Parallel resetting of arterial baroreflex control of renal and cardiac sympathetic nerve activities during upright tilt in rabbits

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Since humans are under ceaseless orthostatic stress, the mechanisms to maintain arterial pressure (AP) against gravitational fluid shift are important. As one mechanism, it was reported that upright tilt reset baroreflex control of renal sympathetic nerve activity (SNA) to a higher SNA in anesthetized rabbits. In the present study, we tested the hypothesis that upright tilt causes a parallel resetting of baroreflex control of renal and cardiac SNAs in anesthetized rabbits. In anesthetized rabbits (n = 8, vagotomized and aortic denervated) with 0° supine and 60° upright tilt postures, renal and cardiac SNAs were simultaneously recorded while isolated intracarotid sinus pressure (CSP) was increased stepwise from 40 to 160 mmHg with increments of 20 mmHg. Upright tilt shifted the reverse-sigmoidal curve of the CSP-SNA relationship to a higher SNA similarly in renal and cardiac SNAs. Although upright tilt increased the maximal gain, the response range and the minimum value of SNA, the curves were almost superimposable in these SNAs regardless of postures. Scatter plotting of cardiac SNA over renal SNA was increased stepwise changes in CSP was close to the line of identity in 0° supine and 60° upright tilt postures. In addition, upright tilt also shifted the reverse-sigmoidal curve of the CSP-heart rate relationship to a higher heart rate, with increases in the maximal gain and the response range. In conclusion, upright posture caused a resetting of arterial baroreflex control of SNA similarly in renal and cardiac SNAs in anesthetized rabbits.

Blood pressure; orthostasis; sympathetic nervous system

Methods

Animals, preparation, and measurements. Japanese White rabbits weighing 2.4–3.3 kg were used. Animals were cared for in strict accordance with the “Guiding Principles for the Care and Use of Animals in the Field of Physiological Science” approved by the Physiological Society of Japan. Animals (n = 8) were initially anesthetized by intravenous injection (2 ml/kg) of a mixture of urethane (250 mg/ml) and α-chloralose (40 mg/ml). Anesthesia was maintained by continuously infusing the anesthetics at a rate of 0.33 ml·kg⁻¹·h⁻¹ using a syringe pump (CFV-3200; Nihon Kohden, Tokyo, Japan). The rabbits were mechanically ventilated with oxygen-enriched room air. Bilateral carotid sinuses were isolated vascularly from the systemic circulation by ligating the internal and external carotid arteries and other small branches originating from the carotid sinus regions. The isolated carotid sinuses were filled with warmed physiological saline, pre-equilibrated with atmospheric air, through catheters inserted via the common carotid arteries. The intracarotid sinus pressure (CSP) was controlled by a servo-controlled piston pump (model ET-126A; Labworks, Costa Mesa, CA). Bilateral vagal and aortic depressor nerves were sectioned in the middle of the neck region to eliminate reflexes from the cardiopulmonary region and the aortic arch. The systemic AP was measured using a high-fidelity pressure transducer (Millar Instruments, Houston, TX) inserted retrograde from the right common carotid artery below the isolated carotid sinus region. Heart rate (HR) was measured with a cardiobrachet (model N4778; San-ei, Tokyo, Japan).

Body temperature was maintained at around 38°C with a heating pad. The left renal sympathetic nerve was exposed retroperitoneally, and the left cardiac sympathetic nerve was exposed through a middle thoracotomy. A pair of stainless steel wire electrodes (Bioflex wire AS633; Cooner Wire) was hooked onto each of these nerves to record renal and cardiac SNAs. The nerve fibers peripheral to electrodes were ligated securely and crushed to eliminate afferent signals. The nerve
and electrodes were covered with a mixture of silicone gel (silicon low viscosity, Kwik-Sil; World Precision Instruments, Sarasota, FL) to insulate and immobilize the electrodes. The preamplified SNA signals were band-pass filtered at 150–1,000 Hz. These nerve signals were full-wave rectified and low-pass filtered with a cutoff frequency of 30 Hz to quantify the nerve activity. After the experiment, an intravenous infusion of hexamethonium bromide (6 mg/kg) abolished the SNA signals, indicating that the signals recorded were postganglionic SNA.

Protocols. After the preparation, the animal was maintained in a 0° supine posture on a tilt bed. To stabilize the posture, the head was fixed full-frontal to the bed by strings, and the body and legs were rigged up in a clothes-like bag. Bilateral CSP was artificially controlled independently of systemic AP. First, actual operating pressure and SNAs under baroreflex closed-loop conditions in the 0° supine posture were obtained. The animal was kept in the 0° supine posture for 10 min while CSP was matched with systemic AP via the servo-controlled piston pump.

Second, the static characteristics of the sympathetic baroreflex system were estimated in the 0° position under baroreflex open-loop conditions. The animal was kept in the 0° supine posture while CSP was decreased to 40 mmHg and then increased stepwise from 40 to 160 mmHg in increments of 20 mmHg. Each CSP step was maintained for 60 s.

Third, actual operating pressure and SNAs under baroreflex closed-loop conditions in the 60° upright tilt position were obtained. The animal was kept supine for 10 min and then tilted upright to 60° within 10 s by inclining the tilt bed to 60° and dropping the lower regions of the rabbit with the fulcrum set at the level of the carotid sinus. The 60° upright posture was maintained for 10 min. CSP was matched with systemic AP via the servo-controlled piston pump. Since the clothes-like bag stabilized the posture of the animals, there was no additional mechanical movement that reduced the quality of measurements. The position of the head remained almost fixed during the tilt to minimize vestibular stimulation. Last, the static characteristics of the sympathetic baroreflex system were estimated during the 60° upright tilt posture. CSP was increased stepwise from 40 to 160 mmHg similarly to the experiment in the 0° position. These SNAs and AP were recorded at a 200-Hz sampling rate using a 12-bit analog-to-digital converter and stored on the hard disk of a dedicated laboratory computer system for later analysis.

Data analysis. The SNA signals were normalized by the following steps. First, for each type of SNA, 0 arbitrary unit (a.u.) was assigned to the postmortem noise level. Second, 100 a.u. were assigned to the average of actual operating SNA values during baseline period in 0° positions. Last, the other SNA signals were then normalized to these values in each experiment.

These SNA and HR values were averaged for the last 10 s of each CSP level. The static relationships between CSP and SNA and between CSP and HR were parameterized by two widely used traditional models (nonlinear reverse-sigmoidal curve, linear regression line), although both models have limited abilities to reproduce the actual data. In the former case, the data were parameterized by a four-parameter logistic equation model as follows:

\[
Y = P_4 + \frac{P_1}{1 + \exp[P_2(CSP - P_3)]}
\]

where Y is SNA or HR, P_1 is the response range of Y (i.e., the difference between the maximum and minimum values of Y), P_2 is the coefficient of gain, P_3 is the midpoint CSP of the logistic function, and P_4 is the minimum value of Y. We calculated the instantaneous gain.
from the first derivative of the logistic function and the maximum gain ($G_{\text{max}}$) from $-P_2P_3/4$ at CSP = $P_3$.

**Statistic analysis.** All data are means ± SD. Effects of the upright tilt on baroreflex parameters were evaluated by repeated-measures analysis of variance. When the main effect was found to be significant, post hoc multiple comparisons were made using Scheffé’s F-test to compare baroreflex controls between renal and cardiac SNAs (3). Differences were considered significant when $P < 0.05$.

**RESULTS**

**Baroreflex control of renal and cardiac SNAs.** Figure 1 shows the representative time series data obtained from one subject. The renal and cardiac SNAs similarly decreased in response to stepwise increase in CSP in the 0° supine posture (Fig. 1A). The 60° upright tilt increased these SNAs at each CSP level (Fig. 1B) compared with the supine posture.

Figure 2 shows the relationship between CSP and SNA in the same data as in Fig. 1A. In Fig. 2, these SNAs were averaged for the last 10 s of each CSP level to investigate the steady-state, not transient, response to a stepwise change in CSP. The 60° upright tilt increased renal (Fig. 2A) and cardiac SNAs (Fig. 2C) at all CSP levels compared with the supine posture. The renal SNA approximately matched the cardiac SNA at all CSP levels in the supine posture (Fig. 2B) and also in the upright tilt posture (Fig. 2D).

When the static relationship between CSP and each SNA was fitted to a nonlinear reverse-sigmoidal curve (Fig. 3), the $r^2$ value was ~0.95. The 60° upright tilt shifted the CSP-renal SNA curve upward to a higher SNA (Fig. 3A). Similarly, the upright tilt shifted the CSP-cardiac SNA curve (Fig. 3C) in the same manner as renal SNA. The CSP-renal SNA curve was almost superimposed on the CSP-cardiac SNA curve in the 0° supine (Fig. 3B) and upright tilt postures (Fig. 3D). However, the model was limited in reproducing the data, since the measured SNAs did not saturate at the CSP levels of 40–60 mmHg.

When the static relationship between CSP and each SNA was fitted to a linear regression line (Fig. 4), the $r^2$ value was 0.82–0.88, lower than when the nonlinear reverse-sigmoidal curve was used. The 60° upright tilt shifted the CSP-renal SNA (Fig. 4A) and the CSP-cardiac SNA lines (Fig. 4C) upward to the higher SNA levels. The CSP-renal SNA line was almost superimposed on the CSP-cardiac SNA line in the 0° supine (Fig. 4B) and upright tilt postures (Fig. 4D).

Averaged data from all animals showed that the 60° upright tilt increased renal (Fig. 5A) and cardiac SNAs (Fig. 5B) at all CSP levels compared with the 0° supine posture. The renal SNA almost matched the cardiac SNA at all CSP levels in the supine (Fig. 5D) and also in the upright tilt posture (Fig. 5E),
indicating that 60° upright tilt shifted the CSP-SNA relationship upward by similar magnitudes in renal and cardiac SNAs.

When the static relationship between CSP and each SNA was fitted to a nonlinear reverse-sigmoidal curve (Fig. 6), the upright tilt shifted the CSP-SNA curve to higher SNA similarly in renal (Fig. 6A) and cardiac SNAs (Fig. 6C). The CSP-SNA relationship was almost superimposed between these SNAs in both the supine (Fig. 6B) and upright tilt postures (Fig. 4D). In both renal and cardiac SNAs, P1 (the range of SNA response to CSP), P4 (the minimum value of SNA), and the maximal gain (at the midpoint of the logistic function) were larger at upright tilt than supine posture (Table 1), whereas P2 (the coefficient of gain) and P3 (the midpoint CSP of the logistic function) were not different between postures (Table 1). In both postures, these parameters of P1–4 and maximal gain were similar in renal and cardiac SNAs (Table 1).

When the static relationship between CSP and each SNA was fitted to a linear regression line (Fig. 7), the upright tilt shifted the CSP-SNA line to higher SNA similarly in renal (Fig. 7A) and cardiac SNAs (Fig. 7C). It increased the slope of regression from \( \frac{1.1002}{1.1006} \) to \( \frac{1.1102}{1.1006} \) a.u./mmHg in renal SNA and from \( \frac{1.1002}{1.1006} \) to \( \frac{1.1102}{1.1006} \) a.u./mmHg in cardiac SNA. The CSP-SNA lines were almost superimposed on their SNAs in the 0° supine (Fig. 7B) and also the upright tilt posture (Fig. 7D). In both SNAs of all animals, the \( r^2 \) value was always lower (0.80–0.89) than when a nonlinear reverse-sigmoidal curve was used (0.92–0.97).

In addition, in both 0° supine and 60° upright tilt postures, scatter plotting of cardiac SNA over renal SNA was approximately close to the line of identity for each subject (Fig. 8A) and the pooled data from all subjects (Fig. 8B), indicating that these SNAs changed in parallel in response to stepwise increase in CSP regardless of posture. The upright tilt did not change operating AP (steady-state AP: 102 ± 4 mmHg in supine posture, 102 ± 5 mmHg in upright tilt posture). The upright tilt increased operating renal (100 a.u in supine posture, 148 ± 19 a.u. in upright posture) and cardiac SNAs (100 a.u in supine posture, 155 ± 21 a.u. in upright posture) by similar magnitudes.

Figure 9 showed the discharge characteristics of the renal and cardiac SNAs obtained from the same animal studied in Fig. 1. These SNAs were similar to some extent regardless of baroreflex condition and posture. In the supine posture (Fig. 9A), first, these SNAs were weakly pulse synchronous and had slower fluctuations with a time cycle of \( \approx 1.7 \) s in the baroreflex closed-loop condition, where CSP was artificially matched with systemic AP. The CSP and AP also had fluctuations with the same time cycle. Second, in the baroreflex open-loop condition, where CSP was fixed at 40 mmHg (the CSP level was chosen because it maximized these SNAs) without pulse, these SNAs had neither a pulse rhythmicity nor the slower fluctuation observed in the closed-loop condition. These discharge characteristics of SNAs were also observed in the 60° upright posture (Fig. 9B), although the amplitude of SNAs were larger at upright tilt than supine posture.
Baroreflex control of HR. In the representative time-series data, HR decreased in response to a stepwise increase in CSP in the 0° supine posture (Fig. 1A) and during 60° upright tilt (Fig. 1B). The upright tilt shifted the CSP-HR relationship upward to a higher HR (Fig. 2E), although HR was averaged for the last 10 s of each CSP level to investigate the steady-state, not transient, response to stepwise change in CSP.

Averaged data from all animals showed that the upright tilt shifted the CSP-HR relationship upward to a higher HR (Fig. 5E). When the static relationship between CSP and HR was fitted to a nonlinear reverse-sigmoidal curve (Fig. 6E), the P1 (the range of HR response to CSP) and the maximal gain (at the midpoint of the logistic function) were larger at upright tilt than in the supine posture (Table 2), whereas P2 (the coefficient of gain), P3 (the midpoint CSP of the logistic function), and P4 (the minimum value of HR) were not different between postures (Table 2). When the static relationship between CSP and HR was fitted to a linear regression line (Fig. 8E), the upright tilt increased the slope of regression from 0.46 ± 0.3 to 0.60 ± 0.3 beats·min⁻¹·mmHg⁻¹. The upright tilt increased operating HR (steady-state HR; 204 ± 11 beats/min in supine posture, 220 ± 12 beats/min in upright tilt posture).

DISCUSSION

Arterial baroreflex control of SNA is considered to have an important role to maintain AP under orthostatic stress against gravitational fluid shift directed toward the lower part of the body (15). In addition, we (8) recently reported that upright tilt resets arterial baroreflex control of renal SNA to increase orthostatic sympathetic activation. However, it remains unknown whether upright tilt resets arterial baroreflex control of SNA innervating cardiovascular organs (i.e., the heart) other than the kidney. One major new finding in this study is that 60° upright tilt resets arterial baroreflex control of SNA to higher SNA similarly in renal and cardiac SNAs. This supports our hypothesis that upright tilt causes a parallel resetting of arterial baroreflex control of renal and cardiac SNAs in anesthetized rabbits.

Some regional differences between renal and cardiac SNAs certainly have been reported under some physiological conditions. First, for example, the dynamic high-pass characteristics in baroreflex control of SNA were greater in cardiac SNA than renal SNA (6, 10). Second, activating left atrial receptors increased cardiac SNA but decreased renal SNA (9). Last, hypoxia reset the AP-SNA relationship to higher AP and SNA in renal SNA but to lower AP and SNA in cardiac SNA (4). These lines of evidence indicate that renal and cardiac SNAs respond differently to specific physiological stimulation and stress (14).

However, our results indicate that upright posture induces a parallel resetting in arterial baroreflex control of renal and cardiac SNAs in the static characteristics. In agreement with previous studies (6, 7), the CSP-renal SNA reverse-sigmoidal curve was superimposable to the CSP-cardiac SNA curve in
The supine posture. This indicates that static nonlinear characteristics in arterial baroreflex control of renal SNA matched those of cardiac SNA in the posture. In addition, since upright tilt posture shifted the CSP-SNA curves upward similarly in renal and cardiac SNAs, the static nonlinear characteristics in arterial baroreflex control of renal SNA also matched those of cardiac SNA in upright tilt posture. These results were consistent with the close correlation between renal and cardiac SNAs during forced CSP changes with supine and upright tilt postures. They might also be consistent with a numerical simulation study indicating parallel responses of renal and cardiac SNAs to physiological pressure perturbations (AP change) (6).

Our results indicate that upright posture resets arterial baroreflex control of HR to a higher HR. This is consistent with the results of baroreflex resetting for cardiac SNA under upright tilt, because the P1 (the response range) and the maximal gain (at the midpoint of the logistic function) were larger in both CSP-HR and CSP-cardiac SNA relationships. The parallelism suggests that cardiac sympathetic efferent was a dominant determinant for HR in the present experimental condition with cutting of vagal nerves. Our results could be consistent with the increase in the baroreflex gain for HR assessed by a neck pressure/suction device in humans (11).

Limitations. The present study has several limitations. First, we excluded the efferent effect of vagally mediated arterial baroreflex and anesthetic agent that could affect baroreflex control of SNA. Second, the vascular isolation of carotid sinus might decrease brain blood flow under, in particular, upright tilt position. Third, we eliminated cardiopulmonary baroreflex by cutting bilateral vagal nerves. Earlier human studies have indicated that nonhypotensive hypovolemic perturbations do not change AP but reduce central venous, right heart, and pulmonary pressures and cause vasoconstriction. These observations have been interpreted as reflexes triggered by cardiopulmonary baroreceptors (5, 12). However, Taylor et al. (17) showed that small reductions of effective blood volume reduce aortic baroreceptive areas and trigger hemodynamic adjustments that are so efficient that alterations in AP escape detection by conventional means. In addition, Fu et al. (2) reported that arterial baroreceptors are consistently unloaded during low levels (i.e., −10 and −15 mmHg) of lower body negative pressure in humans. Accordingly, further studies are needed to understand the relative importance and mutual cooperation of arterial and cardiopulmonary baroreflexes in AP control during orthostatic stress. Fourth, we investigated arterial baroreflex during upright posture in rabbits, which are quadripeds. However, denervation of both carotid and aortic arterial baroreflexes caused postural hypotension of ~50 mmHg during 60° upright tilt in quadripeds [rabbits and rats (16)]. This suggests that even in quadripeds, arterial baroreflex has a very important role in maintenance of AP under orthostatic stress.
Last, although we used two widely used traditional models to analyze the relationship between CSP and SNA, both have limited abilities to reproduce actual data. The nonlinear reverse-sigmoidal curve parameterized by a four-parameter logistic equation model provided high $r^2$ values (0.92–0.97) regardless of SNA type and posture. However, we failed to observe a saturation of SNA at the lowest CSP level in some cases (40 mmHg; Fig. 3, A and B, in upright tilt position). Lots of earlier studies have applied the model to AP and SNA (or HR) data under pharmacological perturbation (i.e., nitroprusside, phenylephrine) (1, 14), although it is difficult to observe clear saturation and/or threshold in the data. In contrary, the simple linear regression line model provided lower $r^2$ values (0.80 – 0.89). The plotted data did not appear to lie on a simple line in individuals (Fig. 4). Accordingly, we cannot conclude whether the relation between CSP and SNA is sigmoid or not. This problem is not the purpose of this study. Importantly, without modeling, our data (Fig. 2 and 5) indicate the parallel resetting of arterial baroreflex control of renal and cardiac SNAs.

In conclusion, upright posture causes a resetting in arterial baroreflex control of SNA in parallel in renal and cardiac SNAs in anesthetized rabbits.

**GRANTS**

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**DISCLOSURES**

No conflicts of interest, financial or otherwise, are declared by the author(s).
Fig. 7. A model of the averaged data shown in Fig. 5 using a simple regression line. Dotted and solid lines show the data in the supine and 60° upright tilt postures, respectively. The upright tilt shifted the baroreflex lines to a higher SNA similarly in renal (A) and cardiac SNAs (C). The lines were superimposed between these SNAs in the supine (B) and upright tilt postures (D). The upright tilt also shifted the baroreflex line of HR upward (E).

REFERENCES

Fig. 8. Scatter plots and regression lines drawn between renal and cardiac SNAs in supine (dotted lines) and upright tilt postures (solid lines) during stepwise changes in CSP for each subject (A) and the pooled data from all 8 subjects (B).
Fig. 9. Discharge characteristics of the renal and cardiac SNAs in supine (A) and 60° upright tilt postures (B) under the baroreflex closed-loop condition, where CSP was artificially matched with systemic AP, and under the open-loop condition, where CSP was fixed at 40 mmHg. Variables were resampled at 200 Hz. Data were obtained from the same animal studied in Fig. 1.

Table 2. Effect of upright tilt on parameters of baroreflex control of HR

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Supine</th>
<th>Upright tilt</th>
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<tbody>
<tr>
<td>P1, beats/min</td>
<td>53 ± 11</td>
<td>67 ± 11*</td>
</tr>
<tr>
<td>P2, beats·min⁻¹·mmHg⁻¹</td>
<td>0.07 ± 0.03</td>
<td>0.07 ± 0.03</td>
</tr>
<tr>
<td>P3, mmHg</td>
<td>93 ± 8</td>
<td>97 ± 12</td>
</tr>
<tr>
<td>P4, beats/min</td>
<td>184 ± 17</td>
<td>191 ± 18</td>
</tr>
<tr>
<td>Gmax, beats·min⁻¹·mmHg⁻¹</td>
<td>-0.9 ± 0.2</td>
<td>-1.2 ± 0.4*</td>
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Values are means ± SD (n = 8) for the parameters of baroreflex control of heart rate (HR). *P < 0.05, supine vs. upright tilt.