view is further supported by Pomoni et al. (21) who reported that ET A receptor immunoreactivity is found in CGRP-containing sensory neurons in DRG, whereas ET B receptor immunoreactivity is seen in DRG satellite cells or Schwann cells.

We observed that infusion of ET-1 increased plasma ET-1 concentration approximately two- to threefold without changing baseline blood pressure. The results are consistent with previous studies by Mortensen and Fink (17). They reported that chronic ET-1 infusion at 5 pmol·kg⁻¹·min⁻¹ (=12 ng·kg⁻¹·min⁻¹), a dose that was about two to three times higher than what has been used in the current study, did not cause an increase in blood pressure in rats fed a normal sodium

![Graph A](https://example.com/graphA.png)

**Fig. 3.** Immunoactive CGRP content in plasma (A) and the dorsal root ganglia (B, DRG) of rats infused with either ET-1 or vehicle, alone or in combination with ABT-627 or A-192621. Values are means ± SE (n = 7–8). *P < 0.05 vs. Con.

**Fig. 4.** Changes in plasma ET-1 concentration in rats infused with either ET-1 or vehicle, alone or in combination with ABT-627 or A-192621. Values are means ± SE (n = 7–8). *P < 0.05 vs. Con. #P < 0.05 vs. ET-1.
diet (2 meq/day). The blood pressure increased in these rats only when a high-salt diet was given (17). Therefore, despite the fact that ET-1 is a potent vasoconstrictor and prohypertensive factor, ET-1-mediated blood pressure regulation appears to be the result of the balance between ETA-mediated vasoconstriction, ET A-stimulated increases in CGRP release and vasodilation, and ET B-mediated vasodilation.

Indeed, several studies (6, 15) have shown that selective or nonselective ET B receptor antagonists increase plasma ET-1. This increase may be due to displacement of ET-1 from receptors into the circulation (15) and/or an antagonism of an ET-1 clearance pathway (6). In the current study, blood pressure increased when ET B receptors were blocked even in rats without receiving ET-1 infusion. This pressor response to ET B blockade may be due to impaired release of endothelial vasodilators (nitric oxide and prostacyclin) and/or increased ET A receptor stimulation via, at least in part, diminished ET-1 clearance (9). Interestingly, the elevation of systolic blood pressure reached a plateau after 4 days of treatment with the ET B antagonist. It is possible that the window of the tail-cuff measurement in this study was not long enough to show further elevation of blood pressure or that additional environmental stress such as high-salt intake would be needed to increase blood pressure further.

It is noted that, whereas plasma CGRP levels were not different between rats treated with ET-1 alone and rats treated with the ET B antagonist with or without ET-1, MAP was higher only in the latter two groups of rats. This raises the question as to, if CGRP played a role in ET-1-mediated blood pressure regulation, why was the blood pressure not at the same level in these three groups of rats? Although the mechanism(s) responsible for this phenomenon is unknown, the following possibilities exist. One is that increased plasma CGRP levels were insufficient in counteracting ET A-mediated
vasoconstriction without the participation of ET\textsubscript{B} as shown in the case in which rats were treated with the ET\textsubscript{B} antagonist with or without ET-1. Another possibility would be that intact ET\textsubscript{B} receptor function is obligatory for CGRP to play a compensatory effect on preventing ET-1-induced increase in blood pressure as shown in the case in which rats were treated with ET-1 alone.

Although the molecular mechanisms by which ET-1 stimulates CGRP release from sensory afferents are largely unknown, several lines of evidence support the hypothesis that ET-1 may exert its actions via protein kinase C (PKC) and/or prostaglandins pathways. It has been shown that ET-1 activates PKC via the ET\textsubscript{A} receptor in cardiac myocyte, as well as in other tissues (25, 32). Also, studies have shown that activators of PKC upregulate CGRP and calcitonin synthesis and release in primary cultures of nodose sensory ganglia and medullary thyroid carcinoma cell lines (2, 16). Alternatively, Wright and Malik (31) demonstrated that activation of the ET\textsubscript{A} receptor promotes prostacyclin synthesis in vascular smooth muscle through a PKC-independent mechanism. It is well established that prostacyclin directly stimulates CGRP release from sensory neurons or sensitizes the sensory neuron responses to other stimuli (11). These findings support the notion that prostacyclin produced by activation of the ET\textsubscript{A} receptor evokes or potentiates CGRP release.

**Perspectives.** The present study shows that the sensory neurotransmitter CGRP may participate in blood pressure regulation in ET-1-treated rats via an ET\textsubscript{A}-dependent mechanism. It is well documented that the endothelin system is activated in certain forms of salt-sensitive hypertension in both humans and animals (12, 23). Moreover, there is accumulating evidence showing that a defect in the sensory nervous system exists in spontaneously hypertensive rats and Dahl-salt hypertensive rats (13, 26). It is conceivable that sensory nerve dysfunction may contribute to the development of hypertension in these models of hypertension. Given the fact that endogenous CGRP may act as a mediator to buffer the elevation of blood pressure, it is reasonable to speculate that agents that modulate the sensory nervous system may be beneficial in treating hypertension as well as in reducing the long-term complications resulting from hypertension.

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