Effects of Nonpeptide and Selective V₁ and V₂ Antagonists on Blood Pressure Short-Term Variability in Spontaneously Hypertensive Rats

Nina Japundžić-Žigon¹*, Sanja Milutinović¹, and Aleksandar Jovanović²

¹Institute of Clinical Pharmacology, Pharmacology and Toxicology, School of Medicine, and ²School of Mathematics, University of Belgrade, 11129 Belgrade, Serbia and Montenegro

Received October 8, 2003; Accepted March 4, 2004

Abstract. Effects of V₁ (OPC-21268) and V₂ (OPC-31260) vasopressin antagonists on blood pressure (BP) short-term variability were investigated in adult spontaneously hypertensive rats (SHR) under basal conditions and after the stimulation of vasopressin release by hemorrhage. BP was recorded intra-arterially and sampled at 20 Hz to be analyzed on a personal computer. BP time spectra were calculated on 30 stationary overlapping 2048 point-time series. Spectral power was estimated in total (0.00976 – 3 Hz), very low frequency (VLF: 0.00976 – 0.195 Hz), low frequency (LF: 0.195 – 0.605 Hz), and high frequency (HF: 0.8 – 3 Hz) regions. Under basal conditions a V₁ antagonist (5 mg/kg, i.v.) decreased BP without affecting BP variability, while combined (V₁ + V₂) blockade or V₂ blockade (1 mg/kg, i.v.) alone did not affect cardiovascular parameters. Mild hemorrhage (5 ml/kg per min) increased HF-BP variability, while moderate (10 ml/kg per min) and massive (15 ml/kg per min) hemorrhage did not affect it. In V₁, but not V₂, antagonist pre-treated SHR HF-BP increased significantly after moderate and massive hemorrhage. V₁ or V₂ antagonist pre-treatment also enhanced VLF-BP variability during massive hemorrhage. Moreover V₁ blockade prevented hemorrhage-induced bradycardia, while V₂ blockade potentiated it. It follows that in adult SHR, vasopressin buffers BP oscillations in HF and VLF frequency domains only in hypovolaemic conditions and that the modulation of the autonomic adjustment of the HR to hemorrhage by vasopressin is preserved.

Keywords: blood pressure variability, spectral analysis, vasopressin antagonist, hemorrhage, hypertension

Introduction

Short-term blood pressure (BP) and heart rate (HR) variability have been shown to depict the activity of regulatory mechanisms involved in fast cardiovascular control (1 – 5). By using spectral methodologies, major components of cardiovascular short-term variability were identified (very low frequency (VLF), low frequency (LF), and high frequency (HF)); and the underlying mechanisms were suggested. For instance, it was shown that HR variability originates from an interplay of cardiac vagus and sympathicus (1 – 4, 6, 8), while BP variability reflects vasoactive mechanisms that modulate peripheral resistance (5 – 7, 9 – 14). Spectral methodologies are now a recognized tool for assessment of fast cardiovascular control (3, 4, 15). In subjects suffering from cardiovascular disease (congestive heart failure, myocardial infarction, and hypertension), changes in HR and BP variability were described (16 – 19). In hypertensive patients, increased BP variability in the VLF and even slower frequencies was found to be significantly correlated to increased incidence of end-organ damage (16). Therefore elucidating the mechanisms that underlie BP variability is of great interest because BP regulation aimed at reducing BP variability may play an important role in reducing the risk of cardiovascular morbidity and mortality.

Several lines of experimental evidence suggest that vasopressin, a neurohypophyseal peptide, contributes to the pathogenesis of hypertension in genetically hypertensive rats – SHR (model of human essential hypertension), and deoxycorticosterone salt hypertensive rats...
mediated by vascular V1 receptor antagonists in SHR, in the doses we employed in this study. We performed pilot experiments to check whether the dose of 5 mg/kg, i.v. of the V1 antagonist and of 1 mg/kg of the V2 antagonist block efficiently vasopressin receptors in vivo and throughout the experiments. We found that 5 mg/kg, i.v. of the V1 antagonist significantly inhibits the hypertensive effect of exogenously applied AVP (50 ng/kg, i.v.; Sigma, St. Louis, MO, USA) at least for 50 min following its application to anesthetized SHR (n = 4). We also found that urine collected 2 h following administration of 1 mg/kg, i.v. of the V2 antagonist in 16-h dehydrated rats (to stimulate vasopressin secretion) increased in volume and decreased in osmolality (<500 mosmol/l). It is noteworthy mentioning that the selectivity and the efficacy of V1 and V2 vasopressin antagonists in SHR, in the doses we employed in this study, were also reported by others (22, 30).

Experimental protocol
The experiments were started around 10 a.m. every day, 1 h after the rat has been connected to matching 30-cm-long extensions. The arterial line was then connected to the pressure transducer (Isotec™; Hugo Sachs, Freiburg, Germany) for direct measurement of pulsating BP, while the venous catheter was connected to a calibrated syringe for i.v. drug injections. The rats were then submitted to 5 different protocols which included at least 7 animals per group. At the beginning of each experiment, baseline values were recorded for 15–20 min.
In protocol 1, the effects of V₁ antagonist, alone or in combination with V₂ antagonist, on BP and HR variabilities were analyzed (n = 7). The rats received three consecutive injections, at 15-min intervals, of vehicle (1 ml/kg, i.v., 20% ethanol saline solution), V₁ antagonist (5 mg/ml per kg, i.v.), and V₂ antagonist (1 mg/ml per kg, i.v.). After each injection, 3 – 5 min elapsed before recording 10-min-long computer files.

In protocol 2, the effect of V₂ antagonist on BP and HR variabilities was explored (n = 7). The rats received two consecutive i.v. injections, at a 15-min interval, of vehicle (water for injection, 1 ml/kg, i.v.) and of V₂ antagonist (1 mg/ml per kg, i.v.). Ten minute-long files were recorded 3 – 5 min after each injection.

Two separate groups of rats (n = 6 in each) received two and three consecutive injections of corresponding vehicles in a volume of 1 ml/kg, i.v. following protocols 1 and 2, respectively.

Protocol 3 was designed to evaluate the effects of graded hemorrhage on BP and HR variabilities (n = 7). Rats were treated with vehicle (water for injection, 1 ml/kg, i.v.) and 3 – 5 min later, hemorrhaged on three occasions. Each time, 5 ml/kg per min of blood was retrieved (or 0.5 ml/100 g rat body weight which corresponds to 8.3% of total circulating blood volume when it is calculated as 6% of body weight). After each hemorrhage, up to a 5-min period was necessary for BP and HR to stabilize and allow 10 min-long recordings.

A separate group of SHR (n = 5) was submitted to graded hemorrhage after injection of vehicle of the V₁ antagonist (1 ml/kg, i.v. of 20% ethanol saline solution) in order to rule out its potential effects on hemorrhage-triggered changes in BP and HR variability. In this control group, no effects of the vehicle on BP and HR parameters were found (not shown).

In protocol 4, the effect of V₁ antagonist on hemorrhage-induced changes in BP and HR variability was investigated (n = 7). Three to five min prior to hemorrhage, rats received 5 mg/ml per kg (i.v.) of V₁ antagonist and then hemorrhaged to be recorded following protocol 3.

In protocol 5, the effect of V₂ antagonist on hemorrhage-induced changes in BP and HR variability was investigated (n = 7). Rats were treated with the V₂ antagonist (1 mg/ml per kg, i.v.) and 3 to 5 min later, hemorrhaged on three occasions following the same procedure as in protocols 3 and 4.

In protocols 3 – 5, SHR were deprived of drinking during experimentation. All rats tolerated hemorrhage very well and all survived. Nevertheless, one rat was never included twice in the study.

**Drugs**

Selective and non-peptide V₁ (OPC-21268) and V₂ (OPC-31260) antagonists were kindly donated by Dr. J.-F. Liard (Otsuka America Pharmaceutical, Inc., Rockville, MD, USA). OPC-21268 was dissolved in a 20% ethanol-saline solution and OPC-31260 was dissolved in water for injection. Pentobarbital sodium solution (Nembutal® solution) was purchased from Sanofi (Budapest, Hungary) and ampicillin from ICN Pharmaceuticals (Belgrade, Serbia and Montenegro).

**Signal processing and spectrum analysis**

Signal processing and the spectrum analysis were described in detail elsewhere (29). Cardiovascular parameters (systolic blood pressure (SBP), diastolic blood pressure (DBP), and HR) were sampled equidistantly at 20 Hz, allowing direct spectrum analysis using fast fourier transform (FFT) over 2048 point time series. Time spectra were calculated over 8192 points of data sets, free of artifacts which correspond to 410-s (approximately equals 6.8 min) recording periods at 20-Hz sampling rate. Previous to FFT calculations, data sets were subjected to linear trend removal and a 15 point Hanning window. Spectra were analyzed up to 3 Hz. BP and HR variability was quantified by calculating the sum of the moduli (mmHg · Hz⁻¹/₂ or bpm · Hz⁻¹/₂) under the volume created by overlapping 30 FFT segments, for the whole spectrum (total volume = 0.00976 – 3 Hz) and in three frequential domains: VLF (0.00976 – 0.195 Hz), LF (0.195 – 0.605 Hz), and HF (0.8 – 3 Hz) domains. Finally, simple statistics (mean, S.D.) of the distribution of the variables of data sets in time domain used for spectral analysis were computed.

Under basal (resting) conditions, the contribution of VLF- and LF-BP spectral components to total BP and HR variability was comparable in SHR and Wistar rats: the VLF and LF-BP component comprised more than

<table>
<thead>
<tr>
<th></th>
<th>VLF %</th>
<th>LF %</th>
<th>HF %</th>
</tr>
</thead>
<tbody>
<tr>
<td>SBP</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Wistar</td>
<td>28.8 ± 1.5</td>
<td>25.7 ± 1.3</td>
<td>38.1 ± 2.7</td>
</tr>
<tr>
<td>SHR</td>
<td>29.1 ± 1.2</td>
<td>25.9 ± 1.5</td>
<td>37.4 ± 2.8</td>
</tr>
<tr>
<td>DBP</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Wistar</td>
<td>32.3 ± 1.6</td>
<td>25.7 ± 1.4</td>
<td>34.8 ± 2.2</td>
</tr>
<tr>
<td>SHR</td>
<td>32.3 ± 1.2</td>
<td>26.6 ± 1.0</td>
<td>35.7 ± 1.2</td>
</tr>
</tbody>
</table>

Values are mean % ± S.E.M. of the total spectral modulus (n = 20 rats in each group).
50% of total BP variability (Table 1), while the HF-HR component contributed more than 60% to total HR variability in both strains (not shown).

Statistics
Data are presented as means ± S.E.M. In order to apply parametric tests for statistical comparisons of spectral changes, moduli were expressed in log values. Statistical significance was then assessed by one way ANOVA repeated measures (protocol 1), paired t-test (protocol 2), and two way ANOVA one factor repetition (protocols 3–5) using software SPSS.

Results
Effects of V₁ and V₂ antagonists on BP, HR, and their variabilities in resting SHR (protocols 1 and 2)
In conscious, resting SHR, administration of the V₁-receptor antagonist significantly reduced SBP and DBP without affecting BP variability, HR, and HR variability.

| Table 2. Effects of vasopressin V₁ and V₂ antagonists on mean values of SBP, DBP, and HR in resting SHR |
|-------------------------------------------------|-----|-----|-----|-----|-----|
|                                                  | Control 1 | V₁ | V₁ + V₂ | Control 2 | V₂ |
| SBP (mmHg)                                       | 174 ± 6.2 | 160 ± 5.2<sup>a</sup> | 165 ± 7 | 172 ± 6.8 | 167 ± 10.1 |
| DBP (mmHg)                                       | 133 ± 5.2 | 121 ± 4.2<sup>a</sup> | 130 ± 7.2 | 123 ± 5.6 | 124 ± 4.6 |
| HR (bpm)                                         | 347 ± 11.0 | 362 ± 12.0 | 362 ± 9.5 | 369 ± 13.0 | 348 ± 12.9 |

|                                                  | Vehicle 1 | Vehicle 1 + V₂ | Vehicle 2 | Vehicle 2 |
| SBP (mmHg)                                       | 173 ± 3.2 | 176 ± 3.2 | 171 ± 4.4 | 172 ± 6.8 | 175 ± 2.1 |
| DBP (mmHg)                                       | 133 ± 3.0 | 133 ± 1.2 | 127 ± 4.2 | 125 ± 5.6 | 124 ± 9.6 |
| HR (bpm)                                         | 347 ± 6.9 | 361 ± 8.2 | 359 ± 9.5 | 357 ± 9.0 | 352 ± 8.9 |

Values are mean ± S.E.M. <sup>a</sup>P<0.05, vs control 1; <sup>b</sup>P<0.05, vs vehicle 1.

Fig. 1. Effects of V₁ and V₂ antagonists on the components of SBP and DBP spectra in SHR under basal conditions. Bars represent the mean spectral volume ± S.E.M. in total, very low frequency (VLF), low frequency (LF), and high frequency (HF) domains of SBP and DBP. Left panels: empty bars, vehicle; hatched bars, V₁ blockade; cross hatched bars, combined V₁ + V₂ blockade. Right panels: empty bars, vehicle; hatched bars, V₂ blockade. <sup>b</sup>P<0.05, vs V₁ blockade.
Addition of the V₂ antagonist (combined blockade) or administration of the V₂ antagonists alone did not affect cardiovascular parameters (Table 2 and Fig. 1).

Effects of graded hemorrhage on BP, HR, and their variability in SHR

Graded hemorrhage (5 – 15 ml/kg per min) induced a volume-dependent decrease in BP and a significant decrease of HR after massive hemorrhage (Table 3). Mild hemorrhage (5 ml/kg per min) enhanced total SBP variability due to the increase of HF-SBP oscillation, while massive hemorrhage (15 ml/kg per min) produced a decrease of VLF-SBP and VLF-DBP variabilities (Fig. 2).

In this and the following protocols, SHR submitted to graded hemorrhage exhibited no changes in HR variability (results not shown).

Effects of V₁ antagonist on hemorrhage-triggered changes in BP and BP variability

In SHR pre-treated with the V₁ antagonist, hemorrhage induced a volume-dependent decrease in BP and the HR increased significantly (Table 3). In BP spectra, the V₁ antagonist pre-treatment potentiated the HF-BP increase by graded hemorrhage, while the decrease of the VLF-BP oscillation induced by massive hemorrhage was prevented (Figs. 2 and 3).

Table 3. Effects of graded hemorrhage on mean values of SBP, DBP, and HR in vehicle, V₁ antagonist, or V₂ antagonist pre-treated SHR

<table>
<thead>
<tr>
<th></th>
<th>Pre-hemorrhage</th>
<th>Mild hemorrhage (5 ml/kg)</th>
<th>Moderate hemorrhage (10 ml/kg)</th>
<th>Massive hemorrhage (15 ml/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vehicle</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SBP</td>
<td>175 ± 9</td>
<td>173 ± 14</td>
<td>155 ± 10</td>
<td>132 ± 10⁺⁺⁺⁺⁺⁺</td>
</tr>
<tr>
<td>DBP</td>
<td>145 ± 9</td>
<td>140 ± 12</td>
<td>122 ± 12</td>
<td>99 ± 11⁺⁺⁺⁺⁺⁺</td>
</tr>
<tr>
<td>HR</td>
<td>343 ± 17</td>
<td>341 ± 14</td>
<td>328 ± 2.8</td>
<td>279 ± 15⁺⁺⁺⁺⁺⁺</td>
</tr>
<tr>
<td>V₁</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SBP</td>
<td>172 ± 5</td>
<td>174 ± 3</td>
<td>172 ± 5</td>
<td>143 ± 9⁺⁺⁺⁺⁺⁺</td>
</tr>
<tr>
<td>DBP</td>
<td>129 ± 8</td>
<td>120 ± 7</td>
<td>117 ± 12</td>
<td>94 ± 14⁺⁺⁺⁺⁺⁺</td>
</tr>
<tr>
<td>HR</td>
<td>347 ± 4</td>
<td>375 ± 5⁺⁺⁺⁺⁺⁺</td>
<td>392 ± 6⁺⁺⁺⁺⁺⁺</td>
<td>361 ± 11⁺⁺⁺⁺⁺⁺</td>
</tr>
<tr>
<td>V₂</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SBP</td>
<td>176 ± 15</td>
<td>176 ± 13</td>
<td>172 ± 15</td>
<td>146 ± 11⁺⁺⁺⁺⁺⁺</td>
</tr>
<tr>
<td>DBP</td>
<td>131 ± 12</td>
<td>130 ± 13</td>
<td>122 ± 15</td>
<td>92 ± 17⁺⁺⁺⁺⁺⁺</td>
</tr>
<tr>
<td>HR</td>
<td>351 ± 8</td>
<td>346 ± 7</td>
<td>308 ± 5⁺⁺⁺⁺⁺⁺</td>
<td>269 ± 19⁺⁺⁺⁺⁺⁺</td>
</tr>
</tbody>
</table>

Inserted marks of significance: ⁺⁺⁺⁺⁺⁺P<0.05, ⁺⁺⁺⁺⁺⁺⁺⁺P<0.01, vs pre-hemorrhage values; ⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺P<0.05, vs nontreated SHR (Bonferroni pairwise comparison).

Discussion

In this paper, the contribution of vasopressin to BP short-term variability in conscious and freely moving SHR with overt hypertension was assessed by pharmacological blockade of vascular V₁ and renal V₂ vasopressin receptors. The results imply that vasopressin, by V₁ and V₂ receptors, buffers BP oscillations in HF and VLF domains only after the stimulation of vasopressin release by hemorrhage. The VLF oscillation is the component of BP short-term variability that comprises most of the spectral power under basal conditions. It was found to arise from the activity of multiple mechanisms in the regulation of the peripheral resistance and local blood flow. These mechanisms involve neurohormones such as the renin-angiotensin system (RAS), thermoregulation, arterial baro-receptor reflex, endothelial NO, and inherent myogenic mechanism (6, 9, 10, 12). Some mechanisms were found to be directly opposed and others were found to act in concert to modulate VLF-BP oscillation. For instance, myogenic mechanisms were found to create VLF-BP oscillations at 0.13 Hz in rat mesenteric and renal arteries, while the baro-receptor reflex and RAS were found to buffer them (6, 10, 12).

Vasopressin exerts its hemodynamic effects through V₁-receptor subtypes located at the site of vascular smooth muscles, contributing thus to overall peripheral
resistance and BP maintenance. It also acts at $V_2$-receptor subtypes located in the collecting ducts of the kidneys to modulate water diuresis in the short-term and the long-term manner (30). Furthermore, $V_1$ receptors have been found in the area postrema, a site of the brain deprived of the blood brain barrier associated with the modulation of the baro-receptor reflex sensitivity (27, 31–34). We have previously reported that $V_1$- and $V_2$-receptor antagonists enhance VLF-BP variability in normotensive rats under basal conditions and after the stimulation of vasopressin release by hemorrhage (29), suggesting that both vascular $V_1$-receptor and renal $V_2$-receptor subtypes are implicated. The present results in SHR with the same doses of vasopressin antagonists
show that although in resting conditions $V_1$ antagonist decreased BP, it modified VLF-BP variability only after massive hemorrhage; and likewise, in normotensive rats, this effect involved $V_1$- and $V_2$-receptor subtypes. This finding suggests that $V_1$ receptors that contribute to BP level in resting SHR and to VLF-BP modulation in hemorrhage are not the same. Maybe $V_1$ receptors located in other vascular beds (mesenteric and/or renal) have to be stimulated by increased vasopressin concentration in hemorrhage (35) to buffer VLF-BP oscillation. Another possibility is that $V_1$ receptors located elsewhere; that is, in the area postrema are recruited by hemorrhage (36 – 38) to buffer VLF-BP variability. The lack of this effect in adult SHR is not surprising and supports the view of Stauss et al. (13) and Daffonchio et al. (49) who refuted the reliability of LF-BP oscillation as a marker of sympathetic outflow to blood vessels in SHR.

The changes of BP and HR after $V_1$ and $V_2$ blockade in SHR under basal conditions and after hemorrhage corroborate well with the general view that vasopressin contributes little to the genesis of genetic hypertension (20, 22 – 26, 41), but plays an important role in the autonomic adjustment to hemorrhage (35 – 37). Under basal conditions, $V_1$-receptor blockade significantly reduced BP, confirming that vasopressin in SHR contributes to the peripheral vascular resistance and BP via the $V_1$-receptor subtype located at the site of the vascular smooth muscle. However, combined $V_1 + V_2$ blockade or $V_2$ antagonist alone had no effect on BP. Furthermore, in SHR, as well as in normotensive rats, hypotensive hemorrhage is a strong stimulus for vasopressin release (35 – 38). The beneficial effect of vasopressin in hypotensive hemorrhage was found to be due to cardiac and renal sympathoinhibition that reduce oxygen demands and conserves circulating blood volume (35 – 38). The sympathoinhibitory effect of vasopressin to the heart was assigned to $V_1$ receptors located in the area postrema (36 – 38). There is also evidence, provided by Fujisawa et al. (50), that $V_2$ receptors may act in opposite manner. Our results that $V_1$ blockade prevented bradycardia while $V_2$ blockade enhanced bradycardia in SHR submitted to hemorrhage suggest that the modulation of the baro-receptor HR response to hemorrhage by vasopressin is preserved in...
 SHR. Similar findings in SHR were reported by Sampey et al. (31).

We cannot totally exclude the possibility that \( V_1 \) and \( V_2 \) antagonists used in this study may have affected other receptors that mediate vasodilatation (51 – 53). It is therefore important to emphasize that the receptor subtype selectivity and the functional separation between \( V_1 \)- and \( V_2 \)-receptor blockade is warranted even at doses ten times higher than the ones we employed (22, 23, 28, 31, 50). This minimizes the possibility that the effects of vasopressin antagonists in this study were due to inhibition of receptors other than the \( \text{V}_1 \)- and \( \text{V}_2 \)-receptor subtypes.

Finally, our study implies that in adult SHR, vasopressin buffers BP oscillations in HF and VLF domains only in hypovolaemic conditions. They also suggest that the modulation of the autonomic adjustment of the HR to hemorrhage by vasopressin is preserved.

Acknowledgments

We are thankful to Mrs. Vera Savić for her skilful technical assistance. This study was supported by the Serbian Ministry of Science, Technologies, and Development (Grant No. 1774).

References

39 Mulvany MJ, Hanssen PK, Aalkjaer C. Direct evidence that the greater contractility of resistance vessels in spontaneously hypertensive rats is associated with a narrowed lumen, a thickened media, and an increased number of smooth muscle cell layers. Circ Res. 1978;43:854–864.