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Endothelin contributes to the hemodynamic effects of vasopressin in spontaneous hypertension

Suchitra M. Balakrishnan, Venkat Gopalakrishnan, J. Robert McNeill *

Department of Pharmacology and Cardiovascular Risk Factor Reduction Unit, College of Medicine, University of Saskatchewan, Saskatoon, Sask., Canada S7N 5E5

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Abstract

Changes in blood pressure, cardiac output, and total peripheral conductance evoked by intravenous infusions of [Arg^8]-vasopressin (vasopressin) were recorded before and after pretreatment with bosentan, a non-selective endothelin antagonist, in conscious unrestrained spontaneously hypertensive rats (SHR) and Wistar–Kyoto rats (WKY). The presser effects of vasopressin were exaggerated in SHR compared to WKY. Pretreatment with bosentan failed to change hemodynamic responses of WKY to vasopressin, but it blunted the increases in blood pressure and the decreases in conductance evoked by vasopressin in SHR. In contrast, bosentan failed to change cardiac output responses of SHR to vasopressin. Except at the highest dose of vasopressin, bosentan abolished the exaggerated pressor responsiveness of the SHR to vasopressin. The results suggest that endothelin contributes to the exaggerated pressor responsiveness of SHR to vasopressin, and that this effect is exerted at the level of the resistance vessels and not on factors that regulate cardiac output.

Keywords: Vasopressin; Endothelin; Spontaneously hypertensive rats (SHR); Hemodynamics; Cardiac output; Radiotelemetry

1. Introduction

Pressor responsiveness to [Arg^8]-vasopressin (vasopressin) is increased in the spontaneously hypertensive rat (SHR) and this effect is presumed to be related to the direct action of the peptide on vascular smooth muscle (Mohring et al., 1981; Datar et al., 1985). However, vasopressin has been reported to dose dependently induce preproendothelin-1 mRNA expression from cultured rat endothelial cells (Imai et al., 1992), and to release endothelin-1 from bovine endothelial cells (Emori et al., 1991), human mesangial cells (Bakris et al., 1991), and from the perfused rat mesenteric artery (Tomobe et al., 1993). As well, infusion of vasopressin (10 ng/kg/min) has been reported to increase only, plasma endothelin-1 levels in conscious dogs (Emmeluth and Bie, 1992). Thus, much evidence suggests that vasopressin releases endothelin-1 from endothelial cells, but the possibility that endothelin-1 contributes to the vascular effects of vasopressin and to the enhanced presser responsiveness of vasopressin in SHR has not been studied. In order to address these issues, and to determine if any indirect contribution of endothelin to the effects of vasopressin on blood pressure were exerted at the level of the resistance vessels or on factors which regulate cardiac output, we recorded blood pressure by radiotelemetry and cardiac output with ultrasonic flow-probes in conscious unrestrained rats.

2. Methods

2.1. Animal care

Recommendations on the care of animals issued by the European Community and by the Canada Council on Animal Care were adhered to during the course of this study. The protocols were approved by the Animal Care Committee at the University of Saskatchewan. In order to conserve animal life, an issue emphasized by the Canada Council on Animal Care, the vasopressin experiments were performed in the same rats as those that had been used in a study involving angiotensin and endothelin-1 infusions (Balakrishnan et al., 1996). The stability of the flow and

* Corresponding author. Tel.: +1-306-9666293; fax: +1-306-9666220.

pressure measurements outlined below in the chronically instrumented rat allow such studies. Importantly, vasopressin was the only agonist infused on any one day and all control hemodynamic variables had returned to control values before an vasopressin infusion was commenced.

2.2. Surgical procedures

SHR and Wistar–Kyoto rats (WKY) were purchased from Taconic Farms (German Town, NY) at six weeks of age and raised in our animal quarters under standardized conditions. Rats were selected randomly for experiments when they reached the age of 1621 weeks. Under anesthesia with sodium pentobarbital (50 mg/kg), the animals were intubated and ultrasonic flow probes (2.5 SB series, Transonic Systems, Ithaca, NY) were implanted on the ascending aorta. A catheter connected to a radiotelemetry capsule (TAT lPA-C40, Data Sciences, MN) was implanted in the femoral artery of each rat for the recording of arterial pressure. Before implantation of the radiotelemetry capsules, the zero of each transducer (TAT lPA-C40, Data Sciences) was verified to be ≤ 4 mm Hg. A fluid filled sensor catheter was inserted into the femoral artery and advanced so that the tip of the catheter was in the abdominal aorta above the iliac bifurcation. The capsule containing the transducer and radiotransmitter was positioned sub-cutaneously in the flank region. A second catheter was implanted into a femoral vein for administration of vasopressin and bosentan. Rats received an i.m. injection of gentamycin (1 mg) preoperatively followed by tobramycin (2 ma) and ticarcillin (15 mg) post-operatively for three days.

2.3. Recording procedures

At least 10 days after instrumentation, blood pressure and cardiac output were recorded in conscious, unrestrained rats. Blood pressure was recorded by placing individual rat cages on top of a receiver (RA 1010, Data Sciences) and data were collected with a computer driven data acquisition system (Dataquest IV; Data Sciences). The waveform sampling rate was set to 250 Hz with a 100 Hz

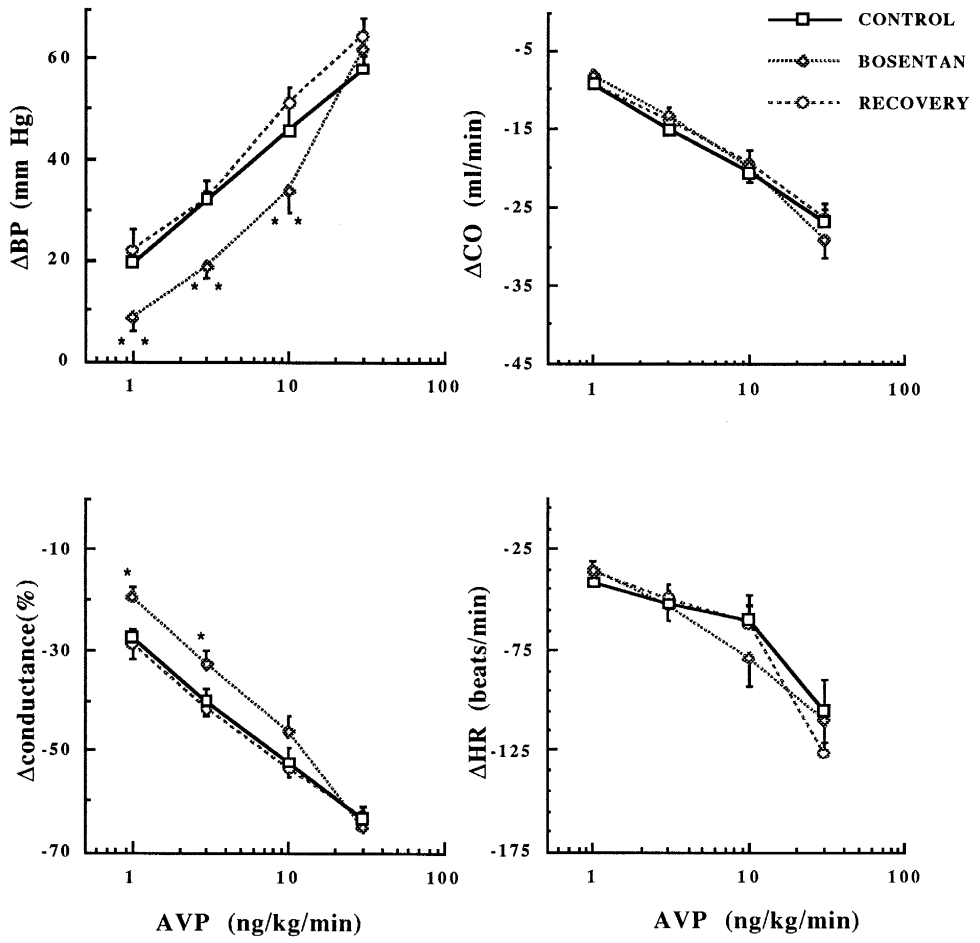

Fig. 1. Changes in blood pressure (blood pressure), cardiac output (CO), total peripheral conductance (percent control) and heart rate (heart rate) in spontaneously hypertensive rats (SHR, $n = 8$) in response to graded infusions of vasopressin-AVP (CONTROL); vasopressin-AVP after pre- treatment with bosentan (B OSENTAN) and vasopressin-AVP after elimination of bosentan (RECOVERY). * $P < 0.05$ and ** $P < 0.01$ compared to control and recovery.

Table 1
Control values

	SHR ($n = 8$)		WKY ($n = 7$)	
	control	bosentan	control	bosentan
BP (mm Hg)	141 ± 2 [a]	136 ± 2	102 ± 2	98 ± 2
CO (ml/min/kg)	225 ± 7 [b]	231 ± 10	254 ± 16	261 ± 14
TPC (ml/min/kg/mm Hg)	1.599 ± 0.048 [a]	1.699 ± 0.063	2.509 ± 0.179	2.663 ± 0.150
Heart rate (beats/min)	297 ± 6	314 ± 8	294 ± 5	308 ± 8

Values for blood pressure (BP), cardiac output (CO), total peripheral conductance (TPC) and heart rate in spontaneously hypertensive rats (SHR) and Wistar–Kyoto rats (WKY) before and after treatment with bosentan (4 determinations in each rat). Values are the means ± sem.
[a] $P < 0.001$; [b] $P < 0.05$ compared to WKY.

filter. Ambient (atmospheric) pressure was periodically monitored so that the telemetered pressure was recorded relative to atmospheric pressure. The pressure waveform was sampled every 30 s with a 5 s sample duration. A 2 min moving average was calculated for mean arterial pressure. Cardiac output was recorded by feeding the signal from the flowmeter (T206, Transonic Systems) to the pen recorder (model 7P03, Grass Instrument, Quincy, MA). The zero flow reference was considered to be the flow during diastole. Total peripheral conductance was calculated as the quotient of cardiac output (ml/min) and blood pressure (mm Hg). Thus, total peripheral resistance is the reciprocal of total peripheral conductance. At the end of each experiment, the animals were sacrificed and an in-situ calibration of the ultrasonic flow probe was performed

2.4. Infusion protocols

The protocol for the infusions of vasopressin before and after treatment with bosentan were as follows. After a 2 h

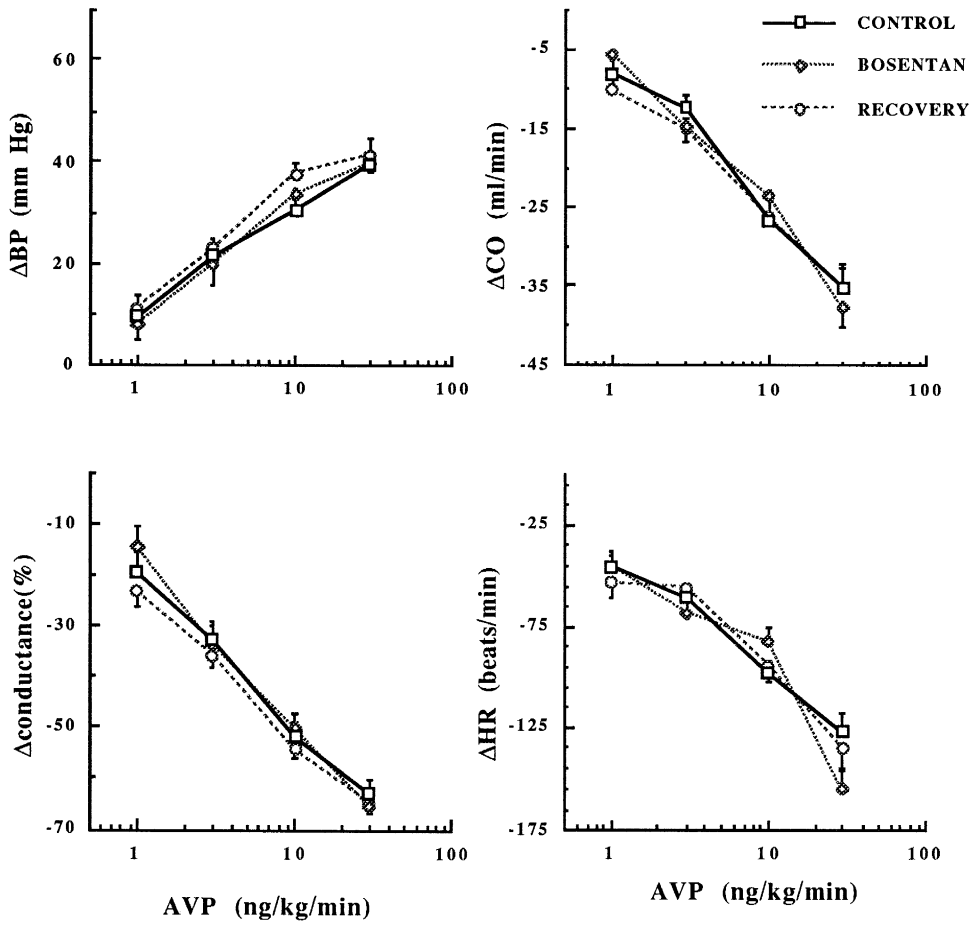

Fig. 2. Changes in blood pressure (blood pressure), cardiac output (CO), total peripheral conductance (percent control) and heart rate (heart rate) in Wistar-Kyoto rats (WKY, $n = 7$) in response to graded infusions of vasopressin-AVP (CONTROL); vasopressin-AVP after pretreatment with bosentan (BOSENTAN) and vasopressin-AVP after elimination of bosentan (RECOVERY).

control period, a single dose of vasopressin was infused intravenously at a rate of 1, 3, 10 or 30 ng/kg/min for 15 min. Only one of these 4 doses of vasopressin was selected for a particular day, and the selection of this dose was randomized to control for any time or sequence related variation. The maximal responses were observed within 10 min. Responses to vasopressin returned to control within 15–20 min after stopping the infusions. After a 3 h recovery period, 30 mg/kg of bosentan was injected intravenously as a bolus. In a previous study, we had shown that this dose of bosentan antagonized the effects of exogenous administration of endothelin-1 in conscious rats (Balakrishnan et al., 1996). The three hr interval between the initial control responses to vasopressin and the responses in the presence of bosentan was chosen because, in preliminary experiments, this interval yielded reproducible results to repeated administration of vasopressin. Thirty min after the bosentan, the infusion of vasopressin at the same dose was repeated. The 30 min period after bosentan was chosen because the response to bosentan had reached a plateau by this time, and it was well before recovery from the effects of bosentan commenced (Balakrishnan et al., 1996). Finally, 24 h later, vasopressin was infused a third time to serve as a post-recovery control.

2.5. Drugs used

Vasopressin (Bachem, Torrence, CA) was dissolved in 0.9% w/v saline. Bosentan (sodium salt), kindly provided by Dr. M. Clozel (F. Hoffman-La Roche, Basel) was dissolved in sterile water.

2.6. Data analyses

All mean values are expressed as means + sem. Control values in both strains, the effect of treatment with bosentan alone and the effect of bosentan treatment on individual doses of vasopressin were compared by analysis of variance (ANOVA). Graphs of the residuals were plotted to detect heterogeneity of variances. Homogeneity of variances was achieved by transforming the data (log or square root). The responses to all doses of vasopressin in the presence or absence of bosentan and strain differences were compared by analysis of variance for repeated measures. Simultaneous multiple comparisons were based on contrasts or Games–Howell multiple comparisons procedures.

3. Results

3.1. Control values

The control values for blood pressure, cardiac output, and total peripheral conductance before infusion of vasopressin both in the absence and in the presence of bosentan are shown in Table 1. Before bosentan, blood pressure was significantly higher in the SHR compared to the WKY. The elevated pressure appeared to be due to the elevated resistance, (i.e. reduced total peripheral conductance) as cardiac output was somewhat lower in the SHR. Bosentan failed to change arterial pressure, cardiac output, total peripheral conductance, or heart rate in either SHR or WKY.

3.2. Responses to vasopressin

The hemodynamic responses of SHR to vasopressin are shown in Fig. 1 and those of WKY are shown in Fig. 2. In the absence of bosentan, presser responsiveness to vasopressin was exaggerated in SHR compared to WKY ($P < 0.05$): a 20 mm Hg rise in pressure was evoked by approximately 1.0 ng/kg/min of vasopressin in SHR (Fig. 1) but the same increase in pressure required 3.0 ng/kg/min in WKY rats (Fig. 2). Except for the highest

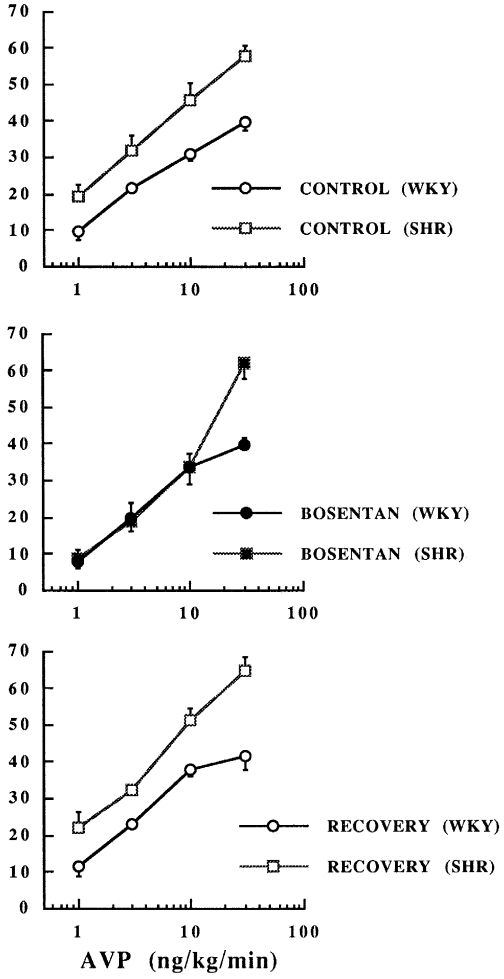

Fig. 3. Changes in blood pressure (blood pressure) in spontaneously hypertensive rats (SHR) and Wistar–Kyoto rats (WKY) in response to graded doses of vasopressin-AVP (CONTROL), vasopressin-AVP after pretreatment with bosentan (BOSENTAN) and vasopressin-AVP after elimination of bosentan (RECOVERY).

dose of vasopressin (30 ng/kg/min), bosentan blunted the increases in blood pressure induced by vasopressin ($P < 0.01$) in SHR. This effect of bosentan was not observed in the WKY ($P > 0.86$). Importantly, except for the highest dose of vasopressin, pre-treatment with bosentan abolished the exaggerated presser responsiveness of SHR to vasopressin (Fig. 3): presser responses of the two strains were not significantly different at doses of 1.0, 3.0, and 10.0 ng/kg/min. There was no significant difference between the control response to vasopressin and the response following elimination of bosentan (recovery) in either strain ($P > 0.65$).

The increases in pressure evoked by vasopressin were associated with dose related decreases in total peripheral conductance, cardiac output, and heart rate (Figs. 1 and 2). At the two lower doses of vasopressin, the fall conductance was greater in SHR than WKY, and bosentan abolished these differences. At the two higher doses of vasopressin, the fall in cardiac output was greater in WKY than SHR, but bosentan had no effect on the cardiac output responses in either strain. Similar to cardiac output, bosentan did not alter the heart rate response to vasopressin in either strain.

4. Discussion

The blunted blood pressure responsiveness of SHR to vasopressin in the presence of bosentan is the major finding reported in this study and the data provides the first direct evidence at the hemodynamic level for an endothelin dependent component which contributes to the presser activity of vasopressin in the SHR. The data also demonstrate for the first time that this contribution of endothelin to the pressor activity of vasopressin was exerted at the level of the resistance vessels as total peripheral conductance responses to vasopressin were enhanced when the endothelin system remained fully functional. In contrast, decreases in cardiac output tended to oppose the blood pressure elevating effect resulting from the vasoconstrictor activity of the peptide and bosentan failed to change cardiac output responses to vasopressin.

The endothelin component which contributes to the vasoconstrictor activity of vasopressin appears to explain, at least in part, the exaggerated pressor activity of vasopressin in SHR. At the three lower doses of vasopressin, presser responsiveness was virtually identical in SHR and WKY in the presence of bosentan (Fig. 3). When the endothelin system was fully functional, presser responsiveness of SHR to vasopressin was exaggerated. Thus, the enhanced presser responsiveness of SHR to vasopressin that had been reported earlier (Mohring et al., 1981; Datar et al., 1985) appears to be related in part to an endothelin dependent component. The endothelin component could be due to increased release of endothelin-1 or to increased expression of endothelin receptors. Unfortunately, data on the plasma concentrations of endothelin-1 would have little relevance since endothelin-1 is primarily secreted abluminally and plasma concentrations are not an accurate reflection of locally released endothelin-1. On the other hand, increased expression of endothelin ET_{B2} receptors in aortic cells of SHR has been reported and this element could well contribute to the exaggerated pressor effect (Batra et al., 1993).

Bosentan failed to reduce presser responsiveness of SHR to the highest dose of vasopressin suggesting other factors may contribute to the exaggerated reactivity of the SHR. Earlier, we reported that impairment of baroreflex activity of SHR to the vasoconstrictor activity of vasopressin contributes to the enhanced presser activity of the peptide (Datar et al., 1985). The data reported in this article is consistent with this notion because, for any given increase in blood pressure, the fall in heart rate was less in SHR. This difference was particularly dramatic at the higher dose range: for a 40 mm Hg change in pressure, the heart rate fell 125 beats per min in WKY but only 55 beats per min in SHR. Thus, both an endothelin component and an impaired baroreflex component appear to contribute to the exaggerated presser activity of SHR to vasopressin.

The contribution of endothelin-1 to the vasoconstrictor effects of vasopressin is not unique to vasopressin as we reported previously that endothelin-1 contributes to the hemodynamic effects of angiotensin II (Balakrishnan et al., 1996) and to the contractile responses of rat mesenteric and tail artery ring preparations evoked by angiotensin II (Chen et al., 1995a,b). In contrast to vasopressin however, bosentan blunted presser responses to angiotensin II in both SHR and WKY. The in vitro data reported from our laboratory showed that angiotensin II-, but not vasopressin-, evoked tension responses in the rat mesenteric artery and rat tail artery were attenuated by pretreatment with either an endothelin converting enzyme inhibitor (Chen et al., 1995a) or an endothelin ETA receptor antagonist (Chen et al., 1995b) in *normotensive* Sprague–Dawley rats.

The conclusions of this study depend on the assumption that bosentan is a selective endothelin antagonist, and also that the doses employed would antagonize the endothelin system in SHR and WKY to a similar extent. The pharmacological characteristics of endothelin have been described in detail by Clozel et al. (1994): the compound competitively inhibited the specific binding of [^{125}I]-labeled endothelin-1 to a variety of cell types, but failed to affect the binding of 40 other peptides including vasopressin, as well as prostaglandins, ions, and major neurotransmitters. Moreover, the failure of bosentan to attenuate responses to vasopressin in the WKY strain in our study suggests that bosentan does not possess an intrinsic ability to block vasopressin receptors and that the ability to attenuate the responses in the SHR is likely related to its ability to block endothelin receptors. Finally, we reported previously that the doses of bosentan employed in this study blocked the

effects of endothelin-1 to a similar extent in SHR and WKY, suggesting that the blood levels achieved with bosentan were sufficient in both strains to effectively block endothelin receptors (Balakrishnan et al., 1996).

In summary, the results suggest that endothelin contributes to the presser activity of vasopressin in the SHR, and this endothelin component contributes to the exaggerated responsiveness of SHR to the peptide. Finally, the contribution of endothelin to the presser activity of vasopressin is exerted at the level of the resistance function of the circulation, and not on factors that regulate cardiac output.

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