Endothelin Blockade Lowers Total Peripheral Resistance in Hemorrhagic Shock Recovery

R.S. Zimmerman, M. Maymind, R.W. Barbee

Abstract To determine whether endothelin (ET) has a role in maintaining circulatory support during hypotensive hemorrhage, we (1) examined cardiac and systemic hemodynamics after a 6-mL hemorrhage in the presence and absence of the ET\textsubscript{A} receptor blocker BQ-123, (2) examined cardiac and systemic hemodynamics during BQ-123 infusion in nonhemorrhaged rats, (3) measured changes in circulating immunoreactive endothelin (IR-ET) after a 6-mL hemorrhage, and (4) infused pathophysiological doses of ET-1 into rats anesthetized with thiobutabarbital. Twenty minutes after hemorrhage, cardiac output and mean arterial pressure had stabilized in part because of an increase in systemic vascular resistance from 0.86±0.04 (baseline) to 1.04±0.05 (20 minutes) mm Hg/mL per minute. The rise in systemic vascular resistance was temporally associated with a significant (24%) increase in circulating IR-ET from 29±2 to 36±3 pg/mL 20 minutes after hemorrhage. During BQ-123 infusion mean arterial pressure at 5, 10, and 20 minutes after hemorrhage was 9±2, 23±4, and 23±3 mm Hg lower than values obtained after hemorrhage alone (P<.05). Mean arterial pressure was unaffected by BQ-123 infusion at baseline and 30 minutes after hemorrhage. Systemic vascular resistance was not affected at baseline by BQ-123 infusion. However, systemic vascular resistance was significantly lower 5, 10, 20, and 30 minutes after hemorrhage during BQ-123 infusion compared with hemorrhage alone at each time point. Infusion of BQ-123 into nonhemorrhaged rats had no effect on mean arterial pressure, systemic vascular resistance, or cardiac output. Infusion of ET-1 at 75 ng/kg per minute for 45 minutes in a separate group of rats resulted in similar changes in IR-ET and resulted in increases in systemic vascular resistance from 0.92±0.07 to 1.76±0.31 mm Hg/mL per minute and mean arterial pressure from 125±3 to 144±4 mm Hg. The following findings together support a role for endothelin in maintaining pressure homeostasis during hemorrhagic shock by enhancing systemic vascular resistance: (1) IR-ET increases during hypotensive hemorrhage in association with an increase in systemic vascular resistance, (2) ET\textsubscript{A} receptor blockade after hypotensive hemorrhage decreases mean arterial pressure and systemic vascular resistance, and (3) pathophysiological infusion of ET-1 increases systemic vascular resistance and mean arterial pressure. (Hypertension. 1994;23:205-210.)

Key Words • cardiac output • cardiovascular system • hypotension • hemorrhage • blood pressure

The endothelin family of peptides (ET-1, ET-2, ET-3) has recently been described.\textsuperscript{1-3} ET-1 is released from endothelial cells\textsuperscript{1} but also exists in other cell types.\textsuperscript{3} Infusion of ET-1 and ET-3 causes potent systemic and renal vasoconstriction.\textsuperscript{4,6} Furthermore, preliminary studies have suggested that this peptide is elevated during heart failure and hypovolemic shock.\textsuperscript{7,8} Two endothelin receptors have been described: ET\textsubscript{A} and ET\textsubscript{B}.\textsuperscript{10} The ET\textsubscript{A} receptor is highly selective for ET-1 and ET-2 isopeptides and has lower affinity for ET-3.\textsuperscript{10} The ET\textsubscript{B} receptor does not discriminate among the three endothelin isopeptides.\textsuperscript{11} Recently, BQ-123, a potent ET\textsubscript{A} receptor blocker, has been described.\textsuperscript{12} We hypothesized that ET-1 release may support the circulation during hypovolemic shock by acting to increase systemic vascular resistance (SVR). To assess the role of this peptide in hypovolemic shock, we measured SVR and mean arterial pressure (MAP) in rats after hemorrhage in the presence and absence of the ET\textsubscript{A} receptor blocker BQ-123. We subsequently measured immunoreactive endothelin levels (IR-ET) after hemorrhage. Finally, we infused ET-1 into normal rats at rates that produced ET-1 increments similar to those seen after hemorrhage to determine if pathophysiological changes in ET-1 could affect MAP and SVR in the rat.

Methods

Male Sprague-Dawley rats (275 to 403 g) were anesthetized with thiobutabarbital (100 mg/kg IP, BYK-Gulden, Konstanz, FRG), and a 200-gauge polyethylene tube was inserted into the trachea to facilitate spontaneous breathing. In group 1 (n=5), a 0.5-mm vinyl catheter (Dural Plastics, Auburn, Australia) was placed in the femoral vein and advanced to the thoracic inferior vena cava for measurement of central venous pressure (CVP). A PE-50 catheter was placed in the femoral artery for measurement of MAP and for blood withdrawal. Pressures were measured continuously with transducers (Gould-Statham, Oxnard, Calif) and recorded on a model 79D polygraph (Grass Instruments, Quincy, Mass). An SV31 catheter was placed in the external jugular vein and advanced to the right atrium for infusion of 5% dextrose. A thermocouple microprobe (Sensortek, Clifton, NJ) was placed in the aortic arch and connected to a Cardiotherm 500 (Columbus Instruments, Columbus, Ohio) for measurement of cardiac output (CO). After a 30-minute recovery period, baseline measurements of CO (average of two readings), CVP, MAP, and heart rate (HR) were obtained. Six milliliters of blood (approximately 25% of the total blood volume) was removed in 1 minute to induce hemorrhagic shock. Measurements of CVP, MAP, HR, and CO were also obtained at 5, 10, 20, and 30 minutes after hemorrhage. SVR was calculated as the ratio of MAP and CO. Stroke volume (SV) was calculated as the ratio of CO and HR. Aortic blood temperature was monitored...
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with saline at 0.01 mL/min for 45 minutes and ET-1 at 75 ng/kg per minute for 30 minutes starting at 15 minutes. The other 6 rats were infused with BQ-123 in saline at 20 μg/kg per minute (0.01 mL/min) for 45 minutes and ET-1 at 75 ng/kg per minute for 30 minutes starting at 15 minutes. MAP was determined every 15 minutes as described above. In a fourth group of rats (n=6) BQ-123 was infused at 20 μg/kg per minute (0.01 mL/min) for 45 minutes in nonhemorrhaged animals. Data were obtained from this group using the same protocol as in group 1.

In a fifth group of 28 rats anesthetized with thiobutabarbital (100 mg/kg IP), a femoral artery line was placed for blood withdrawal. Six milliliters of blood was removed in 1 minute to induce hemorrhagic shock and was used for measurement of IR-ET. A second blood sample (3 mL) for measurement of IR-ET was obtained at 10 (n=5), 20 (n=9), or 30 minutes (n=9) for determination of IR-ET. To serve as a time control, a group of five thiobutabarbital-anesthetized (100 mg/kg IP) rats with a femoral artery line placed for blood withdrawal was studied. Three milliliters of blood was removed for measurement of IR-ET and immediately replaced with 3 mL of heparinized donor blood from rats of the same strain. Three milliliters of blood was collected at 30 minutes for determination of IR-ET.

After determination of IR-ET changes during hemorrhage, a sixth group of five thiobutabarbital-anesthetized (100 mg/kg IP) rats was evaluated. The ET-1 dose necessary to cause a change in IR-ET similar to that observed during hemorrhage was determined. After surgical preparation as described above, ET-1 (Peptides International) (n=5) was infused for 45 minutes at 75 ng/kg per minute at the rate of 0.01 mL/min with an infusion pump (Harvard Apparatus, South Natick, Mass). MAP, CVP, HR, and CO were determined at baseline and 45 minutes. An additional group of four thiobutabarbital-anesthetized (100 mg/kg IP) rats was infused with saline at 0.01 mL/min. Blood for IR-ET determination was obtained at baseline and 45 minutes. Baseline blood samples were immediately replaced with warm heparinized donor blood of rats from the same strain.

A seventh group of four thiobutabarbital-anesthetized rats was evaluated for change in hematocrit after the 6-mL hemorrhage over 1 minute. Blood (25 μL) for hematocrit determination was obtained at 5, 10, 20, and 30 minutes after hemorrhage.

IR-ET was determined by radioimmunoassay with an ET-1-21-specific radioimmunoassay kit (Amersham, UK). Cross-reactivity between ET-1 and big endothelin in this kit is 0.4%; ET-2, 144%; and ET-3, 52%. Blood was collected in chilled EDTA tubes and centrifuged at 4°C for 20 minutes, and the plasma was frozen at ~70°C until assay. One milliliter of plasma was precleared with 1 mL of 0.1% trifluoroacetic acid (TFA) before extraction on a Sep-Pak C18 cartridge (Millipore Corp, Milford, Mass). The cartridge was rinsed with 4 mL of 60% acetonitrile in 0.1% TFA before extraction. Two milliliters of the plasma sample was applied to the column. The columns were washed with 10 mL of 0.1% TFA. The sample was eluted with 3 mL of 60% acetonitrile in 0.1% TFA. The eluate was dried on a Speed Vac (Savant, Hicksville, NY) overnight. Samples were resuspended in 250 μL of assay buffer.

Antiserum for ET-1-21 (100 μL) was incubated with duplicate samples for 4 hours. 125I-ET-3 tracer (100 μL) was subsequently added and incubated for 18 hours. Donkey anti-rabbit serum (250 μL) was incubated for 10 minutes.

Tubes were centrifuged for 10 minutes at 2500g. The supernatant was aspirated and the pellet counted for radioactivity on an Auto-Gamma 5000 series gamma counter (Packard Instrument Co, Downers Grove, Ill), and log-logit analysis was performed (SIECURA II software package, Packard). The intraassay coefficient of variation was 8.8%; the interassay coefficient of variation was 13.8%. The sensitivity was 3 pg/mL. Recovery of radioactive ET-1 was 76±1%. Parallelism was determined for the assay comparing assay standards against spiked plasma from rats 20 minutes after hemorrhage. No difference in slopes was observed (P>0.3).

Data in groups 1, 2, 4, and 7 were analyzed with a one-way analysis of variance with repeated measures. Subsequent comparisons between vehicle and experimental time points were analyzed by Dunnett's test for one-sided comparisons between treatment and control means. Data in group 3 was analyzed with a t test for independent groups. Data in groups 5 and 6 were analyzed by paired Student's t test. A value of P<0.05 was considered significant. Data are expressed as mean±SEM.

These studies were performed after approval by the Ochsner Foundation Animal Care and Use Committee and were performed following the Guide for the Care and Use of Laboratory Animals (NIH, 1985).

**Results**

The hemodynamic effects of hemorrhage are illustrated in Fig 1. MAP decreased from 114±2 to 42±3 mm Hg (P<0.05) 5 minutes after the 6-mL hemorrhage and returned toward baseline at 10, 20, and 30 minutes to 72±6, 73±3, and 73±7 mm Hg, respectively (P<0.05 compared with baseline). HR decreased from 394±17 beats per minute to 352±8 and 372±6 beats per minute 5 and 10 minutes after hemorrhage, respectively.
Effect of BQ-123 on ET-1 Induced Hypertension

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Sal indicates saline; ET-1, endothelin-1; and MAP, mean arterial pressure. *P<.05 compared with Sal-ET-1 group.

(P<.05). At 20 and 30 minutes after hemorrhage, HR returned to baseline values of 391±5 and 400±5 beats per minute, respectively. CO decreased from 136±5 (baseline) to 51±8 mL/min 5 minutes after hemorrhage (P<.05) and remained significantly depressed 10, 20, and 30 minutes after hemorrhage (89±5, 82±3, and 86±6 mL/min, respectively, P<.05). The increase in CO from 5 to 10 minutes after hemorrhage was due to a significant increase in SV from 0.43±0.02 (5 minutes) to 1.04±0.01 (10 minutes) milliliter per beat (P<.05).

CVP decreased from 1.6±0.2 to 1.0±0.3 mm Hg 5 minutes after hemorrhage (P<.05) and remained suppressed at 1.1±0.3, 1.0±0.3, and 1.0±0.2 mm Hg (P<.05) at 10, 20, and 30 minutes, respectively, after hemorrhage. SVR did not increase significantly from baseline levels of 0.86±0.04 mm Hg/mL per minute until 20 minutes after hemorrhage when it increased to 1.04±0.05 mm Hg/mL per minute and then returned to baseline values of 0.85±0.07 mm Hg/mL per minute at 30 minutes. Hematocrit decreased significantly starting from 5 minutes after hemorrhage from 0.43±0.02 to 0.39±0.01 (P<.05), 0.36±0.02 (P<.05), and 0.35±0.02 (P<.05) at 5, 10, 20, and 30 minutes, respectively, after hemorrhage.

The hemodynamic effects of BQ-123 after hemorrhage also are illustrated in Fig 1. MAP was unaffected at baseline and 30 minutes after hemorrhage by BQ-123 infusion. However, MAP was significantly lower during hemorrhage compared with hemorrhage alone). The effects of hemorrhage on HR, CO, and CVP were not significantly altered by BQ-123 infusion. SVR was not affected at baseline by infusion of BQ-123, whereas SVR was reduced to values of 0.57±0.04, 0.59±0.03, 0.65±0.03, and 0.74±0.03 mm Hg/mL per minute 5, 10, 20, and 30 minutes, respectively, after hemorrhage (P<.05 compared with hemorrhage alone). The effects of hemorrhage on HR, CO, and CVP were not significantly altered by BQ-123 infusion. SVR was not affected at baseline by infusion of BQ-123, whereas SVR was reduced to values of 0.57±0.04, 0.59±0.03, 0.65±0.03, and 0.74±0.03 mm Hg/mL per minute 5, 10, 20, and 30 minutes, respectively, after hemorrhage (P<.05 compared with hemorrhage alone).

The effect of BQ-123 on ET-1-induced hypertension is illustrated in the Table. BQ-123 completely blocked ET-1-induced hypertension during the 30-minute ET-1 infusion.

The hemodynamic effects of BQ-123 infusion in the absence of hemorrhage are illustrated in Fig 2. No significant changes in MAP, HR, CO, CVP, or SVR were observed during BQ-123 infusion alone.

The changes in endothelin levels 10, 20, and 30 minutes after hemorrhage are shown in Fig 3. IR-ET levels increased by 1.6±0.4 pmol (4±1 pg/mL) during ET-1 infusion (P<.05). The change in IR-ET was 0.3±1.1 pmol (0.8±2.7 pg/mL, P=NS) during saline infusion in a separate group of four rats.

Discussion

The response to hemorrhage involves a number of physical, neural, and hormonal changes.13-15 Korner et

Fig 2. Bar graph shows effect of BQ-123 infusion on mean arterial pressure (MAP), heart rate (HR), cardiac output (CO), central venous pressure (CVP), and systemic vascular resistance (SVR) in the rat. *P<.05 compared with baseline values.

hemorrhage group (n=9) and 2.4±0.8 pmol (6±2 pg/mL) in the 30-minute hemorrhage group (n=9). In five additional rats not exposed to hemorrhage, IR-ET levels decreased by 0.5±0.4 pmol (1.3±0.9 pg/mL, P=NS) after 30 minutes.

The effects of ET-1 infusion at 75 ng/kg per minute over 45 minutes on MAP, CO, and SVR are illustrated in Fig 4. MAP increased from 124±3 to 143±4 mm Hg (P<.05). CO decreased from 135±10 to 87±14 mL/min (P<.05), and SVR increased from 0.92±0.07 to 1.76±0.31 mm Hg/mL per minute (P<.05) after 45 minutes of ET-1 infusion. IR-ET increased by 1.6±0.4 pmol (4±1 pg/mL) during ET-1 infusion (P<.05). The change in IR-ET was 0.3±1.1 pmol (0.8±2.7 pg/mL, P=NS) during saline infusion in a separate group of four rats.

Fig 3. Bar graph shows change in circulating levels of immunoreactive endothelin-1 (ET-1) after 6-mL hemorrhage in the rat. *P<.05 compared with corresponding baseline values.
produced by hemorrhage can have physiological actions these previous studies whether the observed changes in IR-ET associates the changes in IR-ET with SVR, which was showing that IR-ET is not significantly elevated until 20 present study gives a time course for endothelin release, from that study in several important ways. First, the present study identifies an additional factor—endothelin—that, like the previously identified hormones, may have a role as a circulating hormone. The third finding supporting a pathophysiological role for endothelin is that MAP after hemorrhage with BQ-123 infusion is significantly lower than MAP after hemorrhage without BQ-123. The decrease in MAP during BQ-123 infusion is due to a significant decrease in SVR. The observation that ET-1 blockade lowers MAP and SVR at times when circulating levels of ET-1 were unchanged lends support to the concept that ET-1 acts partly by paracrine actions. Thirty minutes after hemorrhage, BQ-123 had no effect on MAP despite the fact that SVR remained significantly decreased in the BQ-123-infused group. An increase in CO prevented a decrease in MAP at that time.

The third finding supporting a role for endothelin in response to hemorrhagic shock is in agreement with preliminary studies by Vemulapalli et al,9 who also found that hemorrhage increased circulating levels of ET-1. The present study identifies an additional factor—endothelin—that, like the previously identified hormones, may have a role as a circulating hormone. The second finding supporting a pathophysiological role for endothelin is that MAP after hemorrhage with BQ-123 infusion is significantly lower than MAP after hemorrhage without BQ-123. The decrease in MAP during BQ-123 infusion is due to a significant decrease in SVR. The observation that ET-1 blockade lowers MAP and SVR at times when circulating levels of ET-1 were unchanged lends support to the concept that ET-1 acts partly by paracrine actions. Thirty minutes after hemorrhage, BQ-123 had no effect on MAP despite the fact that SVR remained significantly decreased in the BQ-123-infused group. An increase in CO prevented a decrease in MAP at that time.

The observation that BQ-123 did not significantly affect MAP or SVR in the absence of hemorrhage lends further support to the concept that ET-1 is an important factor in maintaining pressure homeostasis after hemorrhage because it illustrates that the lowering of MAP and SVR are not nonspecific effects of the antagonist. Furthermore, this finding suggests that ET-1 has an important role in pathological states such as hemorrhagic shock but is not important in maintaining basal vascular tone.

The third finding supporting a role for endothelin in recovery from hypotensive hemorrhage is that exogenously infused ET-1 at a pathophysiological dose has considerable hemodynamic effects. In the present study we infused doses of ET-1 that produced only small changes in circulating IR-ET, similar to the 24% increase observed during hemorrhage. ET-1 was infused in a volume-replete state in the present study for AHAJournals.org Downloaded from
determination of the effects of ET-1 independent of other hormonal and neurological changes that occur during hemorrhage. It is clear that the body has multiple overlapping physiological responses to hemorrhage, which help maintain volume-pressure homeostasis including release of circulating catecholamines, vasopressin, and aldosterone and stimulation of sympathetic nerves.\(^\text{10-14}\) Infusion of ET-1 in a nonhemorrhaged animal allowed us to observe the effects of ET-1 without interference from the other hemorrhage-induced changes. Circulating levels of ET-1 were measured 45 minutes after initiation of ET-1 infusion because preliminary studies in our laboratory showed that a 45-minute infusion is required to reach a hemodynamic steady state. It was not the purpose of this study to precisely mimic the time course observed after hemorrhage.

Although similar changes in IR-ET were observed during ET-1 infusion (4±1 pg/mL) and during hypotensive hemorrhage (7±2 pg/mL), SVR was more profoundly affected by exogenous ET-1 infusion than endogenous production of endothelin. It is possible that some of the measured changes in IR-ET were ET-2 or ET-3, as the antiserum used in the present study has cross-reactivity with all three endothelin peptides. Previous studies\(^\text{1}\) have shown that these peptides have less pressor activity than ET-1. The observation in the present study that MAP and SVR increase when IR-ET levels are elevated by an amount seen during hemorrhage is further evidence that endothelin may be an important hormonal factor in maintaining volume-pressure homeostasis.

Early studies suggested that ET-3 was the predominant endothelin isopeptide in the rat.\(^\text{1}\) In the present study the ET\(\text{A}\) receptor blocker BQ-123 was used. The ET\(\text{A}\) receptor is found on a variety of smooth muscle types.\(^\text{21}\) Previous studies have shown that the ET\(\text{A}\) receptor is specific for ET-1 and ET-2, with lower affinity for ET-3.\(^\text{10}\) Furthermore, infusion of this blocker prevents the pressor response and hematocrit changes produced by ET-1.\(^\text{22}\) The findings in this study that SVR and MAP were lower after hypotensive hemorrhage in the rat lend support to the concept that ET-1 has an important physiological role in the rat.

In addition to the effects endothelin has on vascular tone, it is possible that endothelin helps maintain MAP after hemorrhage via other endocrine systems that affect volume-pressure homeostasis. Indeed, recent preliminary studies by Lloyd et al\(^\text{17}\) have shown that infusion of antibodies to endothelin during hypotensive hemorrhage in the dog decreases the activation of renin and aldosterone. The findings of Lloyd et al support a role for endothelin release in the stimulation of renin and aldosterone during hypotensive hemorrhage.

Although the present study suggests that hypotensive hemorrhage enhances release of endothelin, it is also possible that endothelin is increased in part because of decreased clearance of endothelin. Recent preliminary studies by Underwood et al\(^\text{18}\) suggest that decreased CO caused by 8 days of thoracic inferior vena cava constriction in the dog decreased the metabolic clearance rate of endothelin by one half. Because hemorrhagic shock also decreases CO and presumably decreases glomerular filtration rate, it is likely that endothelin clearance is also affected in the present study, which may in part account for the increased circulating level of endothelin.

It is possible that the enhanced release of endothelin is related to massive tissue and endothelial cell destruction produced by acute irreversible shock. The 6-mL hemorrhage may have blocked autotransfusion of plasma from the interstitial space to the vascular space because of lack of blood flow through muscle and gastrointestinal capillary beds. This interpretation is unlikely, however, based on two important observations. First, hematocrit decreased from 0.43±0.02 to 0.41±0.01 within 5 minutes after hemorrhage occurred and decreased to 0.35±0.02 thirty minutes after hemorrhage, demonstrating that autotransfusion occurred. Had autotransfusion not occurred, no change in hematocrit would have been observed. Second, Darlington et al\(^\text{20}\) performed a similar study evaluating the effect of 10-, 15-, and 20-mL/kg hemorrhage in rats 4, 7, and 10 days after surgical preparation. The fact that all of the rats subjected to a 20-mL/kg hemorrhage in that study recovered strongly supports the concept that the amount of blood removed in the current study represents reversible hemorrhagic shock. The blood pressure response in the posthemorrhage period in the study of Darlington et al was almost identical to that observed in the current study, suggesting that the difference in time of hemorrhage between the two studies (1 versus 3 minutes) did not significantly alter the neuronal or hormonal response to hemorrhage.

The present study supports a role for endothelin in recovery from hypotensive hemorrhage. Previous studies have demonstrated a similar important role for angiotensin II and vasopressin in recovery from hypotensive hemorrhage.\(^\text{24}\) It is possible that endothelin acts independently of these other hormones or that it is stimulated by release of these hormones.

In conclusion, we postulate a role for endothelin in recovery from hemorrhagic shock. An increase in MAP and CO toward prehemorrhage values is observed 5 to 10 minutes after hemorrhage, and an increase in SVR with an elevation in plasma IR-ET is observed 20 minutes after hemorrhage. A similar change in circulating levels of IR-ET is observed during low-dose infusion of ET-1 in the rat, associated with an increase in MAP and SVR. ET-1 blockade with infusion of BQ-123 lowers blood pressure and SVR during recovery from hemorrhagic shock. These observations strongly support both an endocrine and paracrine role for endothelin in maintaining pressure homeostasis during hemorrhagic shock.

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