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Role of Efferent Sympathoadrenal Effects in Cooling-Induced Hemodynamic Perturbations in Rats: An Investigation by Spectrum Analysis

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Abstract

Cold stress may produce hemodynamic perturbations but the underlying mechanisms are still not clear. Spectral analysis was used in this study to explore that sympathoadrenal activation could be involved in mechanisms of hemodynamic perturbations to cooling. Conscious rats after treatment with a control vehicle (saline) compared with withdrawal of sympathetic influences by ganglion blocker hexamethonium (HEX) or chemical sympathectomy guanethidine (GUA) were challenged by stressful cooling as acute immersing all four extremities in ice water (4 ± 2°C) for 10 min. Plasma nitric oxide (NO) and the appearance of Dichroitic notch (DN) were measured in comparison between treatment groups throughout the experimental course. Hemodynamic indices were telemetrically monitored, and variability of blood pressure and heart rate (BPV; HRV) were assessed over a range of frequencies: very-low frequency (VLF: 0.02-0.2 Hz), low frequency (LF: 0.2-0.6 Hz), high frequency (HF: 0.6-3 Hz), normalized (n)LF, nHF, ratio LF/HF of HRV (LF/HF$_{HRV}$), and total power (TP: ≦3 Hz). Results showed that the concomitant reciprocal changes of spectral powers existed between frequencies of BPV and HRV to the stressful cooling (*i.e.* VLF$_{BPV}$ versus VLF$_{HRV}$, LF$_{BPV}$ versus LF$_{HRV}$, and nLF$_{BPV}$ versus nLF$_{HRV}$) which contribute to the underlying mechanisms of sympathetic efferent influences and myogenic cardiovascular responsiveness. Furthermore, compared with the control vehicle in the stressful cooling, HEX restrained the increase of the pressor, tachycardia and VLF$_{BPV}$, except that VLF$_{HRV}$ was reduced. GUA abolished pressor, however, restrained the increase of the tachycardia, VLF$_{BPV}$ and LF$_{BPV}$. In addition, GUA reversed the downward tendency of nLF$_{BPV}$ into an upward tendency and attenuated both nLF$_{HRV}$ and LF/HF$_{HRV}$. DN was virtually undetectable after HEX management but was apparently noticeable after GUA management. Finally, the increase of plasma NO after cooling was diminished after HEX or GUA management. Taken together, these results substantiate that the spectral changes during stressful cooling are highly relevant to the efferent sympathetic rhythmicity and subsequent NO production.

Key Words: guanethidine, hemodynamic perturbations, hexamethonium, spectral powers, stressful cooling, sympathetic influences

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Introduction

Stressful cooling elicits an immediate increase in arterial blood pressure (BP), heart rate (HR) and greater adrenergic neurotransmissions, which is commonly used in clinical practice in diagnosis on autonomic control of the cardiovascular system in cold pressor test (18); however, there is little substantiated evidence for the underlying mechanisms of this test.

It is well known that when immediately exposed to cooling, a predominant initial response is restricted blood flow due to cooling-induced vasoconstriction. As exposure to cooling persists, the peripheral blood flow passes through a phase of induced vasodilation thought to be a protective mechanism against tissue damage (4, 12, 14).

The interplay between initial vasoconstriction and the subsequently evoked vasodilatation through prolonged cooling is complex (4, 6), which requires intact sympathetic and sensory functions as well as ensuing compensatory baroreflex and releases of humoral substances. The evoked vasodilatation, a myogenic vascular responsiveness, is suggested by reduction of vascular resistance and increases of nitric oxide (NO) production (8, 11, 21, 23).

Spectral analysis in hemodynamic fluctuations has been widely applied to investigate the baroreflex control of cardiovascular homeostasis—a dynamic interplay between ongoing BP perturbation and compensatory cardiovascular response (2, 5, 10, 16). The standard frequencies commonly used for power assessment in BP variability (BPV) and HR variability (HRV) are the following: (a) the high frequency (HF) BPV (HF_{BPV}) reflects the oscillation in cardiac output secondary to mechanic respiratory sinus arrhythmia, whereas HF of HRV (HF_{HRV}) reflects oscillation in respiration and efferent vagal modulation of HR; (b) the low frequency (LF) of BPV (LF_{BPV}) reflects oscillation in efferent sympathetic modulation of vascular resistance, whereas LF of HRV (LF_{HRV}) reflects oscillation in sympathetic modulation of HR; (c) the LF/HF ratio of HRV (LF/HF_{HRV}) reflects the overall balance between efferent sympathetic and vagal modulations of HR; (d) the total power (TP) of BPV (TP_{BPV}) or HRV (TP_{HRV}) reflects the variance of oscillations and overall autonomic activity in vascular bed or the heart; and (e) the very-low frequency (VLF) of BPV (VLF_{BPV}) or HRV (VLF_{HRV}), a heterogeneously frequency band, the physiological background of which is mostly unrevealed. However, this band has been variously ascribed to the thermoregulatory vasomotor modulation, activity of hormonal systems and the autonomic nervous system itself (7, 13, 17, 20).

Since the regulation of sympathetic outflow can be highly patterned in baroreflex mechanisms of hemodynamic changes, the question arises as to how different patterns of sympathetic activation are generated by various kinds of pathophysiological stimuli (3, 9). We have previously successfully developed a rodent model to study cooling stress through a novel measurement of cardiovascular variability, and the results revealed that frequency power of VLF_{BPV} might reflect the myogenic vascular responses to the cooling-induced sympathetic activation (22). In addition, the cooling-induced spectral VLF_{BPV} enhancement has been related to sympathetic activation and subsequent NO production. The present study was performed through pharmacological intervention to explore the underlying mechanisms of cooling-induced spectral power VLF_{BPV} enhancement in which the efferent sympathoadrenal pathways could be involved.

Materials and Methods

Subjects

Adult male Sprague-Dawley rats (BioLASCO, Taiwan, ROC) weighing between 300 and 350 g were used. The experiments were performed according to a protocol approved by the Animal Care Committee of the National Defense Medical Center, Taipei. 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, with light on from 07:00 to 19:00, maintained by manual light control switches as required by the experiment. Rats in the same experimental group were housed together. All rats received food and water *ad libitum*. Experiments were performed between 08:30 and 17:30, with all rats were tested at the same time every day, whenever possible.

Experimental Protocols and Cooling Procedure

Rats were randomly divided into three groups in assessing the effects of withdrawal of the neural sympathetic influences: (a) in the control vehicle intervention by intraperitoneal (i.p.) injection of saline (n = 12) to obtain hemodynamic data in the intact condition; (b) after induction of ganglionic blockade by hexamethonium (HEX) (30 mg/kg intravenous bolus followed by continuous infusion at 1.5 mg/kg/min) (n = 12), and the infusion was continued throughout the experimental course; (c) after sympathetic denervation by i.p. injection of guanethidine sulfate (GUA) (50 mg/kg i.p. seven times a week for 1 week) (n = 12), and the treatment was continued throughout the experimental course. The stressful cooling stimulation consisted of rapidly immersing

all four extremities of an individual rat in ice-cold water (CI).

Prior to the experiments, the rats were adapted to the experimental circumstances for approximately 30 min. Following a complete stabilization of hemodynamics and temperature, an individual rat was quickly placed in a Plexiglas cage with ice-water (depth, 2 cm; temperature, 4°C) and received CI for a period of 10 min. After exposure, the rat was removed from the cage and dried with a cloth in a similar cage for 30 min to facilitate recovery. Hemodynamic changes were monitored continuously *via* a telemetric device in 10-min stages, *i.e.*, 10 min before (PreCI), 20 min after (PostCI), and during the 10-min of the cooling trial (0-3 min: CIIP, the initial ascending pressor period; 7-10 min: CIMP, the maintaining plateau pressor period). Dicrotic notch (DN) was counted manually.

Surgical Intervention

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

Acquisition and Processing of Spectrum Signals

On the day of the experiment, the pulse signals obtained after magnetic activation of the transmitter at least 1 h before starting the test were generated as a calibrated analog signal (UA10; DSI, St. Paul, MN, USA) with a range of ± 5 V and a 12-bit resolution. Individual rat in each group was then placed on the top of a receiver (PhysioTel® RPC-1) for acquisition of telemetric signals. Five receivers were connected to a PC desktop computer *via* a matrix (Dataquest ART Data Exchange Matrix) and the signals were recorded with the Dataquest Acquisition software (Dataquest ART 4.33). A series of successive signals of SBP and inter-beat interval (IBI) throughout the experiments were then digitized at a 500-Hz sampling rate and processed off-line using Matlab software (Terasoft Co. San Diego, CA).

Beat-by-beat fluctuations in SBP and IBI movements were analyzed to quantitate the frequency and power in cardiovascular variability (BPV and HRV) using autoregressive spectral decomposition. Spectral analysis for BPV calculation was based on a software kindly written for us by Professor P.L. Lee, National Central University, Taiwan. Briefly, the acquired SBP signals were pre-processed by applying a band-pass filter (0.1~18 Hz, zero-phase 4^{th}-order) to remove DC components. After finding all the maximum SBP peaks 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 sampling, which allowed a direct spectral analysis of each distribution using a Fast Fourier Transform (FFT) algorithm. On the other hand, spectral analysis for HRV calculation was based on the Chart software developed by PowerLab, ADInstruments, Colorado Springs, CO, USA. Spectral indexes of BPV and HRV were then computed independently to obtain the total power (0.00 to 3.0 Hz, TP) and three major spectral components: 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 modulus of the HR or BP spectrum (ordinates) had units of ms^2 or $mmHg^2$.

NO Assay

Tail venous blood was withdrawn for NO assessments in the following rats: Vehicle-PreCI (n = 6): rats treated with control vehicle and blood was withdrawn before CI (PreCI); Vehicle-CI (n = 6): rats treated with control vehicle and blood was withdrawn immediately after 10 min of a CI trial; HEX-CI (n = 6): rats treated with HEX and blood was withdrawn immediately after a 10-min CI trial. GUA-CI (n = 6): rats treated with GUA and blood was withdrawn immediately after a 10-min CI trial. The plasma NO level was determined indirectly by the content of nitrite and nitrate using enzyme-linked immunosorbent assay (ELISA) kits (CAT No. 780001) supplied by Cayman Chemical Company (Ann Arbor, MI, USA). Results were expressed as μM.

Statistical Analysis

Statistical analyses were performed using the SPSS version 18.0 software. Homogeneity of variance was assessed by the Kolmogorov-Smirnov test. Statistical comparisons were performed by repeated measures two-way ANOVA followed by a *post-hoc* Scheffe's test (SPSS version 18.0). Student's *t*-test was used to detect differences between two groups. A *P*-value < 0.05 was considered statistically significant. The results are expressed as mean ± SEM.

Results

Typical examples of BP tracings and the changes in power spectrum are shown in Figs. 1 and 2 for rats receiving pharmacological interventions. Averaged data are shown in Figs. 3-5.

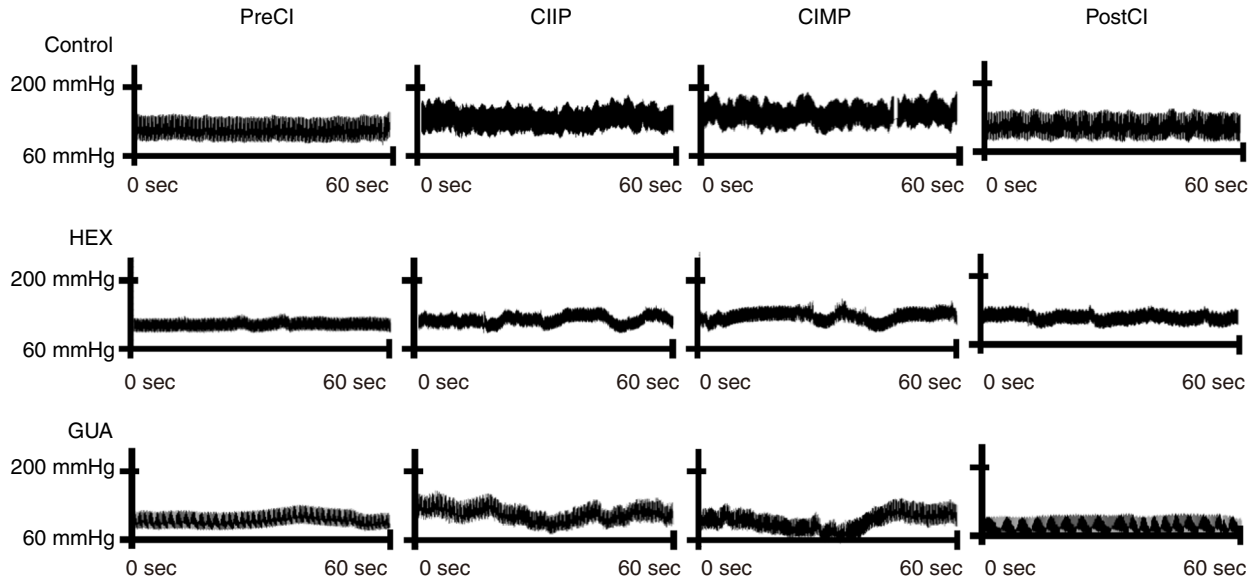

Fig. 1. A typical example of blood pressure tracings in rats treated with saline (Control Vehicle) or withdrawal of sympathetic influences by HEX or GUA before the cooling stimulation. Abbreviations: Stressful cooling as rapid immersion of four extremities in 4°C ice water (CI), 10 min before CI (PreCI), 20 min after CI (PostCI), and during 10 min of CI (0-3 min: CIIP, the initial ascending pressor period; 7-10 min: CIMP, the maintaining plateau pressor period).

Cooling-Induced SBP and HR with Concomitant DN Changes

The changes in cooling-induced SBP and HR are shown in Fig. 3. After ganglionic blockade with the HEX treatment, cooling generally induced pressor (PreCI versus CIIP, $P < 0.001$) and tachycardia (PreCI versus CIIP and CIMP, all $P < 0.001$). However, after sympathetic denervation with the GUA treatment, cooling induced tachycardia at CIIP (PreCI and PostCI versus CIIP, all $P < 0.001$) but without SBP changes comparing with PreCI and PostCI. Compared with the control vehicle, HEX generally significantly increased HR but attenuated SBP in both the PreCI and PostCI stages ($P < 0.01$~0.001), but conversely, GUA generally caused no significantly changes but tended to attenuate both HR and SBP in both the PreCI and PostCI stages. However, during CI exposure, all HEX and GUA generally significantly attenuated SBP and HR at both CIIP and CIMP ($P < 0.001$).

The appearance of DN showed that when compared to the control vehicle during CI exposure, HEX caused DN virtually undetectable at both CIIP (0.03 ± 0.03% versus 3.94 ± 1.48%) and CIMP (0.0 ± 0.0% versus 0.64 ± 0.41%), but conversely, GUA generally increased the appearance of DN (CIIP: 55.73 ± 14.95% versus 3.94 ± 1.48%; CIMP: 33.63 ± 15.38% versus 0.64 ± 0.41%).

Cooling-Induced Spectral Power Changes

The global changes of spectral powers of VLF, LF and nLF in BPV and HRV are selectively shown in Fig. 4. During CI exposure, HEX and/or GUA tended to increase VLF_{BPV} (HEX: PreCI and PostCI versus CIIP, all $P < 0.05$, PreCI versus CIMP, $P = 0.112$; GUA: PreCI versus CIIP, $P = 0.219$, PreCI versus CIMP, $P = 0.188$) (Fig. 4A), LF_{BPV} (HEX: PreCI and PostCI versus CIIP and CIMP, all $P < 0.05$; GUA: PreCI and PostCI versus CIIP, $P < 0.05$) (Fig. 4B), nLF_{BPV} (GUA: PreCI and PostCI versus CIIP and CIMP, $P < 0.05$~0.01) (Fig. 4C) and nLF_{HRV} (HEX: PostCI versus CIMP, $P < 0.05$) (Fig. 4C), and TP_{BPV} (HEX: PreCI–7.59 ± 3.15 and PostCI–8.09 ± 2.0 versus CIIP–28.63 ± 9.94, all $P < 0.01$; GUA: PreCI–3.66 ± 0.81 *vs.* CIIP–31.22 ± 15.7, $P < 0.05$), whereas both HEX and GUA tended to decrease VLF_{HRV} (HEX: PostCI versus CIIP and CIMP, all $P < 0.05$; GUA: PreCI and PostCI versus CIIP, $P = 0.615$~0.688) (Fig. 4A).

Compared to the control vehicle prior to CI initiation (PreCI), HEX significantly attenuated VLF_{HRV} (Fig. 4A; $P < 0.01$), nLF_{BPV} and nLF_{HRV} (Fig. 4C; all $P < 0.05$), LF/HF_{HRV} (0.04 ± 0.01 versus 0.19 ± 0.03, $P < 0.05$), and TP_{HRV} (20.35 ± 8.37 versus 40.25 ± 7.44, $P < 0.05$), whereas GUA significantly attenuated VLF_{BPV} (Fig. 4A; $P < 0.05$), LF_{BPV} (Fig. 4B; $P < 0.01$), nLF_{BPV} (Fig. 4C; $P < 0.01$), nLF_{HRV} (Fig. 4C; $P < 0.05$), and TP_{BPV} (3.66 ± 0.81 versus 6.76 ± 1.68, $P < 0.05$).

In contrast, when compared with the control vehicle during the CI exposure, HEX significantly attenuated VLF_{BPV} and VLF_{HRV} (CIIP: all $P < 0.05$ and CIMP: all $P < 0.01$) (Fig. 4A), LF_{BPV} (CIIP: $P <$

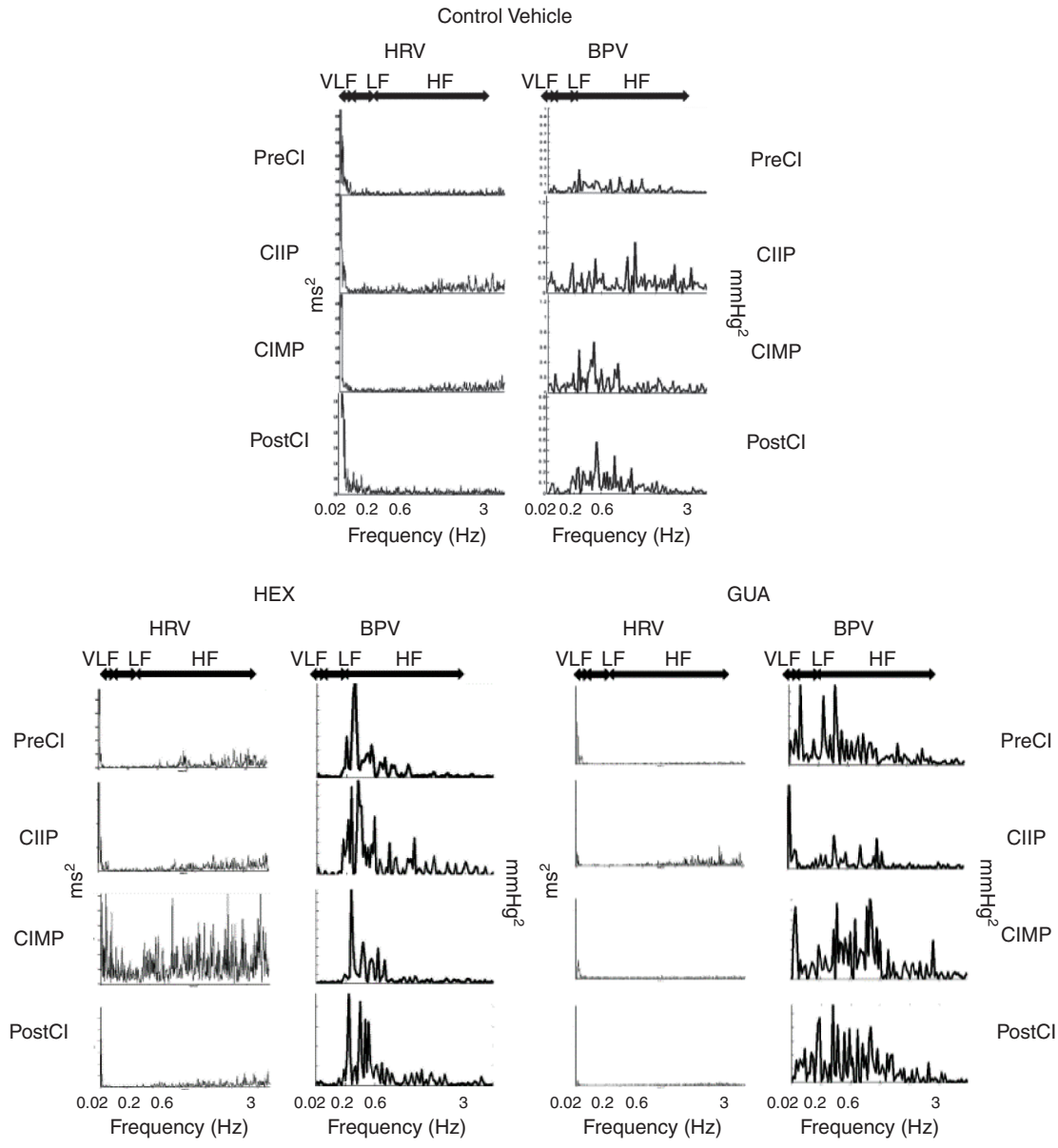

Fig. 2. A typical example of spectral power changes in rats treated with saline (Control Vehicle) or withdrawal of sympathetic influences by HEX or GUA before the cooling stimulation. Blood pressure variability (BPV) and heart rate variability (HRV) were assessed over a range of frequencies: very-low frequency (VLF: 0.02-0.2 Hz), low-frequency (LF: 0.2-0.6 Hz), high-frequency (HF: 0.6-3 Hz). Abbreviations used are as described in the legend to Fig. 1.

0.01) (Fig. 4B), LF/HF_{HRV} (CIMP: 0.08 ± 0.02 versus 0.23 ± 0.07, $P < 0.01$), and TP_{BPV} (CIIP: 28.63 ± 9.94 versus 146.82 ± 46.3, $P < 0.05$ and CIMP: 19.63 ± 20.19 versus 88.51 ± 35.18, $P < 0.05$), whereas GUA significantly increased TP_{HRV} (CIIP: 49.8 ± 8.33 versus 21.15 ± 4.43, $P < 0.05$ and CIMP: 45.64 ± 9.18 versus 25.33 ± 6.56, $P < 0.05$), attenuated VLF_{BPV} (CIIP: $P < 0.05$ and CIMP: $P < 0.05$) (Fig. 4A), LF_{BPV} (CIIP: $P < 0.01$ and CIMP: $P < 0.05$) (Fig. 4B), nLF_{HRV} (CIMP: $P < 0.01$) (Fig. 4C), LF/HF_{HRV} (CIMP: 0.10 ± 0.04 versus 0.23 ± 0.07, $P < 0.05$), and TP_{BPV} (CIIP: 31.22 ± 15.70 versus 146.82 ± 46.30, $P < 0.05$).

NO Levels

After 10 min of a CI trial, the significant elevation of plasma NO in the rats treated with the control vehicle was diminished compared with the vehicle-PreCI and also in the rats treated with either HEX or GUA (Fig. 5; $P < 0.05$).

Discussion

As expected, the pattern of hemodynamic changes under control conditions in the present study was con-

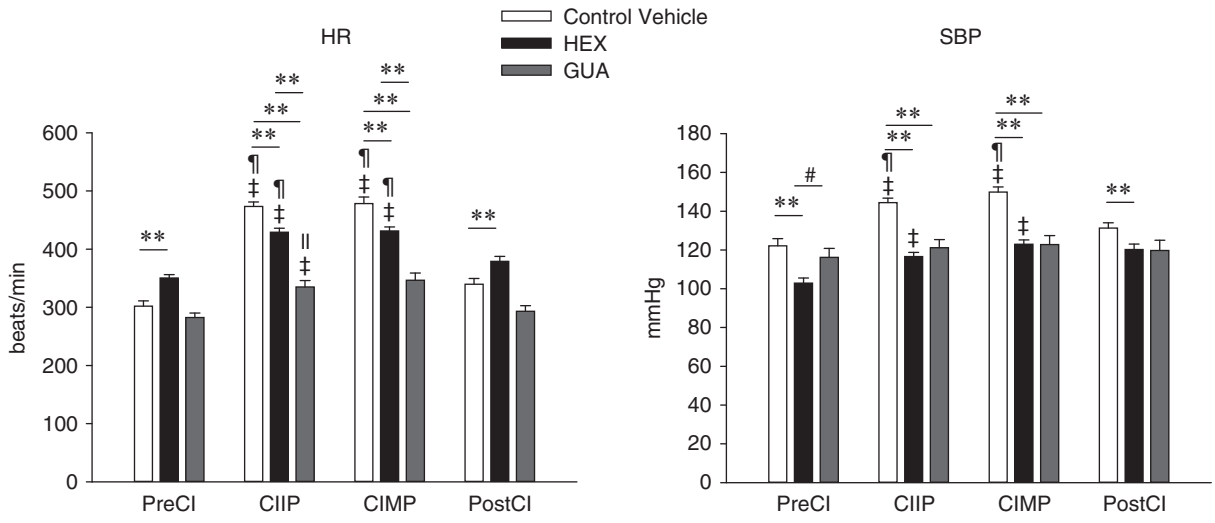

Fig. 3. The effects of stressful cooling on systolic blood pressure (SBP) and heart rate (HR) in rats treated with saline (Control Vehicle) (n = 12) or withdrawal of sympathetic influences by HEX (n = 12) or GUA (n = 12) before the cooling stimulation. Significant differences between PreCI and CI stages ($^{\ddagger}P < 0.001$) or between PostCI and CI stages ($^{\|}P < 0.01$, $^{\P}P < 0.001$) were evaluated by repeated measures two-way ANOVA and *post-hoc* Scheffe's test. Significant differences in rats between control vehicle and HEX or control vehicle and GUA (**$P < 0.01$~0.001) were evaluated by Student's *t*-test. Abbreviations used are as described in the legend to Fig. 1.

sistent to our previous findings (22). Stressful acute cooling significantly perturbed the hemodynamic pattern of the tested rats, as evidenced by pressor, tachycardia and concomitant reciprocal changes in spectral powers between frequencies of BPV and HRV, which contribute to the underlying mechanisms in the activation of sympathetic efferent influences and myogenic cardiovascular responsiveness (13, 17). Owing to the fact that the rhythmic oscillations of the LF band is referred as a marker for the status of sympathetic modulation of cardiovascular tonicity, whereas the VLF band is referred as an indicator of cardiovascular myogenic responsiveness to hemodynamic perturbations (13, 17, 19, 20), we will focus on such two bands in the following discussion.

The Effects of Neural Sympathetic Influences

In sympathetic modulation, the changes were examined after withdrawal of the neural sympathetic influences by HEX or GUA. Under treatment with the control vehicle, results showed that during CI exposure, the absolute value, "LF_{BPV}", markedly increased, whereas the relative unit, "nLF", both of nLF_{HRV} and nLF_{BPV}, were reduced. When compared with the control vehicle, results showed that both HEX and GUA significantly attenuated both SBP and HR throughout the CI trial. However, HEX remained the pressor and tachycardia to the cooling stimulation, whereas GUA abolished the pressor, attenuated but remained the tachycardia to the cooling stimulation. Both HEX and GUA significantly attenuated but remained the increasing tendency of LF_{BPV} to the cooling stimulation, and HEX and GUA both also attenuated nLF_{HRV} and LF/HF_{HRV} during the later cooling period (CIMP). On the other hand, when compared with the control vehicle throughout the experiment course, HEX did not cause much effective nLF_{BPV} changes; however, GUA reversed the downward tendency of nLF_{BPV} into an upward tendency. In general, these results are in keeping with the notion that sympathetic vasoconstrictor system can be involved in the cooling-induced pressor responses, and additional aspects of these results deserve further comments.

Firstly, it is to be noted that our discussion is based on the presumption that neural sympathetic influences on vasculature and the heart were completely eliminated in resting condition (PreCI) after HEX or GUA treatment. However, we have unexpectedly observed pressor and/or tachycardia, which still remained even after removal of the neural influences. During the cooling stimulation of the rats treated with ganglionic blockade (HEX), both pressor and tachycardia largely diminished but still existed. On the other hand, during the cooling stimulation of the rats treated with sympathetic denervation (GUA), both pressor and tachycardia were clearly reduced, and the pressor was totally abolished; however, tachycardia was reduced but still existed.

For these observed changes after HEX treatment, two possible mechanisms may be involved: (a) some

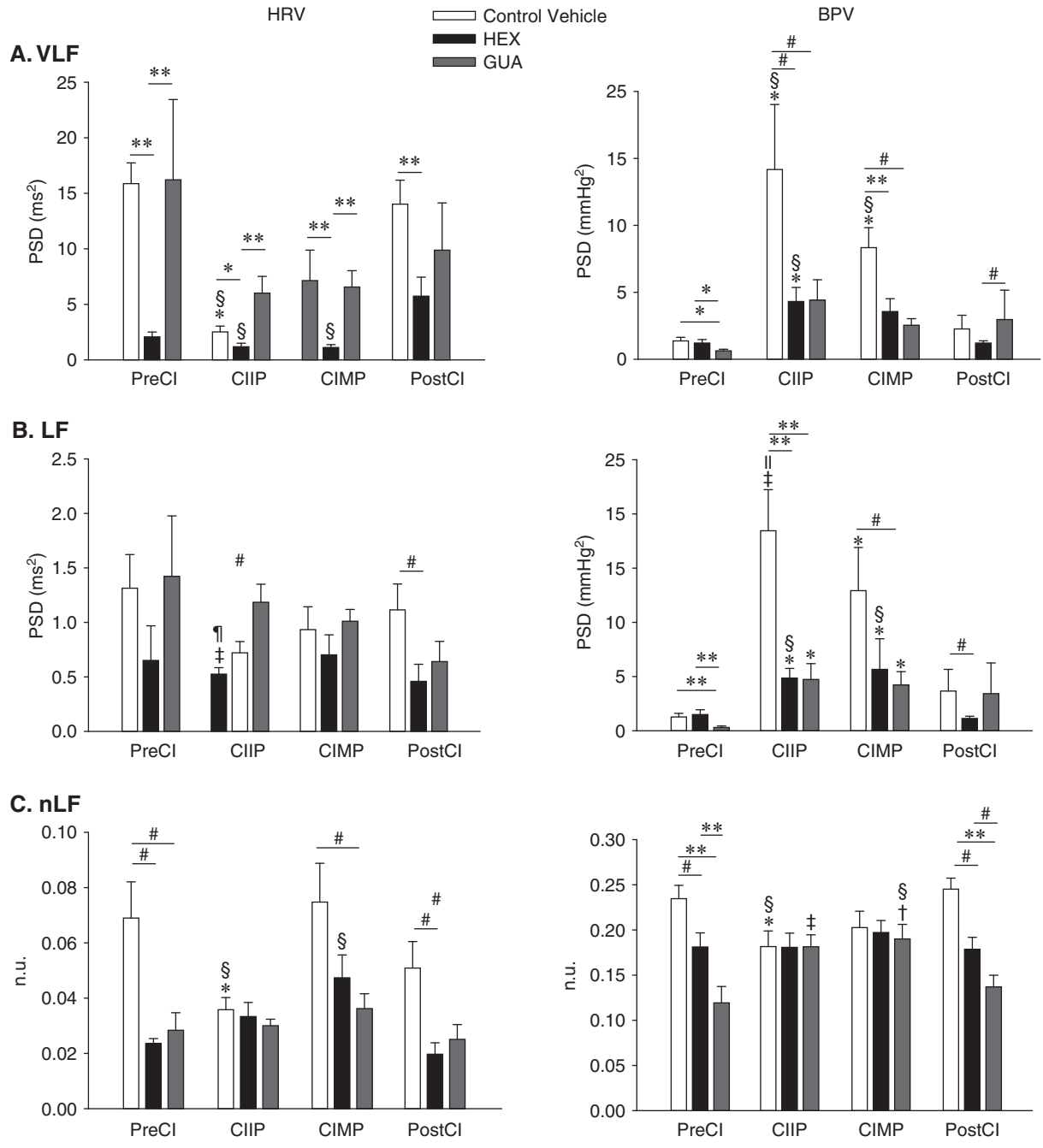

Fig. 4. The power spectral density (PSD) changes in (A) very-low frequency bands (VLF) and (B) low-frequency bands (LF), and (C) The relative unit (n.u.) changes in normalized low-frequency band (nLF) of blood pressure variability (BPV) or heart rate variability (HRV) of rats treated with saline (Control Vehicle) (n = 12) or withdrawal of sympathetic influences by HEX (n = 12) or GUA (n = 12) before the cooling experiments. Significant differences between PreCI and CI stages (*$P < 0.05$) or between PostCI and CI stages ($^{¶}P < 0.001$) were evaluated by repeated measures two-way ANOVA and *post-hoc* Scheffe's test. Significant differences in rats between control vehicle and HEX or control vehicle and GUA ($^{\#}P < 0.05$, **$P < 0.01$~0.001) were evaluated by Student's *t*-test.

unknown effects originated from the central nervous system which might have elicited and augmented the efferent sympathetic rhythmicity to vascular beds and the heart, and such central effects consequently overdrawn the resting blockade effects of HEX; (b) local cooling might have activated the cold-sensitive afferents to some downstream somatosympathetic relaying areas, such as the dorsal root ganglion, where they are not blocked after HEX treatment.

For the observed changes after GUA treatment,

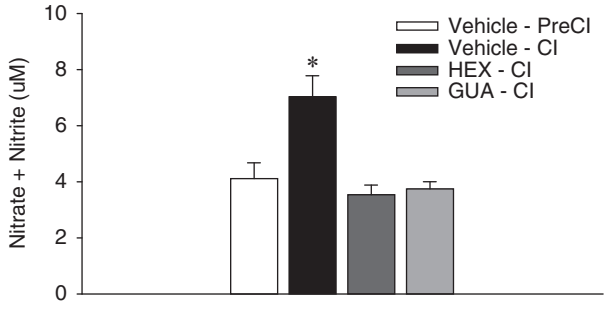

Fig. 5. Plasma nitric oxide (NO) levels of rats treated with saline (Control Vehicle) (n = 6) or withdrawal of sympathetic influences by HEX (n = 6) or GUA (n = 6) before the cooling experiments. Significant differences between treatments were evaluated by Student's *t*-test (*$P < 0.05$). See legend to Fig. 1 for abbreviations used.

a possible explanation is based on the pharmacological features of this drug, *i.e.* once GUA has entered the sympathetic terminals for sympathectomy, it blocks the release of norepinephrine in response to arrival of an action potential; however, spontaneous release is not affected. In addition, GUA is known to have spare effects on central noradrenergic neurons and adrenal medulla (1). Therefore, these two interesting hemodynamic changes observed during the cooling stimulation after GUA treatment can be explained as follows: (a) the pressor had totally abolished and tachycardia still existed but had been reduced, and (b) the downward tendency of nLF_{BPV} of control vehicle was reversed into an upward trend. The former possibility may be explained by the adrenal medulla-spared function of GUA that the remained tachycardia is possible because of the cooling-evoked releases of epinephrine from the adrenal medulla into the circulation. The latter possibility is further discussed in more details below:

The normalized LF (nLF) was used in this study as an index to illustrate the efferent sympathetic influences of either sympathetic vascular outflow (nLF_{BPV}) or sympathetic cardiac outflow (nLF_{HRV}), because this application accords with the principles of baroreflex mechanisms, *i.e.*, the firing rhythmicity is reasonable to be coherence between efferent sympathetic fibers to the targeted resistance vessels and to the heart (5, 10, 16). In other words, our using nLF_{BPV} rather than using LF_{BPV} is more appropriate to signify the compensatory baroreflex effects of sympathetic influences on vascular resistance. In addition, to denote the sympathetic influences on vascular bed, nLF_{BPV} is reported to be specific without the influence of HF_{BPV} (24). As a consequence, we attribute that during the cooling stimulation after GUA treatment, the downward tendency of nLF_{BPV} reversed into an upward tendency is due to an electrical/chemical dissociation phenomenon, *i.e.*, the efferent sympathetic fibers are still intact, action potential still appears, but the efferent sympathetic fibers lose the baroreflex function to compensate the acute stressful cooling, a phenomenon analogous to an engine idling.

The Effects of Cardiovascular Myogenic Responsiveness

In cardiovascular myogenic responsiveness to the cooling stimulation, power change of VLF bends between BPV and HRV was studied after withdrawal of sympathetic influences by HEX or GUA. There was an immediate increase of VLF_{BPV} but a decrease of VLF_{HRV} during treatment with the control vehicle; however, when HEX or GUA was applied compared with the control vehicle treatment, the VLF_{BPV} bands were generally attenuated but remained an increasing tendency to the cooling stimulation. On the other hand, when compared with control vehicle to the cooling stimulation, HEX attenuated VLF_{HRV} throughout the experimental course, but GUA generally did not change much, on the contrary, by a rising propensity in CIIP.

An association between VLF_{BPV} band and vascular myogenic responsiveness has been reported previously (13, 17, 19, 20). The reports suggested that because sympathetic activation and blood flow fluctuations could lead to dynamic BP fluctuations as increase of VLF_{BPV} spectral power, and this increase of VLF_{BPV} spectral power could be due to the increase of vascular myogenic responsiveness. In addition, it is well known that the ongoing blood flow and BP perturbations could produce mechanical shear stress and enhance endothelial releases of NO (19), this secondary humoral substance could then antagonize the initial rising of BP to protect endothelial cells from hemodynamic injuries (8, 15).

Consequently, as for the aforementioned attenuation of VLF_{BPV} after either HEX or GUA treatment, we believe that the attenuation is a consequence of the deterioration of the cooling-evoked sympathetic activation on resistance vessels. As for the still existing rising propensity of VLF_{BPV} to the cooling stimulation, our explanation is that even if the neural sympathetic influences on vascular beds were completely eliminated, there might still be some unknown humoral substances present to enhance the rhythmic oscillation in vasculature.

Nevertheless, our data further showed that, as compared with control vehicle treatment, during the cooling stimulation, both sympathetic withdrawal treatments have concurrently attenuated the spectral powers of LF_{BPV} and VLF_{BPV}; in addition, after HEX or GUA treatment, the effects on NO production were abolished. Since the aforementioned sympathetic activation could

enhance VLF_{BPV} through the shear stress-dependent NO production, our findings are in line with the previous assumption that the spectral power changes in VLF_{BPV} band during stressful cooling are highly relevant to the sympathetic activation and subsequent NO production (13, 17, 19, 20).

Finally, we observed an attenuation of VLF_{HRV} band to the cooling stimulation only after HEX treatment, whereas VLF_{HRV} band was not much changed but slightly increased at CIIP after GUA treatment. To the effects of ganglionic blocker HEX effects, our explanation is because both sympathetic and parasympathetic influences on the cardiac muscles were eliminated. However, to the effect of chemical sympathectomy GUA effects, we speculate here that it was because the adrenal medulla-spared function again, the expedited circulatory epinephrine and β-adrenoceptor activation might have enhanced the rhythmic oscillation in the myocardiac functions. Furthermore, our data proved that DN was virtually undetectable after ganglionic blocker HEX treatment, but DN was apparently noticeable after chemical sympathectomy GUA treatment. Taken together, our findings support that autonomic balance and humoral substance were both involved in the cardiac effects as for the spectral changes of VLF_{HRV} band during the cooling stimulation.

Conclusions

The use of power spectrum analysis has enabled applications in assessing stressful cooling-induced hemodynamic perturbations. The present study has demonstrated that the spectral changes during cooling stimulation are highly relevant to the efferent sympathetic rhythmicity and subsequent NO production.

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