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Role of Beta-Adrenoceptors in Cooling-Evoked Hemodynamic Perturbations of Rats: Investigation by Spectral Analysis

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Abstract

Aim: Rapid immersion of a rat's limbs into 4°C water, a model of cooling stress (CS), can elicit hemodynamic perturbations (CEHP). We have reported that CEHP is highly relevant to the sympathetic activation. This study identifies the role of β-adrenoceptors in CEHP.

Method: Conscious rats were pretreated with the β-adrenoceptor blockade propranolol, (PRO)-only, or following the removal of sympathetic influences using hexamethonium (HEX) or guanethidine (GUA), and then they were subjected to a 10-min CS trial. Cardiovascular indices were monitored via an implanted telemetric device throughout the experimental course. The analyses included measurements of systolic blood pressure (SBP); heart rate (HR); cardiovascular variability (BPV; HRV); spectral coherence at very-low, low, and high frequency regions (VLF: 0.02-0.2 Hz, LF: 0.2-0.6 Hz, and HF: 0.6-3.0 Hz); total power (TP); and dicrotic notch (Dn).

Results: Compared with the vehicle control under the resting (PreCS) and CS conditions respectively, the PRO-only (a) increased the powers for VLF_{BPV}, LF_{BPV}, HF_{BPV}, HF_{HRV}, and TP_{BPV} but decreased the power for VLF_{HRV}, the LF/HF ratio, the Dn under PreCS; (b) increased the powers for LF_{HRV}, HF_{HRV}, and TP_{HRV}, decreased the powers for LF_{BPV}, HF_{BPV}, and TP_{BPV}, the LF/HF ratio, and the Dn, and converted the original negative correlation into positive correlation for VLF_{HRV} with VLF_{BPV} under CS; and (c) weakened the spectral coherence at all frequency regions between BPV and HRV throughout the experimental course. Compared with the control vehicle under PreCS and CS, there were more decreases of SBP (under CS) and HR (under PreCS and CS) after the GUA+PRO than the other interventions (PRO-only and/or HEX+PRO). In addition, the effect on spectral powers of the PRO-only was generally altered when the rats were pretreated with HEX or GUA throughout the experimental course.

Significance: Our findings suggest that PRO may exert vascular effects which are dependent on the sympathetic vasodilator tone. Intact sympathetic efferent pathways are required for the inhibition of CEHP by PRO. Besides, the effects of HEX+PRO versus GUA+PRO indicate a functional role of adrenal medulla to release epinephrine to react the cooling stress.

Keywords: Cooling stress; Sympathetic activation; β-adrenoceptors; Cardiovascular variability

Introduction

Both animal and clinical studies suggest that sympathetic overactivity is a major determinant of pathophysiological conditions, including hypertension and myocardial infarction. β-adrenoceptor (β-ADR) antagonists are largely used in cardiovascular diseases and appear to reduce mortality and morbidity [1]. However, the complexity of the influence of β-ADR blockade on autonomic and cardiovascular function is still largely elusive. Acute immersion of the limbs of a conscious rat into 4°C water induces pressure and tachycardia reactions. Cooling-elicited hemodynamic perturbations (CEHP) represent an ideal model for evaluation of autonomic cardiovascular regulation [2,3]. Generally, CEHP is characterized by hemodynamic instability (irregular blood pressure (BP), heart rate (HR), and vasomotor oscillations), an initial vasoconstriction followed by vasodilatation and a secondary progressive vasoconstriction, thereby providing greater blood flow and tissue perfusion to the cooled areas to avoid damage, as first described by Lewis [4]. The interplay between the initial vasoconstriction and subsequently evoked vasodilatation during prolonged cooling is complex; intact sympathetic and sensory functions, as well as reflex components and humoral factors, are generally required to alter the net vasoconstrictor responses [2,5-7]. A number of humoral substances including the releases of epinephrine from adrenal medulla is considered in mechanisms that determine vascular resistance and myocardial contractility during acute cooling exposure.

The oscillations in BP and HR reflect a dynamic interplay of diverse physiological processes [7-11]. Clinical and experimental evidence suggests that short-term oscillatory variability of BP (BPV) and HR (HRV) has become an increasingly common index for the assessment of autonomic functions and diagnostic and prognostic purposes in various diseases [10,12]. However, the information on the autonomic functions provided by BPV differs from that provided by HRV. Exploration of the associations between BPV and HRV and resultant changes in the underlying frequency powers could be helpful in understanding CEHP. Recently, we performed serial studies to test the responses to acute cooling stimulation (CS) of rats [13-15]. We found that during this test, there were sympathetic activation with

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pressor and tachycardia and concomitant with a significant increase in frequency powers for LF_{BPV} and VLF_{BPV}, such effects were attenuated markedly after pretreatment with the ganglion blocker hexamethonium (HEX) or chemical sympathectomy with guanethidine (GUA) [13]. We postulate the effect of increasing VLF_{BPV} is resulted from an enhanced shear stress secondary to the CEHP of sympathetic activation. However, the significance of the relationship between β-ADR and CEHP is still unclear. To address this question, the present study was carried out by comparing the effects of β-ADR inhibition by propranolol (PRO) in the presence or absence of sympathetic influences.

Materials and Methods

Animals

Adult male Sprague-Dawley rats (BioLASCO, Taiwan (ROC)) weighing between 300 and 350 g were received at the animal center of the National Defense Medical Center (NDMC, Taipei, Taiwan) one week before experiments. The experiments were performed according to a protocol approved by the animal care committee of NDMC. All efforts were made to keep the number of animals used as low as possible and to minimize animal suffering during the experiments. All rats were housed in a temperature- and humidity-controlled holding facility with a 12-hour light/dark cycle (lights on from 07:00 to 19:00) maintained by manual light control switches as required by the experiment. The rats in the same experimental group were housed together. All rats received food and water ad libitum. The experiments were performed between 08:30 and 17:30, with individual rats being tested at the same time every day, when possible.

Experimental protocols and cooling procedure

The rats were randomly divided into four experimental groups for treatment with a similar stressful cooling procedure. The control group rats were given the vehicle (saline, n=12) for baseline comparisons, and the other three groups of rats were given a non-selective beta-blocker, PRO, alone (n=12) or with pretreatment with HEX (n=12) or GUA (n=12). The drug intervention procedures included (a) tail venous infusion of PRO (5 mg/kg/min ml) 30 min prior to the presentation of CS, (b) a tail venous bolus of HEX (30 mg/kg) 20 min after the beginning of the PRO infusion, or (c) intraperitoneal injection of GUA (50 mg/kg) seven times a week for 1 week prior to the experimental sessions. Following a complete stabilization of BP and HR at room temperature, each individual rat was quickly placed in a Plexiglas cage with ice-water (depth=2 cm; temperature=4ºC) to immerse its glabrous palms and soles for a period of 10 min. After this cooling maneuver, the rat was removed from the cage, dried with a cloth, and placed in a similar cage for 30 min to facilitate recovery. The beat-to-beat BP signals were monitored continuously via a telemetric device (TL11M2-M2-C50-PXT, DSI, USA) for 10-min intervals during the three experimental conditions, which included 10 min before (PreCS), 10 min of a cooling maneuver (CS), and 20 min after (PostCS). The entire time course of one experiment required approximately 1 hr., afterward, successive signals were taken for spectral analyses during periods of approximately 5 min (3 to 8 min) in each stage because the mean and variance of the VLF_{BPV} signals were generally stable, and the fluctuations of the systolic blood pressure (SBP) during these periods were also found to be stable. The dicrotic notch (Dn) and counts were handled manually.

Surgical intervention

A telemetry transmitter was implanted intra-abdominally into each rat under anesthesia (sodium pentobarbital, 50 mg/kg). A laparotomy was performed using aseptic procedures, and the catheter of the transmitter was inserted into the abdominal aorta, distal to the kidneys, and fixed in place. The experiments were initiated after the rats had fully recovered from surgery (7 days).

Spectrum signal acquisition and processing: On the day of the experiment, the transmitter was magnetically activated at least 1 hr. before starting the experiment. Pulse signals for calibration were generated as an analog signal (UA10; DSI, St. Paul, MN) with a range of ± 5 V and a 12-bit resolution. Individual rats in each group were then placed on the top of the receivers (PhysioTel® RPC-1) for telemetric signal acquisition. Five receivers were connected to a PC desktop computer via a matrix (Dataquest ART Data Exchange Matrix), and the received signals were recorded with Dataquest Acquisition software (Dataquest ART 4.33). A series of the successive SBP and the inter-beat interval (IBI) signals throughout the experiments were then digitized at a 500 Hz sampling rate and processed off-line using Matlab software (Terasoft Co.). The beat-by-beat oscillatory SBP and IBI signals were analyzed to quantify their frequency and power with respect to cardiovascular variability (BPV and HRV) using autoregressive spectral decomposition. The BPV calculation was based on software kindly written for us by Prof. P.L. Lee, National Central University, Taiwan. Briefly, the acquired SBP signals were pre-processed by applying a band-pass filter (0.1-18Hz, zero-phase 4^{th}-order) to remove the DC components. After identifying all of the SBP peak maxima between two zero-cross points, the extracted beat-by-beat SBP time series were detrended, interpolated and resampled at 0.05 s to generate a new time series of evenly spaced SBP samples, which allowed a direct spectral analysis of each distribution using a Fast Fourier Transform (FFT) algorithm. In addition, the HRV calculation was based on Chart software developed by PowerLab, ADInstruments, USA. The spectral indices of the hemodynamic oscillations were then computed independently to obtain the total power (0.00 to 3.0 Hz, TP) as well as within three major spectral bands: very-low frequency (0.02 to 0.2 Hz, VLF), low frequency (0.20 to 0.60 Hz, LF), and high frequency (0.60 to 3.0 Hz, HF). The normalized LF and HF were also calculated as nLF (or nHF) = LF (or HF)/TP-VLF×100%. The moduli of the BP or HR spectrum (ordinates) had units of $mmHg^2$ or ms^2, respectively. In addition, to examine the strength of the linear link between BPV and HRV oscillations across a given frequency region, further computation was performed on the data using cross-spectrum analysis. An estimated squared coherence value ($K^2_{IBI/SBP}$)>0.58 was considered to indicate that the two variability signals covered significantly at the various frequency regions.

Statistics: The statistical analyses were performed using SPSS (Statistical Package for the Social Sciences) version 18.0 software. The homogeneity of the variance was first confirmed using the Kolmogorov–Smirnov test, and the differences between groups were subsequently compared using Student's t-test or repeated measures two-way ANOVA followed by a post-hoc Scheffe's test as appropriate. Univariate correlations were calculated using Pearson's correlation analysis to estimate the associations between selected frequency bands. The results are expressed as the mean ± SEM. A p-value<0.05 was considered statistically significant.

Results

Typical examples of the BP tracings for representative rats are shown in Figure 1. The averaged data are shown in Table 1S (please see the Data Supplement) and Figures 2-4.

Responses of SBP, HR, and Dn appearance to various drug interventions throughout the experimental course

As shown in Figure 2(I), under the CS condition, inhibition of

β-ADRs by PRO caused increases of SBP of this treatment (CS versus PreCS or PostCS, all P<0.05); in addition, PRO caused decreases of SBP and HR (all P<0.01) compared to that of the control vehicle treatment. During the CS period when compared with the control vehicle, the effect of SBP reduction in response to PRO was similar to the effect by the add-on HEX (PRO or HEX+PRO versus Control Vehicle: all<0.01), but there was a further reduction by the add-on GUA (GUA+PRO) (GUA+PRO versus PRO or HEX+PRO: all P<0.01). However, the effect of HR reduction in response to PRO were similar to the effects by removal of sympathetic influences (PRO or HEX+PRO or GUA+PRO versus Control Vehicle: all P<0.001). During the resting PreCS period, by contrast, the add-on HEX or GUA compared with the control vehicle or the PRO-only had significant increase of SBP (HEX+PRO versus Control Vehicle: P<0.01; HEX+PRO or GUA+PRO versus PRO: all<0.01); however, only the add-on GUA had somewhat decrease of HR compared with the control vehicle treatment (GUA+PRO versus Control Vehicle: P<0.05).

PostCS and CS (||P<0.01, ¶P<0.001) were found using a repeated measures two-way ANOVA and post-hoc Schaffer's test. Significant differences between two experimental conditions (#P<0.05, **P<0.01-0.001) were calculated using Student's t-test. The values are presented as the mean ± SEM. The abbreviations are defined in Figure 1. As shown in Figure 2 (II), Both PRO-only and HEX+PRO generally decreased the appearance of dicrotic notch (Dn) under all experimental conditions compared to the control vehicle, whereas the Dn were much more apparent (with Dn) and significant in the rats subjected to the GUA+PRO intervention (P<0.01).

The effects of PRO alone on frequency power and coherence function

As shown in Figure 3 and Table 1S, under the PreCS condition when compared with the control vehicle, the PRO-only generally increased the spectral powers for VLF_{BPV} (P<0.05), LF_{BPV} (P<0.05), HF_{BPV} (P<0.001), HF_{HRV} (P<0.001), and TP_{BPV} (P<0.01) and decreased those for VLF_{HRV} (P<0.01) and the LF/HF_{HRV} (P<0.01). Under the CS condition when compared with the control vehicle treatment, by contrast, PRO generally increased the spectral powers for LF_{HRV} (P<0.05), HF_{HRV} (P<0.05), and TP_{HRV} (P<0.05) and decreased those for LF_{BPV} (P<0.01), HF_{BPV} (P<0.05), and TP_{BPV} (P<0.05), and the LF/HF ratio (P<0.01). Nevertheless, the original tendency of negative correlation (NC) for the VLF pair observed for the control vehicle (VLF_{HRV} versus VLF_{BPV}: r=-0.32, P=0.39) was converted into a tendency of positive correlation (PC) for this pair (r=0.44, P=0.15) by the PRO-only intervention Figure 3.

The coherence linkage as assessed by the peak coherence values ($K^2_{IBI/SBP}$) between BPV and HRV for three major frequency regions is summarized in Figure 4. When compared with the control vehicle under various experimental conditions, PRO generally has lowered the $K^2_{IBI/SBP}$ values at the VLF region (PRO versus Control Vehicle: PreCS: 0.43 ± 0.03 versus 0.48 ± 0.03; CS: 0.41 ± 0.02 versus 0.46 ± 0.03; PostCS: 0.38 ± 0.01 versus 0.47 ± 0.03), at the LF region (PRO versus Control Vehicle: PreCS: 0.50 ± 0.03 versus 0.58 ± 0.03; CS: 0.51 ± 0.04 versus 0.59 ± 0.03; PostCS: 0.47 ± 0.02 versus 0.57 ± 0.03), and at the HF region (PRO versus Control Vehicle: PreCS: 0.52 ± 0.01 versus 0.75 ± 0.03; CS: 0.54 ± 0.03 versus 0.74 ± 0.03; PostCS: 0.51 ± 0.03 versus 0.69 ± 0.03) (Figure 4).

Comparisons of the responses of frequency power and coherence function for HEX versus GUA superimposed on the PRO intervention

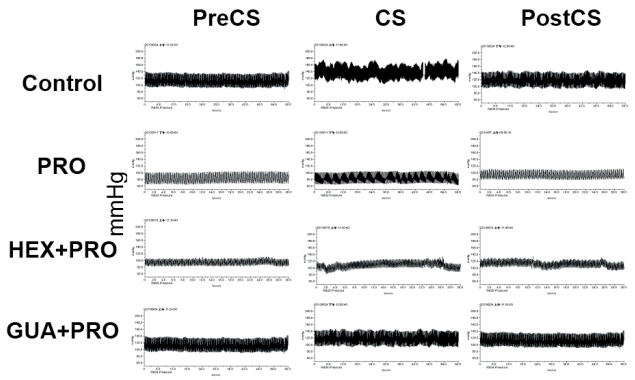

Figure 1: Typical examples of the blood pressure tracings for the four experimental groups of rats treated with the saline vehicle (control) or a non-selective beta-blocker propranolol (PRO) only or with the add-on HEX (HEX+PRO) or GUA (GUA+PRO) before an acute cooling challenge. Abbreviations: before CS (PreCS), after CS (PostCS), and during the cooling stimulus (CS, 4°C ice-water immersion of the palms and soles).

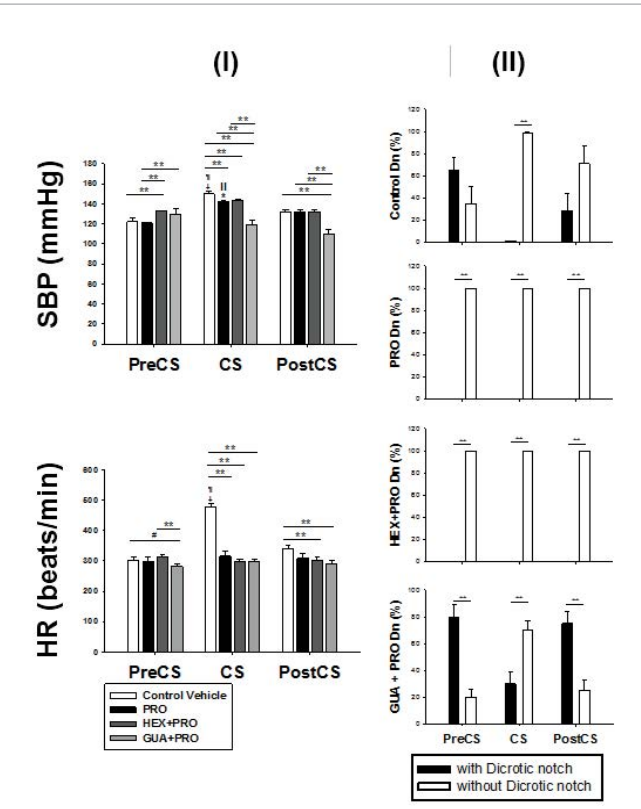

Figure 2 (I): The effects on systolic blood pressure (SBP) and heart rate (HR) and (II) the appearance of dicrotic notch (Dn) in the rats of the four experimental groups throughout the experimental course. Note that PRO and HEX+PRO have equipotent on reduction of the Cooling-Induced Pressor (CIP) and the appearance of Dn, whereas GUA+PRO has abolished CIP but enhanced the appearance of Dn. Significant differences between PreCS and CS (*P<0.05, †P<0.01, ‡P<0.001) and between.

As shown in Figure 3 and Table 1S, the effect of PRO-only on the spectral powers were generally attenuated by HEX+PRO. The parameters affected throughout the experimental course included VLF_{BPV} (PreCS: P<0.05; PostCS: P<0.05), VLF_{HRV} (CS: P<0.05), LF_{BPV} (PreCS: P<0.05),

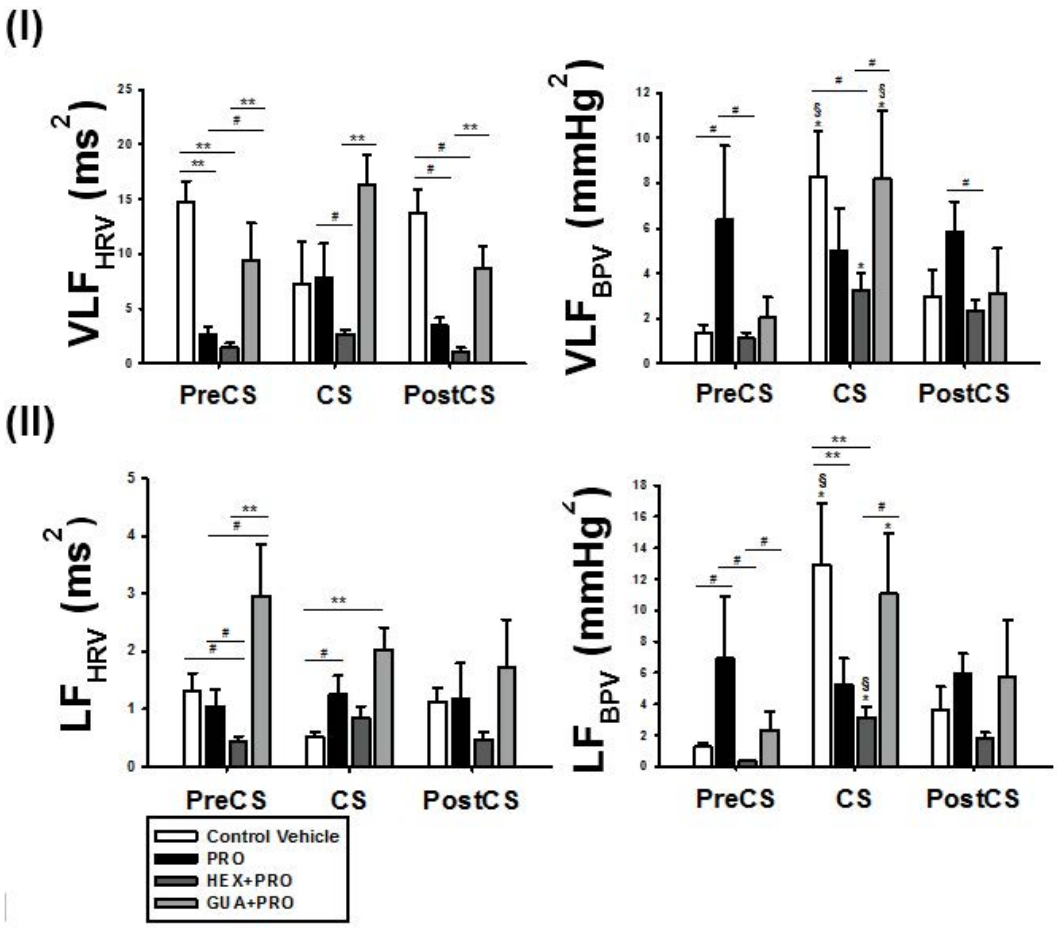

Figure 3: Changes in the spectral powers of the (I) very-low frequency (VLF) and (II) low frequency (LF) for the blood pressure variability (BPV) and heart rate variability (HRV) of the rats in the four experimental groups throughout the experimental course. The moduli of the BP or HR spectrum (ordinates) have units of $mmHg^2$ and ms^2, respectively. Note that PRO or HEX+PRO in changes of the spectral powers for the BPV or the HRV relative to the control vehicle was generally attenuated and/or directed to an opposite direction by GUA+PRO. The values are presented as the mean ± SEM. The statistical analyses, abbreviations, and symbols are defined in Figure 2.

LF_{HRV} (PreCS: P<0.05), HF_{BPV} (PreCS: P<0.05; PostCS: P<0.05), HF_{HRV} (PreCS: P<0.01), and TP_{BPV} (PreCS: P<0.01). The effect of GUA+PRO throughout the experimental course, however, was different as generally attenuated and/or converted to an opposite direction as compared to the effects of PRO-only or HEX+PRO, in which included increases of VLF_{BPV} (GUA+PRO versus HEX+PRO—CS: P<0.01; PostCS: P<0.05), VLF_{HRV} (GUA+PRO versus PRO: PreCS: P<0.05; CS: P<0.001; PreCS: P<0.01 and GUA+PRO versus HEX+PRO—PreCS: P<0.001; CS: P<0.001; Post CS: P<0.001), LF_{BPV} (GUA+PRO versus HEX+PR: PreCS: P<0.05; CS: P<0.05), LF_{HRV} (GUA+PRO versus PRO: PreCS: P<0.05 and GUA+PRO versus HEX+PRO: PreCS: P<0.001), HF_{BPV} (GUA+PRO versus HEX+PRO: PreCS: P<0.05), HF_{HRV} (GUA+PRO versus HEX+PRO: PostCS: P<0.05), TP_{BPV} (GUA+PRO versus HEX+PRO: PreCS: P<0.05; CS: P<0.05), and TP_{HRV} (GUA+PRO versus HEX+PRO: PreCS: P=0.101; CS: P<0.05). Nevertheless, HEX+PRO caused the PC tendency for the VLF pair observed for the PRO-only (r=0.44, P=0.15) reverted back to a NC tendency (r=-0.31, p=0.19) similar to that observed for the control vehicle (r=-0.32, p=0.39). In addition, the original tendency of NC for the LF pair observed for the PRO-only (LF_{HRV} versus LF_{BPV}: r=-0.28, P=0.26) was converted into a tendency of PC for this pair (r=0.47, P<0.05) by HEX+PRO. However, the original NC tendencies for both the VLF pair and LF pair observed in the control vehicle (VLF_{HRV} versus VLF_{BPV}: r=-0.32, P=0.39; LF_{HRV} versus LF_{BPV}: r=-0.39, P=0.20) were converted to PC tendencies (VLF_{HRV} versus VLF_{BPV}: r=0.40, p=0.22; LF_{HRV} versus LF_{BPV}: r=0.26, p=0.18) by GUA+PRO. Compared with the respective $K^2_{IBI/SBP}$ values for PRO under any experimental conditions (Figure 4), there were still no consistent linkage between BPV and HRV at the HF region for HEX+PRO or GUA+PRO ($K^2_{IBI/SBP}$ <0.58). However, the detached VLF and LF by PRO as shown in lower $K^2_{IBI/SBP}$ values were somewhat improved when added on chemical sympathectomy with GUA (PRO versus GUA+PRO: VLF-PreCS: 0.43 ± 0.03 versus 0.59 ± 0.02; CS: 0.41 ± 0.02 versus 0.56 ± 0.02; PostCS: 0.38 ± 0.01 versus 0.51 ± 0.03 and LF-PreCS: 0.50 ± 0.03 versus 0.58 ± 0.04; CS: 0.11 ± 0.04 versus 0.57 ± 0.04; PostCS: 0.47 ± 0.02 versus 0.57 ± 0.04).

		Blood Pressure Variability				Heart Rate Variability			
		Control	PRO	HEX+PRO	GUA+PRO	Control	PRO	HEX+PRO	GUA+PRO
PreCS	VLF (mmHg²)	1.33±0.41	6.36±3.28#	1.11±0.27	2.04±0.89	VLF (ms²) 15.86±3.21	2.61±0.76**	1.44±0.41**	9.37±3.46
	LF (mmHg²)	1.26±0.29	6.93±3.92#	0.34±0.07#	2.34±1.20	LF (ms²) 1.3±0.4	0.85±0.21	0.44±0.09#	3.03±0.93
	HF (mmHg²)	0.88±0.21	12.6±7.94**	1.63±0.27#	4.52±2.44**	HF (ms²) 6.77±1.01	25.3±7.89**	7.61±1.14	46.88±16.05**
	TP (mmHg²)	6.76±1.68	50.06±29.5**	6.48±1.32	16.46±7.94	LF/HF 0.19±0.03	0.07±0.01**	0.05±0.01**	0.07±0.01**
				0.101		TP (ms²) 40.25±7.44	34.46±7.48	25.15±8.12	94.11±30.08#
CS	VLF (mmHg²)	8.28±2.52*§	5.01±1.86	3.23±0.76*#	8.20±0.99*§	VLF (ms²) 7. 13±2.66	7.88±3.05	2.68±0.38*§#	16.33±2.74*§
	LF (mmHg²)	12.93±4.8*§	5.26±1.65#**	3.16±0.65*§**	11.06±3.88	LF (ms²) 0.93±0.25*§	1.25±0.33	0.85±0.20	2.03±0.39**
	HF (mmHg²)	20.17±9.86*§	5.73±2.77#	4.19±1.04*§#	11±3.68	HF (ms²) 5.04±1.32	39.61±9.75#	39.27±12.3*§	48.46±6.21**
	TP (mmHg²)	88.51±35.2‡¶	30.05±10.91#	19.62±3.42#	58.84±18.47	LF/HF 0.23±0.07	0.06±0.01**	0.07±0.01**	0.05±0.01**
						TP (ms²) 25.33±6.56	56.86±12.9#	55.68±11.07#	89.11±10.59**
PostCS	VLF (mmHg²)	2.23±1.23	5.86±1.33	2.32±0.50	3.13±1.99	VLF (ms²) 13.99±3.44	3.42±0.76#	1.09±0.36#	8.67±1.98
	LF (mmHg²)	3.57±1.55	5.91±1.32	1.81±0.36	5.72±3.66	LF (ms²) 1.12±0.29	0.55±0.15	0.46±0.13	1.71±0.88
	HF (mmHg²)	3.2±2.92	6.94±3.12	1.08±0.12	9.32±6.23	HF (ms²) 5.3±1.06	16.58±5.53	13.24±4.76	21.54±5.39**
	TP (mmHg²)	18.13±13.49	36.11±9.5	10.68±2.04	33.82±21.77	LF/HF 0.22±0.05	0.05±0.01**	0.07±0.01**	0.06±0.01**
						TP (ms²) 46.94±13.6	27.51±8.24	42.51±16.68	63.49±19.79

Table 1S. Overall frequency power data for the four rat experimental groups that were treated with the saline vehicle (control, n=12) or a non-selective beta-blocker propranolol (PRO, n=12) alone or with the superimposition of HEX (HEX+PRO, n=12) or GUA (GUA+PRO, n=12) before an acute cooling challenge. Significant differences between PreCS and CS (*P<0.05, †P<0.01, ‡P<0.001) and between PostCS and CS (§P<0.05, ||P<0.01, ¶P<0.001) were determined using a repeated measures two-way ANOVA and post-hoc Schaffer's test. Significant differences between two experimental groups (#P<0.05, **P<0.01-0.001) were calculated using Student's t-test. The values are presented as the mean ± SEM.

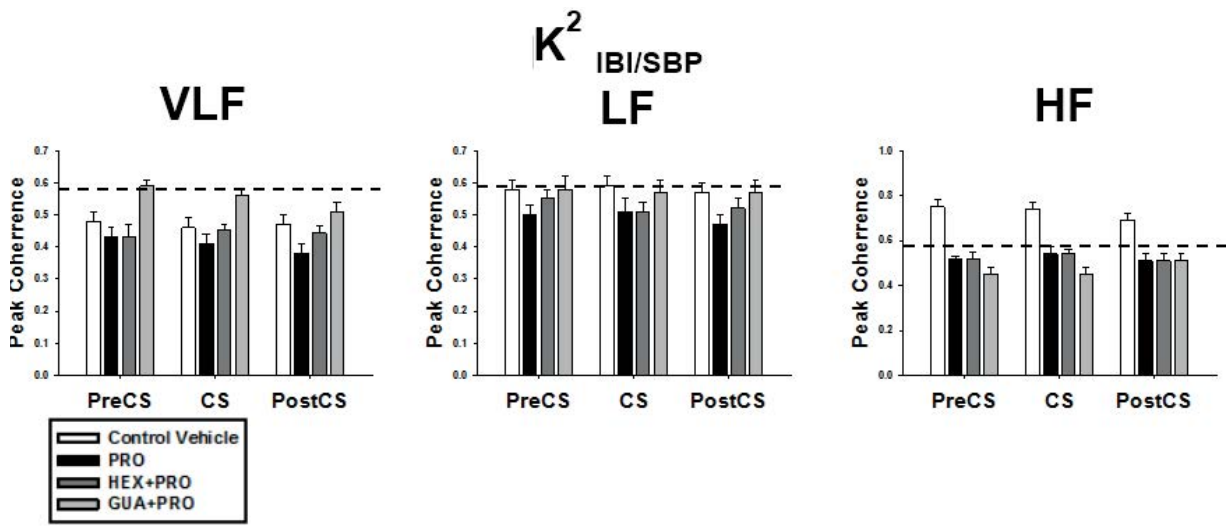

Figure 4: The linkage as assessed by a coherence value ($K2_{IBI/SBP}$) between BPV and HRV at the VLF, LF, and HF regions under various conditions for rats throughout the experimental course. When the coherence value exceeded 0.58 at a frequency region, the two signals were considered to covary significantly at that frequency region. Note that the coherence linkage ($K_{IBI/SBP}$) between BPV and HRV at all frequency regions were detached by PRO. The effects of PRO at VLF and LF regions were somewhat improved by the add-on GUA. The values are presented as the mean ± SEM. The statistical analyses, abbreviations, and symbols are described in Figure 2.

Abbreviations: Before CS (PreCS), after CS (PostCS), and during the cooling stimulus (CS, 4°C ice-water immersion of the palms and soles); spectra: very low-frequency (VLF), low frequency (LF), high frequency (HF), normalized low frequency (nLF), normalized high frequency (nHF), ratio (LF/HF), total power (TP), and dicrotic notch (Dn).

Discussion

PRO exerts several mechanisms within brain and directly upon cardiovascular system for lowering BP [1,16-18]. We previously reported that the sympathetic activation with the enhancement of efferent sympathetic oscillations (LF_{BPV}) and the subsequent CEHP were highly associated with the strength of vasculomyogenic oscillations (VLF_{BPV}) [13,14]. The present study was designed to collect more information about β-ADR role in CEHP.

Responses of PreCS to PRO

The antihypertensive effect of PRO has been extensively studied, however it still remains unclear [17,19]. It is known that rat has a strong β-ADR tone in the peripheral vascular beds [20]. Our present study under PreCS, i.e., a resting condition, showed although PRO-only did not change SBP and HR, it exerts converse effects on BPV and HRV in terms of their spectral powers, they are increased in BPV and decreased for most of the HRV (VLF_{HRV} and LF/HF_{HRV}), except that HF_{HRV} was increased. These PRO effects can be interpreted in several aspects. First, although SBP and HR were unchanged, possibly due to some homeostatic mechanisms. It is noted that LF_{BPV} and VLF_{BPV} were significantly increased because the PRO induced inotropic-blocking effect on the myocardium β_1-ADR. We assumed that this inhibition on cardiac contractility of PRO could attenuate the oscillatory thoracic hemodynamics which led to a decrease of VLF_{HRV} (Figure 3-left upper panel: PreCS) and an increase of HF_{HRV} (Table 1S). In fact, the inhibition on cardiac contractility might reduce the ventricular output which leads to a dynamic unloading of the arterial baroreceptors. Therefore, the efferent sympathetic discharges (oscillations) were activated as LF_{BPV} power was enhanced (Figure 3-right lower panel: PreCS) by reducing the afferent input of arterial and cardiopulmonary baroreceptors. Consequently, the secretion of epinephrine from adrenal medulla might be upraised and further increased the VLF_{BPV} power (Figure 3-right upper panel: PreCS) [21]. Second, we assumed that skin and muscle vessels their α-ADRs were activated because of β2-ADR-blocking effects and/or sympathetic activation due to the inotropic-blocking effect of PRO. The stimulation of renal vascular α-ADRs might also activate the renin-angiotensin-aldosterone system (RAAS) [22] and other humoral influences and thus oscillate LF_{BPV} and VLF_{BPV} seen here [11,23].

On the other hand, when compared with the PRO-only under PreCS, the add-on HEX or GUA had significantly increased the SBP, but when compared with control vehicle, only the add-on GUA had decreased the HR. In other words, the effects of PRO-only on most spectral powers of BPV and HRV were generally attenuated by HEX+PRO whereas differently affected by GUA+PRO, which GUA+PRO had attenuated and/or reversed the HEX+PRO effects to an opposite direction.

Compared with control vehicle, the increase of SBP in both HEX+PRO and GUA+PRO (Figure 2-left upper panel: PreCS) appear to be related to the relative vascular tone. We assumed there is a heightened vasodilatation effect after sympathetic tonus is removed by HEX or GUA, thus PRO may raise BP in the condition of higher peripheral vasodilator tonus [20]. Compared with PRO-only, however, HEX+PRO attenuated most spectral powers, particularly with respect to LF_{BPV}, the result indicated that uprising of efferent sympathetic discharges by reducing the afferent input of baroreflex loop is responsible for the PRO effect. Compared with HEX+PRO, however, GUA+PRO reversed the HEX+PRO effects on spectral powers of LF_{BPV} and LF_{HRV} (Figure 3-right and left lower panels: PreCS) and myocardium oscillations (VLF_{HRV}) (Figure 2 left upper panel: PreCS). These observations suggest the possibilities of the efferent sympathetic discharges in response to the PRO inotropic-blocking effect and the sparing effects of GUA on adrenal medulla [24]; the released epinephrine in plasma might activate RAAS as the renal vascular α-ADRs were virtually unopposed by PRO [22]. Thus, GUA+PRO seen here has further strengthened the oscillations concomitant with LF_{BPV}, LF_{HRV}, and VLF_{HRV} [22,23,25]. The same rationale can be applied to interpret the observation that GUA+PRO significantly decreased HR (Figure 2-left lower panel: PreCS). Because GUA on epinephrine releases, α2-ADR unopposed, and thus, vessels become constricted by PRO. This vasoconstriction in turn lead to a baroreflex compensation, i.e., increase of HF_{HRV} power, decrease of LF/HF ratio, and dysregulation of oscillatory thoracic hemodynamics, i.e., increase of HF_{BPV} power and disruption of oscillatory respiration movement (HF: $K2_{IBI/SBP}$<0.58) (Table 1S and Figure 4).

Responses of CS to PRO

Compared with control vehicle under CS, the PRO-only reduced the cold-induced pressor (CIP), abolished the cold-induced tachycardia (CIT), and increased the spectral powers for HF_{HRV} and TP_{HRV}, decreased the spectral powers for LF_{BPV}, HF_{BPV}, and TP_{BPV}, and decreased the LF/HF ratio and the Dn. Furthermore, the original NC of Pearson's correlation by control vehicle was converted into PC for VLF_{HRV} with VLF_{BPV} by PRO. On the other hand, when compared with PRO under CS, HEX+PRO reduced CIP was similar to that by PRO-only, but GUA+PRO has further reduced and abolished such CIP effect (Figure 2-left middle panel: PreCS). However, the reduction of CIT by PRO was similar to the condition of HEX+PRO or GUA+PRO (Figure 2-left lower panel: PreCS). In respect of frequency powers (Table 1S and Figure 3), the effects of PRO were generally attenuated by HEX+PRO, and the effects of PRO and HEX+PRO were generally attenuated or even converted by GUA+PRO. Nevertheless, the tendency of PC for the VLF pair by PRO was reverted back to NC as the effect of control vehicle by HEX+PRO. In addition, the tendency of NC for the LF pair by PRO was converted into PC by HEX+PRO. However, the original tendency of NC for both VLF pair and LF pair by control vehicle were converted into PC by GUA+PRO (Figure 1S).

CEHP is a key manipulation for the above observation. Thus, high tonicity of cardiovascular system is common to all interventions containing the non-selective β-ADR-blocking effects of PRO. The pharmacological properties of PRO are worth to be particularly addressed. Note the dosage we selected caused both central and peripheral β-ADR blockades. For the central effect, it is possible that systemic application of PRO would lead to sufficient β1- and β2-ADR blockade in the brain, as the central effect of PRO has been evidenced in this study by the concurrent decreases of LF_{BPV} for the reduction of CIP, and in spite of the increase of LF_{HRV}, the increase of HF_{HRV} with decrease of LF/HF for reduction of CIT [16,17,26]. For the periphery effect [19], PRO is known to inhibit β-adrenergic transmission to the heart and attenuate presynaptic β2-ADR to inhibit synaptic NE releases at the postganglionic adrenergic neurons [27]. The peripheral effects of PRO may explain, together with its central effects, the decreases of SBP and HR. Nevertheless, our data here demonstrated an attenuation of PRO effects on the spectral power of VLF_{BPV}. Since VLF_{BPV} is known to represent vasculomyogenic oscillations and sympathetic activation is important for generating this power [11,13-15,21], in addition, the cooling-induced vasculomyogenic activation is noted depending on peripheral α2-ADR tone [15], we consider in this study, therefore, a weak attenuation of PRO on VLF_{BPV} indicates an example of how VLF_{BPV} is governed by activations of ADRs [21], in which β-ADR is not the key factor.

On the other hand, when compared with the PRO-only, HEX+PRO did similar effects to reduce CIP and CIT but not for both LF and VLF for BPV and HRV. These results confirm our speculation that the β-ADR-blocking effects of PRO require intact sympathetic efferent pathways. In addition, when compared with HEX+PRO, GUA+PRO significantly reduced CIP. In respect of spectral powers, the effects of GUA+PRO on both LF and VLF for BPV and HRV appeared strikingly different from those of HEX+PRO (Figure 3-right and left: CS). These two observations can be used to exemplify the role or PRO in terms of the sympathoadrenal activation and the evoked efferent sympathetic oscillations.

Compared with HEX+PRO, however, GUA+PRO significantly reduced CIP. In addition, respecting frequency powers the effects of GUA+PRO on both LF and VLF for BPV and HRV were strikingly different from that by HEX+PRO (Figure 3-right and left: CS). These results are intriguing to us and need to be further explained.

For the first observation (i.e., significant reduction of CIP), states of acute sympathoadrenal activation to stressful cooling together with the PRO blockade on presynaptic β2-ADR, a direct action of circulating epinephrine on presynaptic α2-ADR might further strength the inhibition of synaptic NE release at postganglionic adrenergic neurons [28,29], thus PRO has reduced the vascular resistance as the CIP attenuated. The second observation (i.e., the disparity of changes in spectral powers between GUA+PRO and HEX+PRO) can be explained as pharmacological properties of GUA. Compared with HEX+PRO upon CS, the reversing effects on LF_{BPV} and VLF_{BPV} by GUA+PRO is assumed because of GUA inhibition on the sympathetic NE release, however, the CS-evoked enhancement of efferent sympathetic oscillations were still remained under CS. In addition, as showing in Figure (Figure 3-right upper panel: CS), we found HEX+PRO eliminated but GUA+PRO reconstructed the CS-evoked VLF_{BPV}, these opposite effects implicated again that in process of vasculomyogenic oscillations, β-ADR does not play an important role.

Finally, we observed a detached frequency oscillation between BPV and HRV at all frequency regions ($K_{BPV/SBP}$ <0.58) throughout the experimental course by PRO, but at LF and VLF regions, the disruption was improved by GUA+PRO (Figure 4). The disruptions of efferent sympathetic oscillations (LF) and respiratory rhythmus (HF) by PRO were consistent with our assumption that PRO may reduce the afferent input from baroreceptors. However, the coherence reinforcement on LF and VLF by GUA+PRO could attribute to the pharmacological significance of GUA [24], i.e., sympathetic activation with firing discharges and neurotransmitter releases are dissociable. Finally, activation of RAAS might be also involved the reinforcement of VLF coherence.

Conclusion

By using telemetric system and spectral and cross-spectral analyses in conscious rats, the present study investigated the dynamic and functional role of PRO on CHEP. The major finding of the study was that PRO did exert different pattern of effects on cardiovascular indices at presence (i.e., stressful cold challenge) or absence (i.e., controlled resting condition) of sympathetic influences. The results support the hypothesis that epinephrine originated from adrenal medullary is an endogenous agonist mediating stimulation of oscillatory sympathetic discharges. However, the effect of β-ADR on vasculomyogenic oscillations might be less powerful than the effect of α2-ADR under stressful cooling challenge. Future studies are suggested to identify the roles of sympathoadrenal activation towards a better understanding of the CEHP mechanism.

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