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Role of vasopressin V1 antagonist in the action of vasopressin on the cooling-evoked hemodynamic perturbations of rats

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ABSTRACT

We aimed to investigate the role of arginine vasopressin (AVP) acting via the AVPV1 receptor in the autonomic cardiovascular responses to cold stress (CS). The study was conducted on adult male Sprague-Dawley rats with telemetry transmitters implanted to monitor heart rate (HR) and systolic blood pressure (SBP) throughout the experiment course. Rats were divided into four groups and were given, respectively, saline (control group), AVPV1 antagonist (V1880) alone, and V1880 following the removal of sympathetic outflows using hexamethonium (HEX + V1880) or guanethidine (GUA + V1880). Rats were subjected to the CS stimuli (rapid immersion of the rat's limbs into 4 °C water). Hemodynamic responses were recorded at baseline (PreCS), during CS, and after CS. Data analysis was performed using descriptive methods and spectral and cross-spectral analysis of blood pressure variability (BPV) and heart rate variability (HRV). Key results showed that at PreCS, inhibition of AVPV1 increases SBP and HR as well as very-low-frequency BPV and low-frequency BPV, which is attenuated by hexamethonium (effect on SBP only) and guanethidine (effect on both SBP and HR). HEX + V1880 results in increased high-frequency BPV and attenuated very-low-frequency HRV, while GUA + V1880 results in increased high-frequency HRV and attenuated very-low-frequency HRV. During CS, we observed that SBP and HR, as well as very-low-frequency BPV and low-frequency BPV, were similar in the control group and the group with AVPV1 inhibition, while AVPV1 inhibition results in attenuated high-frequency BPV. Furthermore, we observed that changes produced by AVPV1 inhibition alone were affected differently by HEX + V1880 and GUA + V1880, particularly in low-frequency HRV and very-low-frequency HRV. The results support that AVPV1 mediates autonomic cardiovascular responses at both baseline and CS stimuli conditions are associated with central mechanism engagement.

1. Introduction

Arginine vasopressin (AVP) is a neurohypophysial nonapeptide that has been traditionally recognized for its role as a vasoconstrictor hormone and its antidiuretic effect by acting on two receptor subtypes V1 (V1a and V1b) and V2 (Koshimizu et al., 2012; Mavani et al., 2015; Wolfe et al., 2018). V1a receptors show a widespread distribution within the brain, whereas V1b receptors are less prominent centrally but highly expressed within the anterior pituitary. In addition to AVP's well-known role in cardiovascular regulation, it has also been demonstrated to have other interesting effects on behavior, such as the release of AVP within the lateral septum for controlling learning and memory (Jiang et al., 2017).

On the other hand, recent evidence suggests that AVP may have analgesic effects (Jasnic et al., 2015; Mavani et al., 2015; Oluyomi and Hart, 1992; Yang et al., 2007). Studies have shown that AVP can increase the pain threshold to painful stimuli (antinociception), and AVP antagonists could invert such an effect. Given that morphological and electrophysiological studies have indicated that V1a binding sites are present in all laminae of the central gray in the spinal cord, this kind of receptor seems to facilitate inhibitory transmission (Liu et al., 2003). Animal studies have also indicated that the V1a receptor subtype mediates the inhibitory action of AVP on reflex circuits to input from sensory stimuli. Evidence also shows that nociception and mood

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regulation of AVP/oxytocin occurs in the dorsal root ganglia, and primary afferent neurons appear to be activated by the V1a receptors (Han et al., 2018; Peng et al., 2015).

When exposed to cold stress (CS), thermoregulatory networks can stimulate a cascade of orchestrated stressful hormones and produce pressor and tachycardia responses (Daanen, 2003). As in the noxious cold pain CS condition, rapid immersion of the extremities into 4 °C water will evoke hemodynamic perturbations (CEHP), which can be a useful animal model for the assessment of autonomic cardiovascular regulation. Concurrent processing of the signals of rhythmic variations in blood pressure (BPV) and heart rate (HRV) is useful in the study of cardiovascular regulatory mechanisms affected by the autonomic nervous system (Japundzic et al., 1990; Liu et al., 2015a). We previously conducted a series of experiments to investigate CEHP and have provided a rationale that increased low-frequency BPV (LFBPV) indicates sympathetic arousal, while the subsequently increased very-low-frequency BPV (VLFBPV) indicates strengthened vascular myogenic activity (Lin et al., 2017a, 2017b; Liu et al., 2015a).

The purpose of this study was to evaluate the role of putative AVPV1 in the autonomic regulation of cardiovascular responses to CS. We examined whether AVPV1 antagonist treatment with or without CS causes rhythmic variations in systolic blood pressure (SBP) and heart rate (HR) by using the telemetric system and spectral and cross-spectral analyses in conscious rats.

2. Materials and methods

2.1. Animals

Adult male Sprague-Dawley rats weighing between 300 and 350 g were received at the Laboratory Animal Center of the National Defense Medical Center (NDMC, Taiwan, ROC) one week before the experiments. The studies were performed according to a protocol approved by the Institutional Animal Care and Use Committee (IACUC) of NDMC. All efforts were made to reduce the number of experimental animals and their suffering in experiments. Rats from the same experimental groups were housed together in an ambient thermoneutral environment (23 °C) and humidity-controlled holding facility with a 12-h light/dark cycle (lights-on from 07:00 to 19:00) maintained by manual light control switches, and they received food and water ad libitum.

2.2. Experimental design

The time sequence of the dosing drugs is shown in Fig. 1. Rats were randomly divided into four experimental groups, all of which received a similar stressful cooling procedure in the experimental rooms at ambient temperature (23 °C). The control group rats were given 0.4 mL vehicle solution (normal saline [NS], 0.9% NaCl/1 mL, $n = 12$) via an intraperitoneal injection 15 min before the CS trial. The other three groups of rats were given V1880 ([deamino-Pen1, O-Me-Tyr2, Arg8]-Vasopressin, $n = 12$), a potent AVPV1 antagonist alone (n = 12), or V1880 following the removal of sympathetic outflows using the ganglion blocker hexamethonium (HEX + V1880, $n = 10$) or chemical sympathectomy guanethidine (GUA + V1880, n = 12), respectively, that is, (a) an intraperitoneal injection of V1880 (0.2 mg/kg/1 mL) 15 min prior to the presentation of a CS trial, (b) a tail venous bolus injection of hexamethonium (30 mg/mL/kg) followed by continuous infusion (1.5 mg/kg/min) 5 min before the V1880 injection throughout the CS trial (around 2 mL), or (c) an intraperitoneal injection of guanethidine (50 mg/kg/day × 7 days) including an additional dose 15 min before the V1880 injection in the testing day. All chemicals were purchased from Sigma-Aldrich Corp (St. Louis, MO, USA).

2.3. Cooling procedure

All rats were brought to an adjacent room and given the same acute cooling procedure after they had adjusted to the experimental environment. A maximum of five rats were tested at the same time every day. All experiments were performed between 08:30 and 11:30.

Following a complete stabilization of SBP and HR at room temperature, every rat was quickly placed in a Plexiglas test cage ($35 \times 18 \times 25\ cm^3$) to immerse its glabrous palms and soles for 10 min in the 4 °C water (CS). After this cooling procedure, the rat was removed from the cage, dried with a cloth, and placed in a similar cage for 20 min to facilitate recovery. Beat-to-beat SBP signals were recorded continuously via telemetric monitoring equipment (TL11M2-M2-C50-PXT, Data Sciences International, St. Paul, Minnesota, USA) including 10 min for baseline before the CS trial (PreCS), 10 min during the CS trial, and 40–50 min after the CS trial (PostCS). Afterward, successive signals from a 5-min period (3 to 8 min) at each trial were submitted to spectral analyses because the mean and variance of VLFBPV signals were stable and the oscillations of SBP during this period were observed to be steady.

2.4. Surgical intervention and spectrum signal acquisition and processing

The telemetry transmitter was implanted intra-abdominally into each rat under anesthesia (sodium pentobarbital, 50 mg/kg). A laparotomy was performed under aseptic conditions to insert a catheter containing the transmitter into the abdominal aorta, distal to the kidneys, and fixed. Experiments were started after the rat had fully recovered from surgery (14 days). The SBP signal-processing and spectral and cross-spectral analyses used the methods reported in our previous study (Liu et al., 2015a). Briefly, signals of the SBP and HR oscillations

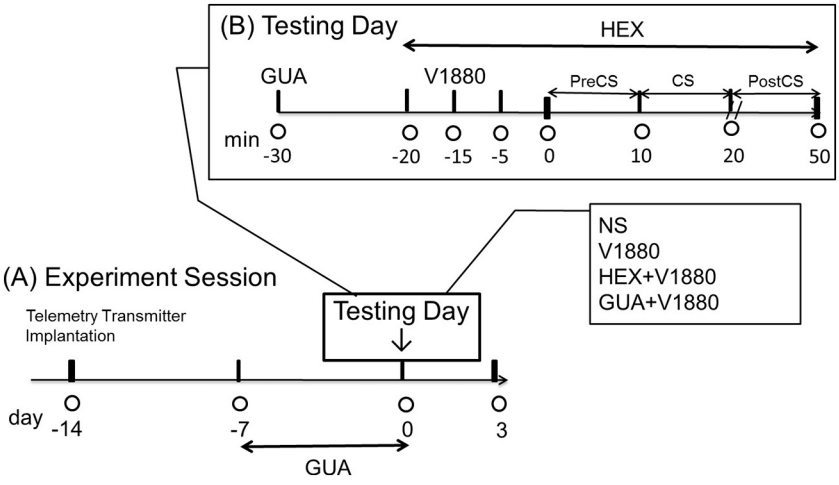

Fig. 1. General protocol for (A) the implantation of the telemetry device in rats 14 days before the testing day and (B) the sequence of testing-day procedures in the following order: PreCS, CS, and PostCS, for a rat in a Plexiglas cage. The control group rats were given 0.4 mL vehicle solution (normal saline, 0.9% NaCl/1 mL, $n = 12$) via intraperitoneal injection. The other three groups of rats were given (a) an intraperitoneal injection of a potent vasopressin V1 receptor antagonist alone (0.2 mg/kg/1 mL) 15 min prior to the presentation of cold stimuli (V1880, n = 12), (b) superimposition of a tail venous bolus injection of hexamethonium (30 mg/mL/kg) followed by continuous infusion (1.5 mg/kg/min) 5 min before the V1880 injection throughout the CS trial (HEX + V1880, $n = 10$), or (c) an intraperitoneal injection of guanethidine (50 mg/kg/day × 7 days) including an additional dose 15 min before the V1880 injection in the testing day (GUA + V1880, $n = 12$). CS, cold stress (4 °C water immersion of the palms and soles); PreCS, before CS; PostCS, after CS.

were computed independently to obtain the total power (TP; 0.00 to 3.0 Hz) and spectral power of the three major frequency regions: very low frequency (VLF; 0.02 to 0.2 Hz), low frequency (LF; 0.20 to 0.60 Hz), and high frequency (HF; 0.60 to 3.0 Hz). The ratio of low-frequency power to high-frequency power (LF/HF ratio) of HRV, which is commonly used as a measure of sympathovagal balance, was also calculated. The modulus of the spectral density for each frequency had units of BPV (mm Hg^2) and HRV (ms^2). On the other hand, the squared coherence function was computed as the square of the cross-spectrum normalized by the product of the spectra of the BPV and HRV signals. When the peak coherence value ($K^2_{HR/SBP}$) exceeded 0.58 within a frequency range, the 2 signals were considered to covary significantly at that frequency.

2.5. Statistical methods

Statistical analyses were performed using SPSS 18.0 for Windows (Chicago, Illinois, USA). Data were tested for normality using Kolmogorov and Smirnov distributions. Comparisons between groups were performed by the within-group design to fit the multifactor analysis of variance (ANOVA) with a within-subject factor, TRIAL (3 conditions along the experimental procedures, i.e., PreCS, CS, and PostCS, according to a repeated-measurement design), and a between-subject factor, GROUP (4 treatments: NS, V1880, HEX + V1880, and GUA + V1880). If necessary, post hoc comparisons were carried out with Tukey or Student's *t*-tests where appropriate. Data are presented as the mean value per group ± standard error of the mean (SEM). Results were considered statistically significant at $p < .05$.

3. Results

Averaged data are shown in Figs. 2-4 and Table S1 (please see the Data Supplement).

3.1. Response of SBP and HR

For SBP and HR (Fig. 2A, B and Table S1) throughout the experiment course, the ANOVA (See Table 1) showed a significant effect of TRIAL (SBP and HR), a significant effect of GROUP (SBP and HR), and a significant interaction between TRIAL and GROUP (SBP and HR). When compared with the control saline (NS), inhibition of AVPV1 (V1880) significantly increased SBP in PreCS ($p < .05$) and resulted in a tendency toward increased SBP in CS (Fig. 2A). The higher SBP levels in response to V1880 were attenuated by the superimposition of ganglionic blockade with HEX (HEX + V1880) and were also markedly attenuated by the superimposition of chemical sympathectomy with GUA (GUA + V1880) in all experimental conditions (all $p < .05$). On the other hand, V1880 caused a significant increase in HR (Fig. B) compared with NS in PreCS only ($p < .05$). However, this effect was not affected by HEX but was significantly attenuated by GUA compared with NS in all experimental groups (GUA + V1880 versus NS and HEX + V1880: all $p < .05$). Furthermore, when comparing CS with PreCS or PostCS, CS on V1880, as on NS, still somewhat evoked the cold-induced pressor (Fig. A: cold pain–related vasoconstriction [CIP]; PreCS or PostCS versus CS: $F(1, 11) = 73.70$, $p < .05$) and cold-induced tachycardia (Fig. B: [CIT]; PreCS or PostCS versus CS: $F(1, 11) = 76.38$, $p < .05$) responses.

3.2. Response of frequency powers and coherence functions

For frequency powers (Fig. 3A: VLF; Fig. 3B: LF; Fig. 3C: HF; Table S1: LF/HFHRV and TP) throughout the experiment course, the ANOVA (See Table 1) showed a significant effect of TRIAL (VLFHRV, LFHRV, HFBPV, HFHRV, and LF/HFHRV), a significant effect of GROUP

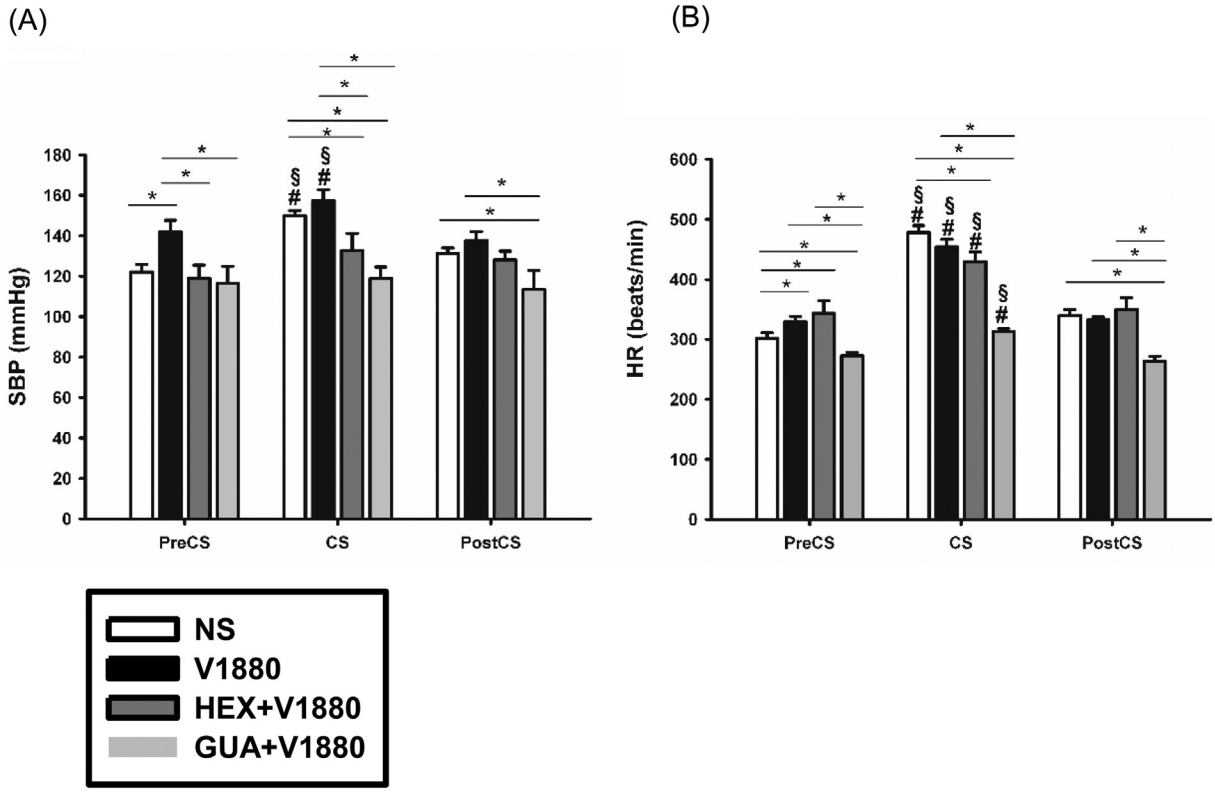

Fig. 2. Effects on (A) systolic blood pressure and (B) heart rate of rats in the four experimental groups throughout the experiment course. Data are presented as mean value per group ± standard error of the mean (SEM). Note that statistical significance shows the differences between experimental groups (*$p < .05$). #$p < .05$ compared with the same parameter for CS versus PreCS. §$p < .05$ compared with the same parameter for CS versus PostCS. CS, cold stress (4 °C ice-water immersion of the palms and soles); PreCS, before CS; PostCS, after CS; SBP, systolic blood pressure; HR, heart rate.

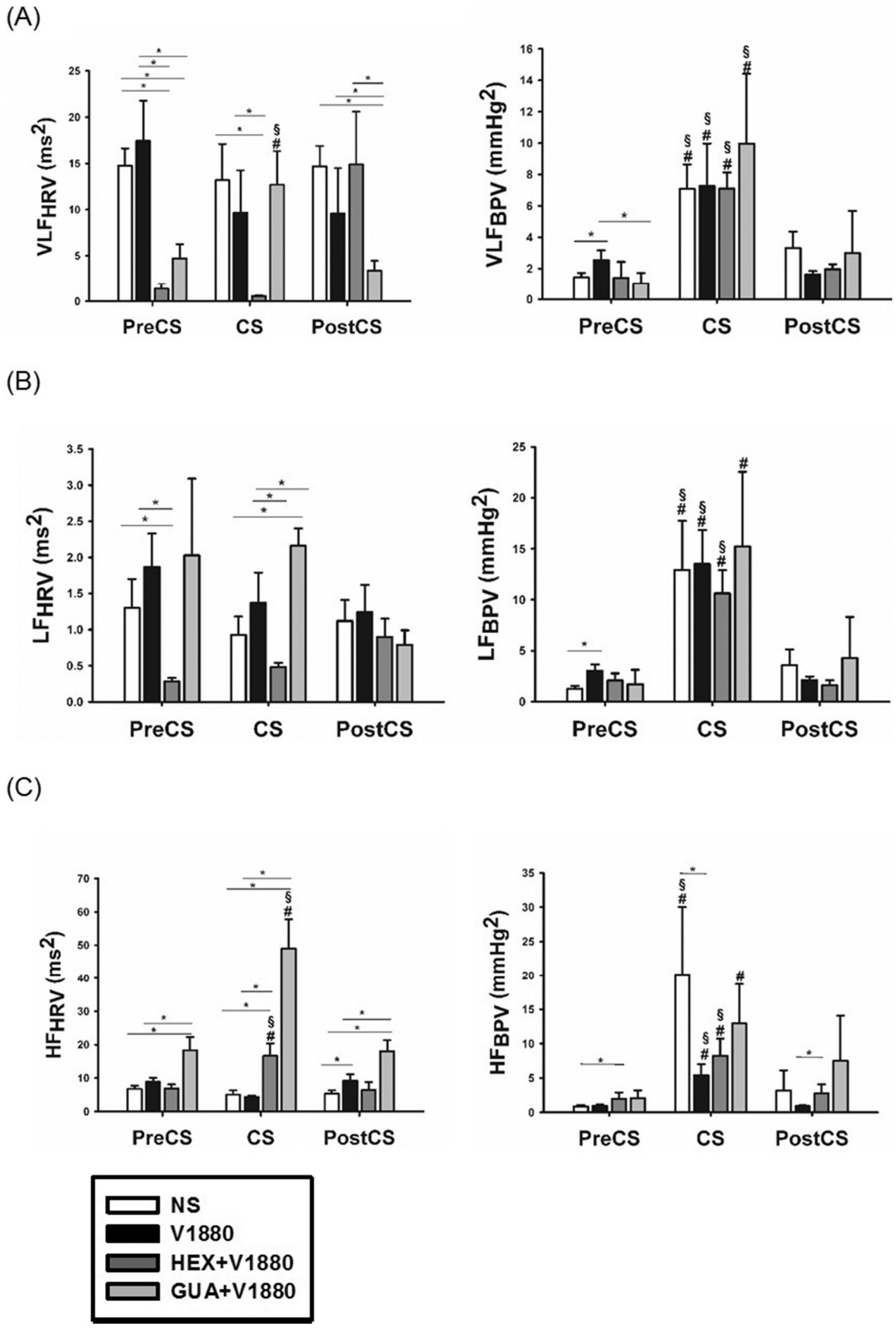

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Fig. 3. Changes in the mean spectral powers in regions of (A) very low frequency, (B) low frequency, and (C) high frequency for blood pressure variability and heart rate variability of rats in the four experimental groups throughout the experiment course. Data are presented as the mean value per group ± standard error of the mean (SEM). Note that statistical significance shows the differences between experimental groups (*$p < .05$). #$p < .05$ compared with the same frequency power for CS versus PreCS. §$p < .05$ compared with the same frequency power for CS versus PostCS. CS, cold stress (4 °C ice-water immersion of the palms and soles); PreCS, before CS; PostCS, after CS; VLF, very low frequency; LF, low frequency; HF, high frequency; BPV, blood pressure variability (mm Hg^2); HRV, heart rate variability (ms^2).

(VLFBPV, VLFHRV, LFBPV, HFBPV, HFHRV, TPBPV, and TPHRV), and a significant interaction between TRIAL and GROUP (VLFHRV, HFBPV, and HFHRV). When compared with NS in PreCS, V1880 increased LFBPV and VLFBPV (all $p < .05$) and also showed a tendency toward increased HFHRV, LFHRV, and VLFHRV. However, when compared with NS in CS, V1880 decreased HFBPV ($p < .05$) but showed a tendency toward increased LFHRV and decreased VLFHRV.

When compared with V1880 in PreCS, HEX + V1880 attenuated LFHRV and VLFHRV (all $p < .05$); showed a tendency toward attenuated LFBPV, VLFBPV, TPHRV, and the LF/HFHRV ratio; but also demonstrated a tendency toward increased HFBPV and TPBPV by V1880. When compared with V1880 in CS, however, HEX + V1880 attenuated LFHRV and VLFHRV, increased HFHRV (all $p < .05$), showed a tendency toward attenuated LFBPV and the LF/HFHRV ratio by V1880, and also showed a tendency toward increased HFBPV, TPBPV, and TPHRV by V1880.

On the other hand, when compared with GUA + V1880 throughout the experiment course, in general, the pattern of spectral power changes in HEX + V-1880 was different from the changes in GUA + V1880. When compared with V1880, GUA + V1880 increased HFHRV in PreCS and CS, decreased VLFBPV and VLFHRV in PreCS (all $p < .05$), showed a tendency toward decreased LFBPV and the LF/HF ratio in PreCS, and increased VLFBPV, VLFHRV, LFBPV, LFHRV, and HFBPV in CS.

Fig. 4 shows the strength of a relationship between BPV and HRV signals as the peak coherence value ($K^2_{HR/SBP}$) of a specific frequency region. When compared with NS throughout the experiment course, V1880 had a low coherence value in the HF region (PreCS, 0.69 ± 0.03 versus 0.75 ± 0.03; CS, 0.68 ± 0.03 versus 0.74 ± 0.03; PostCS, 0.72 ± 0.03 versus 0.69 ± 0.03) but a high coherence value in the VLF and LF regions (VLF: PreCS, 0.58 ± 0.03 versus 0.48 ± 0.03; CS, 0.59 ± 0.03 versus 0.46 ± 0.02; PostCS, 0.56 ± 0.03 versus 0.47 ± 0.03; and LF: PreCS, 0.62 ± 0.03 versus 0.58 ± 0.03; CS, 0.65 ± 0.03 versus 0.59 ± 0.03; PostCS, 0.64 ± 0.03 versus 0.57 ± 0.03). On the other hand, when compared with NS throughout the experiment course, both HEX + V1880 and GUA + V1880 had high coherence values in the VLF region (HEX + V1880: PreCS, 0.56 ± 0.03 versus 0.48 ± 0.03; CS, 0.55 ± 0.03 versus 0.46 ± 0.03; PostCS, 0.51 ± 0.03 versus 0.47 ± 0.03; and GUA + V1880: PreCS, 0.55 ± 0.03 versus 0.48 ± 0.03; CS, 0.56 ± 0.03 versus 0.46 ± 0.03; PostCS, 0.54 ± 0.03 versus 0.47 ± 0.03), HEX + V1880 had a low coherence value in the LF and HF regions (LF: PreCS, 0.52 ± 0.03 versus 0.58 ± 0.03; CS, 0.54 ± 0.03 versus 0.59 ± 0.03; PostCS, 0.54 ± 0.03 versus 0.57 ± 0.03; and HF: PreCS, 0.48 ± 0.02 versus 0.75 ± 0.03; CS, 0.50 ± 0.03 versus 0.74 ± 0.03; PostCS, 0.51 ± 0.03 versus 0.69 ± 0.03), and GUA + V1880 also had a low coherence value in the HF region (PreCS, 0.58 ± 0.03 versus 0.75 ± 0.03; CS, 0.57 ± 0.03 versus 0.74 ± 0.03; PostCS, 0.62 ± 0.03 versus 0.69 ± 0.03).

4. Discussion

In this study, we sought to investigate the role of AVPV1 on cardiovascular regulation. We focused on the discussion of spectral powers (HF, LF, and VLF) in respective HRV and BPV changes because of their specific indication in cardiorespiratory activity, sympathetic outflows, and myogenic vascular oscillations. The overall results indicated that the effects of AVPV1 antagonist on autonomic cardiovascular responses in both baseline PreCS and noxious cold pain CS are associated with central mechanism engagement.

4.1. Responses of V1880 in baseline condition

In PreCS, we observed a tendency toward an increase in both SBP

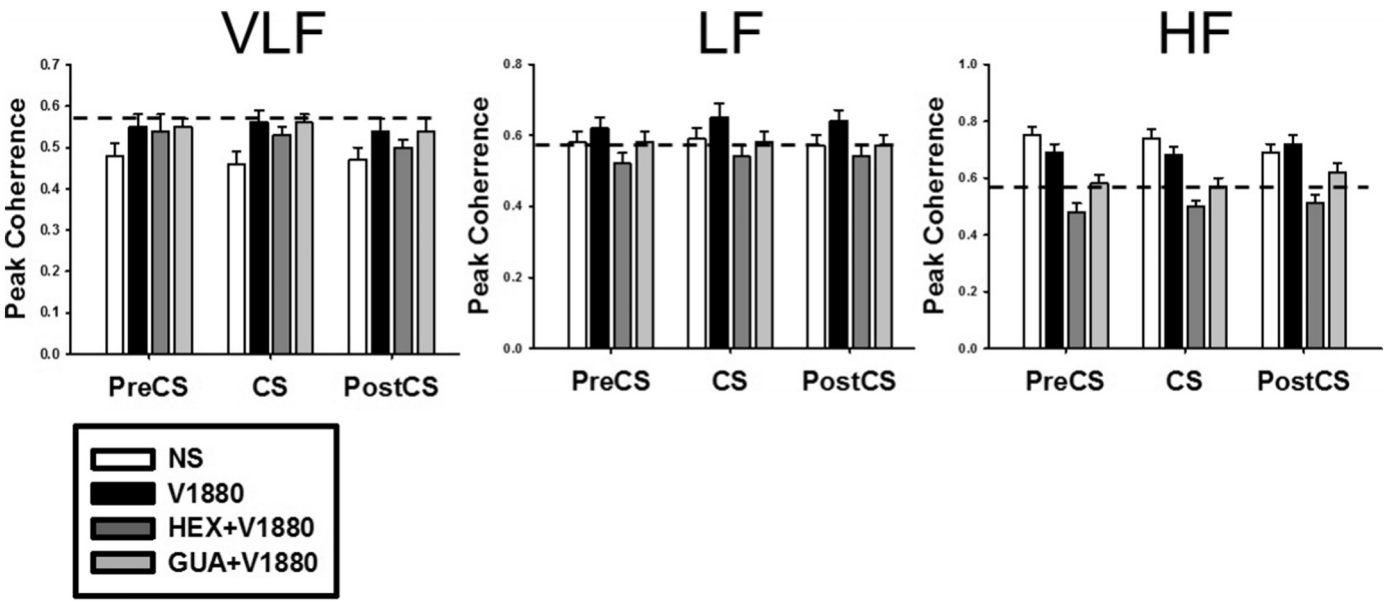

Fig. 4. The relationship between heart rate and systolic blood pressure oscillations as assessed by peak coherence value ($K^2_{HR/SBP}$) between blood pressure variability and heart rate variability in the VLF, LF, and HF regions of rats in the four experimental groups throughout the experiment course. Data are presented as the mean value per group ± standard error of the mean (SEM). CS, cold stress (4 °C ice-water immersion of the palms and soles); PreCS, before CS; PostCS, after CS; $K^2_{HR/SBP}$, peak coherence value; VLF, very low frequency; LF, low frequency; HF, high frequency.

Table 1
The effects of between-subject and within-subject factors on study variables.

Hemodynamic parameter		df	F	*p*
SBP	Trial	3,42	23.42	< 0.05
	Group	2,42	41.34	< 0.05
	Trial & Group	6,84	25.66	< 0.05
HR	Trial	3,42	18.64	< 0.05
	Group	2,42	19.38	< 0.05
	Trial & Group	6,84	16.23	< 0.05
Frequency power		**df**	**F**	***p***
VLFHRV	Trial	3,42	12.12	< 0.05
	Group	2,42	29.72	< 0.05
	Trial & Group	6,84	18.43	< 0.05
VLFBPV	Trial	3,42	2.66	0.68
	Group	2,42	32.46	< 0.05
	Trial & Group	6,84	7.41	0.44
LFHRV	Trial	3,42	31.63	< 0.05
	Group	2,42	1.26	0.47
	Trial & Group	6,84	2.22	0.39
LFBPV	Trial	3,42	1.66	0.69
	Group	2,42	39.84	< 0.05
	Trial & Group	6,84	5.80	0.62
HFHRV	Trial	3,42	19.56	< 0.05
	Group	2,42	25.40	< 0.05
	Trial & Group	6,84	21.36	< 0.05
HFBPV	Trial	3,42	37.50	< 0.05
	Group	2,42	42.11	< 0.05
	Trial & Group	6,84	40.62	< 0.05
TPHRV	Trial	3,42	4.33	0.60
	Group	2,42	18.46	0.05
	Trial & Group	6,84	0.75	0.43
TPBPV	Trial	3,42	1.86	0.29
	Group	2,42	53.20	< 0.05
	Trial & Group	6,84	0.58	0.82
LF/HFHRV	Trial	3,42	29.77	< 0.05
	Group	2,42	2.37	0.36
	Trial & Group	6,84	2.56	0.28

Data were analyzed using multifactor analysis of variance (ANOVA) with a within-subject factor, TRIAL (3 conditions along the experimental procedures, i.e., PreCS, CS, and PostCS according to a repeated-measurement design), and a between-subject factor, GROUP (4 treatments: NS, V1880, HEX + V1880, and GUA + V1880). VLF, very low frequency; LF, low frequency; HF, high frequency; TP, total power; LF/HF, the ratio of LF to HF; BPV, blood pressure variability; HRV, heart rate variability; df, degree of freedom; F, between-group variability/within-group variability; *p*, probability value.

and HR accompanied by increased LFBPV, LFHRV, VLFBPV, and VLFHRV for the V1880 rats when compared with the NS rats. Conversely, we observed that HEX + V1880 attenuated the effects of V1880 on SBP, LFHRV, VLFBPV, and VLFHRV, whereas GUA + V1880 attenuated the effects of V1880 on SBP, HR, LFBPV, VLFBPV, and VLFHRV. In addition, we observed a high coherence value between BPV and HRV in the LF region for the V1880 rats when compared with the NS rats. When compared with V1880, we observed that HEX + V1880 increased HFBPV, whereas GUA + V1880 increased HFHRV. Furthermore, we observed that HEX + V1880 and GUA + V1880 showed a tendency toward attenuation of the LF/HFHRV ratio and the coherence value in both the LF and HF regions of V1880.

Contrary to our expectations that AVP would be a potent vasoconstrictor and that V1a would mediate this vasoconstrictor effect, we observed that peripheral administration of V1880 increased rather than decreased SBP in PreCS. Another observation that requires explanation is that after V1880 treatment, the concurrent SBP and HR increases accompanied by sympathetic activation were associated with an increase of LFBPV. We discuss the possible mechanisms of this observation below.

It is well known that in normal physiological conditions in vivo, a relatively large amount of plasma AVP is required to raise BP, because of its effects on the brain (Johnston, 1985; Liard, 1984) and association with decreased cardiac output via inhibition of the sympathetic activity, potentiating baroreflex, or both (Abboud et al., 1990). Plasma AVP might act on AVPV1 without crossing the blood-brain barrier (Ufnal and Skrzypecki, 2014) or the circumventricular organs such as the area postrema, to sensitize the brain baroreflex neurons to the afferent inputs, thereby augmenting the baroreflex inhibition of sympathetic activity and renin secretion (Applegate et al., 1987; Veelken et al., 1989). It has been shown that systemically administered AVP could exert a tonic enhancement effect on the baroreceptor-mediated bradycardia and sympathoinhibition through the area postrema containing AVPV1 (Hasser and Bishop, 1990; Hay et al., 1991; Iovino et al., 2012; Jurzak and Schmid, 1998; Zhang et al., 1992).

Since V1880 would not be expected to cross the blood-brain barrier, we thus speculate that in PreCS, diffusion of V1880 into the area postrema might shift the autonomic cardiovascular activity via antagonism of AVPV1 to relieve the tonic inhibition of baseline sympathetic activity. Consequently, V1880 causes sympathetic activation in the form of a tendency to increase LFBPV and LFHRV and the LF/HFHRV ratio and then to raise SBP and HR. We believe that this sympathetic activation to increase LFBPV and subsequently to increase VLFBPV is caused by vasoconstriction and heightening of myogenic vascular oscillation (Lin et al., 2017b). On the other hand, our data further show a tendency of HEX + V1880 or GUA + V1880 to attenuate the increase in SBP, LFBPV, VLFBPV, and LF/HFHRV ratio caused by V1880 alone; such results did support our speculation that the effect of V1880 on sympathetic activity to increase SBP and HR is associated with brain structure engagement.

However, it could be argued that V1880 should cause bradycardia rather than the observed tachycardia. In this regard, we consider the tachycardia of V1880 to be due to the effect of the vasodilation of plasma AVP on AVPV2 that was initially masked by AVPV1 vasoconstrictor activity. The effect of AVPV2 vasodilation would increase cardiac output, HR, and plasma renin activity, a baroreflex effect of the decreased peripheral resistance. In addition, AVPV2 directly on the heart might activate HR and cardiac output (Elliott et al., 1985; Schwartz et al., 1985).

Furthermore, our data showed a tendency toward an increase in HFBPV of V1880 by HEX + V1880 or GUA + V1880. HFBPV reflects BP changes induced by respiration (Liu et al., 2015a; Milutinovic et al., 2006). Multiple pieces of evidence have indicated that plasma AVP inhibits breathing by activation of AVPV1 through a neural pathway between the area postrema and the nucleus tractus solitaries (Jurzak and Schmid, 1998; Yang et al., 2006; Zera et al., 2018). We thus speculate that V1880 combined with sympathetic removal might relieve the inhibitory effect on the respiratory system, hence elevating phrenic nerve activity and subsequent respiratory oscillation, causing the increases of HFBPV. This interpretation supports the notion that HFBPV depends on the oscillatory cardiac output that is generally counteracted by the sympathetic tonicity (Japundzic et al., 1990).

Finally, we observed that the coherence value for the V1880 rats increased in the LF region but decreased in the HF region. This finding suggests that the V1880 rats become more sensitive to sympathetic arousal and that their baroreflex feedback loop was intact. We also observed that both HEX + V1880 and GUA + V1880 decreased the coherence values in the LF and HF regions of V1880 rats. This observation further supports our speculation that brain structures engaged the V1880 effects on sympathetic arousal.

4.2. Responses of V1880 in the noxious cold pain condition

In CS, however, we observed a tendency toward increased SBP and CIP concurrent with the cold-induced increase in HFBPV, LFBPV, VLFBPV, and TPBPV for both V1880 and NS rats. When compared with V1880, we observed that HEX + V1880 attenuated SBP, LFHRV, and VLFHRV, whereas GUA + V1880 attenuated SBP and HR but showed a

tendency toward increased LFHRV. Nevertheless, there was a tendency to increase HFBPV and HFHRV and decrease the LF/HFHRV ratio as well as weakening coherence in both the LF and HF regions for both HEX + V1880 and GUA + V1880 when compared with V1880.

Similar to the baseline PreCS, it seemed unlikely that V1880 increased rather than decreased SBP in CS. When compared with the NS rats, except for the significantly decreased HFBPV for the V1880 rats, most SBP and HR and other spectral powers were not much different between these two groups of rats. Here, we propose two possible mechanisms for these results.

Previous studies indicated that plasma AVP level did not change, but the centrally released AVP (neuronal AVP) level increased and was sensitive to CS (Edelson and Robertson, 1986; Jasnic et al., 2015; Wu and Childs, 1990). As mentioned above, AVP might reduce its vasoconstrictor activity through AVPV1 in the area postrema by exciting the brain baroreflex neurons to increase the baroreflex-mediated sympathoinhibition. We therefore propose that one of the possible underlying mechanisms is the functional role of AVPV1 in the brain against the CEHP reactions. We speculate that CS-evoked increases in neuronal AVP might exaggerate the central inhibition on sympathetic arousal of CS and that such an effect is inverted by V1880, which thus augments the sympathetic activation to enhance SBP and CIP. Conversely, V1880 might attenuate the CS-induced vasoconstriction effect to decrease SBP and CIP. In agreement with this speculation, there are only mild changes in SBP, CIP, and the spectral powers of LF and VLF of BPV and HRV as effects of V1880.

On the other hand, baroreflex-mediated sympathoinhibition has known to affect the pain regulatory process. The degree of hypertension has known to correlate positively with the pain threshold and negatively with the perception of the intensity of the painful stimulus (Bruehl and Chung, 2004; Reyes del Paso et al., 2011). Furthermore, AVP has been thought to be an endogenous analgesic substance whose antinociceptive effect is mediated by AVPV1 expressed in the dorsal root ganglia and primary afferent neurons (Han et al., 2018; Peng et al., 2015). Another mechanism we proposed, therefore, is that CS-induced pain perception and rise in neuronal AVP could trigger pain inhibition through AVPV1. CIP might stretch the baroreceptors, which in turn might trigger pain inhibition to buffer the cold-evoked sympathetic arousal. We speculate that in the event of lack of pain inhibition and/or impaired baroreflex effect, the direct pressor effect of pain might predominate in CS. Administration of V1880 thus might attenuate the pain-inhibitory effect of CIP and/or rise in CS-induced neuronal AVP, consequently elevating the pain sensitivity to CS of those V1880 rats. As expected, our data did show that the SBP and sympathetic arousal indices, LFBPV and LFHRV, in CS were slightly high in V1880 but low in NS rats.

Furthermore, we observed an increase in both the HR and HFBPV of the NS rats in CS. This observation is in line with previous reports indicating that with rapid cooling of the skin, cold and pain lead to sympathetic overdrive, which predominately activates the cardiac and respiratory pumps, resulting in a potential respiratory sinus arrhythmia (Tipton et al., 2017). For those two mechanisms proposed above, the increases in both HR and HFBPV in CS point out a possible central AVPV1 mechanism for the CS-evoked increase of respiratory frequency (HFBPV); therefore, V1880 might inhibit such activity, resulting in attenuation of HFBPV. This speculation is supported by an earlier work demonstrating that endogenously released AVP by acute stress could increase the respiration rate via the AVPV1 effect (Milutinovic et al., 2006).

Finally, we turn to examine the effects of endogenous AVP on the genesis of CEHP by removal of the sympathetic influences via superimposed HEX or GUA on V1880. Compared with our previous studies in which HEX/GUA only was given to abolish sympathetic outflows (Liu et al., 2015b), the overall difference between HEX + V1880 and GUA + V1880 in the present spectral and cross-spectral analyses support the notion that AVP through AVPV1 activates the central sympathoadrenomedullary outflow in CS (Lin et al., 2017a, 2017b; Okada et al., 2002).

5. Conclusions

The present study provides the first evidence of the role of AVPV1 in CEHP genesis using spectral and cross-spectral analyses. We suggest that in the baseline PreCS, central AVPV1 inhibition might release the intrinsic tonic restraint of sympathetic activity, elevate the afterload, and thus induce the spontaneous hemodynamic perturbations. In the noxious pain CS condition, however, neuronal AVP (through AVPV1) might cause pain inhibition to attenuate CIP. Future studies of the interaction between AVP and other substantial factors in CEHP genesis could provide potential therapeutic benefit for cold injury.

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Declaration of Competing Interest

None.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.npep.2019.101939.

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