J Physiol Sci (2010) 60:221–225
DOI 10.1007/s12576-009-0084-0

SHORT COMMUNICATION

Sympatho-inhibitory response of the heart as a result of short-term acupuncture-like stimulation of the rat hindlimb is not augmented when sympathetic tone is high as a result of hypercapnia

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Received: 9 October 2009 / Accepted: 29 December 2009 / Published online: 20 January 2010

Abstract This study examined whether the sympatho-inhibitory response of the heart to acupuncture-like stimulation of a hindlimb in anesthetized rats depends on the sympathetic tone. Observed reductions in both cardiac sympathetic nerve activity and heart rate following short-term acupuncture-like stimulation of a hindlimb were not augmented when the sympathetic tone was high as a result of hypercapnia.

Keywords Sympathetic tone · Cardiac sympathetic nerve · Acupuncture

Introduction

Acupuncture has been used clinically for treatment of some cardiac dysfunctions including cardiac arrhythmias such as supraventricular tachycardia [1–5]. There have been studies reporting that acupuncture causes a reduction of heart rate in healthy human subjects [6–10]. By recording cardiac sympathetic efferent nerve activity in anesthetized rats, we have recently demonstrated that reductions in heart rate in response to acupuncture-like stimulation are the result of reduced sympathetic outflow to the heart, but not increased parasympathetic vagal outflow [11, 12]. Simultaneous depressor response induced by acupuncture-like stimulation has clarified that the induced bradycardia could not be a secondary effect of pressor response (i.e., baroreflex) [11]. Cardiovascular depression has also been reported in unanesthetized spontaneous hypertensive rats (SHR) in response to inhibited sympathetic vasoconstrictor activity, as induced by acupuncture-like stimulation of the sciatic nerve [13, 14]. In these studies, the magnitude of the depressor response was found to be greater in SHR than in normotensive controls (Wistar Kyoto rats; WKY). Sympathetic tone is known to be higher in SHR or stroke-prone SHR than in WKY [15, 16]. Thus, it is likely that the magnitude of sympatho-inhibitory response to acupuncture-like stimulation depends upon pre-existing sympathetic tone. Hypercapnia is known to increase cardiac sympathetic nerve activity [17]. In this study, we experimentally produced an increase in basal activity of the cardiac sympathetic nerve by hypercapnia, and examined how the sympatho-inhibitory response of the heart to acupuncture-like stimulation of a hindlimb is modulated by sympathetic tone.

Materials and methods

All experiments were performed on a total of 11 adult male Wistar rats (body weight 320–440 g). All animal experiments were conducted with the approval of, and in accordance with, the Guidelines for Animal Experimentation prepared by the Animal Care and Use Committee of the Tokyo Metropolitan Institute of Gerontology. Of the 11 experimental rats, 6 were used to record cardiac sympathetic nerve activity, and the remaining 5 were used to record heart rate. Rats were anesthetized initially with pentobarbital (50 mg/kg, i.p.), and maintained using a continuous infusion of pentobarbital in saline (8 mg/h, i.v.), as described previously [12]. General experimental conditions, including methodology for recording cardiac sympathetic nerve activity and heart rate, and methodology

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for acupuncture-like stimulation of a hindlimb have been described previously [12]. In order to avoid vagal contamination during nerve activity recording from the cardiac sympathetic nerve, we ensured that the vagal nerves were cut at the cervical level. Gallamine triethiodide (20 mg/kg, i.v.) was also used during nerve recording to help stabilize nerve activity recordings. However, during heart rate recording, we neither cut the vagal nerve nor injected gallamine. In 2 of 5 rats used to record heart rate, after taking the heart rate responses to acupuncture-like stimulation under each condition, vagal nerves were cut bilaterally at the cervical level to examine the vagal nerve contribution under the hypercapnic condition $(\text{at } 9.0\%\, F_{\mathrm{ET_{CO_2}}})$. Animals were artificially ventilated (model 683, Harvard, MA, USA) with room air. End-tidal (i.e., approximately mean alveolar) CO_2 concentrations $\left[F_{\mathrm{ET_{CO_2}}}(\%)\right]$ were measured with a gas monitor (Microcap, Oridion Medical, Jerusalem, Israel). Under control conditions, $F_{\mathrm{ET_{CO_2}}}$ was held at 3.0% by controlling respiratory rate (60–80 cycles/min) and tidal volume (2.6–3.0 ml). Hypercapnia was induced by adjusting the concentration of CO_2 in the gas mixture which contained 21% O_2 in N_2 and flowed into the input line of the respirator as described previously [17]. $F_{\mathrm{ET_{CO_2}}}$ was increased to 5.0, 7.0, and 9.0%, usually in this order. After changing the level of $F_{\mathrm{ET_{CO_2}}}$, we waited for more than 20 min until the basal level of both cardiac sympathetic nerve activity and heart rate had became stable. At this point, we delivered acupuncture-like stimulation to a hindlimb for 1 min. The stimulation point was within an area of 1 cm^2 about 5 mm below and lateral to the anterior tubercle of the tibia of a hindlimb. This area seems to include an area corresponding to the zsusanli point (ST36) in humans. Each stimulation was applied three times under each hypercapnic condition. We usually waited more than 5 min between each stimulation. For each rat, results from 3 trials were averaged.

Data were expressed as the mean ± standard error of the mean (SEM). Statistical comparisons were carried out using repeated-measures ANOVA followed by Dunnett's multiple comparison test or the paired *t*-test. A *p* value of <0.05 was considered to be statistically significant.

Results

Cardiac sympathetic nerve activity

The basal spontaneous efferent activity of the cardiac sympathetic nerve under control conditions $(F_{\mathrm{ET_{CO_2}}}3.0\%)$ was 420 ± 71 impulses/5 s. Basal cardiac sympathetic efferent nerve activity increased in proportion to the increase in $F_{\mathrm{ET_{CO_2}}}$ from control conditions (3.0%) to 5.0%, 7.0%, and finally to 9.0% (Fig. 1b). At 5.0% $F_{\mathrm{ET_{CO_2}}}$, the basal activity

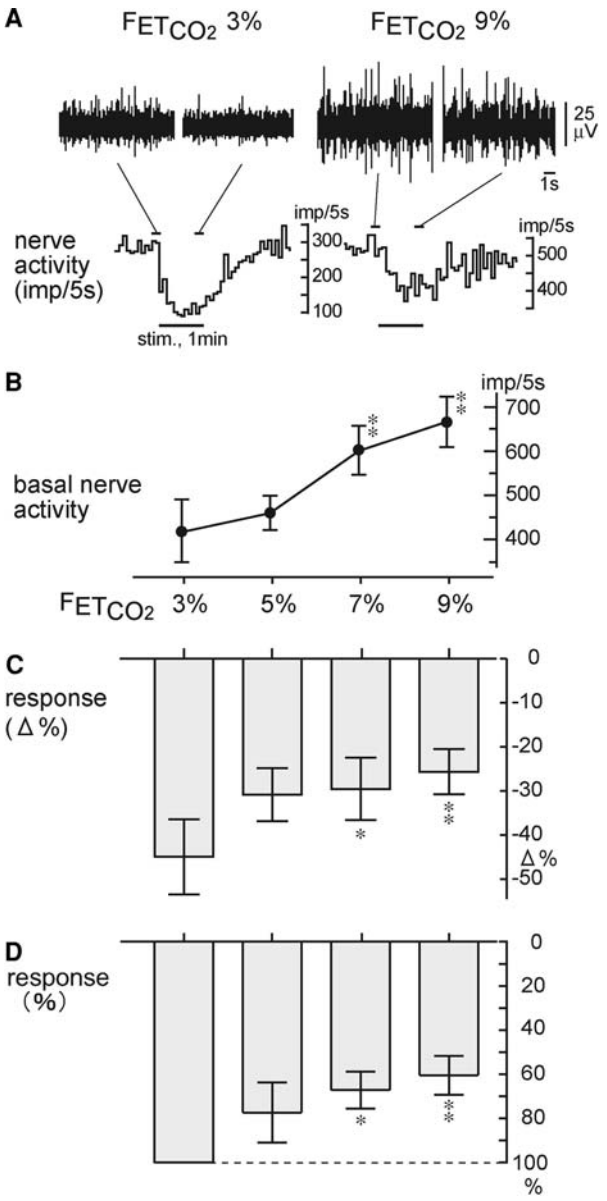

Fig. 1 Cardiac sympathetic nerve activity changes elicited by acupuncture-like stimulation of a hindlimb during hypercapnia. **a** *Upper traces* sample recordings of cardiac sympathetic nerve activity. *Lower traces* recordings of nerve activity expressed as a histogram of impulses per 5 s. Acupuncture-like stimulation was applied for 1 min as indicated by the *bar* below each trace. **b** Summary of basal nerve activity during hypercapnia. **c**, **d** Summary of responses of cardiac sympathetic nerve activity. **c** Changes in nerve activity in the 10-s period just before the end of the acupuncture-like stimulation were expressed as a percentage of the pre-stimulus basal values for the 10-s period. **d** The cardiac sympathetic nerve responses under control conditions $(F_{\mathrm{ET_{CO_2}}}3.0\%)$ were taken as 100%. Each *point* or *column* and *vertical bar* depicts the mean ± SEM for 6 rats. For each rat, the results of 3 trials were averaged. $^{*}p < 0.05$, $^{**}p < 0.01$; significantly different from the magnitudes of the responses under control conditions $(F_{\mathrm{ET_{CO_2}}}3.0\%)$ using one-way repeated measures ANOVA followed by Dunnett's multiple comparison test. The background noise level of nerve recording was about 10 μV when checked at the end of the experiment (after the rat had been killed)

increased slightly relative to controls but the difference was not statistically significant. At 7.0 and 9.0% $F_{ET_{CO_2}}$, the basal activity increased significantly reaching 603 ± 55 and 667 ± 57 impulses/5 s, respectively (Fig. 1b).

Under control conditions ($F_{ET_{CO_2}}$3.0%) cardiac sympathetic nerve activity decreased by 45 ± 9% of pre-stimulus value following acupuncture-like stimulation of a hindlimb. The magnitude of decrease in cardiac sympathetic nerve activity by acupuncture-like stimulation was less pronounced when cardiac sympathetic nerve activity was high as a result of hypercapnia ($F_{ET_{CO_2}}$5.0 − 9.0%, Fig. 1a–d). At 5.0% $F_{ET_{CO_2}}$, the sympatho-inhibitory response was slightly smaller than responses under control conditions but the difference was not statistically significant. At 7.0 and 9.0% $F_{ET_{CO_2}}$, the magnitude of sympatho-inhibitory response was 30 ± 7% and 26 ± 5% (67 ± 8% and 60 ± 9% of the control response), respectively, and were significantly smaller when compared with the control responses (Fig. 1c, d).

In addition to normalization to the pre-stimulus value, cardiac sympathetic nerve activity was normalized as a percentage of the basal value (maximum value) at 9.0% $F_{ET_{CO_2}}$ for the entire experiment. In such a calculation, the decrease in cardiac sympathetic nerve activity as a result of acupuncture-like stimulation at 3.0% $F_{ET_{CO_2}}$ was 28 ± 7%, which was comparable with that at 9.0% $F_{ET_{CO_2}}$.

Heart rate

The basal heart rate under control conditions ($F_{ET_{CO_2}}$3.0%) was 383 ± 9 beats/min and did not change significantly when $F_{ET_{CO_2}}$ was increased to 5.0, 7.0, and 9.0% (Fig. 2b).

Under control conditions ($F_{ET_{CO_2}}$3.0%) heart rate decreased by 34 ± 5 beats/min following acupuncture-like stimulation of a hindlimb. The magnitude of the reduction in heart rate as a result of acupuncture-like stimulation was less pronounced in proportion to the increase in $F_{ET_{CO_2}}$ from the control conditions (3.0%) to 5.0%, 7.0%, and 9.0% (Fig. 2a–d). At 5.0% $F_{ET_{CO_2}}$, the bradycardiac response was slightly smaller than responses under control conditions but the difference was not statistically significant. At 7.0 and 9.0% $F_{ET_{CO_2}}$, the magnitude of the reduction in heart rate was 20 ± 4 beats/min and 7 ± 3 beats/min (58 ± 5% and 19 ± 7% of the control response), respectively, and were significantly smaller than the control responses (Fig. 2c, d). The heart rate response to acupuncture-like stimulation of a hindlimb was not affected by bilateral vagotomy when examined under hypercapnic condition (at 9.0% $F_{ET_{CO_2}}$) ($n = 2$).

When $F_{ET_{CO_2}}$ was increased to 9.0% from the control conditions of 3.0%, the basal level of mean arterial pressure increased significantly (from 117 ± 8 to 130 ± 8 mmHg), while the magnitude of the reduction in mean arterial pressure induced by acupuncture-like stimulation attenuated significantly (from 39 ± 7 to 11 ± 7 mmHg).

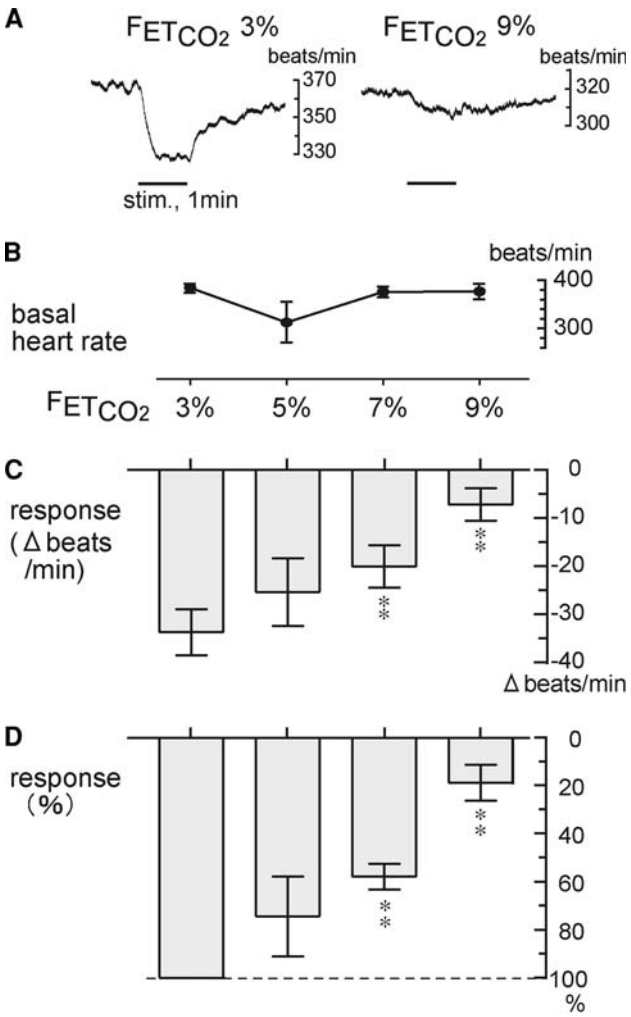

Fig. 2 Heart rate changes elicited by acupuncture-like stimulation of a hindlimb during hypercapnia. **a** Sample responses of heart rate. **b** Summary of basal heart rate during hypercapnia. **c**, **d** Summary of responses of heart rate. **c** Changes in heart rate at the end of the acupuncture-like stimulation were compared with pre-stimulus basal values and plotted as absolute values. **d** Heart rate responses under control conditions ($F_{ET_{CO_2}}$3.0%) were taken as 100%. Each *point* or *column* and *vertical bar* depicts the mean ± SEM for 5 rats. Other details are the same as in Fig. 1

Discussion

Acupuncture-like stimulation (manual acupuncture and electroacupuncture) has been shown to inhibit sympathetic nerve activity and reduce heart rate [11, 12] and blood pressure [13, 14, 18, 19], in experimental animals. Yao et al. [13, 14] demonstrated that the magnitude of depressor response as a result of the inhibition of sympathetic vasoconstrictor activity induced by acupuncture-like

stimulation was greater in SHR than in normotensive controls (WKY). Sympathetic tone is known to be higher in SHR or stroke-prone SHR than in WKY [15, 16]. Therefore, it has been considered that the magnitude of sympatho-inhibitory response to acupuncture-like stimulation depends upon pre-existing sympathetic tone. In contrast, our results in this work showed that the magnitude of sympatho-inhibitory response in the heart following acupuncture-like stimulation was not augmented during acutely increased sympathetic tone induced by hypercapnia. This result suggests that the magnitude of sympatho-inhibitory response to acupuncture-like stimulation does not always depend on pre-existing sympathetic tone. Concerning the reason for the discrepancy between our results in this work and the reports of Yao et al. [13, 14], we speculate that condition of chronically increased sympathetic tone in SHR, and condition of acutely increased sympathetic tone as a result of hypercapnia, are different and these two different conditions might differently modulate the effects of acupuncture.

Comparison of response magnitudes is a difficult problem when basal values are different. In this study, the magnitudes of sympatho-inhibitory response to acupuncture-like stimulation were significantly less pronounced under hypercapnic conditions than under the control condition $(3.0\% F_{ET_{CO_2}})$ when the changes in nerve activity were normalized to the pre-stimulus value. On the other hand, when the nerve activity was normalized to the basal activity (maximum value) at 9.0% $F_{ET_{CO_2}}$ for the entire experiment, the magnitudes of sympatho-inhibitory responses to acupuncture-like stimulation were similar under both the control and hypercapnic conditions. In either calculation, the sympatho-inhibitory response to acupuncture-like stimulation was not augmented during acutely increased sympathetic tone induced by hypercapnia.

Our previous reports [11, 12] showed that the decrease in heart rate by acupuncture-like stimulation of a hindlimb was accompanied by a decrease in cardiac sympathetic nerve activity, and was abolished by bilateral sympathectomy but not by bilateral vagotomy under the condition of about 3.0% $F_{ET_{CO_2}}$. These results indicate that heart rate changes are the result of decreases in sympathetic outflow to the heart rather than increases in parasympathetic outflow. In this study, we observed that under the hypercapnic condition $(\text{at } 9.0\% F_{ET_{CO_2}})$, bilateral vagotomy had no effect on the heart rate response to acupuncture-like stimulation of a hindlimb. This suggests that reduction of acupuncture-induced bradycardiac response under hypercapnic conditions is because of reduction of sympatho-inhibitory response.

Our results in this work, reporting an increase in basal cardiac sympathetic nerve activity as a result of hypercapnia, are in accordance with a previous report [17]. The almost constant basal heart rate observed during hypercapnia despite the increase in basal cardiac sympathetic outflow is suggested to be the result of the inhibitory effect of hypercapnia acting directly on the cardiac pacemaker cells [20, 21], which is also in accordance with a previous report [17].

Makeham et al. [22] reported that the sympatho-excitatory reflex induced by hindlimb somatic afferent stimulation is inhibited during acutely increased splanchnic sympathetic tone induced by hypercapnia. Our results in this work demonstrated that not only sympatho-excitatory reflex as reported by Makeham et al. [22] but also sympatho-inhibitory reflex induced by somatic afferent stimulation decreased during increased sympathetic tone induced by hypercapnia.

This study demonstrated that the sympatho-inhibitory response of the heart rate following short-term acupuncture-like stimulation of a hindlimb was less pronounced when sympathetic tone was high as a result of hypercapnia. This study suggests that the efficacy of the sympatho-inhibitory effects of acupuncture on heart rate become small in the clinical state of hypercapnia.

Acknowledgments The authors are grateful to Professor Yuko Sato of the University of Human Arts and Sciences for her encouragement in the performance of this study.

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