

# Cooling-Evoked Hemodynamic Perturbations Facilitate Sympathetic Activity with Subsequent Myogenic Vascular Oscillations via Alpha2-Adrenergic Receptors

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Received June 1, 2016

Accepted December 9, 2016

On-line February 28, 2017

## Summary

This study extends our previous work by examining the effects of alpha2-adrenoceptors under cold stimulation involving the increase of myogenic vascular oscillations as increases of very-low-frequency and low-frequency of the blood pressure variability. Forty-eight adult male Sprague-Dawley rats were randomly divided into four groups: vehicle; yohimbine; hexamethonium+yohimbine; guanethidine+yohimbine. Systolic blood pressure, heart rate, power spectral analysis of spontaneous blood pressure and heart rate variability and spectral coherence at very-low-frequency (0.02 to 0.2 Hz), low-frequency (0.2 to 0.6 Hz), and high-frequency (0.6 to 3.0 Hz) regions were monitored using telemetry. Key findings are as follows: 1) Cooling-induced pressor response was attenuated by yohimbine and further attenuated by hexamethonium+yohimbine and guanethidine+yohimbine, 2) Cooling-induced tachycardia response of yohimbine was attenuated by hexamethonium+yohimbine and guanethidine+yohimbine, 3) Different patterns of power spectrum reaction and coherence value compared hexamethonium+yohimbine and guanethidine+yohimbine to yohimbine alone under cold stimulation. The results suggest that sympathetic activation of the postsynaptic alpha2-adrenoceptors causes vasoconstriction and heightening myogenic vascular oscillations, in turn, may increase blood flow to prevent tissue damage under stressful cooling challenge.

## Key words

Cold stimulation • Power spectral analysis • Sympathetic activation • Alpha2-adrenoceptors • Myogenic vascular oscillations

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## Introduction

Acute immersion of the limbs of a conscious rat into 4 °C water induces pressor and tachycardia reactions. Cooling-elicited hemodynamic perturbations exemplify an ideal model for evaluation of autonomic cardiovascular regulation (Robertson *et al.* 1979, Velasco *et al.* 1997). It is characterized by hemodynamic instability (irregular blood pressure, heart rate, and cardiovascular 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 (Lewis 1926, Daanen 2003).

The interplay between the initial vasoconstriction and subsequently evoked vasodilatation during prolonged cooling is complex. Intact sympathetic and sensory functions as well as ensuing compensatory baroreflex and releases of humoral substances are known involving the cooling-elicited hemodynamic perturbations (Folkow *et al.* 1963, Daanen 2003, Johnson and Kellogg 2010). Cold and or adrenergic stimulation can induce changes in the gene expression of transcription factors and their cofactors that regulate the

expression of target genes (Watanabe *et al.* 2008). The arterial and venous beds where the coexistence of postsynaptic  $\alpha_1$ - and  $\alpha_2$ -adrenoreceptors ( $\alpha_2$ -ADRs) are known involved in vasoconstriction evoked by sympathetic activation (Elsner *et al.* 1986, McGillivray-Anderson and Faber 1991).

Spectral analysis of blood pressure variability (BPV) and heart rate variability (HRV) using frequency domain approaches has been widely applied to investigate the oscillations of hemodynamic parameters manifested the baroreflex control of cardiovascular homeostasis (Akselrod *et al.* 1985, Japundzic *et al.* 1990, Pagani *et al.* 1996, Stauss 2007, di Rienzo *et al.* 2009, Novakova 2013). Cardiovascular conditions common to dysautonomia usually display a bad prognostic sign with excessive BPV and weakened HRV.

In our previous studies, we used telemetry in conscious rats for measuring power spectral density and coherence relationship between BPV and HRV of cooling elicited hemodynamic perturbations found new and exciting results. We theorized that very-low-frequency BPV ( $VLF_{BPV}$ ) power might reflect the myogenic vascular responsiveness to a cold stimulation trial (Liu *et al.* 2015a, Liu *et al.* 2015b, Liu *et al.* 2015c). We also observed sympathetic activation and vasoconstriction increased the low-frequency BPV ( $LF_{BPV}$ ) and subsequent  $VLF_{BPV}$  powers owing to the activation of  $\alpha_2$ -ADRs (Liu *et al.* 2015d). To clarify the significance of  $\alpha_2$ -ADRs in the progression of cooling elicited hemodynamic perturbations, we compared by using a rather selective antagonist, yohimbine (YOH), to the superimposition of sympathetic removal using ganglion blocker hexamethonium (HEX) or chemical sympathectomy guanethidine (GUA) in the present study.

## Methods

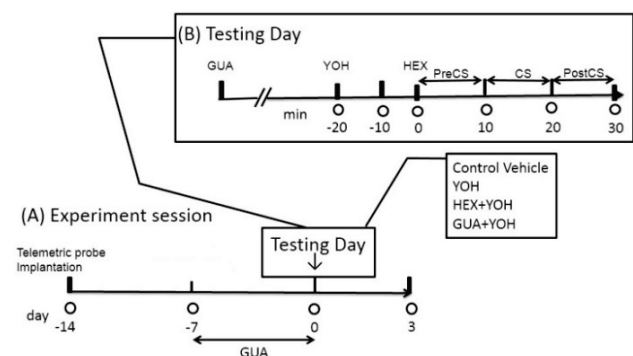
### Animals

Adult male Sprague-Dawley rats weighing between 300 and 350 g were received at the Laboratory Animal Center (LAC) of the National Defense Medical Center (NDMC, Taiwan) one week before the experiments. The experiments were performed according to a protocol approved by the Institutional Animal Care and Use Committee (IACUC) 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. It took 1.5 h to complete the test of a rat. Eight rats were tested daily with four rats being tested at the same time every day. Total experiments were performed between 08:30 and 11:30.

### Experimental protocols and cooling procedure

The timing of the experimental protocols is shown in Figure 1. 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 (0.9 % NaCl,  $n=12$ ) for baseline comparisons, and the other three groups of rats were given the  $\alpha_2$ -ADR antagonist (YOH,  $n=12$ ) alone or with the superimposition of HEX (HEX+YOH,  $n=12$ ) or GUA (GUA+YOH,  $n=12$ ): (a) an intraperitoneal infusion of YOH (2.5 mg/kg/2 ml) 30 min prior to the presentation of cold stimulation, (b) a tail venous bolus of HEX (30 mg/kg/1 ml) 20 min after the beginning of the YOH infusion, or (c) an intraperitoneal injection of GUA (50 mg/kg) seven times a week for 1 week containing a dose 30 min before the cold stimulation trial.



**Fig. 1.** General protocol for (A) implantation of telemetry device in rat 14 days before the testing day and (B) the sequence of test day procedures in the following order, PreCS, CS, and PostCS, for a rat in a Plexiglas cage. After three days at the end of the study, the rats are sacrificed. The experimental groups were 0.9 % NaCl solution (Control Vehicle), yohimbine alone (YOH), hexamethonium superimposed on YOH (HEX+L-NAME), and guanethidine superimposed on YOH (GUA+YOH). CS, cold stimulation (4 °C ice-water immersion of the palms and soles); PreCS, 10 min before CS; PostCS, 20-30 min after CS.

Following a complete stabilization of blood pressure and heart rate at room temperature, each rat was quickly placed in a Plexiglas cage with ice-water (depth=2 cm; temperature=4 °C) to immerse its glabrous palms and soles for a period of 10 min. The Plexiglas

cage was placed on top of the telemetry receiver. Four telemetry receivers were located in four separate but identical Plexiglas cages at the same time for one experiment course approximately 1.5 h of four rats. After a cooling trial, 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 systolic blood pressure 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 cold stimulation (PreCS), 10 min of a cold stimulation (CS), and 20 min after cold stimulation (PostCS). Successive signals during a period of approximately 5 min (3 to 8 min) in each condition were taken for spectral analysis because during this period, the mean and variance of VLF<sub>BPV</sub> and blood pressure were stable. The dirotic notch (Dn) and counts were handled manually.

#### *Surgical intervention and spectrum signal acquisition and processing*

A telemetry transmitter was implanted intra-abdominally into each rat under anesthesia (sodium pentobarbital, 50 mg/kg). The experiments were initiated after the rats had fully recovered from surgery (7 days). The systolic blood pressure signal processing and spectral and cross-spectral analyses were adopted from our previous study (Liu *et al.* 2015b). Briefly, the spectral indices of the hemodynamic oscillations were computed independently to obtain the total power (0.00 to 3.0 Hz, TP) and three major frequency regions: 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 calculated as:

$$nLF \text{ (or } nHF) = LF \text{ (or } HF) / (TP - VLF) \times 100 \%$$

The modulus of the spectral density for each frequency had units of blood pressure: mmHg<sup>2</sup> and heart rate: ms<sup>2</sup>. In addition, to examine the strength of the linear link between BPV and HRV signals across a given frequency region, further computation was performed on the data using cross-spectrum analysis. When the peak coherence value ( $K^2_{HR/SBP}$ ) exceeded 0.58 within a frequency range, the two signals were considered to covary significantly at that frequency.

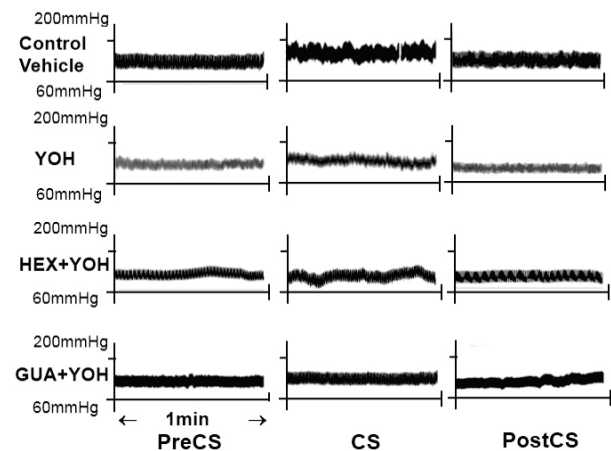
#### *Statistics*

The statistical analyses were conducted with SPSS 18.0 for Windows (Chicago, IL, USA). The

homogeneity of the variance was first confirmed using the Kolmogorov-Smirnov test. Data were then analyzed by the multiple way of analysis of variance (ANOVA) with a within-subject factor, "Trial" (three conditions: PreCS, CS, and PostCS) and a between-subject factor, "Group" (four treatments: Control Vehicle, YOH, HEX+YOH, and GUA+YOH). Subsequent Tukey post hoc test was used to assess the differences in within-subject and between-subject data and Student's t-test was used to compare the differences between with and without Dn data for the various treatment options, respectively. Univariate correlations were calculated using Pearson's correlation analysis to provide the associations between selected frequency bands. The results are expressed as the mean  $\pm$  standard error of mean (SEM). The statistical significance of probability level was set at 0.05.

## Results

Typical examples of the arterial blood pressure tracings are shown in Figure 2. Averaged data are shown in Figures 3-5.



**Fig. 2.** Typical examples of the blood pressure tracings for rats treated with the saline vehicle (control), the YOH alone (YOH) or the superimposition of HEX (HEX+YOH) or GUA (GUA+YOH) before cooling stimulation. Timeline is 0-60 s of the fourth minute (4 to 5 min). Abbreviations: before CS (PreCS), after CS (PostCS), and during the cooling stimulus (CS, 4 °C rapid ice-water immersion of the palms and soles).

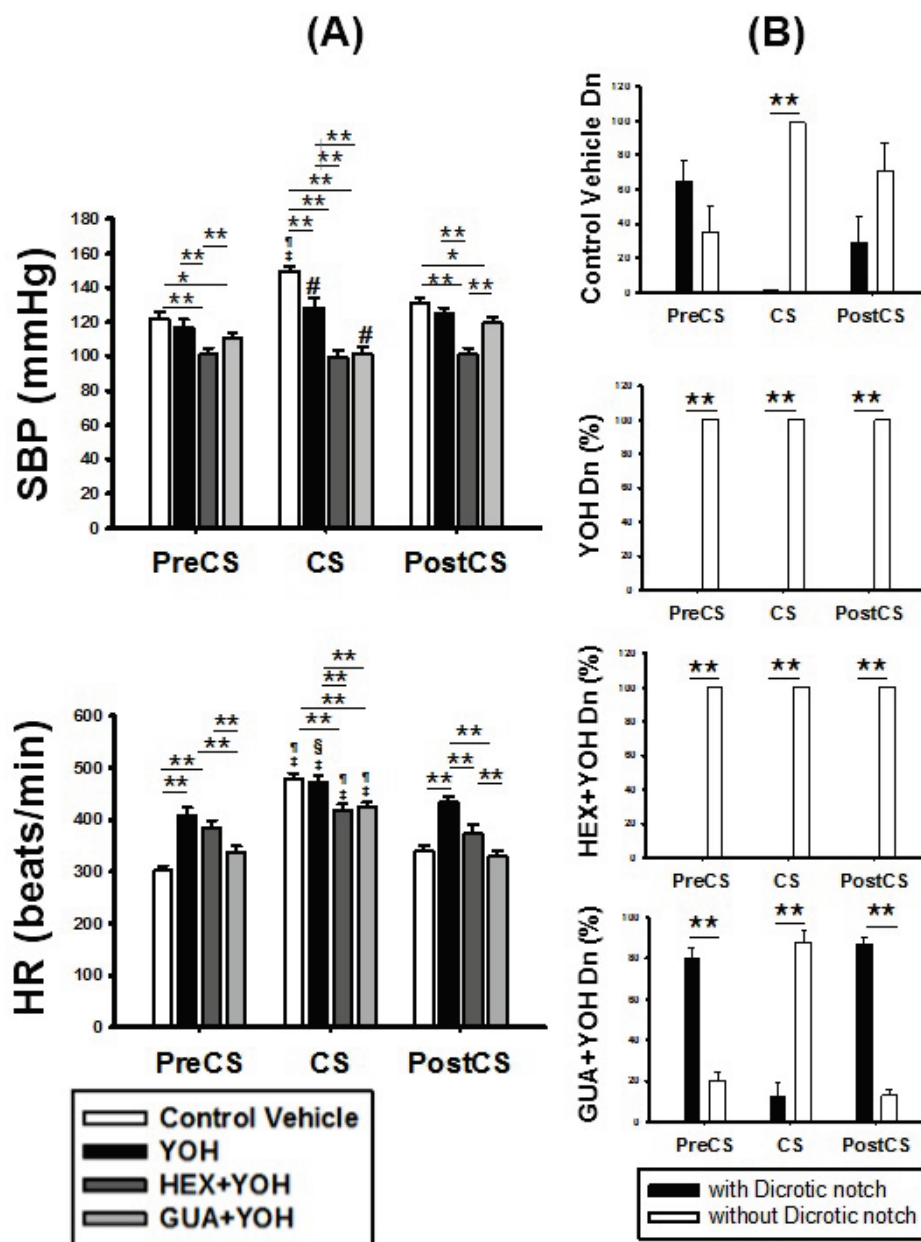
#### *Responses of systolic blood pressure, heart rate, and dirotic notch appearance to different treatment groups of rats throughout the experiment course*

As shown in Figure 3A, when compared to the control vehicle, inhibition of  $\alpha_2$ -ADR by YOH alone has

increased heart rate but did not affect systolic blood pressure before and after the cold stimulation (PreCS and PostCS). On the contrary, YOH alone rather prevented systolic blood pressure increase but not heart rate induced by the stressful cooling challenge (CS). When compared CS with its counterparts (PreCS or PostCS), YOH alone has decreased the control cooling-induced pressor and tachycardia responses. On the other hand, when compared to HEX+YOH or GUA+YOH during PreCS and CS, the effects on systolic blood pressure and heart rate by YOH alone were attenuated by HEX+YOH and

also by GUA+YOH. When compared CS with its counterpart of PreCS or PostCS, the effects of attenuation of control cooling-induced pressor and tachycardia responses by YOH alone were further attenuated by HEX+YOH and also by GUA+YOH. In addition, the effect of cooling-induced pressor by YOH was reversed into a cooling-induced depressor status by GUA+YOH.

When compared to the control vehicle of all experimental conditions (Fig. 3B), both YOH alone and HEX+YOH, in general, caused the dicrotic notch disappeared, whereas GUA+YOH, it is observable.

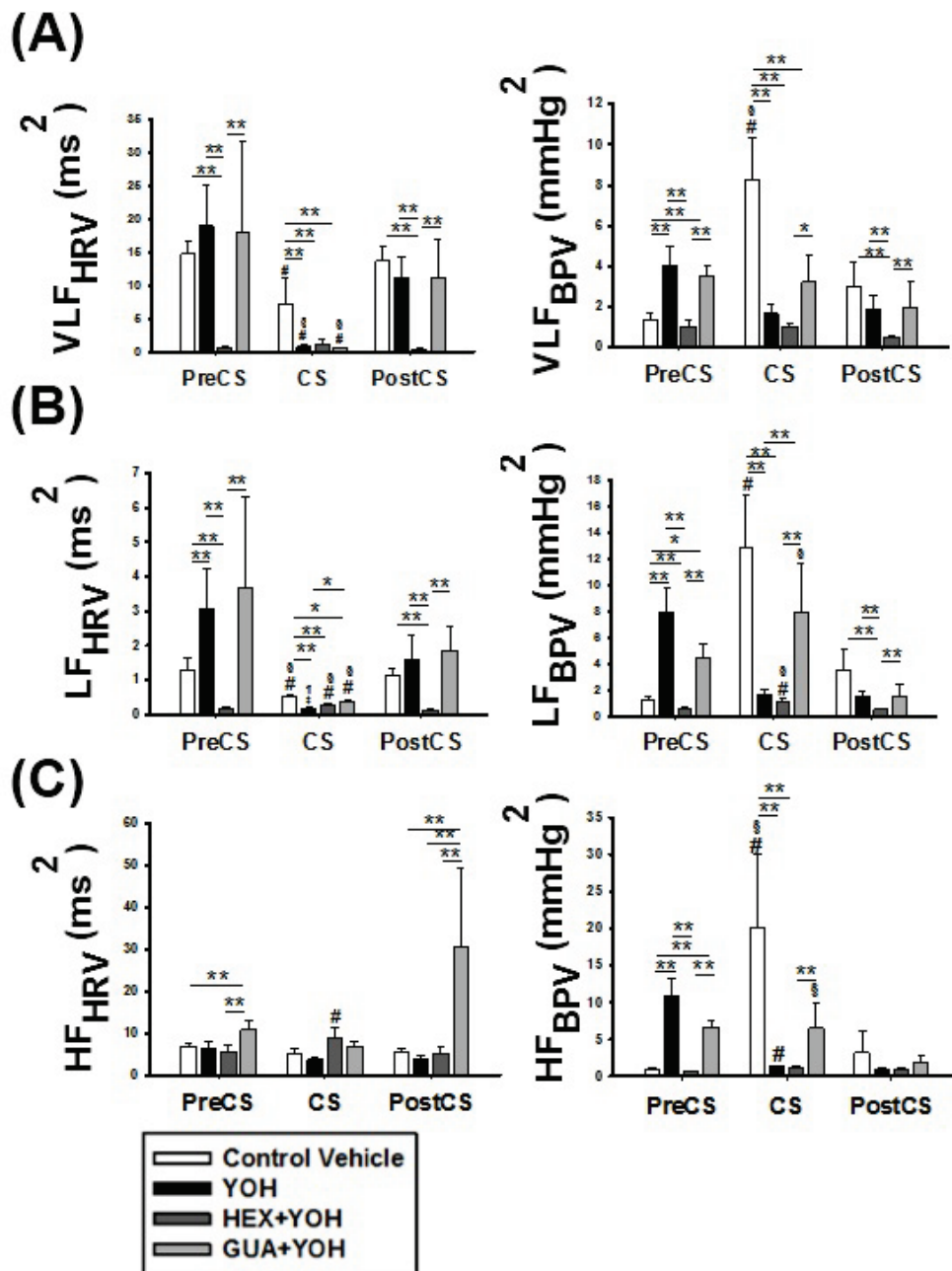


**Fig. 3.** Effects on (A) systolic blood pressure (SBP), heart rate (HR), and (B) dicrotic notch (Dn) of the four rat groups throughout the experimental course. Significant differences between PreCS and CS (<sup>#</sup>P<0.05, <sup>†</sup>P<0.01, <sup>\*</sup>P<0.001), between PostCS and CS (<sup>§</sup>P<0.05, <sup>||</sup>P<0.01, <sup>¶</sup>P<0.001), and between groups (<sup>\*</sup>P<0.05, <sup>\*\*</sup>P<0.01-0.001) were assessed by the multiple way of analysis of variance (ANOVA) and *post hoc* comparisons. The values are presented as the mean  $\pm$  SEM. The abbreviations are defined in Fig. 1.

*Comparisons of the changes in spectral powers for HEX versus GUA superimposed on YOH intervention*

When compared to the control vehicle before the cold stimulation (Fig. 4: PreCS), YOH alone has increased the spectral powers of HF<sub>BPV</sub>, LF<sub>BPV</sub>, and

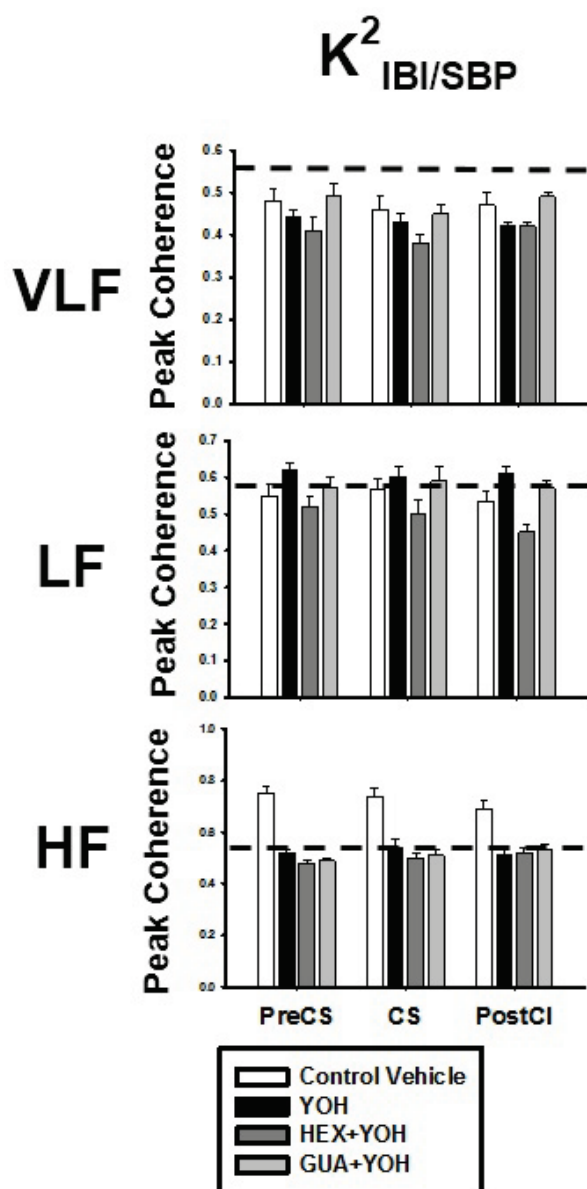
VLF<sub>BPV</sub>. However, when compared to the control vehicle under the stressful cooling challenge (Fig. 4: CS), YOH alone has decreased the spectral powers of HF<sub>BPV</sub>, LF<sub>BPV</sub>, LF<sub>HRV</sub>, VLF<sub>BPV</sub>, and VLF<sub>HRV</sub>.



**Fig. 4.** Changes in spectral powers of (A) very-low-frequency (VLF), (B) low-frequency (LF), and (C) high-frequency (HF) for blood pressure variability (BPV) and heart rate variability (HRV) of the four rat groups throughout the experimental course. The modules of the BP or HR spectrum (ordinates) have units of mmHg<sup>2</sup> and ms<sup>2</sup>, respectively. The statistical analyses, abbreviations, and symbols are defined in Figure 2.

On the other hand, when compared to HEX+YOH before the cold stimulation (Fig. 4: PreCS), the effects on spectral powers by YOH, in general, were attenuated by HEX+YOH. The affected spectral powers

included HF<sub>BPV</sub>, LF<sub>BPV</sub>, LF<sub>HRV</sub>, VLF<sub>BPV</sub>, and VLF<sub>HRV</sub>. However, when compared between YOH alone and HEX+YOH under the stressful cooling challenge (Fig. 4: CS), a general equipotent attenuation of most spectral



**Fig. 5.** The relationship between HR and SBP rhythmic oscillations as assessed by coherence values ( $K^2_{IBI/SBP}$ ) between BPV and HRV at the VLF, LF, and HF regions of the four rat groups throughout the experimental course. The values are presented as the mean  $\pm$  SEM. The statistical analyses, abbreviations, and symbols are described in Figure 2.

powers between them except  $HF_{HRV}$  that was slightly, but not significantly increases by HEX+YOH.

Furthermore, when compared to GUA+YOH throughout the experiment course (Fig. 4), the effects on spectral powers by YOH, in general, were attenuated by GUA+YOH as HEX+YOH did, though the affected profiles between GUA+YOH and HEX+YOH were different. When compared to HEX+YOH, GUA+YOH increased spectral powers for  $VLF_{BPV}$ ,  $VLF_{HRV}$ ,  $LF_{BPV}$ ,  $LF_{HRV}$ ,  $HF_{BPV}$ , and  $HF_{HRV}$ .

Nevertheless, we observed there were trends towards negative correlations between LF pair ( $LF_{HRV}$  versus  $LF_{BPV}$ ) ( $r=-0.39$ ,  $P=0.20$ ) and between VLF pair ( $VLF_{HRV}$  versus  $VLF_{BPV}$ ) ( $r=-0.32$ ,  $P=0.39$ ) by control vehicle. However, both YOH alone and HEX+YOH have reversed the control vehicle original negative correlation trend of the LF pair into positive correlation trend (YOH:  $r=0.51$ ,  $P<0.05$ ; HEX+YOH:  $r=0.23$ ,  $P=0.42$ ). On the other hand, YOH alone remained the control vehicle original negative correlation trend of the VLF pair ( $r=-0.16$ ,  $P=0.19$ ). However, HEX+YOH reversed the negative correlation of that pair into a trend of positive correlation ( $r=0.16$ ,  $P=0.43$ ). Furthermore, we observed the original negative correlation trends for LF and VLF pairs by control vehicle have remained presented by GUA+YOH (LF pair:  $r=-0.17$ ,  $P=0.61$ ; VLF pair:  $r=-0.26$ ,  $P=0.22$ ).

#### *Response of coherence linkage to different treatment groups of rats throughout the experiment course*

The linear relationships as assessed by the peak coherence values ( $K^2_{HR/SBP}$ ) between BPV and HRV for the three major frequency regions are summarized in Fig. 5. When compared with the control vehicle throughout the experiment course, YOH generally showed large  $K^2_{HR/SBP}$  ( $>0.58$ ) at the LF region but small  $K^2_{HR/SBP}$  ( $<0.58$ ) at the HF region. However, HEX+YOH decreased the large coherence value at the LF region by YOH, in contrast, GUA+YOH had it remained large. On the other hand, HEX+YOH and GUA+YOH remained the small coherence value at the HF region by YOH. Nevertheless, we observed there were small coherence values at the VLF region throughout the experiment course for all treatments.

## Discussion

Norepinephrine and epinephrine through  $\alpha_2$ -ADRs at several sites that participate in cardiovascular regulations, wherever located, these sites govern the central adrenergic neurons to inhibit sympathetic outflow, the peripheral sympathetic neurons to inhibit catecholamine release, and the resistance and capacitance vessels to enhance vasoconstriction (Timmermans and van Zwieten 1981, Goldberg *et al.* 1983, Elsner *et al.* 1986, Grossman *et al.* 1991, McGillivray-Anderson and Faber 1991). We previously reported that cooling-elicited hemodynamic perturbations is highly relevant to the sympathetic activation and found

that effect of  $\beta$ -ADR on myogenic vascular oscillations is less powerful than the effect of  $\alpha$ 2-ADR under the stressful cooling challenge (CS) (Liu *et al.* 2015a, Liu *et al.* 2015d). Here we extend our studies on the effect by  $\alpha$ 2-ADRs in the progression of cooling elicited hemodynamic perturbations and based on our findings, focus on discussion of the resulting oscillatory changes in LF and VLF for both BPV and HRV, because those spectral power changes are known to reflect sympathetic activity and myogenic vascular oscillations.

The results of increased HR,  $LF_{BPV}$ , and successive  $VLF_{BPV}$  elevation at rest in PreCS by YOH alone in the present study agreed quite well with the previous reports that YOH acts as a central mediator to elevate sympathetic outflows under normal circumstances (Grossman *et al.* 1991, Kuo and Keeton 1991). The results suggested that YOH has elevated the sympathetic oscillations ( $LF_{BPV}$ ) by a decrease of central  $\alpha$ 2-ADRs tonicity (Goldberg *et al.* 1983, Grossman *et al.* 1991, Liu *et al.* 2015d) and then augmented ADR effects on vasoconstriction to elevate the myogenic vascular oscillations ( $VLF_{BPV}$ ) (Liu *et al.* 2015a, Liu *et al.* 2015c, Liu *et al.* 2015d). To investigate such possibilities further, we compared the effects of YOH alone to the presence and absence of HEX or GUA discussed in the following.

Compared with the YOH alone in PreCS (Fig. 3 and Fig. 4: PreCS), we found that both systolic blood pressure and heart rate were decreased by the superimposition of HEX or GUA. On the other hand, the spectral powers for indication of sympathetic oscillations,  $LF_{BPV}$  and  $LF_{HRV}$ , and myogenic vascular and cardiac oscillations,  $VLF_{BPV}$  and  $VLF_{HRV}$ , were decreased by HEX+YOH, in general, but not much affected by GUA+YOH. We suggest the distinct pattern of spectral power effects between HEX+YOH and GUA+YOH could be due to the sparing effects of GUA on adrenal medulla (Abercrombie and Davies 1963). In the case of GUA+YOH, the release of epinephrine from adrenal medulla might activate the renin-angiotensin-aldosterone system and produce a positive chronotropic effect *via*  $\beta$ -ADR, which is different to the effects of HEX+YOH.

However, when compared with the vehicle control under the stressful cooling challenge (CS) (Fig. 3 and Fig. 4: CS), we found that YOH decreased systolic blood pressure and most of the spectral powers particular LF and VLF for both BPV and HRV. We also found that YOH alone decreased most frequency powers for both BPV and HRV when compared CS with respective

PreCS. The explanation lies in the fact that baroreflex compensation for the control cooling-induced pressor response might reduce  $LF_{BPV}$  power, whereas the vasodilation-increased  $LF_{BPV}$  after inhibition of peripheral postsynaptic  $\alpha$ 2-ADRs by YOH is unable to offset the CS-induced  $LF_{BPV}$  reduction. In addition, it is possible that there were some reflexatory mechanisms still existed to decrease  $LF_{BPV}$  and  $LF_{HRV}$  due to the remained non-sympathetic vasoconstrictors even after blockade of  $\alpha$ 2-ADRs under CS. These results provide an insight that sympathetic activation in the progression of cooling elicited hemodynamic perturbations as an increase of  $LF_{BPV}$  power to stimulate  $\alpha$ 2-ADRs may well increase the myogenic vascular oscillations as the elevation of  $VLF_{BPV}$  power (Radaelli *et al.* 2006, Liu *et al.* 2015d). We therefore suggest that YOH decreased  $VLF_{BPV}$  power under CS is primarily generated by the peripheral rather than the central  $\alpha$ 2-ADR effects. Once again, we examined the validity of this suggestion compared the effects with and without sympathetic influences after inhibition of  $\alpha$ 2-ADRs in the following.

We observed that both the control cooling-induced pressor and the cooling-induced tachycardia responses attenuated by YOH alone were further attenuated by HEX+YOH and also by GUA+YOH. We also observed HEX+YOH similar to YOH attenuated most spectral powers specifically LF and VLF for both BPV and HRV when compared with the control vehicle under CS; however, those attenuation effects for GUA+YOH were different. The attenuation of GUA+YOH was apparently less potent than those effects of HEX+YOH or YOH alone (Fig. 4: right panel: CS). Here again, we attribute these results to the fact that pharmacological properties of GUA. As aforementioned above, the attenuation of  $LF_{BPV}$  by GUA+YOH might be owing to the baroreflex compensation for the remained vasoconstriction effects induced by CS. On the other hand, the attenuation of  $VLF_{BPV}$  by GUA+YOH might be owing to the sparing effect of GUA on sympathetic outflow to release epinephrine from adrenal medulla (Abercrombie and Davies 1963) under CS. The released epinephrine may circulate to the vascular smooth muscle *via* activation of  $\alpha$ 2-ADRs (Timmermans and van Zwieten 1981, Elsner *et al.* 1986, McGillivray-Anderson and Faber 1991) to elevate myogenic vascular oscillations as an increase of  $VLF_{BPV}$  (Radaelli *et al.* 2006, Langager *et al.* 2007, Liu *et al.* 2015d), whereas YOH attenuated such effects. Nevertheless, we observed between  $LF_{BPV}$  and  $LF_{HRV}$ , the high coherence value ( $K^2_{IBI/SBP} > 0.58$ ) by

YOH alone has remained unchanged by GUA+YOH but weakened by HEX+YOH under CS (Fig. 5). However, the positive correlation trend for the LF pair under CS observed in YOH alone was changed back to a negative correlation tendency after superimposed GUA (GUA+YOH) but not HEX (HEX+YOH). These results indicated that both YOH and the superimposition of GUA still have remained an integral baroreflex feedback loop under CS. However, the superimposition of HEX abolished such feedback mechanism.

Finally, we observed both YOH and HEX+YOH reduced, but GUA+YOH enhanced, the magnitude of the appearance of Dn throughout the experiment course (Fig. 3B). Overall these data demonstrated that  $\alpha$ 2-ADRs affect the appearance of Dn in the pressure wave. A higher presence of Dn suggests the increased vascular resistance by modifying reflected pressure waves in conduit artery (Politi *et al.* 2016), and also provides additional information about the myogenic vascular responses to the hemodynamic perturbations.

In this study, we used spectrum to analyze the sympathetic modulation of vascular tone. The autonomic modulation of cardiovascular system function is reflected in cardiovascular variability and large portions of this variability are generated by cardiovascular control mechanisms aimed at maintaining homeostasis. However, spectral indices obtained by recording BPV or HRV do not reflect exact quantitative neural signals. Blood pressure fluctuations elicited by sympathetic modulation of vascular tone occur in the LF<sub>BPV</sub> band, whereas myogenic vascular function affects VLF<sub>BPV</sub> and also LF<sub>BPV</sub>, thus LF<sub>BPV</sub> does not exclusively reflect sympathetic modulation of vascular tone. The real world is very complicated and difficult to develop an idea

surrogate. Experiment design always simplified because of the limitation of material and techniques and for better manipulation. Therefore, spectral analysis is still a popular method to use in the assessment of autonomic cardiovascular regulation. In our quoted Pagani *et al.*'s study (1996), LF is a marker of the sympathetic modulation of vasomotor activity. Our previous report also indicated that LF reflected the activity of sympathetic modulation.

In conclusion, our current study provides new evidence that postsynaptic  $\alpha$ 2-ADRs may contribute to the stressful cooling-induced efferent sympathetic activation and hemodynamic perturbations. The results indicated that under stressful cooling challenge, sympathetic activation causes hemodynamic perturbations *via* an activation of postsynaptic  $\alpha$ 2-ADRs, which in turn may increase myogenic vascular oscillations, blood flow, and tissue perfusion to prevent tissue damage. Future studies of the substantial factors for vasoconstriction and vasodilatation in cooling-induced hemodynamic perturbations have potential therapeutic benefit for cold injury.

### Conflict of Interest

There is no conflict of interest.

### Acknowledgements

This work was funded by grants from the Ministry of Science and Technology (MOST 102 & 103-2320-B-350-001) and the Cheng Hsin General Hospital-National Defense Medical Center cooperative research project (CH-NDMC-105-4), Taipei, Taiwan, R.O.C. We wish to thank Miss Chan-Fan Young for her assistance in telemetry technique and spectral analysis for experiments.

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