Evaluation of Autonomic Nervous System Function Using Heart Rate Variability Analysis During Transient Heart Rate Reduction Caused by Acupuncture

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ABSTRACT

Objective: Human studies have demonstrated that heart rate (HR) decreases during acupuncture stimulation, and pharmacologic studies have shown that this autonomic nervous system (ANS) response is parasympathetic-dominant. It has become clear that significant changes occur in the ANS after acupuncture, based on HR variability (HRV). However, it is inconclusive, according to HRV analysis, if acupuncture induces a significant change in autonomic function during stimulation. The aim of this study was to investigate ANS function using HRV analysis during HR reduction induced by manual acupuncture stimulation to the muscles.

Materials and Methods: In this study, electrocardiograms of 25 adult men were analyzed. After resting for 20 minutes, participants underwent 15–20-mm deep acupuncture stimulation at the *Shousanli* (LI 10) point at 1 Hz for 2 minutes. Instantaneous HR was recorded. The index of parasympathetic nervous activity high-frequency (HF) normalized units (HFnu) and the ratio of sympathovagal balance (low frequency [LF]/HF) were calculated by HRV analysis.

Results: HR during acupuncture was significantly lower, compared to HR both before and after acupuncture. HFnu during acupuncture were significantly higher, compared to HFnu both before and after acupuncture. The LF/HF ratio during acupuncture was significantly lower, compared to the ratio before acupuncture, and remained low after acupuncture, compared to before acupuncture.

Conclusions: Acupuncture stimulation to the muscle can effectively reduce HR, increase HFnu, and decrease LF/HF that depends on autonomic regulation of both sympathovagal balances.

Keywords: acupuncture, heart rate, autonomic nervous system function, heart rate variability, nociceptive stimulus, human

INTRODUCTION

A TRANSIENT DECREASE IN HEART RATE (HR) during acupuncture has been reported.^{1–4} Pharmacologic studies have demonstrated clearly that HR reduction is due to the effect of acupuncture through inhibition of the sympathetic nervous system,² activation of the parasympathetic nervous system,^{1,4} or both.³

Using HR variability (HRV) analysis, the clinical effects that acupuncture induces in the autonomic nervous system (ANS) have been demonstrated. Changes after acupuncture, compared to before acupuncture, have been verified. Reviews have been conducted based on these studies.^{5,6} It has become clear that significant changes occur in the ANS after acupuncture.⁵ Furthermore, several studies investigating changes in ANS function have reported changes during

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acupuncture stimulation.^{7–10} However, whether or not acupuncture induces a significant change in autonomic function during stimulation remains inconclusive.⁶

Previous systematic reviews and meta-analyses of the literature on studies using functional magnetic resonance imaging (fMRI)¹¹ revealed that most of these studies indicate that acupuncture can modulate the activity within specific areas of the brain. However, these studies on acupuncture neuroimaging are very heterogeneous in terms of study hypotheses, methodologies, and quality. It is clear from the descriptive view on the data that verum acupuncture tends to be associated with greater activation in the basal ganglia, brainstem, cerebellum, and insula, and seems to be associated with greater deactivation in the so-called “default mode network” and limbic brain areas, such as the amygdala and the hippocampus. Regarding the mechanism of the effect of acupuncture stimulation on autonomic nerves’ function, Napadow et al.¹² demonstrated a correlation between the activity in different regions of the brainstem (detected by fMRI) and the indices of HRV analysis during electroacupuncture (EA) stimulation. Beissner et al.¹³ also suggested that “acupuncture stimulation is a nociceptive stimulus,” based on their results from human fMRI. These results suggest that there is a response in which the HR decreases transiently and the ANS becomes parasympathetic-dominant induced by acupuncture stimulation to nociceptors of both skin and muscle in humans.

The aim of this study was to confirm the presence of this parasympathetic-dominant state using HRV analysis during HR reduction during deep-muscle acupuncture stimulation.

MATERIALS AND METHODS

Subjects

This study included 25 healthy male volunteers with no medication, ages 21.0 ± 0.98 years (mean $\pm$ standard deviation [SD]). The study was carried out with the approval of the ethics committee of Teikyo Heisei University, Tokyo, Japan (Approval No. 27-093).

Method of Acupuncture Stimulation

The site of acupuncture stimulation was the *Shousanli* (LI 10) point on the left forearm (situated above the radial nerve between the extensor carpi radialis longus and the extensor carpi radialis brevis on the radial side). The acupuncture stimulation was applied by the sparrow pecking method; that is, the acupuncture needle was inserted and moved vertically at the frequency of 1 Hz at a depth of 15–20 mm for 2 minutes. No. 18 stainless steel needles (40 mm $\times$ 0.18 mm, SEIRIN Co., Japan) were used. One acupuncturist carried out stimulations in all subjects.

Experimental Protocol

Three electrodes for electrocardiogram (ECG) measurement were fixed on the chest and abdomen, and measurements commenced after specifying the site of acupuncture stimulation. After resting for 20 minutes, each subject was confirmed to be in a resting state and underwent acupuncture stimulation for 2 minutes. Measurements ended 15 minutes after the end of the stimulation. The experiment was carried out while each subject was in a semireclining position at an angle of 120° for resting. The measurements were taken during natural spontaneous breathing, and were performed between 11:00 AM and 16:00 PM.

Analysis of ECG Measurements

For each subject, disposable Ag-AgCl electrodes were attached to the right subclavian area (–), on the left side of the chest (+), and on the abdomen (Earth). A 60-Hz low-pass filter and a time constant of 0.3 seconds were used. After amplification with a bio-amplifier (Model ML132, AD Instruments, Australia), the readings were fed into a computer using an analogue-to-digital converter (Power Lab AD Instruments, Australia). The sampling rate was 1 k/s. The ECG R waves were simultaneously detected by software (Lab Chart™ 5.4.2, AD Instruments, Australia), and the instantaneous HR was calculated based on the R–R interval. Analysis was performed at 4–2 minutes before acupuncture, 2 minutes before acupuncture, immediately after the start of acupuncture, immediately after acupuncture completion, and 2 minutes after acupuncture completion. The mean of the HR values at 4–2 minutes and 2 minutes before starting acupuncture was considered the baseline of before acupuncture. The HR value after the initiation of acupuncture was considered the value during acupuncture, and the mean of the HR values immediately after completion and 2 minutes after completion of acupuncture was considered the HR value after acupuncture. Furthermore, during the 4-minute period prior to acupuncture stimulation, the mean values of the instantaneous HR/minutes were also calculated.

In HRV analysis (Lab Chart™ 8.0.4, AD Instruments, Australia), the power spectrum was obtained using the fast Fourier transform. The frequency band analyzed was 0.04–0.50 Hz; 0.04–0.15 Hz power was defined as the low-frequency (LF) component, and 0.15–0.50 Hz power was defined as the high-frequency (HF) component. The ratios of LF and HF to the total power values were defined as normalized units of LF (LFnu) and normalized units of HF (HFnu). Values before acupuncture, during acupuncture, and after acupuncture were calculated for LFnu, HFnu, and the LF/HF ratio. The HFnu was used as the index for vagal activity and the LF/HF ratio was used as the index of sympathovagal balance. The HRV indices were calculated in the same sampling time as HR simultaneously.

Statistical Analysis

To confirm the stability of HR before acupuncture, a one-way repeated-measures analysis of variance (ANOVA) was carried out on values obtained every minute. One-way repeated-measures ANOVA was carried out on the values of indices before, during, and after acupuncture. Multiple comparisons were carried out using Tukey's method in case there were significant differences. Correlations between HR and HRV indices were determined using Spearman's rank test. JSTAT statistical analysis software (Japan) was used. A P -value of <0.05 was considered significant. All data were expressed as means $\pm$ standard deviation.

RESULTS

Changes in HR

There was a transient decrease in HR during acupuncture (Figs. 1A and 2A). No significant differences were observed in HR values measured every minute before acupuncture (P =not significant [NS]; one-way repeated-measures ANOVA; Fig. 2A). The mean HR decreased from 58.3 ± 5.97 beats per minute (bpm) before acupuncture to 55.4 ± 6.93 bpm during acupuncture, and recovered to 58.0 ± 6.29 bpm

after acupuncture. This change was significant ($P < 0.0001$; one-way repeated-measures ANOVA). There were significant differences between the HR values before and during acupuncture ($P < 0.01$) and between the HR values during and after acupuncture ($P < 0.01$). There was no significant difference between the HR values before and after acupuncture. This confirmed the transient decrease in HR during acupuncture (Fig. 2B).

Changes in the Power Spectrum as Determined by HRV Analysis

The power spectrum measured every 2 minutes by HRV analysis indicated that HF power increased during acupuncture and decreased after acupuncture (Fig. 1B).

The value of LFnu decreased from $46.7 \pm 16.82\%$ before acupuncture to $26.1 \pm 16.16\%$ during acupuncture, and increased to $37.4 \pm 13.74\%$ after acupuncture. A significant change was noted in LFnu as a result of acupuncture ($P < 0.0001$; one-way repeated-measures ANOVA). There were significant differences between the values before and during acupuncture ($P < 0.01$), and during and after acupuncture ($P < 0.01$), but there was no significant difference between the values before and after acupuncture. Therefore, a transient decrease in LFnu during acupuncture was confirmed (Fig. 3).

FIG. 1. Changes in the heart rate (HR) and HR variability (HRV) power spectrum by acupuncture stimulation (typical case). (A) Original waveform. (B) HRV power spectrum (analytical interval: 2 minutes). (A) HR decreased during acupuncture and recovered after acupuncture was completed. (B) In the HRV power spectrum, high-frequency power increased during acupuncture and decreased after acupuncture. min, minutes; acp, acupuncture; LF, low-frequency components; HF, high-frequency components.

FIG. 2. Changes in heart rate (HR) induced by acupuncture stimulation. **(A)** Average changes in HR measured every minute. **(B)** Changes in the average HR before, during, and after acupuncture. **(A)** The average values for HR measured every minute before acupuncture were stable, with no significant differences noted ($P=0.1323$). **(B)** HR decreased during acupuncture and returned to baseline level after acupuncture ($P<0.0001$; one-way repeated-measures analysis of variance). **(B)** Before acupuncture versus during acupuncture: $P<0.01$; during acupuncture versus after acupuncture: $P<0.01$; before acupuncture versus after acupuncture: P =not significant. min, minutes; NS, not significant; SD, standard deviation; acp, acupuncture; ANOVA, analysis of variance.

The HFnu value increased from $53.7 \pm 16.02\%$ before acupuncture to $69.5 \pm 15.43\%$ during acupuncture, and decreased to $60.6 \pm 13.36\%$ after acupuncture. A significant change was noted in HFnu as a result of acupuncture ($P<0.0001$; one-way repeated-measures ANOVA). There were significant differences between the values before and during acupuncture ($P<0.01$) and during and after acupuncture ($P<0.05$), but there was no significant difference between the values before and after acupuncture. Therefore, a transient increase in HFnu during acupuncture was confirmed (Fig. 4).

The value of the LF/HF ratio decreased from 1.11 ± 0.777 before acupuncture to 0.47 ± 0.488 during acupuncture, and increased to 0.75 ± 0.467 after acupuncture. A significant change was noted in the LF/HF ratio as a result of acupuncture ($P<0.0001$; one-way repeated-measures ANOVA). There was a significant difference between the LF/HF ratios before and during acupuncture ($P<0.01$), but there was no significant difference between the ratios during and after acupuncture. There was a significant difference between the

LF/HF ratios before and after acupuncture ($P<0.05$). The LF/HF ratio decreased during acupuncture and increased after completion of acupuncture. However, it was clear that, for up to 4 minutes after acupuncture was completed, the LF/HF ratio did not return to baseline (Fig. 5).

Correlation Between HR and HRV Indices

There was no significant correlation between the change in HR and LFnu due to acupuncture ($r_s=-0.18$; $P=0.37$). There was also no significant correlation between the change in HR and HFnu ($r_s=0.22$; $P=0.37$). Furthermore, there was no significant correlation between the change in HR and change in the LF/HF ratio ($r_s=-0.079$; $P=0.69$).

DISCUSSION

LI 10 was selected as the point for acupuncture based on a previous study that demonstrated that the application of

FIG. 3. Changes in normalized units of low-frequency (LFnu) components induced by acupuncture stimulation. The value of LFnu decreased during acupuncture and increased to baseline level after acupuncture was completed ($P < 0.0001$; one-way repeated-measures analysis of variance). Before acupuncture versus during acupuncture: $P < 0.01$; during acupuncture versus after acupuncture: $P < 0.01$; before acupuncture versus after acupuncture: $P =$ not significant. NS, not significant; ANOVA, analysis of variance; acp, acupuncture.

FIG. 4. Changes normalized units of high-frequency (HFnu) components induced by acupuncture stimulation. The value of HFnu increased during acupuncture and decreased to baseline level after acupuncture was completed ($P < 0.0001$; one-way repeated-measures analysis of variance). Before acupuncture versus during acupuncture: $P < 0.01$; during acupuncture versus after acupuncture: $P < 0.05$; before acupuncture versus after acupuncture: $P =$ not significant. NS, not significant; ANOVA, analysis of variance; acp, acupuncture.

FIG. 5. Changes in low-frequency/high-frequency (LF/HF) components induced by acupuncture stimulation. Compared to the baseline level, the LF/HF ratio decreased during acupuncture and remained low after acupuncture was completed ($P < 0.0001$; one-way repeated-measures analysis of variance). Before acupuncture versus during acupuncture: $P < 0.01$; during acupuncture versus after acupuncture: $P = \text{not significant}$; before acupuncture versus after acupuncture: $P < 0.05$. NS, not significant; ANOVA, analysis of variance; acp, acupuncture.

acupuncture reduced HR.² The present study also demonstrated that HR decreased significantly as a result of applying acupuncture stimulation to LI 10. Measurement over a minimum of 5 minutes is considered desirable in order to obtain adequate reliability in HRV analysis. However, in the present study, acupuncture was performed for 2 minutes to avoid overstimulation with the sparrow pecking method. Consequently, HRV analysis was carried out at 2-minute intervals. Although this might not have been an adequate sample size, the data were analyzed, and the results of statistical analysis are provided in Figures 1–5.

The changes in the indices of autonomic nervous function measured by HRV analysis confirmed the presence of a relative parasympathetic-dominant state during acupuncture. As suggested by Beissner et al.,¹³ who based their conclusion on the premise that “acupuncture stimulation is a nociceptive stimulus” (using the results of human fMRI), the current authors believe that the mechanism of autonomic reaction during acupuncture is probably best understood as a response to nociceptive stimuli. Previous studies on animals have indicated that nociceptive deep-muscle stimulation has caused reactions that included a decrease in HR and a change in the ANS to a parasympathetic-dominant state. These reactions have also occurred in decerebrated animals.¹⁴ The ventrolateral periaqueductal gray and hypothalamus contributed to these reactions.^{14–17}

In a human study, Napadow et al.¹² reported a correlation between the activity in different regions of the brainstem (detected by fMRI) and the indices of HRV analysis during

EA stimulation. Napadow et al.’s data suggested that fMRI activity in the hypothalamus, dorsal raphe nucleus, periaqueductal gray, and rostroventral medulla indicated a significant correlation with LF/HF ratio calculated from simultaneous HRV data. The acupuncture in this study might have been perceived mainly as noxious deep-muscle stimulation. Similar to the results of previous animal studies, the current authors determined that HR transiently decreased and that autonomic nervous function exhibited parasympathetic dominance during acupuncture stimulation; this might be the result of a reflex response of the hypothalamus and brainstem.

Moreover, up to 4 minutes after the completion of acupuncture, the LF/HF ratio had not increased to baseline. This suggests that, although the acupuncture-related changes in ANS function might be mainly due to reflexes and that additional mechanisms could also exist.

Pharmacologic studies have determined that HR reduction is due to the relative parasympathetic-system dominance that occurs as a result of acupuncture stimulation.^{1–4} However, the current study results indicated that there was no correlation between the degree of change in the HR and the HRV indices. HRV analysis was applied as an indirect measurement technique to evaluate autonomic function based on fluctuations in the heartbeat. First, the current authors considered that the correlation between the change of HR and HRV indices might be observed. That did not occur. The tonus of autonomic activities might differ in each human being. Therefore, there is the limitation of HRV analysis as an indirect measurement

of the state of autonomous function to analyze the correlation between HR and HRV indices. The current authors are considering a study that will be done with the same subjects. These subjects, with the same autonomic tone, might enable a correlation between HR reduction and HRV indices to be obtained in the future.

CONCLUSIONS

HRV analysis indicated that HFnu was transiently significantly increased and that the LF/HF ratio was significantly decreased with acupuncture. As a result, a transient reduction in HR and a parasympathetic-dominant state during acupuncture were confirmed using HRV analysis. There was no correlation between the HR and HRV indices. The current authors suggest that the primary mechanism for the transient decrease in HR during acupuncture is an autonomic reflex.

AUTHOR DISCLOSURE STATEMENT

No competing financial interests exist.

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