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

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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

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 alpha1- and alpha2-adrenoreceptors (α2-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 a*l. 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 α2-ADRs (Liu *et al*. 2015d). To clarify the significance of α2-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 (HEX) or chemical sympathectomy guanethidine (GUA) in the present study (Liu *et al*. 2015a, Liu *et al*. 2015c).

Material and 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 hours 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 Fig. 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 α2-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.

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^{o}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 hours 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_{BPV} and blood pressure were stable. The dicrotic 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 (or nHF) = LF (or HF)/(TP-VLF)×100%. The modulus of the spectral density for each frequency had units of blood pressure: $mmHg^2$ and heart rate: ms^2. 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 ± 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 Fig. 2. Averaged data are shown in Fig. 3-5.

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

As shown in Fig. 3 A, when compared to the control vehicle, inhibition of α2-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. 3 B), both YOH alone and HEX+YOH, in general, caused the dicrotic notch disappeared, whereas GUA+YOH, it is observable.

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_{BPV}, LF_{BPV}, and VLF_{BPV}. However, when compared to the control vehicle under the stressful cooling challenge (Fig. 4: CS), YOH alone has decreased the spectral powers of HF_{BPV}, LF_{BPV}, LF_{HRV}, VLF_{BPV}, and VLF_{HRV}.

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_{BPV}, LF_{BPV}, LF_{HRV}, VLF_{BPV}, and VLF_{HRV}. However, when compared between YOH alone and HEX+YOH under the stressful cooling challenge (Fig. 4: CS), a general equipotent attenuation of most spectral 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, both 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 α2-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 β-ADR on myogenic vascular oscillations is less powerful than the effect of α2-ADR under the stressful cooling challenge (CS) (Liu *et al*. 2015a, Liu *et al*. 2015d). Here we extend our studies on the effect by α2-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 α2-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 β-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 α2-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 α2-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 α2-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 α2-ADR effects. Once again, we examined the validity of this suggestion compared the effects with and without sympathetic influences after inhibition of α2-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 barorereflex 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 α2-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 3 (B)). Overall these data demonstrated that α2-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_{BPV} band, whereas myogenic vascular function affects VLF_{BPV} and also LF_{BPV}, thus LF_{BPV} 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, LF is a marker of the sympathetic modulation of vasomotor activity (Pagani *et al*. 1996). Our previous report also indicated that LF reflected the activity of sympathetic modulation.

In conclusion, our current study provides new evidence that postsynaptic α2-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 α2-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.

Acknowledgments

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References

ABERCROMBIE GF, DAVIES BN: The action of guanethidine with particular reference to the sympathetic nervous system. *Br J Pharmacol Chemother* **20**: 171-177, 1963.

AKSELROD S, GORDON D, MADWED JB, SNIDMAN NC, SHANNON DC, COHEN RJ: Hemodynamic regulation: investigation by spectral analysis. *Am J Physiol* **249**: H867-875, 1985.

DAANEN HA: Finger cold-induced vasodilation: a review. *Eur J Appl Physiol* **89**: 411-426, 2003.

DI RIENZO M, PARATI G, RADAELLI A, CASTIGLIONI P: Baroreflex contribution to blood pressure and heart rate oscillations: time scales, time-variant characteristics and nonlinearities. *Philos Trans A Math Phys Eng Sci* **367**: 1301-1318, 2009.

ELSNER D, STEWART DJ, SOMMER O, HOLTZ J, BASSENGE E: Postsynaptic alpha 1- and alpha 2-adrenergic receptors in adrenergic control of capacitance vessel tone in vivo. *Hypertension* **8**: 1003-1014, 1986.

FOLKOW B, FOX RH, KRONG J, ODELRAM H, THOREN O: Studies on the Reactions of the Cutaneous Vessels to Cold Exposure. *Acta Physiol Scand* **58**: 342-354, 1963.

GOLDBERG MR, HOLLISTER AS, ROBERTSON D: Influence of yohimbine on blood pressure, autonomic reflexes, and plasma catecholamines in humans. *Hypertension* **5**: 772-778, 1983.

GROSSMAN E, REA RF, HOFFMAN A, GOLDSTEIN DS: Yohimbine increases sympathetic nerve activity and norepinephrine spillover in normal volunteers.

Am J Physiol **260**: R142-147, 1991.

JAPUNDZIC N, GRICHOIS ML, ZITOUN P, LAUDE D, ELGHOZI JL: Spectral analysis of blood pressure and heart rate in conscious rats: effects of autonomic blockers. *J Auton Nerv Syst* **30**: 91-100, 1990.

JOHNSON JM, KELLOGG DL, Jr.: Local thermal control of the human cutaneous circulation. *J Appl Physiol (1985)* **109**: 1229-1238, 2010.

KUO YJ, KEETON TK: Is the sympathoexcitatory effect of yohimbine determined by brain yohimbine concentration? *Naunyn Schmiedebergs Arch Pharmacol* **344**: 308-313, 1991.

LANGAGER AM, HAMMERBERG BE, ROTELLA DL, STAUSS HM: Very low-frequency blood pressure variability depends on voltage-gated L-type Ca2+ channels in conscious rats. *Am J Physiol Heart Circ Physiol* **292**: H1321-1327, 2007.

LEWIS T: The Blood Vessels of the Human Skin. *Br Med J* **2:** 61-62, 1926.

LIU YP, LIN YH, CHEN YC, LEE PL, TUNG CS: Role of Beta-Adrenoceptors in Cooling-Evoked Hemodynamic Perturbations of Rats: Investigation by Spectral Analysis. *J Hyperten (Open Access)* ***4(4)***: doi:10.4172/2167-1095.1000209, 2015a.

LIU YP, LIN YH, CHEN YC, LEE PL, TUNG CS: Spectral analysis of cooling induced hemodynamic perturbations indicates involvement of sympathetic activation and nitric oxide production in rats. *Life Sci* **136**: 19-27, 2015b.

LIU YP, LIN YH, LIN CC, LIN YC, CHEN YC, LEE PL, TUNG CS: Role of Efferent Sympathoadrenal Effects in Cooling-Induced Hemodynamic Perturbations in Rats: An Investigation by Spectrum Analysis. *Chin J Physiol* **58**: 312-321, 2015c.

LIU YP, LIN YH, LIN CC, LIN YC, LEE PL, TUNG CS: Role of alpha 2-adrenoceptors in hemodynamic instability in rats under cooling challenge: Investigation by spectral analysis. *Journal of Advanced Clinical Pharmacology* **1 (2)**: 15-26, 2015d.

McGILLIVRAY-ANDERSON KM, FABER JE: Effect of reduced blood flow on alpha 1- and alpha 2-adrenoceptor constriction of rat skeletal muscle microvessels. *Circ Res* **69**: 165-173, 1991.

NOVAKOVA Z: From the first spectral analysis of blood pressure variability in the world to the present time: contribution of the Department of Physiology of the Faculty of Medicine, Masaryk University, Brno. *Physiol Res* **62**: 341-350, 2013.

PAGANI M, LUCINI D, RIMOLDI O, FURLAN R, PIAZZA S, PORTA A, MALLIANI A: Low and high frequency components of blood pressure variability. *Ann N Y Acad Sci* **783**: 10-23, 1996.

POLITI MT, GHIGO A, FERNANDEZ JM, KHELIFA I, GAUDRIC J, FULLANA JM, LAGREE PY: The dicrotic notch analyzed by a numerical model. *Comput Biol Med* **72**: 54-64, 2016.

RADAELLI A, CASTIGLIONI P, CENTOLA M, CESANA F, BALESTRI G, FERRARI AU, DI RIENZO M: Adrenergic origin of very low-frequency blood pressure oscillations in the unanesthetized rat. *Am J Physiol Heart Circ Physiol* **290**: H357-364, 2006.

ROBERTSON D, JOHNSON GA, ROBERTSON RM, NIES AS, SHAND DG, OATES JA: Comparative assessment of stimuli that release neuronal and adrenomedullary catecholamines in man. *Circulation* **59**: 637-643, 1979.

STAUSS HM: Identification of blood pressure control mechanisms by power spectral

analysis. *Clin Exp Pharmacol Physiol* **34**: 362-368, 2007.

TIMMERMANS PB, van ZWIETEN PA: Mini-review. The postsynaptic alpha 2-adrenoreceptor. *J Auton Pharmacol* **1**: 171-183, 1981.

VELASCO M, GOMEZ J, BLANCO M, RODRIGUEZ I: The cold pressor test: pharmacological and therapeutic aspects. *Am J Ther* **4**: 34-38, 1997.

WATANABE M, YAMAMOTO T, MORI C, OKADA N, YAMAZAKI N, KAJIMOTO K, KATAOKA M, SHINOHARA Y: Cold-induced changes in gene expression in brown adipose tissue: implications for the activation of thermogenesis. *Biol Pharm Bull* **31**: 775-784, 2008.

Figure legends

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.

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 0-60sec 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).

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 (#P<0.05, †P<0.01, ‡P<0.001), between PostCS and CS (§P<0.05, ||P<0.01, ¶P<0.001), and between groups (*P<0.05, **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 ± SEM. The abbreviations are defined in Fig. 1.

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 module of the BP or HR spectrum (ordinates) have units of mmHg2 and ms2, respectively. The statistical analyses, abbreviations, and symbols are defined in Fig. 2.

Fig. 5. The relationship between HR and SBP rhythmic oscillations as assessed by coherence values ($K2_{IBI/SBP}$) 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 ± SEM. The statistical analyses, abbreviations, and symbols are described in Fig. 2.

Figure 1

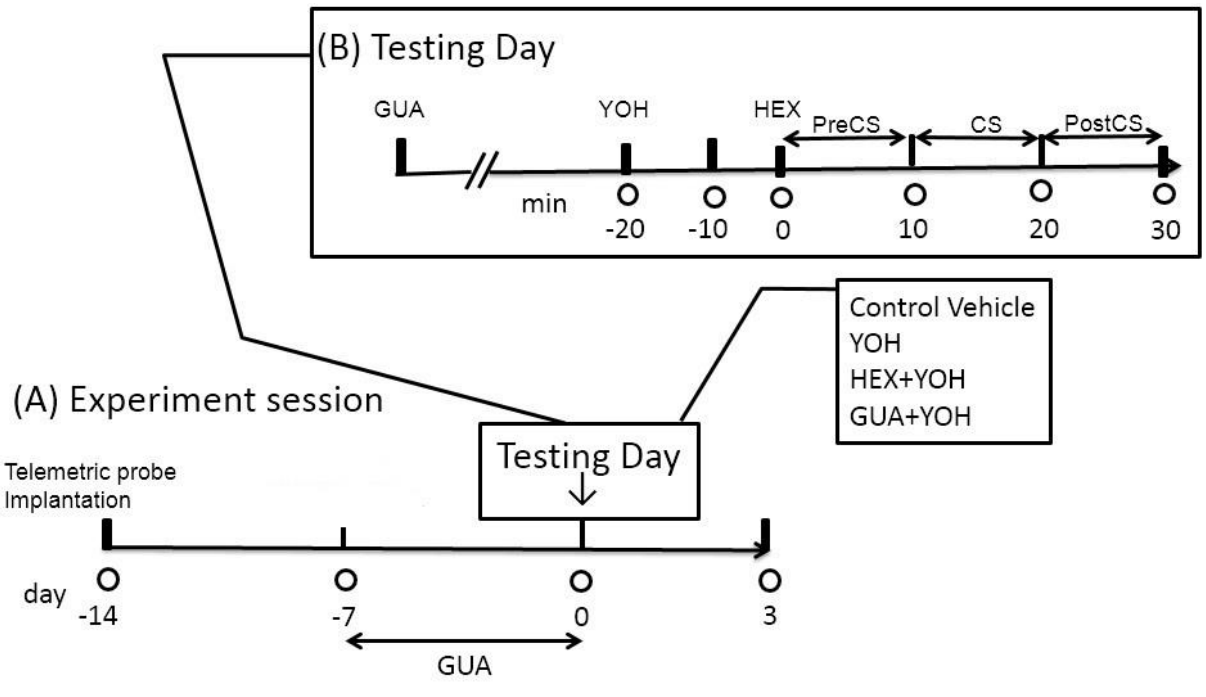


Figure 2

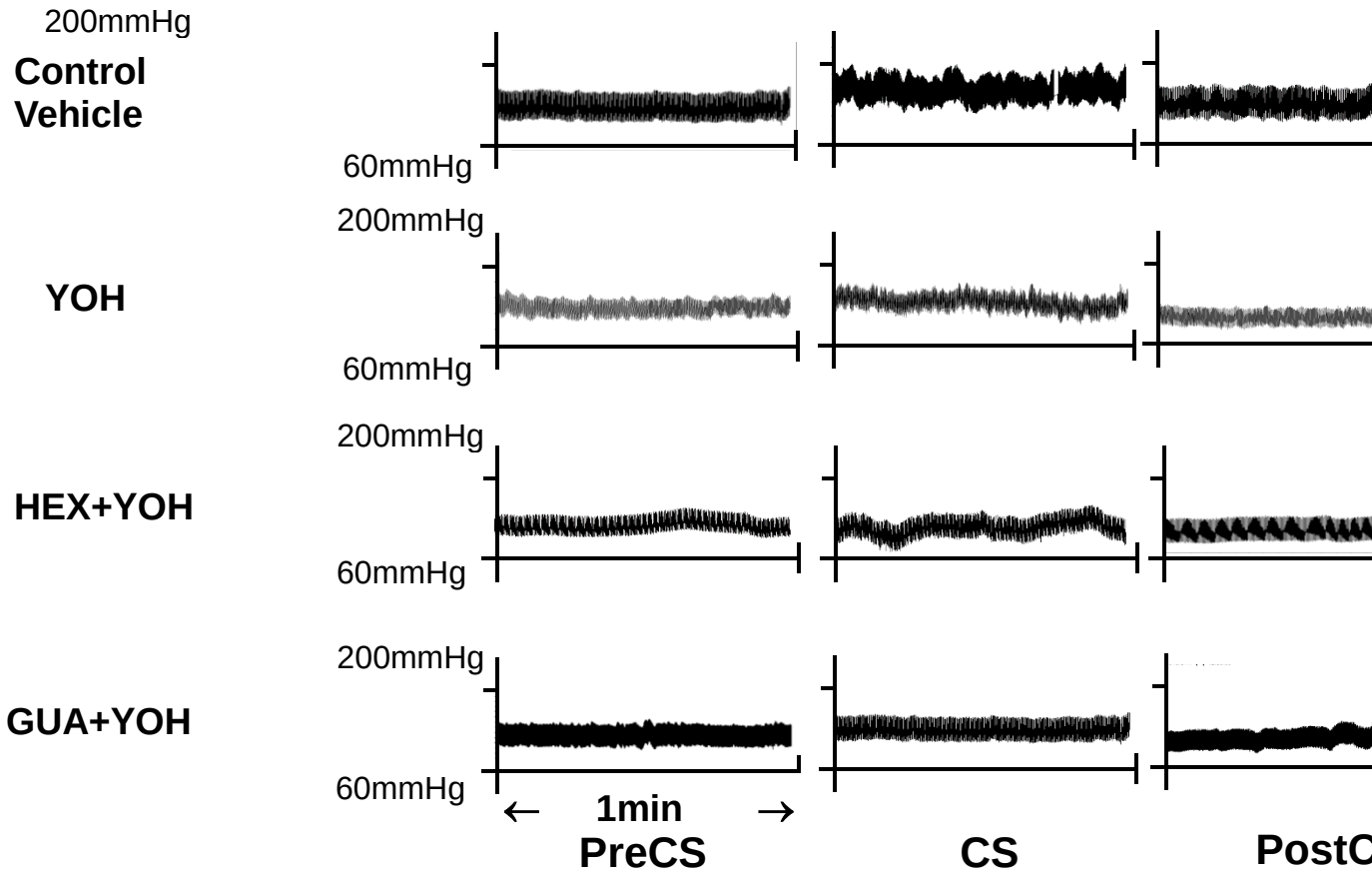

200mmHg
Control
Vehicle
60mmHg
200mmHg
YOH
60mmHg
200mmHg
HEX+YOH
60mmHg
200mmHg
GUA+YOH
60mmHg
← 1min →
PreCS
CS
PostC

Figure 3

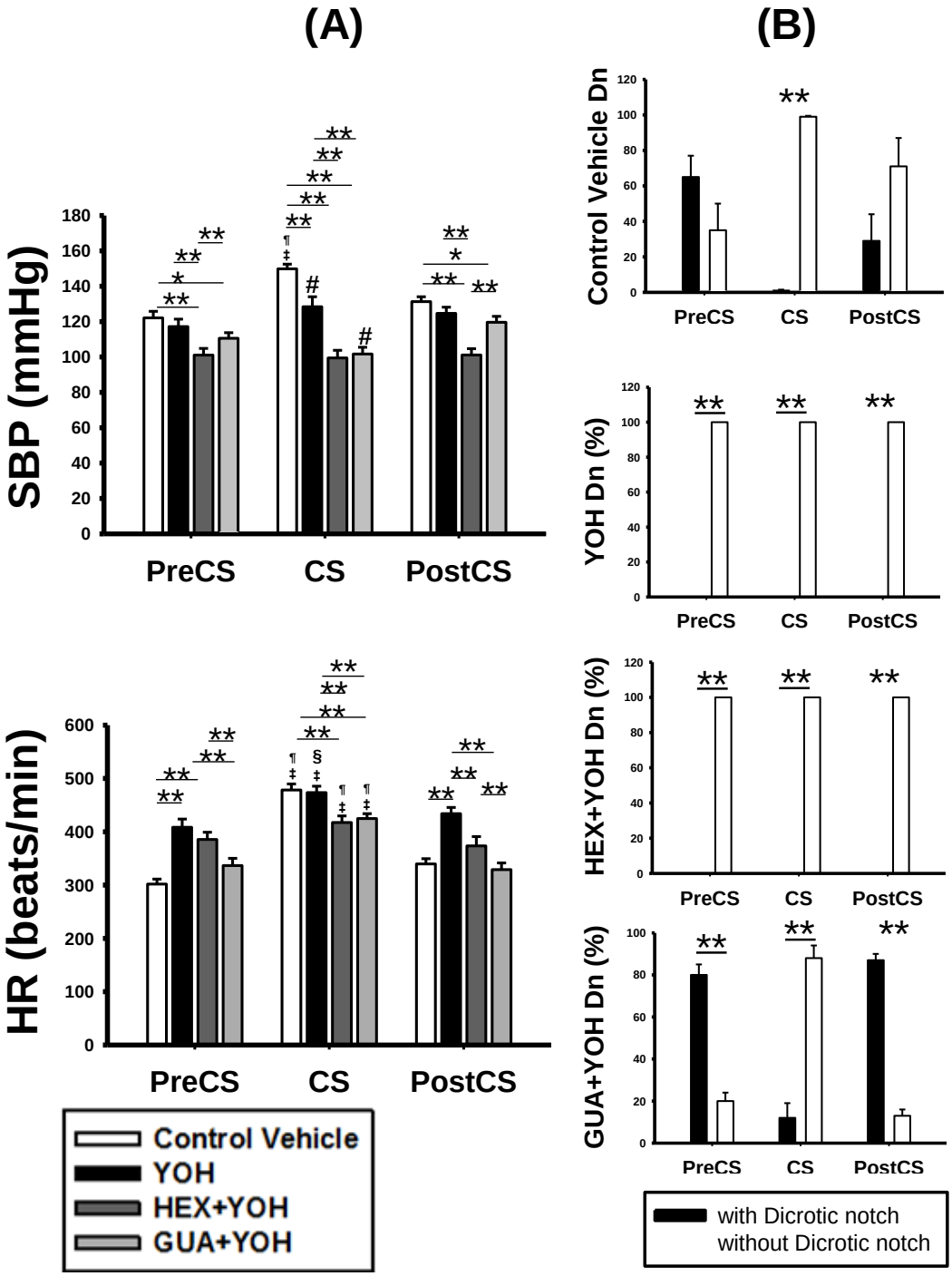

(A)
(B)
SBP (mmHg)
HR (beats/min)
PreCS
CS
PostCS
Control Vehicle
YOH
HEX+YOH
GUA+YOH
Control Vehicle Dn
YOH Dn (%)
HEX+YOH Dn (%)
GUA+YOH Dn (%)
with Dicrotic notch
without Dicrotic notch

Figure 4

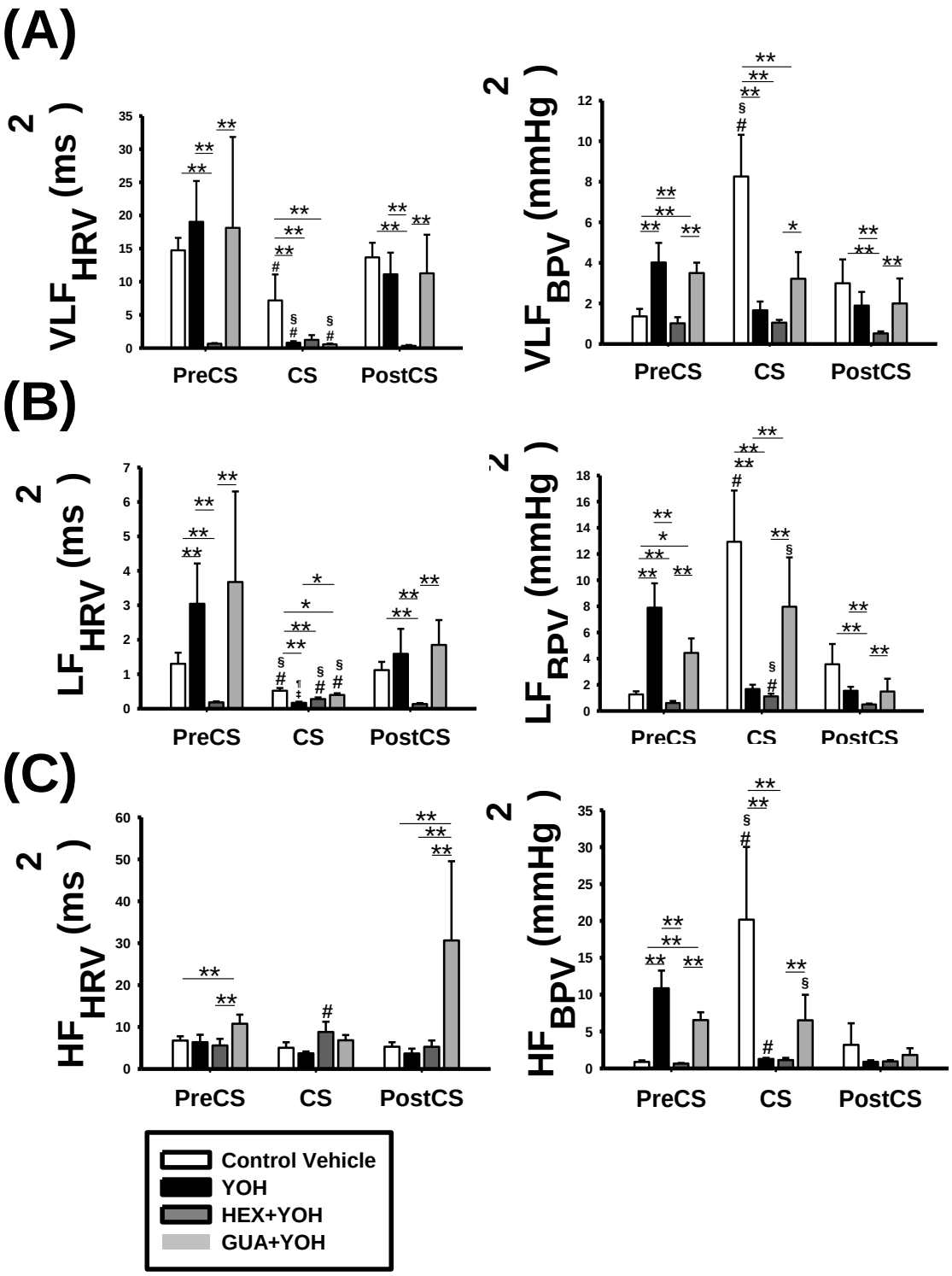
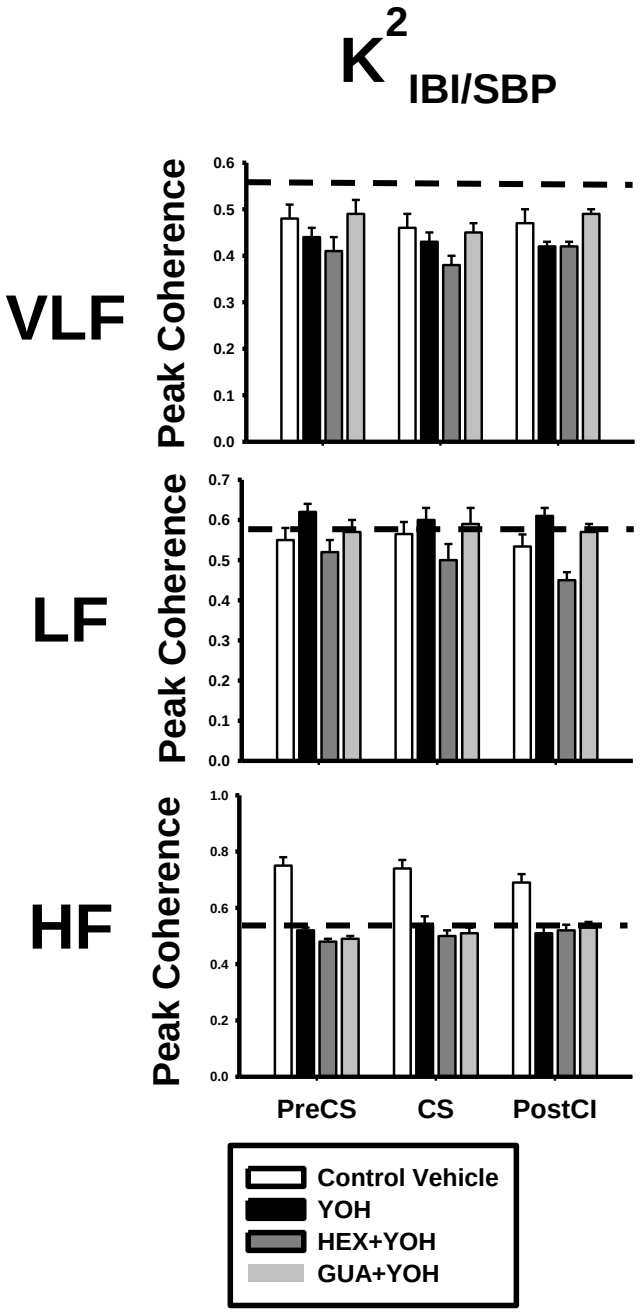

(A)
VLF HRV (ms 2)
VLF BPV (mmHg 2)
(B)
LF HRV (ms 2)
LF RPV (mmHg 2)
(C)
HF HRV (ms 2)
HF BPV (mmHg 2)
PreCS
CS
PostCS
Control Vehicle
YOH
HEX+YOH
GUA+YOH

Figure 5


K2 IBI/SBP
VLF
LF
HF
Peak Coherence
0.0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
1.0
PreCS
CS
PostCI
Control Vehicle
YOH
HEX+YOH
GUA+YOH