Role of cardiopulmonary and carotid sinus baroreceptors in regulating renal sympathetic nerve activity during water immersion in conscious dogs

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The cardio pulmonary baroreceptors have been thought to modulate the baroreflex control of sympathetic nerve activity and systemic arterial pressure (8). Acute loading of cardiopulmonary baroreceptors induced by head-down tilt and saline loading decreased sympathetic baroreflex gain in humans (3). Chronic loading of cardiopulmonary baroreceptors induced by extracellular and plasma volume expansion has been implicated in the modulation of baroreflex control of sympathetic nerve activity (7, 18). However, the detailed mechanisms underlying how cardiopulmonary baroreceptor loading modulates baroreflex control of sympathetic nerve activity remain incompletely understood. We are conscious that no attempt has been made to generate a baroreflex stimulus-response curve for sympathetic nerve activity during cardiopulmonary baroreceptor loading.

Head out water immersion (WI) has been used as an investigative tool causing the acute loading of cardiopulmonary baroreceptors without administration of either drugs or fluid (13). In dogs, WI resulted in a step increase in central venous, left atrial, and arterial pressures (9, 12, 13). In the present study, we attempted to generate the entire baroreflex stimulus response curve for renal sympathetic nerve activity (RSNA) during cardiopulmonary baroreceptor loading that was induced by WI in conscious dogs.

Since WI increases arterial pressure, suggesting an acute resetting of arterial baroreceptors per se (2, 4, 22) may occur, we also attempted to generate the entire stimulus (pressure)-response (baroreceptor activity) curve for carotid sinus nerve activity during WI.

To achieve these aims, dogs were chronically instrumented for the measurements of central and carotid sinus pressures, as well as activity of carotid sinus and renal nerves. Artificial changes in carotid arterial pressure were made by intravenous administration of vasoactive drugs to generate the entire stimulus response curves of Pca-CSNA and Pca-RSNA in the same dog.

We hypothesized that WI could result in either an acute shift in baroreflex control of RSNA or an acute resetting of arterial baroreceptors per se or both.

MATERIALS AND METHODS

Animal care and training. Nine female mongrel dogs with a mean weight of 9.5 ± 0.8 kg (SE) were used in this study. All animals were fed a standard kennel ration (CD-5; Clea Japan, Tokyo, Japan). Dogs were selected that were temperamentally amenable to WI. They were trained to be immersed to the midcervical level while standing for 1-h periods daily over 1–2 wk prior to the investigation. Once the training was completed, the dogs showed no agitation throughout the experimental period.

This study was conducted in accordance with the Guiding Principles for the Care and Use of Animals in the Field of Physiological Sciences and was approved by the Council of the Physiological Society of Japan (19) with the prior approval of the Animal Care Committee of Nara Women’s University and University of Occupational and Environmental Health.

Instrumentation of animals. Two weeks before the experiment, the dogs underwent surgery under pentobarbital sodium anesthesia (30 mg/kg iv) to implant a catheter, positioned with its tip in the inferior vena cava via the femoral vein, for measurement of central venous pressures (13, 14). The catheter was filled with heparin sodium solution (1,000 IU/ml, Sigma), exteriorized to the interscapular space, and then protected by a fitted jacket.

At least 2 days before the experiment, the dogs were again anesthetized, and then electrodes for measurement of RSNA and CSNA were implanted using the same procedure and instruments as previously described (13–15).

The left kidney was exposed retroperitoneally through a left flank incision. Briefly, after the left renal plexus was identified, one of the postganglionic nerves was isolated carefully for a distance of 10–15...
Carotid arterial (Pca) and central venous (Pcv) pressures were measured with Statham strain gauge transducers (P23ID), according to our previous reports (13, 14). The zero pressure reference level for the pressures was taken to be the level of the tricuspid valve (two-thirds of vertical distance between spinal column and sternum). The Pca, Pcv, and integrated RSNA and CSNA were continuously monitored and sampled for analog-to-digital conversion at 1-ms intervals. The mean values of the data converted over 1 s were calculated simultaneously, displayed on a computer screen, and stored on a hard disk, which were used for further analyses.

The background noise for the CSNA recording was determined when nerve activity was eliminated by decreasing Pca with an intravenous infusion of sodium nitroprusside (300 μg) during the control period; the background noise for the RSNA recording was determined when nerve activity was eliminated by increasing Pca with an intravenous infusion of phentolamine hydrochloride (150 μg) during the control period. The background noise was then subtracted from the integrated CSNA and RSNA data.

To quantify the CSNA and RSNA responses, the upper plateaus of CSNA and RSNA, which were attained in response to the injection of phenylephrine hydrochloride and sodium nitroprusside, respectively, during the control period, were taken as 100%.

Experimental protocol. At 0840 on the day of the experiment, the dog was positioned in a sling frame assembly in the normal quadruped position. After the connection of the instruments for the measurement of pressures and nerve activities, ~60 min was allowed for the stabilization of circulatory function.

The WI experiment consisted of a 60-min control period (pre-WI) in air, 120 min of WI, and a 60-min recovery period in air. The dog was immersed to the midcervical level and water temperature was kept constant at 37°C to ensure a thermonutral condition. The time control experiment consisted of a 60 min control period (pre-sham WI) in air, 120 min of sham WI in air, and a 60 min recovery period in air. Sham WI was carried out by placing a plate above the immersion tank such that the dogs stand in air under the same conditions of WI. An interval of at least 2 days was allowed for recovery between WI and time control experiment, and the experiments were performed in random order. The dogs were held by a sling frame assembly, which was loosely applied and allowed them to move freely.

The steady-state levels of Pca, Pcv, RSNA, and CSNA were measured by averaging the values taken over 5 min at 25–30 min of control period and 55–60 min of WI period. Pharmacological manipulation of Pca was carried out after measuring the steady-state level during control and WI periods. The increases and decreases in Pca

mm. A bipolar stainless-steel wire electrode (AS633; Cooner Wire Co., Chatsworth, CA) was hooked onto the renal nerve, and the wires of the electrode and the isolated nerve were embedded in two-component silicone rubber (604; Wacker-Chemie, Munich, Germany), and the gel was allowed to harden. Then, the silicone rubber was cut to a size $\sim 10 \times 5 \times 5$ mm and fixed to the surrounding tissue using glue containing alpha-cyanoacrylate (Aronalpha, Tohwa Gousei Kagaku, Tokyo, Japan). In this preparation, neither distal nor proximal end of the renal nerve was sectioned.

After closing the incision, a ventral midline neck incision was made, and then the carotid sinus nerve was identified. The carotid sinus nerve was isolated for a distance of ~10 mm and then the electrode, which was the same as used for RSNA, was hooked on the carotid sinus nerve using exactly the same procedure as for RSNA. The superior thyroid artery was cannulated with a polyethylene catheter (PE-50, 0.58 mm ID × 0.965 mm OD; Clay Adams, Parsippany, NJ), and the tip of the cannula was inserted rostrally into the common carotid artery until it lay immediately caudal to the bifurcation.

The electrodes and catheter were exteriorized to the interscapular space and protected by the fitted jacket. Penicillin (50,000 U im; Viccillin, Meiji seiyaku, Tokyo, Japan) or sodium cefazolin (500 mg im; Cefamezin, Fujisawa, Tokyo, Japan) was given daily for 5 days after each operation. The catheters were flushed twice a week and kept filled with heparin sodium solution (1,000 IU/ml; Sigma, St. Louis, MO, USA).

Postoperative care. Animals were examined four times per day for 3 days following the surgery, and two times per day thereafter (14, 16). The examination score sheet was divided into three categories: category A included posture, activity, breathing, coat, and eye; category B included body temperature, body weight, food intake, drinking, urine volume, fecal volume, and hydration status; category C (at request) included chest sound, nociceptive mechanical threshold test by palpation, hematocrit, and heart rate. An electrically heated blanket and special foods were provided after the surgery. For the control of postoperative pain, a nonsteroidal anti-inflammatory drug (diclofenac sodium; Voltaren; 0.5-3 mg/kg 12 hourly for short-term, indomethacin; Inteban; 0.2-1 mg/kg for long term) was given. The hydration status was controlled by the infusion of Ringer solution intravenously on the first postoperative day. The stitches were removed a week after the surgery.

Measurements. The RSNA and CSNA were amplified using a differential amplifier with a band-pass filter 30 Hz and 2 kHz (VC-11, Yokogawa-Denki, Tokyo; see Fig. 1). The eight-channel thermal head paper recorder (ORP 1200, Yokogawa-Denki, Tokyo; see Fig. 1). The mean values of the data converted over 1 s were calculated simultaneously, displayed on a computer screen, and stored on a hard disk, which were used for further analyses.

Fig. 1. Typical recordings from an individual dog of carotid arterial pressure (Pca), central venous pressure (Pcv), carotid sinus nerve activity (CSNA), integrated CSNA, renal sympathetic nerve activity (RSNA) during pharmacological manipulation of Pca during prewater immersion period. A bolus intravenous infusion of phenylephrine (150 μg) and nitroprusside (300 μg) was given to generate the stimulus-response curves for CSNA and RSNA. Data are presented at two different recording speeds.
were achieved using bolus intravenous doses of phenylephrine hydrochloride (150 μg) given over 10 s and sodium nitroprusside (300 μg) over 10 s (Fig. 1). The corresponding responses in CSNA and RSNA, together with Pca, were recorded, and data were then fitted into the sigmoidal logistic equation. The average lowest and highest Pca achieved after the pharmacological manipulation was 56 ± 2 mmHg and 165 ± 3 mmHg, respectively (Figs. 2 and 3).

Data analysis. A logistic sigmoid function as described by Kent et al. (10) was used to analyze baroreflex curves: RSNA or CSNA = A1/[1 + exp(A2 (X - A3))] + A4, where RSNA or CSNA is the dependent variable; X is Pca; A1 is the range of response of the dependent variable (upper plateau minus lower plateau); A2 is the gain coefficient (i.e., slope); A3 is the Pca at the midrange of the curve (centering point); A4 is the lower plateau of dependent variable (20, 23). In each animal, Pca and CSNA or RSNA data were fitted to the logistic function to generate parameters A1, A2, A3, and A4 using graphics software (GraphPad, SPSS Chicago, IL). We calculated the upper plateau for CSNA and RSNA, saturation pressure for Pca (Pca,sat), threshold pressure for Pca (Pca,thr), operating range for Pca, maximal gain according to the previous reports (17, 20); upper plateau = A1 + A4; Pca,thr = -0.2/A2 + A3; Pca,sat = 0.2/A2 + A3; operating range = Pca,sat - Pca,thr; and maximal gain = A1/A3/A4.

Pca,thr and Pca,sat are the Pa at which CSNA or RSNA was within 5% of its upper or lower plateau, respectively. The operating range implies the range of Pa over which CSNA and RSNA responded.

To avoid the effects of uneven density of Y (CSNA or RSNA) axis data along the X (Pa) axis, all data were averaged over each 5.0-mmHg bin of Pa. Mean values of RSNA, CSNA, and Pa within every 5.0-mmHg bin of Pa were used for curve fitting. Baroreflex response curves were constructed, and their parameters were calculated for each trial of the pharmacological manipulation of arterial pressure in each animal and then averaged across the animals. The averaged A1, A2, A3, and A4 were then used to generate average baroreflex curves (Figs. 2 and 3).

Statistics. Statistical analysis was performed by ANOVA for repeated measures. When the F values were significant (P < 0.05), individual comparisons were made using the Fisher’s least significant difference test (21). Values are reported as means ± SE.

RESULTS

Steady-state levels of Pca, Pcv, RSNA, and CSNA. WI increased Pca and Pcv by 7.8 ± 3.4 mmHg (0.05 < P < 0.10) and 10.4 ± 0.7 mmHg (P < 0.05) relative to the pre-WI level, respectively (Table 1). The increase in Pca during WI reached statistical significance (by 9.6 ± 3.6 mmHg, P < 0.05) when it was compared with the corresponding time control experiment (sham WI). WI decreased RSNA significantly (P < 0.05) by 28.4 ± 7.0% max. The average level of CSNA increased during WI from 35.6% max during pre-WI to 39.2% max, but it did not reach a statistical significance.

Table 1. Steady-state hemodynamic parameters

<table>
<thead>
<tr>
<th>Condition</th>
<th>Pca, mmHg</th>
<th>Pcv, mmHg</th>
<th>RSNA, %</th>
<th>CSNA, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water immersion</td>
<td></td>
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<tr>
<td>Pre-WI</td>
<td>106.4 ± 3.3</td>
<td>-0.3 ± 0.5</td>
<td>44.0 ± 8.9</td>
<td>35.6 ± 4.9</td>
</tr>
<tr>
<td>WI</td>
<td>114.2 ± 3.4</td>
<td>10.1 ± 0.7†</td>
<td>15.2 ± 6.0†</td>
<td>39.2 ± 3.0</td>
</tr>
<tr>
<td>Time control</td>
<td></td>
<td></td>
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<tr>
<td>Pre-sham WI</td>
<td>104.2 ± 4.4</td>
<td>0.4 ± 0.6</td>
<td>44.6 ± 7.1</td>
<td>34.2 ± 5.2</td>
</tr>
<tr>
<td>Sham WI</td>
<td>104.6 ± 3.6</td>
<td>0.1 ± 0.5</td>
<td>44.1 ± 9.3</td>
<td>36.3 ± 6.3</td>
</tr>
</tbody>
</table>

Values are expressed as means ± SE (n = 9). WI, water immersion; Pca, carotid arterial pressure; Pcv, central venous pressure; RSNA, renal sympathetic nerve activity; CSNA, carotid sinus nerve activity. *P < 0.05 vs. pre-WI; †P < 0.05 vs. control at correc.
baroreceptor loading may modify the baroreflex control of RSNA. Although the four parameters describing Pca-RSNA curve changed significantly (Tables 2 and 3), the suppression of Pa,sat by 39 mmHg may be a primary effect on the Pca-RSNA curve. The fall in Pa,sat led to a decrease in the operating range (Pca,sat – Pca,thr) from 96 mmHg during pre-WI to 53 mmHg because Pca,thr remained constant. The reduction in the operating range by approximately half due to WI was accompanied by no changes in response range, leading to significant increases in gain coefficient and maximum gain because the gain is dependent on the ratio of response to operating range. It is, therefore, likely that the loading of the cardiopulmonary baroreceptors modulates baroreflex control of RSNA by exerting a primary influence on Pca,sat, which, in turn, reduces the operating range by half, and thereby causes significant increases in the gain coefficient and maximum gain.

The shift in Pca-RSNA curve observed in the present study during WI explains well the sustained reductions of sympathetic nerve activity that has been consistently reported in dogs and humans (11, 13). In dogs, RSNA decreased in a sustained fashion by some 43% with significant increases in systemic and central venous pressure (13). In humans, WI decreased RSNA significantly with no changes (5, 11) or a 7–10 mmHg increase (1, 6) in systemic arterial pressure. As shown in Fig. 2, the Pca-RSNA curve during WI became lower compared with that during pre-WI. Therefore, RSNA would be maintained at a lower level despite an increased or unchanged Pca. This view is consistent with our previous report in cardiac denervated dogs (14); WI decreased RSNA transiently in cardiac denervated dogs while RSNA decreased in a step manner throughout 2 h of WI in intact dogs. It is, therefore, concluded that cardiopulmonary baroreceptors play a dominant role in inducing the sustained reduction of sympathetic nerve activity during WI in dogs and in humans.

### Table 2. Logistic model parameters describing Pca-RSNA and Pca-CSNA curves

<table>
<thead>
<tr>
<th></th>
<th>A1(%)</th>
<th>A2(%/mmHg)</th>
<th>A3(mmHg)</th>
<th>A4(%)</th>
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<tbody>
<tr>
<td><strong>Pca-RSNA</strong></td>
<td></td>
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<tr>
<td>Pre-WI</td>
<td>103.8±1.9</td>
<td>0.046±0.006</td>
<td>102.8±7.0</td>
<td>0.3±1.5</td>
</tr>
<tr>
<td>WI</td>
<td>100.7±1.9</td>
<td>0.076±0.003†</td>
<td>85.4±7.0</td>
<td>0.7±1.4</td>
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<tr>
<td><strong>Pca-CSNA</strong></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Pre-sham WI</td>
<td>103.7±2.8</td>
<td>0.047±0.005</td>
<td>100.3±5.5</td>
<td>0.2±1.3</td>
</tr>
<tr>
<td>Sham WI</td>
<td>99.7±1.1</td>
<td>0.048±0.005</td>
<td>101.1±6.6</td>
<td>0.5±2.5</td>
</tr>
</tbody>
</table>

Values are expressed as means ± SE (n = 9). A1, response range; A2, gain coefficient; A3, pressure at the midrange of the curve (centering point); A4, minimum response. *P < 0.05 vs. pre-WI; †P < 0.05 vs. time control at corresponding period.
It should be emphasized that WI relocated the operating pressure to near the \( \text{Pca}_{, \text{sat}} \) (Fig. 4), as well as decreasing the operating range. These facts could provide some new insight into baroreflex control of systemic arterial pressure during cardiopulmonary baroreceptor loading.

From the viewpoint of feedback theory, the system becomes stable if the operating pressure is located near the centering pressure, where the gain is maximal and is a point at which a system could respond equally to an increase or a decrease in pressure. Indeed, the operating pressure in the pre-WI period was located near the centering pressure observed in the present study, and during non-REM sleep and treadmill exercise reported previously (16, 17). The cardiopulmonary baroreceptor loading seems to move the baroreflex feedback system into an unstable state. Because WI relocated the operating pressure to near the \( \text{Pca}_{, \text{sat}} \), it caused a blunted sympathetic response against the changes in arterial pressure because baroreflex sympathetic gain is low around \( \text{Pca}_{, \text{sat}} \). Furthermore, the operating range decreased by half during WI, which would be disadvantageous if arterial pressure should be regulated.
pressure changed rapidly because arterial pressure would be out of the operating range before the onset of compensatory sympathetic responses could become effective. In other words, the baroreflex feedback control system may be modulated in a way resembling a "Flip-Flop" or "On-Off" type regulation, which would fail to drive linear compensatory feedback mechanisms when arterial pressure changed rapidly and then failed to maintain systemic arterial pressure within a certain range.

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