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Effects of Nonpeptide and Selective V_1 and V_2 Antagonists on Blood Pressure Short-Term Variability in Spontaneously Hypertensive Rats

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Abstract. Effects of V_1 (OPC-21268) and V_2 (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 V_1 antagonist (5 mg/kg, i.v.) decreased BP without affecting BP variability, while combined ($V_1 + V_2$) blockade or V_2 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 V_1, but not V_2, antagonist pre-treated SHR HF-BP increased significantly after moderate and massive hemorrhage. V_1 or V_2 antagonist pre-treatment also enhanced VLF-BP variability during massive hemorrhage. Moreover V_1 blockade prevented hemorrhage-induced bradycardia, while V_2 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

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(20, 21). In SHR, plasma concentration of vasopressin was found to be elevated (20), V_1 receptor antagonists significantly reduce BP (22, 23), and vascular responsiveness to exogenously applied arginine-vasopressin is potentiated in comparison to normotensive controls (24). In addition, in normotensive rats, chronic stimulation of V_1 receptors results in sustained hypertension (25, 26).

The main physiological functions of vasopressin in BP control, water balance, and ACTH release are mediated by vascular V_1 (V_{1a}), renal V_2, and pituitary V_3 (V_{1b}) receptors, respectively (27). Random screening of chemical compounds resulted in development of orally active and highly selective antagonists at V_1, V_2, and V_3 receptor sites with no agonist properties (28). By studying the effects of novel V_1 (OPC-21268) and V_2 (OPC-31260) antagonists on BP short-term variability in normotensive rats, we have provided evidence that vasopressin contributes to fast control of BP and buffers BP short-term variability under basal and hypovolaemic conditions (29).

This study is a continuation of the previous study in normotensive rats. The aim is to elucidate the role of vasopressin in the modulation of BP short-term variability in SHR. In order to assess the respective roles of V_1 and V_2 receptor subtypes, we analyzed the effects of non-peptide and selective V_1 (OPC-21268) and V_2 (OPC-31260) vasopressin antagonists on the components of BP short-term variability in conscious freely moving adult SHR, under basal conditions and after the stimulation of vasopressin release by hemorrhage.

Materials and Methods

General

Experiments were carried out in conscious, unrestrained 6-month-old male SHR (Farma VMA, Belgrade, Serbia and Montenegro) weighing 320 – 350 g; chronically instrumented with right femoral artery and right jugular vein catheters, housed in controlled laboratory conditions (temperature, 23 ± 1°C; humidity, 65 ± 3%; light darkness cycle, 12/12 h); with food and tap water provided ad libitum.

All experiments were conducted 48 h after surgery, in a quiet surrounding, in rats exhibiting normal behavior (food and water intake), housed separately in plexiglas cages (25 × 25 × 25) cm. Blood withdrawal and drug injections were done using catheter extensions to avoid animal manipulations.

All procedures in this study conformed to the EEC Directive 86/609 and were approved by the Ethical Committee on Animal Experimentation of the Belgrade School of Medicine. They also comply with guiding principles for the care and use of laboratory animals approved by The Japanese Pharmacological Society.

Surgery

Under pentobarbital anesthesia (50 mg/kg, i.p.), a polyethylene tubing pre-filled with heparinized saline was inserted into the right femoral artery (ID-0.58, ED-0.96) and the jugular vein (ID-0.38, ED-1.09) and tunneled subcutaneously to exit between the scapulae. Postoperatively, the rats received one injection of ampicillin (7 mg, i.m.) and were housed separately in plexiglas cages to recover from surgery.

Pilots

In our previous study in Wistar rats, in dose-response experiments, we established that the least dose of vasopressin V_1 antagonist that affects BP variability is 5 mg/kg, i.v. (29). We also found that under basal conditions the V_2 antagonist does not affect BP variability in a dose of 1 mg/kg, i.v., although it produces water diuresis. Therefore, further increase of the dose of V_2 antagonist was not possible as it would affect circulating blood volume. In order to investigate whether vasopressin modulates BP variability in SHR we used the same doses as in normotensive rats. In SHR, we performed pilot experiments to check whether the dose of 5 mg/kg, i.v. of the V_1 antagonist and of 1 mg/kg of the V_2 antagonist block efficiently vasopressin receptors in vivo and throughout the experiments. We found that 5 mg/kg, i.v. of the V_1 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 V_2 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 V_1 and V_2 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 V_1 antagonist, alone or in combination with V_2 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), V_1 antagonist (5 mg/ml per kg, i.v.), and V_2 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 V_2 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 V_2 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 V_1 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 V_1 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 V_1 antagonist and then hemorrhaged to be recorded following protocol 3.

In protocol 5, the effect of V_2 antagonist on hemorrhage-induced changes in BP and HR variability was investigated (n = 7). Rats were treated with the V_2 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 V_1 (OPC-21268) and V_2 (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 ($mmHg \cdot Hz^{-1/2}$ or $bpm \cdot Hz^{-1/2}$) 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 1. Contribution of spectral components to total systolic and diastolic blood pressure (SBP, DBP) variability in Wistar and SHR rats

	VLF %	LF %	HF %
SBP			
Wistar	28.8 ± 1.5	25.7 ± 1.3	38.1 ± 2.7
SHR	29.1 ± 1.2	25.9 ± 1.5	37.4 ± 2.8
DBP			
Wistar	32.3 ± 1.6	25.7 ± 1.4	34.8 ± 2.2
SHR	32.3 ± 1.2	26.6 ± 1.0	35.7 ± 1.2

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 V_1 and V_2 antagonists on BP, HR, and their variabilities in resting SHR (protocols 1 and 2)

In conscious, resting SHR, administration of the V_1-receptor antagonist significantly reduced SBP and DBP without affecting BP variability, HR, and HR variability.

Table 2. Effects of vasopressin V_1 and V_2 antagonists on mean values of SBP, DBP, and HR in resting SHR

	Control 1	V_1	$V_1 + V_2$	Control 2	V_2
SBP (mmHg)	174 ± 6.2	160 ± 5.2[a,†]	165 ± 7	172 ± 6.8	167 ± 10.1
DBP (mmHg)	133 ± 5.2	121 ± 4.2[a,†]	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	Vehicle 1 + Vehicle 2	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. [a]$P<0.05$, *vs* control 1; [†]$P<0.05$, *vs* vehicle 1.

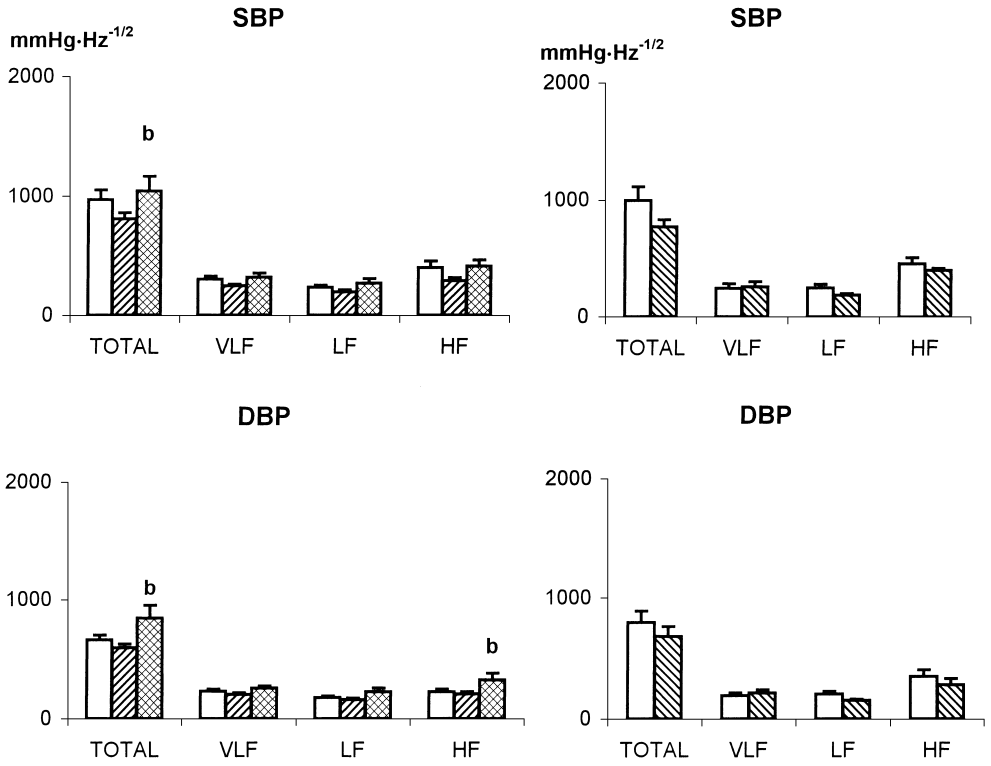

Fig. 1. Effects of V_1 and V_2 antagonists on the components of SBP and DBP spectra in SHR under basal conditions. Bars represent the mean spectral volume ± S.E.M. in total, very low frequency (VLF), low frequency (LF), and high frequency (HF) domains of SBP and DBP. Left panels: empty bars, vehicle; hatched bars, V_1 blockade; cross hatched bars, combined $V_1 + V_2$ blockade. Right panels: empty bars, vehicle; hatched bars, V_2 blockade. [b]$P<0.05$, *vs* V_1 blockade.

Addition of the V_2 antagonist (combined blockade) or administration of the V_2 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 V_1 antagonist on hemorrhage-triggered changes in BP and BP variability

In SHR pre-treated with the V_1 antagonist, hemorrhage induced a volume-dependent decrease in BP and the HR increased significantly (Table 3). In BP spectra, the V_1 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).

Effects of V_2 antagonist on hemorrhage-triggered changes in BP and BP variability

The V_2 antagonist pre-treated SHR submitted to graded hemorrhage exhibited a volume-dependent decrease in BP and greater bradycardia than nontreated SHR (Table 3). Similarly to the V_1 pre-treated SHR, in the V_2 pre-treated SHR, VLF-BP oscillations did not decrease after massive hemorrhage (Figs. 2 and 3).

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 V_1 and renal V_2 vasopressin receptors. The results imply that vasopressin, by V_1 and V_2 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 V_1-receptor subtypes located at the site of vascular smooth muscles, contributing thus to overall peripheral

Table 3. Effects of graded hemorrhage on mean values of SBP, DBP, and HR in vehicle, V_1 antagonist, or V_2 antagonist pre-treated SHR

	Pre-hemorrhage	Mild hemorrhage (5 ml/kg)	Moderate hemorrhage (10 ml/kg)	Massive hemorrhage (15 ml/kg)
Vehicle				
SBP	175 ± 9	173 ± 14	155 ± 10	132 ± 10^{aa}
DBP	145 ± 9	140 ± 12	122 ± 12	99 ± 11^{aa}
HR	343 ± 17	341 ± 14	328 ± 2.8	279 ± 15^{aa}
V_1				
SBP	172 ± 5	174 ± 3	172 ± 5	143 ± 9^{a}
DBP	129 ± 8	120 ± 7	117 ± 12	94 ± 14^{a}
HR	347 ± 4	375 ± 5^{a}	392 ± 6^{aa,†}	361 ± 11†
V_2				
SBP	176 ± 15	176 ± 13	172 ± 15	146 ± 11^{aa}
DBP	131 ± 12	130 ± 13	122 ± 15	92 ± 17^{aa}
HR	351 ± 8	346 ± 7	308 ± 5^{a,†}	269 ± 19^{aa}

Inserted marks of significance: $^{a}P<0.05$, $^{aa}P<0.01$, *vs* pre-hemorrhage values; $^{\dagger}P<0.05$, *vs* nontreated SHR (Bonferroni pairwise comparison).

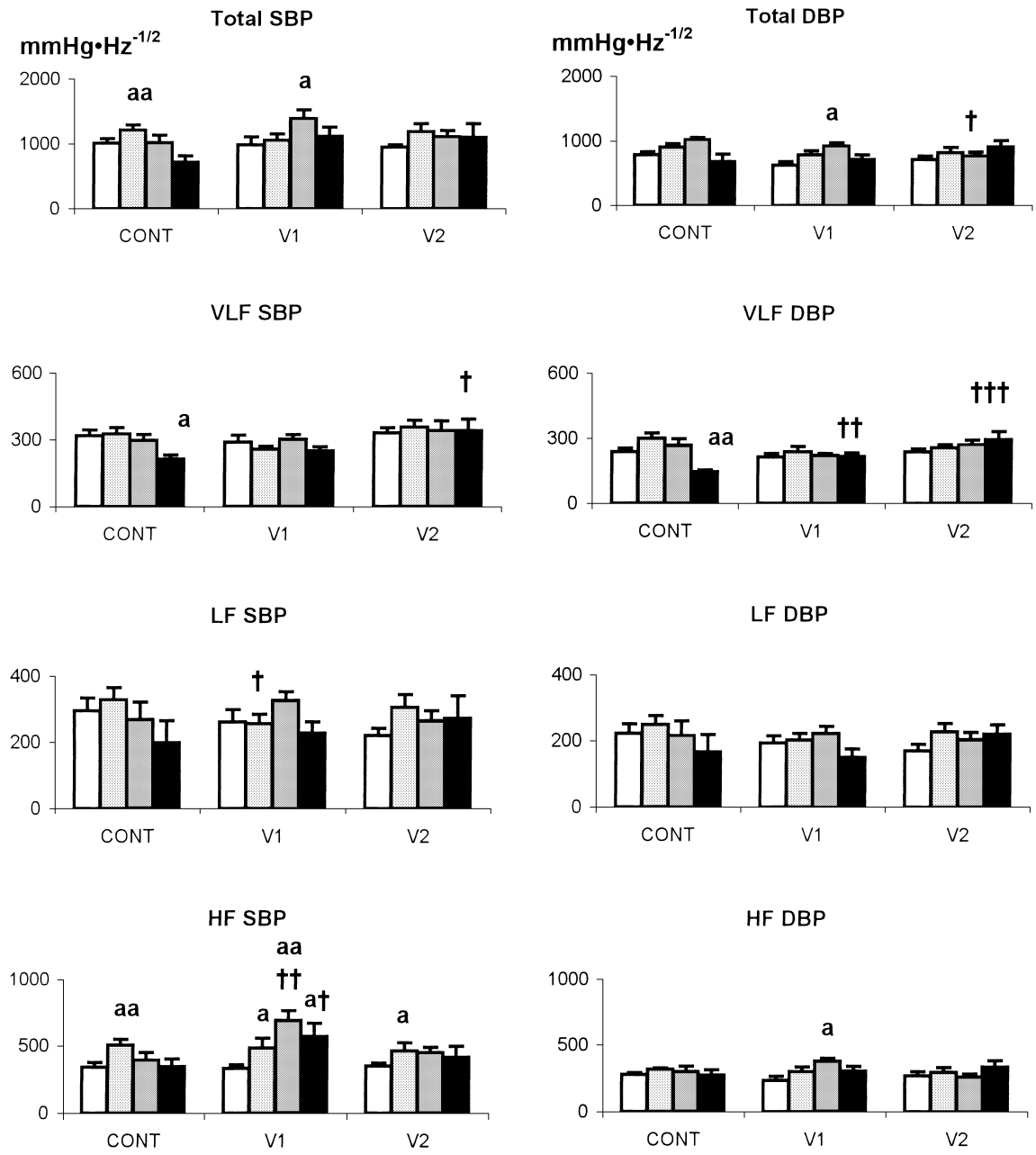

Fig. 2. Effects of graded hemorrhage on total spectral modulus and spectral components of SBP (left panel) and DBP (right panel) in vehicle, V_1 antagonist, or V_2 antagonist pre-treated SHRs. Bars represent the mean spectral volume ± S.E.M. Empty bars, resting; light grey bars, mild hemorrhage; dark grey bars, moderate hemorrhage; black bars, massive hemorrhage; CONT, vehicle pre-treated SHR; V_1, V_1 antagonist pre-treated SHR; V_2, V_2 antagonist pre-treated SHR. $^{a}P<0.05$, $^{aa}P<0.01$, *vs* pre-hemorrhage values; $^{\dagger}P<0.05$, $^{\dagger\dagger}P<0.01$, $^{\dagger\dagger\dagger}P<0.001$, *vs* nontreated SHR (Bonferroni pairwise comparison).

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

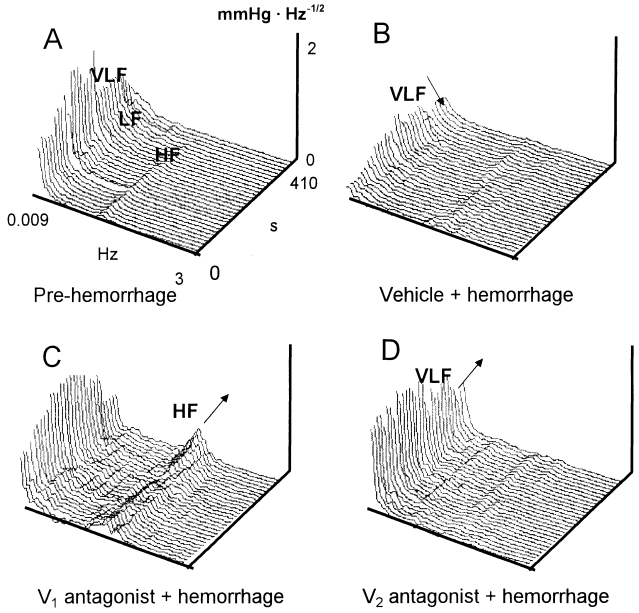

Fig. 3. Effects of massive hemorrhage on SBP time spectra in three typical experiments in SHRs. SBP spectrum of one SHR before (A) and after (B) hemorrhage without vasopressin receptor blockade. Panels C and D show SBP spectra of two other SHRs hemorrhaged under V_1 or V_2 blockade, respectively. Arrows indicate significant changes shown in Fig. 2 (left panel, black bars).

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 invoved 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. Nonetheless, the mechanism(s) by which vasopressin buffers VLF-BP oscillation in SHR is beyond the scope of this paper. It is likely that the genesis of VLF-BP oscillation differs in SHR and normotensive rats and that mechanism(s) involved in its modulation are altered. For instance, changes in sympathetic outflow, renin secretion, blood vessel wall flexibility, baro-receptor reflex sensitivity, and NO production have been associated with hypertension (16, 17, 20, 21, 24, 39 – 44). Moreover, vasopressin, by contributing to cardiovascular homeostasis, interacts with the above-mentioned mechanism(s). It is therefore possible that the outcome of these interactions also depends on changes of the number (gene expression) and/or the affinity of V_1 and/or V_2 receptors that occur in hypertension (42 – 44).

The HF-BP oscillation has been shown to reflect the mechanical perturbations of the circulation by negative intrathoracic pressure associated with inspiration (7). During hemorrhage, this effect is enhanced because greater distension of unloaded thoracic vessels occurs (29, 45, 46). The main vasoconstrictor mechanisms activated by hemorrhage such as the sympathicus, RAS and vasopressin were found to buffer the HF-BP increase induced by hemorrhage (29, 45 – 47). Thus the potentiation of HF-BP increase by hemorrhage in V_1-receptor antagonist pretreated SHR (Fig. 2) was an expected finding in this study.

It is interesting to note that in our experiments, hemorrhage that is usually associated with sympathetic activation (11, 45, 46, 48) failed to modify LF-BP variability in SHR. In normotensive and young SHR, hemorrhage was found to increase 0.4-Hz oscillation (component of LF-BP oscillation) by inducing a positive feed-back response in the arterial baro-receptor reflex loop (14). 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 V_1- and 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.

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