

Volume Expansion Enhances Plasma Endothelin-1

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We hypothesized that acute volume expansion by saline infusion triggers the release of endothelin-1. Bolus intravenous saline infusion (8 mL/min) in six groups of conscious Wistar rats and spontaneously hypertensive rats did not change mean arterial pressure or heart rate ($n = 8$ to 12). At 1 min after infusion, the plasma endothelin-1 level was significantly increased in Wistar rats and in spontaneously hypertensive rats by 42% and 61%, respectively (unpaired data). In 12 Wistar rats, the endothelin-1 level increased from 0.68 ± 0.13 to 1.19 ± 0.17 fmol/mL (mean \pm SEM, $P < .0001$, paired data). Thus, acute

volume load by rapid saline infusion increases plasma endothelin-1 levels. Vasoconstriction induced by endothelin-1 may counteract enhanced circumferential stretch created by volume expansion. *Am J Hypertens* 2003;16:1057-1061 © 2003 American Journal of Hypertension, Ltd.

Key Words: Endothelin, enzyme-linked immunoassay, vasoconstriction, hypervolemia, hoop (circumferential) stretch.

Endothelin-1 (ET-1) is a potent vasoconstrictor peptide hormone of 21 amino acids. It is predominantly produced by vascular endothelial cells.¹ Many factors are known to stimulate endothelin release, such as hormones, growth and metabolic factors, hypoxia, and mechanical forces acting on the vascular wall.² Among the mechanical forces, vascular shear stress and circumferential stretching may play a predominant role in endothelin release.^{3,4} The circumferential expansion of the blood vessels during the cardiac cycle is also known as hoop stretch.

Fyhrquist et al⁵ showed that in anesthetized Wistar rats, basal and volume-stimulated atrial natriuretic peptide (ANP) release was decreased by pretreatment with endothelin antibodies. They suggested that endothelin physiologically modulates ANP release. Furthermore, the specific blockade of the endothelin ET_A receptors blunts the peak ANP response to rapid infusion of saline in conscious Wistar rats.⁶ Thus, endothelin appears to stimulate the ANP response to acute volume overload through the ET_A receptor.

At present, despite good evidence of the involvement of endothelin in the physiologic ANP response to acute volume load, plasma endothelin concentrations have not been measured under such conditions. Different rat models of hypertension based on chronic volume overload such as DOCA-salt or 1-kidney, 1-clip hypertension (Goldblatt

hypertension) (1K1C Goldblatt rats) present increased plasma ET-1 levels.⁷⁻⁹ The present study investigates the effect of acute volume load by rapid saline infusion on plasma ET-1 levels in conscious normotensive Wistar and spontaneously hypertensive rats (SHR).

Methods

Experimental Animals

Male Wistar rats and SHR of 8 weeks of age, weighing 220 to 246 g, were purchased from Iffa Credo (L'Arbresle, France). Rats were housed in transparent plastic cages in a quiet room at 22°C and maintained on standard rat chow containing 0.22% sodium and 0.20% potassium and tap water ad libitum. The Governmental Animal Care Committee approved the study protocols.

Twenty-four hours before the study day, rats were anesthetized by inhalation of 2% halothane in oxygen. For arterial blood pressure (BP) measurements and blood sampling, polyethylene (PE) catheters were inserted into a femoral artery. Catheters consisted of a 6-cm PE-10 intravascular segment that was welded to a 25-cm PE-50 tubing. For saline infusions into the femoral vein, similar catheters and tubings were used but the tip of the intravascular segment was replaced by a less rigid 2-cm silicone tubing of 0.3-mm internal and 0.6-mm external diameter (Ulrich Swiss, St. Gallen, Switzerland). The

Received June 3, 2003. First decision June 19, 2003. Accepted July 9, 2003.

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This work was supported by the Cardiovascular Research Founda-

tion, Lausanne, Switzerland.

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PE-50 tubings of both the intra-arterial and the intravenous catheters were threaded under the skin and exteriorized at the back of the neck. The catheterized rats were housed individually in plastic cages and kept in a quiet place at 22°C.

Study Protocol

On the study day, conscious rats were placed in a plastic tube for partial immobilization. After a 20-min rest, mean arterial pressure (MAP) and heart rate (HR) were monitored using the Notocord computerized system (Paris, France). Then 2 mL of arterial blood was collected for endothelin measurement from control Wistar rats (group A, $n = 11$) and control SHR (group B, $n = 8$).

To four different groups of Wistar rats and SHR, 8 or 10 mL of 0.9% saline were rapidly infused in 1 min (Sage Instruments, Cambridge, MA). At 1 min after the end of the saline infusion, arterial plasma ET-1 was measured, and MAP and HR were registered. In Wistar rats, one group (C, $n = 11$) received 8 mL of saline without being bled for endothelin measurement before the saline infusion. Another group (D, $n = 12$) consisting of two lots of 7 and 5 Wistar rats received 10 mL of saline after an initial 2-mL bleeding for endothelin measurement. In SHR, one group (E, $n = 11$) received 10 mL of saline without initial bleeding for endothelin measurement and another group (F, $n = 9$) received 10 mL of saline after an initial 2-mL bleeding.

Blood Sampling

Two milliliters of blood were collected from the femoral artery of conscious rats through an indwelling catheter. Blood was immediately transferred into prechilled glass tubes containing tripotassium ethylenediaminetetraacetate to achieve by mixture a final plasma concentration of 7 mmol/L. Blood was centrifuged at 4°C for 10 min at 1660 g. Plasma (1.2 mL) was stored in polypropylene tubes at -20°C.

Determination of Dilution Factor

Hematocrit (Hct) was assessed in heparinized capillary tubes (Baxter Healthcare Corp., Deerfield, IL) after 10 min of centrifugation in a bench-top microcentrifuge (Hettich, L'Abergement, Switzerland). The plasma dilution factor (DF) due to saline infusion was defined as the ratio of the relative plasma volume after infusion ($100 - \text{Hct}_A$) over the relative plasma volume before infusion ($100 - \text{Hct}_B$): $DF = (100 - \text{Hct}_A)/(100 - \text{Hct}_B)$.

Measurement of ET-1

Plasma immunoreactive ET-1 was measured by liquid phase extraction and subsequent ELISA, as previously described in detail.⁹ Briefly, endothelin was extracted from 1 mL of plasma by acetone-HCl (recovery 55% to 75%) and specifically quantitated by a sandwich-type ELISA. The detection limit in plasma was 0.05 fmol/mL.

Endothelin concentrations after saline infusion were corrected for plasma dilution by multiplying measured levels with the DF, which varied between 1.04 and 1.26.

Statistical Analysis

For comparison of hemodynamic means, a one-way analysis of variance was performed followed by Tukey's multiple comparison test. Endothelin concentrations before and after saline infusion were evaluated by unpaired or paired Student *t* test as appropriate. The significance level was $P < .05$.

Results

Endothelin Response to Acute Volume Load

Fig. 1 demonstrates the effect of saline infusion in Wistar rats (left panel) and SHR (right panel) for unpaired and paired data (upper and lower panel, respectively).

In normotensive Wistar rats, plasma ET-1 was 42% higher after saline infusion (8 mL) at 1.26 ± 0.13 fmol/mL (group C) as compared to control rats at 0.89 ± 0.09 fmol/mL (group A) ($P < .05$). Fig. 1 also demonstrates individual increases in plasma ET-1 after saline infusion (10 mL) in the 12 rats where ET-1 was measured both before and after saline infusion (group D). Plasma ET-1 increased from 0.68 ± 0.13 to 1.19 ± 0.17 fmol/mL ($P < .0001$). The average increase was 84%. This increase in ET-1 after 10 mL of saline was greater than the enhancement found after infusion of 8 mL of saline ($P < .05$, unpaired *t* test, rank order comparisons for 8-mL infusions).

In the SHR, plasma ET-1 was 61% higher after saline infusion at 1.05 ± 0.14 fmol/mL (group E) as compared to control SHR at 0.65 ± 0.03 (group B) ($P < .05$). Fig. 1 (lower right panel) also depicts individual changes in plasma ET-1 after saline infusion in the nine SHR whose ET-1 was measured both before and after saline infusion (group F). Mean plasma ET-1 concentration increased from 0.64 ± 0.03 to 0.81 ± 0.03 fmol/mL ($P < .02$, paired *t* test). The average increase was 28%. Among the nine SHR of this group F, six increased and two decreased ET-1 levels in response to saline infusion, whereas one rat kept ET-1 unchanged.

BP and Heart Rate Response

Table 1 shows effects of blood sampling and saline infusion on BP and heart rate in Wistar rats and SHR. After the initial 2-mL bleeding, Wistar rats tended to increase heart rate and fairly maintain BP, whereas SHR had reduced heart rate ($P < .05$) and their BP decreased ($P < .05$). Saline infusion reversed these hemodynamic changes toward baseline. Immediately after the initial blood sampling, BP in SHR was considerably lower than in unbled control SHR ($P < .01$). No such difference was found in Wistar rats.

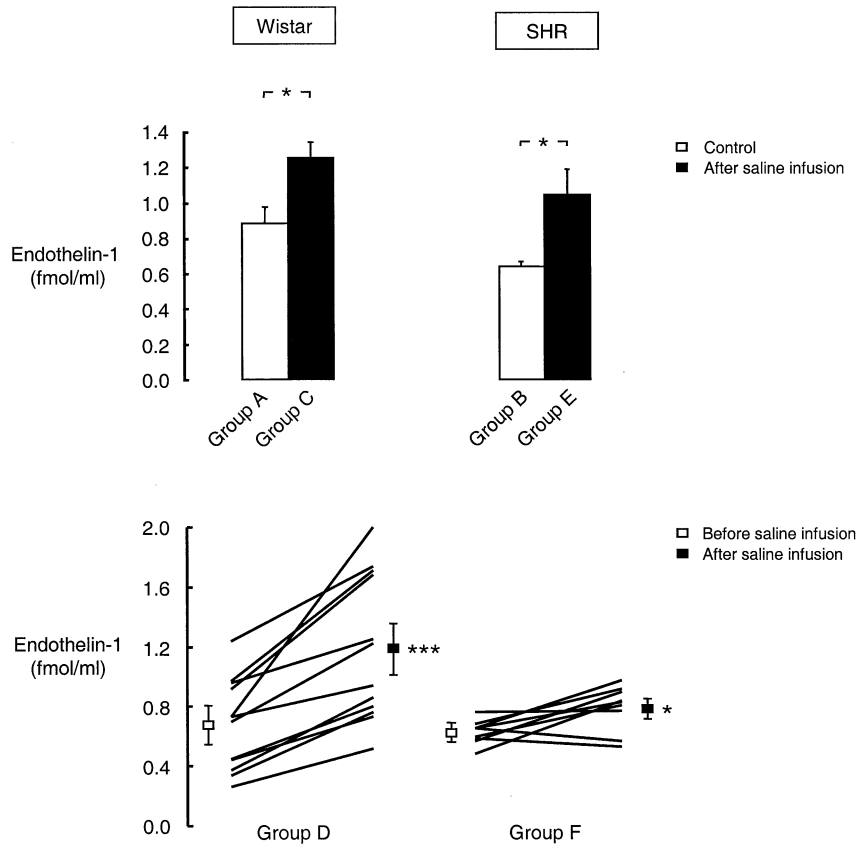


FIG. 1. (Upper left panel) Intravenous 1-min infusions of 8 mL of physiologic saline in Wistar rats enhances plasma immunoreactive endothelin-1 by 42% ($*P < .05$, unpaired t test). (Upper right panel) Intravenous 1-min infusions of 10 mL of physiologic saline in SHR enhances plasma endothelin-1 by 61% ($*P < .05$, unpaired t test). (Lower left panel) Plasma endothelin-1 levels in 12 normotensive Wistar rats before and after 1-min intravenous infusions of 10 mL of physiologic saline: endothelin-1 levels were increased in all rats at the end of the infusion ($P < .0001$). The lowest five levels represent a complete second lot of Wistar rats. (Lower right panel) Plasma endothelin-1 levels in nine SHR before and after 1-min intravenous infusions of 10 mL of physiologic saline: endothelin-1 levels were significantly increased at the end of the infusion despite the lack of response in three rats ($P < .02$). Mean \pm SEM, $n = 8$ to 12, $*P < .05$, $***P < .0001$.

Discussion

Acute volume load by rapid infusion of saline increases plasma ET-1 levels in Wistar rats and in SHR. In Wistar rats, a net volume enhancement of 8 mL led to similar final ET-1 levels of 1.2 fmol/mL in two differently tested groups of rats (C without initial bleeding, D with 2 mL of initial bleeding). Nevertheless, the 10-mL saline infusion

after an initial 2-mL bleeding increased the endogenous ET-1 by 84% (paired data, 12/12 rats increased plasma ET-1), ie, more than the 8-mL saline infusion without blood sampling at baseline (+42%, unpaired data). This enhanced ET-1 response may be due to various reasons. The baseline 2-mL hemorrhage certainly stimulated renal renin secretion and hence increased angiotensin II levels, which are known to trigger ET-1 release.¹⁰ Kowano et al¹¹

Table 1. Blood pressure and heart rate in Wistar rats and SHR (mean \pm SD, $n = 8$ to 12)

Rat Species	Blood Pressure (mm Hg)			Heart Rate (min^{-1})		
	Baseline	Blood Sampled*	Saline Infused	Baseline	Blood Sampled*	Saline Infused
Wistar						
Not bled	119 \pm 3	119 \pm 3	117 \pm 2	446 \pm 8	446 \pm 8	428 \pm 8
Bled	121 \pm 3	113 \pm 4	108 \pm 3	448 \pm 4	475 \pm 5	437 \pm 3
SHR						
Not bled	154 \pm 2	156 \pm 1	153 \pm 1	398 \pm 5	387 \pm 7	401 \pm 8
Bled	152 \pm 8	118 \pm 10†‡	137 \pm 6	410 \pm 9	332 \pm 34†	365 \pm 19

SHR = spontaneously hypertensive rats.

* Ten μl of blood for hematocrit measurement in all rats; two ml of blood for endothelin-1 measurement in "bled" rats.

† $P < .05$ versus baseline, ‡ $P < .01$ versus not bled.

have measured concomitantly increased plasma renin activity and ET-1 concentrations in anesthetized Wistar rats after withdrawing a similar volume of blood (7 mL/kg). In our conscious rats, 10 mL of saline was infused after a 2-min period of hypovolemia. The enhanced ET-1 response may, therefore, be due to both the 2-mL hemorrhage at baseline and to the larger volume of saline infused (10 v 8 mL).

Contrasting with previous results,⁹ baseline ET-1 levels in Wistar rats were not higher than in SHR. Very low ET-1 concentrations in one lot of rats enhanced the scatter of ET-1 levels in Wistar rats, whereas SHR had consistently low plasma ET-1. In SHR, all infusions consisted of 10 mL of saline. The infusions increased the low endogenous plasma ET-1 levels by merely 28% when 2 mL of blood were collected before saline infusion, and by 61% without prior blood sampling. In six of nine SHR, saline infusion actually increased plasma ET-1, whereas two SHR responded with a slight decrease in ET-1 and in one SHR, the ET-1 level remained unchanged.

Our findings in conscious rats are consistent with the modulatory effect of endothelin on ANP release after rapid saline infusion, as previously demonstrated by the use of endothelin antibodies⁵ or ET_A receptor blockers.⁶ The rapid volume expansion by saline infusion triggers a compensatory release of ANP that is lost in the presence of endothelin antibodies or ET_A receptor blockers.^{5,6} Circulating endothelin may directly stimulate atrial myocytes to secrete ANP or indirectly by vasoconstriction and volume centralization. It was therefore likely that saline infusion would stimulate endothelin release. Our measurements in Wistar rats and SHR verify this hypothesis. We used the same infusion technique as did Fyhrquist et al⁵ to obtain conclusive results. Baertschi et al⁶ had also observed an ET-1-mediated increase in plasma ANP after rapid saline infusion (6.6 mL/min) and even to slow infusions (3.3 mL/8 min). It would be of interest to confirm our finding that circulating ET-1 also increased after the more physiologic slow infusions. Nevertheless, previous work in rats also suggested⁷⁻⁹ that higher plasma ET-1 levels prevailed in rat models in which BP predominantly depended on enhanced circulating volume (DOCA-salt, 1K1C). Lower plasma ET-1 levels were found in SHR,⁹ where enhanced vasoconstriction rather than volume expansion maintains high BP. Clinical studies are now awaited to confirm our findings in humans.

Interestingly, our SHR responded to a 2-mL blood withdrawal with a decrease in mean arterial pressure of 40 mm Hg. Unlike Wistar rats, SHR were unable to maintain BP by a rapid baroreflex increase in heart rate. One minute after blood sampling, heart rate of SHR was actually decreased. Albeit SHR have a reduced baroreflex sensitivity, it also appears that SHR as a vasoconstrictor model of hypertension may respond more drastically to a 2-mL blood loss. The circulating volume before blood sampling may be reduced in SHR and therefore a relatively large blood loss is sensed. Furthermore, an already maximally

constricted vasculature may have little functional reserve to respond to vasoconstrictor hormones such as norepinephrine, angiotensin II, or ET-1.

We did not measure circumferential stretch (hoop stretch) in the present experiments. But our results are compatible with the release of ET-1 in response to augmented circumferential stretch due to volume expansion. Lauth et al¹² reported upregulated preendothelin-1 mRNA together with enhanced vascular endothelial ET-1 content in response to increased intraluminal pressure in rabbit carotid arteries. Ziegler et al⁴ found in an artificial device for shear and stretch modeling that shear stress transiently increased ET-1 mRNA. The hoop stretch concept would make sense. Stretched endothelial cells release ET-1 to induce vasoconstriction, which protects endothelial cells against further stretching. High and low circulating levels of ET-1 in different rat models of hypertension could be easily explained by expanded volume or predominant vasoconstriction.

In conclusion, rapid infusion of saline increases ET-1 concentrations in the circulating blood of Wistar rats and SHR. The volume-induced stretching of endothelial cells may cause a release of the protecting vasoconstrictor ET-1.

Acknowledgment

We thank Catherine Amustutz for excellent technical assistance.

References

1. Shimada K, Matsushita Y, Wakabayashi K, Takahashi M, Matsubara A, Iijima Y, Tanzawa K: Cloning and functional expression of human endothelin converting enzyme cDNA. *Biochem Biophys Res Commun* 1995;207:807-812.
2. Tamirisa P, Frishman WH, Kumar A: Endothelin and endothelin antagonism: roles in cardiovascular health and disease. *Am Heart J* 1995;130:601-610.
3. Harrison VJ, Ziegler T, Bouzourène K, Suciuc A, Silacci P, Hayoz D: Endothelin-1 and endothelin-converting enzyme-1 gene regulation by shear stress and flow-induced pressure. *J Cardiovasc Pharmacol* 1998;31(Suppl 1):S38-S41.
4. Ziegler T, Bouzourène K, Harrison VJ, Brunner HR, Hayoz D: Influence of oscillatory and unidirectional flow environments on the expression of endothelin and nitric oxide synthetase in cultured endothelial cells. *Throm Vasc Biol* 1998;18:686-692.
5. Fyhrquist F, Sirvio M-L, Helin K, Sajnamaa O, Metsarinne K, Paakkari I, Asko J, Tikkanen I: Endothelin antiserum decreases volume stimulated and basal plasma concentration of atrial natriuretic peptide. *Circulation* 1993;88:1172-1176.
6. Baertschi AJ, Pedrazzini T, Aubert JF, Roatti A, Pence RA: Role of endothelin receptor subtypes in volume-stimulated ANF secretion. *Am J Physiol Heart Circ Physiol* 2000;278:H493-H499.
7. Larivière R, Thibault G, Schiffrin EL: Increased endothelin-1 content in blood vessels of deoxycorticosterone acetate-salt hypertensive but not in spontaneously hypertensive rats. *Hypertension* 1993; 21:294-300.
8. Li J-S, Knafo L, Turgeon A, Garcia R, Schiffrin EL: Effect of endothelin antagonism on blood pressure and vascular structure in renovascular hypertensive rats. *Am J Physiol* 1966;271 (Heart Circ Physiol 40):H88-H93.

9. Abdel-Sayed S, Nussberger J, Aubert JF, Gohlke P, Brunner HR, Brakch N: Measurement of endothelin-1 in experimental hypertension and in healthy subjects. *Am J Hypertens* 2003;16:515-521.
10. Scott-Burden T, Resink TJ, Hahn AWA, Vanhoutte PM: Induction of endothelin secretion by angiotensin II: effects on growth and synthetic activity of vascular smooth muscle cells. *J Cardiovasc Pharmacol* 1991;17(Suppl 7):S96-S100.
11. Kawano Y, Yoshimi H, Matsuoka H, Omae T: Changes in plasma endothelin-1 concentration during blood volume depletion and expansion: role of the cardiopulmonary baroreflex. *Hypertens Res* 1995;18:43-46.
12. Lauth M, Berger M-M, Cattaruzza M, Hecker M: Elevated perfusion pressure up regulates endothelin-1 and endothelin B receptor expression in the rabbit carotid artery. *Hypertension* 2000;35:648-654.