Endothelin-1 in hypertension in the baroreflex-intact SHR: a role independent from vasopressin release

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Rossi, Noreen F., Donal S. O’Leary, Dixon Woodbury, and Haiping Chen. Endothelin-1 in hypertension in the baroreflex-intact SHR: a role independent from vasopressin release. Am J Physiol Endocrinol Metab 279: E18–E24, 2000.—This study sought to identify whether central endothelin (ET) receptor activation contributes to the elevated pressure in spontaneously hypertensive rats (SHR) and whether an ET-stimulated vasopressin (AVP) release mediates the increased pressure. In Wistar Kyoto (WKY) rats, intracerebroventricular ET-1 induced a dose-dependent pressor response that was shifted rightward in SHR. ETA antagonism decreased mean arterial pressure in baroreflex-intact SHR (P < 0.01), consistent with inhibition of endogenous ET-1, and blocked the pressor response to exogenous ET-1 in both strains. ET-1 increased AVP only after sinoaortic denervation (P < 0.05). Contrary to WKY, sinoaortic denervation was required to elicit a significant pressor response with 5 pmol ET-1 in SHR. Sinoaortic denervation permitted ET-1 to increase AVP in both strains, and peripheral V1 blockade decreased pressure in denervated but not intact rats. After nitroprusside normalized pressure in SHR, the pressor and AVP secretory responses paralleled those in WKY. Thus endogenous ETA receptor mechanisms contribute to hypertension, independent of AVP, in baroreflex-intact SHR. Although blunted in the hypertensive state, the arterial baroreflex buffers the ET-1-induced pressor and AVP secretory responses in both strains. ANTIDIURETIC HORMONE; ARTERIAL BAROREFLEX; BLOOD PRESSURE; SPONTANEOUSLY HYPERTENSIVE RAT; WISTAR KYOTO RAT

INITIALLY IDENTIFIED AS POTENT VASOACTIVE PEPTIDES (38), THE ENDOTHELINS (ET) ARE NOW RECOGNIZED FOR THEIR ROLE IN CENTRAL REGULATION OF CARDIOVASCULAR FUNCTION (4, 5, 17, 21, 22, 31, 32). NEURONAL AND NONNEURONAL ET MRNAS AND PEPTIDES HAVE BEEN LOCALIZED TO HIPOTHALAMONEUROHYPOPHYSIAL AND MEDULLARY STRUCTURES (14, 20, 33). BOTH ETA AND ETB RECEPTORS EXIST WITHIN HIPOTHALAMIC NUCLEI AS WELL AS THE NUCLEUS TRACTUS SOLITARIUS, AREA POSTREMA, VENTROLATERAL MEDULLA, AND OTHER AREAS IMPLICATED IN CARDIOVASCULAR REGULATION AND VASOPRESSIN (AVP) RELEASE (1, 5, 12). THIS DISTRIBUTION OF ET AND ITS BINDING SITES SUGGESTS THAT ET ACTS AS A NEUROPEPTIDE WITHIN THE CENTRAL NERVOUS SYSTEM. ET-1 INJECTIONS INTO THE LATERAL CEREBRAL VENTRICLE CONSISTENTLY INCREASE SYSTEMIC ARTERIAL PRESSURE IN RATS, BUT THE HEART RATE RESPONSE VARIES (17, 21, 27, 28, 31). IN CONTRAST, INTRACISTERNAL INJECTION OR APPLICATION OF ET-1 TO THE VENTRAL MEDULLA EVOKES A BIPHASIC RESPONSE, WITH AN INITIAL INCREASE FOLLOWED BY A DECREASE IN ARTERIAL PRESSURE (6, 14). UNDER BOTH PARADIGMS, THE PRESSOR EFFECT IS ACCOMPANIED BY AUGMENTED SYMPATHETIC OUTPUT OR ELEVATED PLASMA CATECHOLAMINES (12, 15, 21, 27) AND CAN BE INHIBITED BY SYMPATHETIC BLOCKADE (6, 21, 27). THUS THE DATA SUPPORT A PRIMARY ROLE FOR ENHANCED SYMPATHETIC ACTIVITY MEDIATING THE PRESSOR EFFECT OF CENTRAL ET.

INCREASED SYMPATHETIC ACTIVITY IN CONCERT WITH IMPAIRED BAROREFLEX FUNCTION HAS BEEN IMPlicated IN THE DEVELOPMENT OF ELEVATED ARTERIAL PRESSURE IN SPONTANEOUSLY HYPERTENSIVE RATS (SHR) (3, 7, 8). SOME STUDIES HAVE SHOWN THAT CENTRAL ET INCREASES ARTERIAL PRESSURE DUE TO STIMULATION OF AVP RELEASE (15, 31); HOWEVER, ARTERIAL PRESSURE RISES EQUALLY IN NORMOTENSIVE LONG EVANS ANIMALS AND IN THE BRATTELBOOR STRAIN DEVOID OF CENTRAL AVP (27). ABLATION OF TISSUE IN THE ANTEROVENTRAL THIRD VENTRICLE REGION AVERS THE RISE IN ARTERIAL PRESSURE IN SEVERAL MODELS OF HYPERTENSION. HOWEVER, LESIONS OF THE ANTEROVENTRAL THIRD VENTRICLE FAIL TO REDUCE ARTERIAL PRESSURE IN THE SHR RAT (2, 4), NOR DO THEY BLOCK ET-INDUCED HYPERTENSION (28). IT IS NOT YET KNOWN TO WHAT EXTENT CENTRAL ET-INDUCED INCREASES IN SYMPATHETIC EFFERENT ACTIVITY OR AVP RELEASE, OR BOTH, MAY MEDIATE SHR HYPERTENSION.

THE PRESENT STUDIES WERE UNDERTAKEN TO TEST THE HYPOTHESIS THAT CENTRAL ET-1 MEDIATES, AT LEAST IN PART, THE ELEVATED BLOOD PRESSURE OBSERVED IN SHR, BUT THAT THIS INCREASE IS NOT DUE TO RELEASE OF AVP AND ACTIVATION OF V1 VASCULAR RECEPTORS.

METHODS

Adult male SHR or the normotensive Wistar Kyoto (WKY) rats, 11–12 wk of age (~225–250 g), were obtained from Harlan Sprague Dawley (Indianapolis, IN). They were housed under controlled conditions (21–23°C; lights on, 0700–1900) and had free access to water and standard rat chow (101 μmol Na/g of chow). The rats were cared for in accordance with the principles of the National Research Council’s Guide for the Care and Use of Laboratory Animals.

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Surgical Procedures

Sinoaortic denervation. The rats were anesthetized with pentobarbital sodium (40 mg/kg body wt ip). Sinoaortic denervation (SAD) or sham operation was performed by the method of Krieger (10). A ventral midline incision was made in the neck, and the sternocleidomastoid muscle was retracted. The aortic depressor nerve and accompanying fibers were identified. Denervation was accomplished by surgically sectioning the cervical sympathetic trunks, the aortic depressor nerve, and the superior laryngeal nerve. The carotid baroreceptors were denervated by cutting the carotid sinus nerve and stripping the area of the carotid sinus. Sham-operated animals underwent identical dissection, but the nerves were left intact.

Catheter placement. Carotid arterial and jugular venous catheters were placed, tunneled subcutaneously, and exteriorized at the back of the neck (27). Catheter patency was maintained by flushing with 50 μl heparin sodium, 1,000 U/ml. The rats were returned to their cages to recover.

Stereotaxic surgery. Three days after SAD or sham operation, rats that met testing criteria for baroreflex status (see Verification of SAD) were anesthetized with pentobarbital sodium (40 mg/kg body wt iv). Each rat was positioned within a cranial stereotaxic instrument (Kopf, Tujunga, CA), and a 22-gauge stainless steel guide cannula (Plastics One, Roanoke, VA) was inserted into the right lateral cerebral ventricle (coordinates to bregma: −0.3 anteroposterior; 1.3 mediolateral; +2.5 dorsoventral) and affixed with cranialplastic cement. A dummy cannula was placed into the guide cannula to maintain patency. The rat was then allowed to recover.

Verification of SAD

To assess baroreflex status, testing was performed on day 3 in conscious rats. The change in heart rate was ascertained in response to a 40-mmHg pressor stimulus evoked by 2.5–5 μg/kg phenylephrine or a 40-mmHg depressor stimulus elicited by 2–5 μg/kg nitroprusside in 200 μl of saline intravenously. Arterial pressure and heart rate were continuously monitored for 10 min before injection, during the stimulus, and for 10 min afterward. Only rats displaying >95% blunting of reflex changes in heart rate were considered SAD. Partially denervated animals were not used in experimental protocols. One hour after baroreflex testing, SAD and sham-operated rats underwent cannula placement (see Stereotaxic surgery).

Protocols

On postoperative days 4–7, each rat was conditioned to remain for 120 min within a Plexiglas study chamber (Brain tree Scientific, Braintree, MA). All protocols were performed on conscious conditioned rats on postoperative day 8. After the rat was positioned within the chamber, the arterial catheter was connected to a pressure transducer (Grass Instruments), and a 28-gauge infusion cannula whose internal tip projected 1 mm below the guide cannula was inserted. Systemic arterial pressure and heart rate were monitored continuously and recorded to disc by use of the Hemodynamic Monitoring Package (Biotech Products, Greenwood, IN).

Protocol 1: Dose-response relationship to central ET-1 in SHR and WKY rats. Systemic arterial pressure was assessed in response to graded step doses of intracerebroventricular ET-1 in intact, awake SHR and WKY rats. All doses were administered in 10 μl of artificial cerebrospinal fluid (CSF). Twenty minutes elapsed between injections. Subsequent experiments were performed using 5 pmol ET-1 to avoid a maximum pressor response in the SHR that might risk provoking cerebral hemorrhage and to use the dose to which the two strains displayed significantly different pressor responses (see Fig. 1).

Protocol 2: Hemodynamic and AVP responses to ET-1 in intact and SAD SHR and WKY rats. This series of experiments was designed to compare the effect of SAD on the pressor and AVP secretory responses to exogenous ET-1 and selective ET\(_A\) antagonism of endogenous ET-1 in both strains. Experiments were performed in conscious SAD and sham-denervated rats of both strains (27, 28). After a 30-min baseline period, a blood sample (800 μl) was collected over 30 s into prechilled heparinized tubes (25). The rat was then transfused via the venous catheter with an equal volume of donor blood that had been allowed to remain at room temperature for 30 min to allow intrinsic AVP to be catabolized. (Random AVP levels of donor blood ranged from 0.1 to 0.18 pg/ml.) Then the rat received a 10-μl intracerebroventricular injection over 10 s of one of the following dissolved in artificial CSF: 1) artificial CSF, 2) 5 pmol ET-1, 3) 5 pmol ET-1 with 40 nmol cyclo-D-Asp-Pro-D-Val-Leu-D-Trp (BQ-123), or 4) 40 nmol BQ-123, as determined in earlier experiments (26–28). Ten minutes and 60 min after the intracerebroventricular injection, blood was again collected and the rat transfused as before. All rats had free access to water during the experiment.

Protocol 3: Hemodynamic and AVP responses to ET-1 in intact and SAD SHR after normalization of arterial pressure. WKY and SHR are not congenic strains. Biological variability in WKY rats may be greater than initially recognized, so use of WKY rats as controls for SHR is less than ideal (11). Thus experiments were carried out in SHR treated such that their arterial pressure was identical to that in the normotensive WKY rats. After a 30-min baseline period, SAD and sham-operated SHR received a continuous intravenous infusion of nitroprusside (20–80 μg · kg\(^{-1}\) · min\(^{-1}\) or vehicle) to achieve a mean arterial pressure (MAP) equivalent to that of WKY rats. Nitroprusside infusion was continued, and MAP and
heart rate were allowed to equilibrate for a second 30-min period followed by intracerebroventricular injection and blood collection as in protocol 1.

Protocol 4: Pressor response to ET-1 after antagonism of vascular V₁ vasopressinergic receptors. This set of experiments was performed to identify whether the hypertensive response to central ET-1 is due to stimulation of AVP release into the circulation, which in turn induces the rise in arterial pressure. After a 30-min baseline period, each rat received a 50-µl bolus intravenous injection of either vehicle (150 mM NaCl) or V₁ antagonist, 1.5 50-g bolus intravenous injection of either vehicle (150 mM NaCl) or V₁ antagonist, 1.5 µg/kg [1-β-mercapto-β-γ-cyclopentamethylenepropionic acid], 2-(Methyl)tyrosine) vasoressin (exogenous AVP), followed by constant infusion of either vehicle or 0.75 µg·kg⁻¹·h⁻¹ V₁ antagonist at 2.7–3.3 µl·kg⁻¹·min⁻¹. Five minutes later, either artificial CSF or 10 pmol ET-1 were injected centrally. At this dose, the V₁ antagonist blocks the pressor effect of exogenous aqueous AVP infused at 1.2 µg·kg⁻¹·h⁻¹ from 27 ± 3 mmHg to 1 ± 2 mmHg (P < 0.0005) (27). A maximally stimulating dose of ET-1 was used for these experiments to permit comparison with published data (26–28).

Each rat was used for only one protocol and was euthanized with pentobarbital sodium, and cannula placement was verified with methylene blue.

Analytical and Statistical Methods

The Hemodynamic Monitoring Package (Biotech Products) calculated and displayed MAP and heart rate beat by beat for each experiment. Values were recorded on computer disc for off-line analysis. Data were sampled at 6 Hz with a DAP 3216a/415 data acquisition processor as the hardware platform. For protocols 2 and 3, reported values are the running average of the last 3 min before each blood sample. Baroreflex gain for sham and SAD rats was calculated as the change in heart rate divided by the change in MAP. Plasma osmolality was measured by freezing point depression (Precision Systems 5004, Sudbury, MA). Plasma AVP concentration was assessed using methods previously reported (25).

Values for MAP, heart rate, plasma osmolality, and plasma AVP concentrations at 10 and 60 min were compared with baseline values by ANOVA and the Tukey-Kramer analysis for multiple comparisons within groups. The F ratio and modified statistic were used for nonsimultaneous comparisons among groups. All data are reported as means ± SE. A significant difference among the means was assigned at P < 0.05.

RESULTS

Baseline MAP was higher in the intact SHR compared with WKY rats, 156 ± 8 vs. 128 ± 2 mmHg (n = 5 and 5, respectively, P < 0.025), but heart rate did not differ significantly, 391 ± 22 vs. 406 ± 23 beats/min. ET-1 induced a dose-dependent rise in MAP in both strains. The response was shifted to the right in SHR (Fig. 1). In WKY rats, MAP was significantly elevated compared with artificial CSF at doses >5 pmol (P < 0.05), whereas in the SHR strain, 10 pmol of ET-1 were required to achieve an equivalent rise in MAP (P < 0.01 vs. artificial CSF). Only at the 5-pmol level did the pressor response differ between the two strains.

Baroreflex gain was attenuated in the sham-operated SHR compared with WKY rats (Table 1). SAD abolished pressor-induced bradycardia in both strains. The tachycardic response to a 40-mmHg decrease in MAP was also eliminated (data not shown).

Table 2 shows the baseline parameters for all rats on day 8. MAP was significantly higher in the sham-operated SHR compared with sham-operated WKY rats. MAP was also higher in the SAD WKY rats compared with sham-operated WKY rats. In contrast, MAP was higher in the SAD SHR compared with SAD WKY rats but did not differ from intact SHR. No differences were observed in the other parameters.

As depicted in Fig. 2, MAP rose significantly after ET-1 in sham WKY rats at the end of 10 min. The pressor response was blocked by ETₐ antagonism. Pulse pressure did not change. The increase in MAP after ET-1 was significantly augmented by SAD. In contrast, sham SHR displayed little, if any, change in MAP at this dose of ET-1. MAP did rise significantly in the SHR after SAD. Notably, BQ-123 not only blocked the pressor response to ET-1 in SAD SHR but also significantly decreased MAP in both sham and SAD SHR compared with artificial CSF. The ETₐ antagonist inhibited the ET-1-induced pressor response in WKY rats but did not decrease MAP compared with artificial CSF in sham WKY rats. Although MAP decreased 9.2 ± 1.4 mmHg from baseline after BQ-123 in SAD WKY rats, this value did not achieve significance compared with the group that received artificial CSF. Plasma AVP levels increased significantly after ET-1 after SAD in both strains. This response was blocked by BQ-123.

The change in heart rate was 56 ± 16 beats/min after ET-1 compared with −3 ± 6 beats/min with artificial CSF in sham denervated WKY rats (P < 0.01). A

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<th>Table 1. Verification of sinoaortic denervation on day 3</th>
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<td><strong>Baseline</strong></td>
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Values are means ± SE. HR, heart rate; MAP, mean arterial pressure; WKY-S, Wistar Kyoto sham; WKY-D, WKY sinoaortic denervated; SHR-S, spontaneously hypertensive rats sham; SHR-D, SHR denervated. *P < 0.01 vs. WKY-S; †P < 0.005 vs. baseline; ‡P < 0.001 vs. sham.
similar tachycardia was observed in SAD WKY rats after ET-1, 74 ± 33 vs. −11 ± 4 beats/min with artificial CSF (P < 0.05). BQ-123 prevented the tachycardia: 4 ± 9 and −10 ± 7 beats/min in sham and SAD groups, respectively. ET-1 did not induce a significant change in heart rate in either sham or SAD SHR; 18 ± 22 vs. 4 ± 16 beats/min (artificial CSF vs. ET-1, sham) and 4 ± 4 vs. 0 ± 17 beats/min (artificial CSF vs. ET-1, SAD).

After 60 min, the effect of ET-1 was sustained in the SAD WKY rats. MAP remained elevated above baseline by 11.6 ± 2.7 mmHg. The depressor effect of BQ-123 was prolonged but highly variable, −5.0 ± 4.1 mmHg. Neither value differed significantly from artificial CSF, −0.6 ± 1.4 mmHg. In SAD SHR, the change in MAP did not differ with ET-1 (4.9 ± 2.7 mmHg) compared with artificial CSF (3.4 ± 1.7 mmHg). By the end of 60 min, the changes in plasma AVP values dissipated in the SAD rats. No other differences occurred between the strains.

Data from the set of experiments in SHR whose systemic arterial pressure was normalized before injection with ET-1 are displayed in Table 3. Baroreflex gain was 1.51 ± 0.06 beats · min⁻¹ · mmHg⁻¹ in the sham-operated rats (n = 34) and −0.06 ± 0.02 beats · min⁻¹ · mmHg⁻¹ in the SAD rats (n = 40, P < 0.0001). Heart rate and MAP were comparable among the groups before nitroprusside treatment. With nitroprusside infusion, MAP decreased to values observed in the intact WKY strain. MAP values were comparable in sham and SAD SHR and, by design, were significantly lower than in their respective vehicle-treated controls. Plasma osmolality did not differ among the groups. Plasma AVP concentrations rose to similar levels in both nitroprusside-treated groups consistent with the decline in arterial pressure.

Figure 3 shows that, when MAP was brought down to a value observed in WKY rats, ET-1 elicited a significant rise in MAP in SAD SHR (P < 0.05 vs. that of ET-1-treated SAD SHR in Fig. 2). The ET-1-induced increase in MAP in SHR with normalized arterial pressure paralleled that seen in the respective sham and SAD WKY rats. Despite the higher plasma AVP concentration associated with nitroprusside treatment, ET-1 induced a further rise in plasma AVP levels in the SAD SHR (Fig. 3B). SAD SHR treated with nitroprusside displayed significantly higher plasma AVP values than SAD SHR that remained hypertensive (P < 0.05 vs. ET-1-treated SAD SHR in Fig. 2). ETₐ antagonism blocked both the pressor and the AVP responses. BQ-123 given alone did not change MAP in nitroprusside-treated SHR. Heart rate was not significantly changed in any of the groups (data not shown).

The V₁ antagonist significantly attenuated the pressor response to central ET-1 only after SAD in both SHR and WKY rats (Fig. 4).

**DISCUSSION**

Five major findings emerge from the present data. 1) The pressor response to central ET-1 is shifted to the right in SHR. 2) ETₐ blockade significantly decreases...
baseline arterial pressure only in SHR. 3) ET-1-induced AVP release achieves pressor levels only after SAD. 4) V1 antagonism attenuates the ET-1-induced pressor response in SAD but not in sham-operated rats. 5) Normalization of MAP with nitroprusside in SHR augments their pressor response and enhances plasma AVP levels after SAD.

ET-1 induces a dose-dependent increase in MAP in WKY rats comparable to that observed in other normotensive strains (21, 22, 27, 28). The rightward shift in the dose-response relationship seen in the SHR is consistent with the higher plasma ET-1 values (9) and with diminished tissue levels of ET-1 and ET receptor binding in the hypothalamus and ventrolateral medulla of SHR compared with WKY rats (5). Together, these observations suggest that endogenous ET-1 may act at central nervous system loci outside the bloodbrain barrier, such as the subfornical organ (30), to increase systemic arterial pressure and to downregulate hypothalamic ET receptors in SHR.

Additional support for this contention is provided by the present data showing that ETA antagonism decreases arterial pressure in both sham and SAD SHR but that it has no effect on normotensive WKY rats with intact baroreceptor afferents. Admittedly, systemic arterial pressure in SHR is not normalized with BQ-123 treatment, because the decline in MAP averages ~10 mmHg. Nonetheless, this accounts for approximately one-third of the difference between MAP in SHR and WKY rats at this age. Thus endogenous activation of central ETA receptors at least partly mediates the hypertension in SHR. Studies in anesthetized rats were not able to demonstrate a decrease in arterial pressure after ETA inhibition in SHR, except in the stroke-prone substrain (19). The apparent disparity with the present study may be due to the enhanced sympathetic efferent activity typically associated with anesthesia, which may mask the effect of ETA antagonism.

Several laboratories (6, 21–24) including our own (27) have shown that the pressor effect of central ET-1 is due to enhanced sympathetic outflow. Increased activity of the sympathetic nervous system has long been recognized to play a role in SHR hypertension (8). Some reports have also suggested that AVP mediates the pressor response to ET-1 (17, 18, 21). However, ET-1 prompts equivalent increases in the Long Evans rat and its mutant counterpart, the Brattleboro rat, which lacks circulating AVP (27). The current data show that AVP also does not mediate the pressor response to ETA receptor activation in baroreflex-intact SHR and WKY rats, because V1 blockade does not alter the pressor response. In addition, plasma AVP levels do not change with the ETA receptor agonist or antagonist. Notably, lesions of the anteroventral third ventricular region do not prevent hypertension in SHR (2, 4) and not only fail to block, but actually augment, ET-1-induced pressor activity, even though AVP release is suppressed (28).

After SAD renders AVP release independent of systemic hypertension, plasma AVP values achieve pressor levels with ET-1 administration. V1 receptor antagonism is then able to attenuate the rise in arterial pressure in both strains. Thus circulating AVP contributes to the ET-1 pressor response only under conditions in which arterial baroreflex buffering of the pressor response and AVP secretion is impaired.

Normalization of arterial pressure in both shamoperated and arterial baroreflex-impaired SHR by infusion of nitroprusside is associated with a pressor response to ET-1 closely paralleling that in the respective WKY groups. Hence, the lower baseline arterial pressure itself, rather than the strain, is responsible for the enhanced pressor response. Only the SAD SHR given nitroprusside display a significantly enhanced pressor response, consistent with lack of reflex modulation of arterial pressure.

A parallel pattern is exhibited by the AVP response. Nitroprusside administration alone evokes an increase in plasma AVP levels in sham SHR equivalent to that in SAD SHR. Regulation of AVP release is under both arterial and cardiopulmonary baroreflex control, and the cardiopulmonary reflex remains intact after SAD. Because nitroprusside decreases left atrial and ventricular pressures (23), the cardiopulmonary baroreceptors are unloaded, and AVP is released in both sham

| Table 3. Baseline values in nitroprusside-treated SHR, day 8 |
|---------------------------------|-----------------|-----------------|-----------------|-----------------|
|                                | Baseline        | SNP/Vehicle     |                |                |
|                                | HR, beats/min   | MAP, mmHg       | HR, beats/min  | MAP, mmHg      | P\text{\textsubscript{osm}}, mosmol/kgH\textsubscript{2}O | P\text{\textsubscript{AVP}}, pg/ml |
| SHRS-Vehicle                   | n = 16          |                 | n = 18         |                 |
| Baseline                       | 391 ± 19        | 156 ± 6         | 421 ± 11       | 156 ± 6        | 300 ± 2         | 2.5 ± 0.4        |
| SHRS-SNP                       |                 |                 |                |                |                |                |
| SHRS-SNP                       | 393 ± 10        | 158 ± 5         | 376 ± 29       | 124 ± 3\textsuperscript{†} | 303 ± 2         | 14.3 ± 3.0\textsuperscript{‡} |
| SHRS-SNP                       | 407 ± 12        | 150 ± 3         | 415 ± 21       | 156 ± 5        | 303 ± 1         | 2.4 ± 0.3        |
| SHRS-SNP                       | 416 ± 10        | 161 ± 4         | 440 ± 12       | 122 ± 2\textsuperscript{†} | 306 ± 1         | 14.5 ± 3.0\textsuperscript{†} |

Values are means ± SE. SNP, sodium nitroprusside. *P < 0.001 vs. baseline same strain and treatment; ‡P < 0.01 vs. SHR-S vehicle; †P < 0.001 vs. SHR-D vehicle.
and SAD SHR. These data confirm findings in intact and SAD dogs (34). In addition, others have shown that intravenous or intracarotid administration of nitroprusside does not exert a direct central effect on AVP secretion independent of its actions to unload the baroreceptors (20, 34). Despite the already high levels of AVP, ET-1 prompted a further rise in plasma AVP in the SAD SHR, some values exceeding 35 pg/ml. Accordingly, acute central ET-1 administration can be a very powerful central stimulus for AVP release, but only when arterial baroreflex inhibition of AVP secretion is averted.

In addition, SHR with intact arterial baroreceptor afferents display a minimal increase in circulating AVP beyond that induced by the drop in pressure associated with nitroprusside treatment alone. Because the 30-min duration of nitroprusside pretreatment is long enough for baroreceptor resetting to occur (3), it is likely that the similarity of AVP responses to ET-1 by baroreflex-intact WKY rats, as well as hypertensive and normotensive sham-operated SHR, is due to baroreflex buffering of AVP secretion.

Although BQ-123 blocks the pressor effect of exogenous ET-1 in nitroprusside-treated SHR rats, ETA antagonism does not elicit a further decline in arterial pressure below the postnitroprusside values. Several mechanisms may account for this finding. Nitric oxide donors inhibit ET-1 production (11). Because a primary site of action of central ET-1 is the subfornical organ, which lies outside the blood-brain barrier, it is possible that intravenously administered nitroprusside may lead to decreases in endogenous ET-1 production. In addition, recent data suggest that blockade of nitric oxide formation unmasks ETA receptor-associated increases in sympathetic efferent activity in SHR but not WKY rats (11). It may be inferred, therefore, that ETA inhibition in nitroprusside-treated SHR rats does not produce a decline in arterial pressure, because nitric oxide may decrease endogenous ET-1 production or its action on the sympathetic outflow, or both. Further studies would be required to establish whether these mechanisms are involved.

In summary, endogenous ETA receptor mechanisms contribute to the hypertension observed in SHR. The elevation in arterial pressure is independent of AVP secretion. The arterial baroreflex buffers the ET-1-induced pressor and AVP secretory responses in both WKY and SHR strains but is blunted when the baseline pressure is increased. Similar mechanisms may play a role in essential hypertension.

![Figure 3](https://example.com/fig3.png)

Fig. 3. Changes from values after treatment with either vehicle or sodium nitroprusside (see Table 3) for MAP (A) and plasma AVP (B) at the end of period 1 after icv injection of artificial CSF (open bar), 5 pmol ET-1 (solid bar), 5 pmol ET-1 with 40 nmol BQ-123 (gray bar), or 40 nmol BQ-123 (hatched bar) in the following groups of rats: SHR-S (n = 4, 4, 4, and 4), SHR-D (n = 5, 6, 5, and 4), sodium nitroprusside-treated SHR-S (n = 6, 4, 4, and 5), and SHR-D (n = 5, 4, 5, and 4). Data are means ± SE. *P < 0.05, **P < 0.01 vs. artificial CSF for same group; †P < 0.05 vs. ET-1 for same group; ‡P < 0.05 vs. ET-1 in SHR-S group. All comparisons are by ANOVA.

![Figure 4](https://example.com/fig4.png)

Fig. 4. Change in MAP in sham (S) and sinoaortic denervated (D) SHR and WKY rats after bolus iv injection and infusion of V1 antagonist given icv artificial CSP (open bar) or 10 pmol ET-1 (solid bar) or after intravenous saline vehicle followed by icv injection of 10 pmol ET-1 (hatched bar). Data are means ± SE. *P < 0.01 vs. V1 antagonist plus artificial CSF; †P < 0.05 vs. V1 antagonist plus ET-1 for same group. All comparisons are by ANOVA.
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REFERENCES


