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Noriyuki Yamamoto[1] and Masaaki Hashimoto[2]

[1]Department of Health Sciences, Japanese Red Cross Hokkaido College of Nursing, Kitami 090-0011, Hokkaido, Japan, [2]Department of Physiology, Asahikawa Medical University School of Medicine, Asahikawa, 078-8510, Hokkaido, Japan

Running head: Stabilization of BP fluctuation by immersion in CO_2-water with NaCl

Name and address for correspondence:

Noriyuki Yamamoto

Japanese Red Cross Hokkaido College of Nursing, 664-1 Akebono-cho, Kitami, Hokkaido 090-0011, Japan

Tel: +81-157-66-3390 Fax: +81-157-66-3390 E-mail: yama@rchokkaido-cn.ac.jp

Abstract

Bathing in hot springs containing high concentrations of carbon dioxide (CO_2) remarkably influences cardiovascular function more than bathing in fresh water. The CO_2-ennriched water in hot springs generally contains many salts among which interactions remain unknown. We separately evaluated the actions of individual factors in CO_2-water and confirmed that CO_2 and NaCl have combined effects on blood pressure fluctuations in anesthetized rats. The animals were equipped with sensor probes to monitor body temperature, skin blood flow and arterial blood pressure, and immersed in bathwater (35°C) containing CO_2 with NaCl, KCl or sucrose. The effects of these factors on cardiovascular functions were evaluated using power-spectral analysis of the fluctuation in blood pressure and heart rate. Compared with immersion in tap water, heart rate and skin vascular resistance were reduced during immersion in CO_2-water as reported irrespective of the ingredients. In terms of the very low frequency range (0.02 ~ 0.195 Hz), the power of blood pressure fluctuation during immersion was significantly reduced when the CO_2-water contained more than 1.5 % NaCl but was not influenced by other ingredients with a similar osmotic pressure and the same specific gravity. The results indicated that coexistent CO_2 and sodium ions in bathwater reduce blood pressure fluctuations, and suggested that this combination effect of CO_2 and salt contributes to the sedative effect on human cardiovascular functions while bathing in CO_2-hot springs.

Key words: BP fluctuation, hemodynamic, power spectrum density, CO_2 balneotherapy, NaCl

INTRODUCTION

Although balneotherapy has a long history, how bathing in hot springs confers beneficial effects remains unknown (Matz et al. 2003). Bathing in hot springs that are rich in free carbon dioxide (CO_2, $\geq$ 1g CO_2/L: CO_2-hot spring) seems to consistently confer benefits upon patients with cardiovascular diseases (Savin et al. 1995; Toriyama et al. 2002). However, few studies have analyzed the mechanism of action of these benefits compared with the effect of such bathing upon physiological functions.

When humans bathe in CO_2-hot springs, reddening of the immersed skin is remarkably independent of the water temperature (McClellan 1963). Additionally, the effects now include skin vascular dilation, increased blood flow to the skin, reduced heart rates and plasma adrenalin levels, all of which suggest global reduction of sympathetic nervous system activities (Diji 1959; Ito et al. 1989; Schnizer et al. 1985). An experimental analysis of the physiological mechanism underlying these responses to bathing in artificial CO_2-hot springs has recently been undertaken using experimental animals (Hashimoto and Yamamoto 2004; Nishimura et al. 2002; Yorozu 1985, 1984). An apparatus has been developed to create artificial CO_2-hot springs using common tap water and high pressure CO_2 (Kamo 1985) Such artificial spring water containing a high concentration of CO_2 (CO_2-water) is similar to water in natural hot spring in terms of inducing a warm sensation and effects on cardiovascular functions (Hashimoto and Yamamoto 2004; Nishimura et al. 2002). Furthermore, we compared artificial

CO_2-water with tap water in terms of effects on the physiological functions of anesthetized rats, and found that the heart rate was reduced during immersion in CO_2-water, which was probably caused by the inhibition of sympathetic nerve activity (Hashimoto and Yamamoto 2004). These results indicated that the experimental system using an animal model and artificial CO_2-hot spring water would be useful to investigate the physiological mechanism of bathing in CO_2-hot springs.

The artificial CO_2-water contains less mineral ingredients but still mimics the effects of natural CO_2-hot springs on the physiological functions of human and experimental animals; thus the high CO_2 concentration appears to play a key role in the induction of these effects (Hashimoto and Yamamoto 2004; Nishimura et al. 2002). However, CO_2-hot springs contain many different mineral ingredients (salts) depending on geographic location. Among them, NaCl is a common ingredient that evokes physiological reactions by ionic electrochemical and osmotic potentials when applied extracellularly (Chahine et al. 2005; Durbin 1976; Lai et al. 1991; Lotmar 1961; Schafer et al. 1975). Furthermore, NaCl in bath water might modify human cardiac function (Miyajima et al. 2000). Thus, the effect of a high CO_2 concentration combined with salts must be evaluated in order to understand the mechanism of action of CO_2-hot spring water through the skin. However, the influence of percutaneously applied salts with a high CO_2 concentration has not been systematically investigated *in vivo*.

Here, we investigated the effects of a high concentration of CO_2 and ionic salts in artificial CO_2-hot spring water on the cardiovascular functions of experimental animals.

MATERIALS AND METHODS

The Committee for Animal Experiments at Asahikawa Medical University approved the study, which proceeded according to the Guiding Principles for the Care and Use of Animals approved by the Council of the Physiological Society of Japan.

Animals

Male Wistar rats (n = 40, 260 ~ 390 g) were housed in wire cages at room temperature (26°C), relative humidity of 60 % and a 12:12 h light:dark cycle with free access to food and water. The animals were separated into NaCl (n = 20), sucrose (n = 15) and KCl (n = 5) groups. The animals were anesthetized with urethane (ethyl carbamate; Tokyo Chemical Industry, Tokyo, Japan, 1.39-1.60 $g \cdot kg^{-1}$ body weight, i.p.) throughout the study, and the fur on the lower half of the body was sheared to the axillary level. To firmly attach a disk-type flowmetry-probe (type C, Advance, Tokyo, Japan) to the skin, hair stumps of 1 cm^2 on the abdominal side were shaved using a razor blade. The probe was then attached to the skin surface using a small spacer to allow bath water into the gap between the probe and skin, and connected with a laser Doppler flow meter (ALF-21N, Advance, Tokyo, Japan) to measure skin blood flow (BF_{skin}). A 24-G Surflo tube (Terumo, Tokyo, Japan) was inserted into the left femoral artery and connected with a transducer (Life-kit DX360, Nihon Koden, Tokyo, Japan) to measure blood pressure (BP). Beat-to-beat mean arterial BP and HR were calculated from pulsatile changes in blood pressure on-line using a polygraph system (Nihon Koden, Tokyo, Japan). All incisions in the skin were closed and

sealed using an acrylic adhesive (Alon Alpha; Toa Chemical, Tokyo, Japan) to prevent water infiltration. A copper-constant thermocouple was then attached to the shaved, immersed skin and another was inserted into the colon to measure body temperature.

Immersion

Bath water containing high concentrations of CO_2 (965-1,400 ppm; 35°C) was generated using a cylinder of high pressure CO_2 and tap water (~ 20 ppm; 35°C) that was thermostatically controlled (tap water) using an MRE-Spa (Mitsubishi Rayon Engineering, Tokyo, Japan). The CO_2 concentration in the bath water was measured using either a CO_2 probe with a pH/ion meter (model 290A, Orion Research, Beverly, USA) or the pH of the water converted into the CO_2 concentration. The feet of healthy humans turn red after a 10 minute immersion in this CO_2-water, but do not obviously change after immersion in tap water at the same temperature (Hashimoto and Yamamoto 2004). To evaluate the effect of immersion in CO_2-water on the skin vascular system, the BP divided by BF_{skin} was determined as an index of vascular resistance (VR_{skin}).

The animals were loosely taped to plastic lattice-plates that were placed in a head-up position of about 30° to the horizontal in a polycarbonate animal cage (30 x 20 x 15 cm) used as a bathtub containing 1) tap water, 2) CO_2 water, 3) tap water containing NaCl (0.1, 0.4, 1.5 or 4.0%), 4) CO_2 water containing NaCl of the same concentrations as 3), 5) tap water containing sucrose (4.0% and 20%), 6)

CO_2 water containing sucrose of the same concentration as in 5), 7) tap water containing 4.0% KCl, and 8) CO_2 water containing 4.0% KCl. The temperature of the bath water was maintained at 35°C throughout the study by placing the bathtub in a water-bath incubator (BT-25; Yamato Scientific Co., Tokyo, Japan). The animals were immersed in one type of water for 30 min. At the end of this period, the bath water was quickly siphoned off and replaced by another sample with a different CO_2 concentration at the same temperature. To prevent the inhalation of CO_2 that diffused from the surface of the bath water, the anesthetized rats were provided with fresh air (300 $ml \cdot min^{-1}$) through a facemask during the study.

Data analysis

In the immersion experiments, all signals were captured and stored every second using a personal computer (PC9801, NEC, Tokyo, Japan), and simultaneously visualized using an R-66 multi-pen recorder (Rika Denki, Tokyo, Japan) on chart paper for back up. For statistical analysis, data were stored for about 20 min starting from 10 min after the exchange of bath water and were averaged to determine a representative value of each parameter. The fluctuation of BP and HR during water immersion was evaluated using power spectrum density in fast Fourier transform (FFT) mode. A series of 1,024 data points was re-sampled every second for FFT-analysis using commercially available software (Chart5; A/D Instruments, Australia), and calculated using Hamming window settings and the half-overlap method. To evaluate the effect of the bathwater ingredients, the power spectra were classified into two regions of the frequency range: VLF (very low frequency, 0.02 ~ 0.195 Hz) and LF (low frequency, 0.195 ~ 0.5 Hz),

and the power densities of each frequency region were integrated. The frequency ranges of VLF and LF roughly corresponded to those previously reported (Cerutti et al. 1994; Janssen et al. 1995; Japundzic et al. 1990; Julien et al. 1995; Persson et al. 1992). All numerical data generated in the experiments are shown as means ± SEM. Data were statistically analyzed using a two-way ANOVA with repeated measures followed by pair-wise comparisons using the Newman-Keuls post-hoc test. Values of $p < 0.05$ were considered statistically significant.

RESULTS

Baseline levels of colon and skin temperatures, HR, BP and BF_{skin} did not significantly differ among the animal groups. The temperatures of the colon and skin of all animals under room air conditions before starting the first immersion were 34.3 ± 0.3°C (means ± SEM, n = 40) and 32.5 ± 0.2°C, respectively. At this time, the HR was 361 ± 6 beats·min^{-1}, the BF_{skin} was 4.7 ± 0.5 ml·min^{-1}·100 g^{-1} and BP was 98 ± 3 mmHg. Compared with immersion in tap water, the BF_{skin} during CO_2-water immersion significantly increased by 34.9 ± 9.3% and the HR decreased by 2.6 ± 0.7%, while BP did not change. Although these parameters obviously fluctuated in some animals after each exchange of bath water, this influence disappeared within 10 min. The temperatures of the colon and skin during immersion in CO_2-water were 36.0 ± 0.1°C and 34.9 ± 0.1°C, respectively, and did not differ from the values observed during tap water immersion. None of the rats immersed in water under the present experimental conditions shivered, irrespective of the CO_2 concentration of the bath water.

Figure 1 is a representative recording of the effects of immersion and NaCl in bath water on hemodynamic parameters. The HR and BF_{skin} were significantly increased by immersion in tap water, while BP was not. The HR reduction and BF_{skin} increase during immersion in CO_2-water compared with tap water, as previously reported (Hashimoto and Yamamoto 2004), were similar in all animals irrespective of the presence of NaCl in the water. However, the amplitude of BP fluctuation was smaller in CO_2-water containing 4.0% NaCl than in control tap water containing the same amount of NaCl.

Figure 2 shows that the mean values of HR and BP of rats immersed in bath water containing various NaCl concentrations were not affected. At all NaCl concentrations, the mean HR during immersion in CO_2-water was significantly lower than that in tap water (control, 2.6 ± 0.7%; 0.1% NaCl, 3.9 ± 0.8; 0.4% NaCl, 2.9 ± 0.7; 1.5% NaCl, 1.8 ± 0.8, 4.0% NaCl, 2.5 ± 1.0).

Figure 3 represents the power spectral density (PSD) of FFT analysis of BP-fluctuations in one rat immersed in tap and CO_2-water containing 0% or 4.0% NaCl. Clear power peaks rarely appeared in the spectrum over the entire LF range. The frequency region below 0.02 Hz in the spectra contained a huge power density since it is affected by a DC level shift of BP. The PSD between bath water containing various NaCl concentrations did not significantly differ within a frequency range below 0.02 Hz and over 0.2 Hz. In contrast, the PSD levels of the VLF range were apparently lower in CO_2-water containing 4.0% NaCl than no NaCl. Therefore, the effects on BP fluctuations caused by bath water ingredients in the following analysis were evaluated using the integrated PSD (iPSD) of this frequency range.

Figure 4 shows the dose-response relationship between NaCl concentrations and VLF power (iPSD) of BP-fluctuations. The NaCl concentrations did not influence the iPSD of BP-fluctuation of the rats immersed in tap water under our experimental conditions. The iPDS did not significantly differ between immersion in tap water and in CO_2-water containing up to 0.4% NaCl. At NaCl concentrations of 1.5% and 4.0%, compared with tap water, the BP-iPSD was significantly decreased by 63.3 ± 4.5% (1.5% NaCl) and 66.2 ± 3.5% (4.0% NaCl) during immersion in CO_2-water.

Figure 5 shows the effects of CO_2 and NaCl in the bath water on the HR variability power spectra. Both power densities of VLH and of LF were apparently smaller during immersion in CO_2-water than in tap water irrespective of the NaCl concentration (Fig. 5A). Therefore, the power density of the spectra (HR-iPSD) was integrated from 0.02 to 0.5 Hz (Fig. 5B), and the effect of the NaCl on HR variability was evaluated. Statistically significant effects on HR-iPSD of NaCl were undetectable compared with bath water containing 0% and 4.0% NaCl. However, the iPSD was significantly smaller (0% NaCl, 29.7 ± 6.5 %; 4.0%-NaCl, 31.3 ± 5.3%) during immersion in CO_2-water than in tap water.

Figure 6 summarizes the effects of bathwater ingredients (4.0% NaCl, 20% sucrose and 4.0% KCl) on HR, BP, BF_{skin} and VR_{skin} and an index of vascular resistance, BP/BF_{skin} None of these factors elicited prominent effects on these cardiovascular parameters under our experimental conditions. However, the effects of bath water CO_2 on heart activity and peripheral blood flow were reconfirmed irrespective of these factors. Compared with immersion in tap water, HR during immersion in CO_2-water

was significantly decreased under all tested circumstances (control, 2.6 ± 0.7%; 4.0% NaCl, 2.5 ± 1.0%; 20% sucrose, 2.4 ± 1.0%; 4.0% KCl, 2.7 ± 0.8%). None of the bath water ingredients including CO_2 influenced BP. The BF_{skin} was significantly larger during immersion in CO_2-water than in tap water (control, 34.9 ± 9.3%; 4.0% NaCl, 41.7 ± 16.2%; 20% sucrose, 55.4 ± 29.6%; 4.0% KCl, 41.3 ± 9.5%). The VR_{skin}, which indicates blood vessel tone, was significantly smaller in the presence of each factor when CO_2 was also present in the bathwater (control, 13.4 ± 5.5%; 4.0% NaCl, 19.1 ± 5.7%; 20% sucrose, 27.0 ± 11.9%; 4.0% KCl, 20.6 ± 8.8%).

Figure 7 summarizes the influence of BP fluctuations in the physical characteristics of bath water, such as specific gravity, osmotic pressure and cation species, on the iPSD. Except for the CO_2-water containing 4.0% NaCl, none of the other factors (4.0% or 20% sucrose, and 4.0% KCl) affected BP fluctuation in any of the immersion conditions. The mean decrease in BP-iPSD in CO_2-water was over 55% of that in tap water at the same salt concentration.

DISCUSSION

Studies of the CO_2-hot springs used in balneotherapy have focused on analyzing the action mechanism of CO_2 because of its remarkable effects on the cardiovascular functions of humans and experimental animals (Hashimoto and Yamamoto 2004; Komoto et al. 1988; McClellan 1963; Nishimura et al. 2002). A recent study using artificial CO_2-hot spring water and laboratory animals has revealed that CO_2, the principal ingredient of CO_2-hot springs, works percutaneously and reduces heart rate compared

with tap water baths (Hashimoto and Yamamoto 2004). The HR reduction in CO_2-water seems to be produced by inhibiting the cardiac sympathetic nerve activity induced by neuronal information generated in the skin and transported through the spinal cord to the brain (Hashimoto and Yamamoto 2004; Yamamoto and Hashimoto 2007). The present results support the notion that the inhibition of cardiac activity while bathing in CO_2-water is due to modulation of the sympathetic nervous system.

Power spectral analysis of HR and BP fluctuations is a convenient and useful tool for detecting cardiovascular events (Task 1996). Theoretically, the maximal frequency of the power spectrum analysis (Nyquist frequency) is higher when the sampling frequencies of HR and BP are higher. In the present study, the high frequency (HF, > about 0.7 Hz) component was undetectable from the present data set recorded every second (Nyquist frequency, 0.5 Hz). However, since most of the power of arterial blood pressure fluctuations is below 0.6 Hz (Nafz et al. 1997) in the rat, the Nyquist frequency of 0.5 Hz applied in the present study does not interfere with interpretations of the results. Compared with previous studies of conscious animals, the small power over whole frequency ranges in the present study might be due to anesthesia (Akselrod et al. 1987). However, the effects of bath water factors on the power spectrum were sufficiently detectable.

As a common ingredient of CO_2-hot springs, NaCl might be a percutaneous stimulation factor affecting cardiovascular functions (Miyajima et al. 2000). Though the results of human head-out studies of immersion in water containing 0 to 7% NaCl have shown an increase in HR compared with

pre-immersion levels, it seems to be irrelevant to the NaCl concentration within this range (Miyajima et al. 2000). We found here that HR was higher during, than before immersion (Fig. 1), and was not affected by NaCl in the bath water, findings that were similar to those from humans. The HR was also reduced during immersion in CO_2-water compared with tap water irrespective of the NaCl concentration (Fig. 2A). Although an inhibitory effect of salts on the cardiac parasympathetic system was implied (Miyajima et al. 2000), we assumed that inhibition of the cardiac sympathetic system by bathwater CO_2 makes a larger contribution to at least the HR reduction mechanism than NaCl (Hashimoto and Yamamoto 2004).

The combination of CO_2 and NaCl in bathwater did not affect the mean values of HR, BP, BF_{skin} and VR_{skin}, which reflected cardiovascular functions under our conditions. The power spectral analysis of BP fluctuation might be a suitable way of detecting a subtle change in the cardiovascular system. Suppression of the sympathetic nerve activity by treatment with sympathetic β-blockers or sympathectomy reduces the low frequency (about 0.2 ~ 0.6 Hz) power of BP fluctuations (Cerutti et al. 1991; Julien et al. 1995; Persson et al. 1992; Stauss et al. 1995). Furthermore, the suppression of myogenic oscillation originating in the blood vessel wall by an α-adrenergic blockade reduces the spectral power of the LF and VLF ranges (Cerutti et al. 1991; Japundzic et al. 1990; Stauss et al. 1995). Blood pressure itself influences blood pressure oscillation through a feedback loop including the sino-aortic nerves (Cerutti et al. 1994). These findings suggest that the stiffness of the blood vessel walls might be involved through sympathetic elements of the baroreflex control loop in blood pressure

oscillations. The present results agree with these observations, and suggest that attenuation of sympathetic nerve activity diminished the VLF, as well as the LF power of BP fluctuations (Persson 1996). A blockade of parasympathetic nerve activity enhances the LF as well as the HF power of BP fluctuations (Cerutti et al. 1991; Japundzic et al. 1990). A similar level of HR reductions in rats immersed in CO_2-water and in CO_2-NaCl water disagrees with the notion that this combined effect on BP fluctuations is due to parasympathetic regulation, because inactivation of the parasympathetic system activates heart functions.

In the spectrum analysis of HR variability, the power ratio of the lower to the higher frequency component (LF/HF) is thought to be an index of cardiac sympathetic nerve activity in humans (Task 1996) and in experimental animals (Kuwahara et al. 1994). Although the frequency domains allocated within the LF and HF differ among studies, a frequency below 0.5 Hz is not found in the HF range in any study (Carson et al. 2002; Cerutti et al. 1991; Japundzic et al. 1990; Kuwahara et al. 1994; Ohnuki et al. 2001; Souza et al. 2001). A sympathetic blockade always decreases LF power and the HR reduction during immersion in CO_2-water is caused by sympathetic inhibition (Hashimoto and Yamamoto 2004). Considering those results, the decrease in LF power during immersion in CO_2-water in the present study was caused by inhibition of the sympathetic nervous system.

Stimulation received by the skin modulates cardiac sympathetic nerve activity (Drescher et al. 1982; Sato et al. 1997). For example, since the tactile stimulation of rat skin decreases mean arterial BP

and HR (Lund et al. 1999), the specific gravity of bath water augmented by NaCl might have caused HR stabilization. However, this is unlikely because sucrose and potassium solutions of the same specific gravity, and 20% sucrose solutions with a larger specific gravity did not influence HR (Fig. 7). Additionally, sodium ions appeared to play a more important role in the present phenomenon than either potassium or chloride ions, since the latter were not effective at similar concentrations to the sodium ions (Fig. 7).

If the skin NaCl was increased, nerve information that modulates BP fluctuation might be generated. When humans or experimental animals are immersed head-out for 30 - 60 min in hypertonic salt water, small mineral ions such as potassium and sodium can permeate the skin without a detectable increase in blood plasma levels (Shani et al. 1985). On the other hand, Matsuka and co-workers (Matsuka and Spigelman 2004) reported that NaCl water (360 mM) modulates the signal propagation and amplitude of the signal wave in sensory nerve fibers between dorsal root ganglia and the periphery. Considering that afferent nerve signals might be generated in the skin and that cardiovascular functions are modulated by a somatosensory mechanism (Drescher et al. 1982; Lund et al. 1999; Sato et al. 1997) (although we did not measure salt levels in the skin here), the combined effect of NaCl and CO_2 in the skin on BP fluctuation could have been induced by information conducted through the sensory nerves and the spinal cord (Yamamoto and Hashimoto 2007).

Compared with bathing in plain tap water, CO_2-water probably reduces the workload of the

heart by reducing post-heart load and HR. We previously showed that the effect of CO_2-water on heart function was due to the inhibition of sympathetic nerve activity (Hashimoto and Yamamoto 2004). In addition, the present study shows that fluctuations of sympathetic origin in the cardiovascular system might be controlled by adding a sufficient amount of NaCl to CO_2-water. This is the first study to clarify the combined effects of CO_2 and other ingredients in hot springs on cardiovascular functions. Hyperthermic treatment increases not only blood flow to the skin, but also energy metabolism. Our findings suggested that for example, blood circulation to the skin of diabetic patients with cardiac failure could be improved using CO_2-water baths without augmenting the heart load evoked by hyperthermia. In addition to cutaneous vasculature, vascular proliferation in the skeletal muscle might also be affected (Irie et al. 2005). The physiological mechanisms underlying these phenomena remain to be clarified.

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FIGURE LEGENDS

Fig. 1. Representative recordings of mean arterial blood pressure (BP), heart rate (HR) and skin blood flow (BF_{skin}) of anesthetized rat before (room air) and during immersion in tap water (tap) and CO_2-water (CO_2).

Levels of HR and BF_{skin} were low in CO_2-water compared with tap water irrespective of NaCl concentration. Fluctuation in BP is minimal during immersion in CO_2-water with 4.0% NaCl, compared with other BP recordings. Bath water was maintained at 35°C and CO_2 concentrations were 965 ~ 1,400 ppm (CO_2-water) and ~20 ppm (tap water).

Fig. 2. Effects of bathwater NaCl concentrations (0 ~ 4.0%) on heart rate (HR) and mean arterial blood pressure (BP).

Open and closed columns show mean and standard error of mean (SEM) of HR and BP in anesthetized rats during immersion in tap water and CO_2-water, respectively. Paired data of immersion tap water and in CO_2-water were generate from the same animal. Numbers in parentheses below each column pair show numbers of animals used in experiments. *, $P < 0.05$.

Fig. 3. Representative power spectrum density (PSD) acquired by FFT analysis of blood pressure (BP) fluctuations in one rat immersed in water without (A) and with (B) 4.0% NaCl.

Solid and broken lines, immersion in CO_2-water and tap water, respectively.

Fig. 4. Effects of NaCl concentrations (0 ~ 4.0%) in bath water on integrated power spectrum density (iPSD) of BP-fluctuations.

Value of iPSD was determined by integration of area under PSD line through frequency range between 0.02 and 0.195 Hz. Columns with bars shows means and SEM of BP iPSD of same animal groups shown in Fig. 2 during immersion in tap water (open columns) and in CO_2-water (closed columns). Numbers in parentheses below each column pair show numbers of animals used the experiments. *, $P < 0.05$.

Fig. 5. Effects of bathwater CO_2 and NaCl on HR variability.

A: Representative recordings of power spectrum density of HR variability of anesthetized rat immersed in CO_2-water (thin lines) and tap water (thick lines) with (broken lines) or without (solid lines) 4.0% NaCl.

B: Integrated power spectrum density (HR iPSD) of frequency range between 0.02 and 0.5 Hz calculated as described in Fig. 4. Columns with bars show mean and SEM of HR iPSD of the same group of 12 animals shown in Figs. 2 and 4. *, $P < 0.05$; **, $P < 0.01$.

Fig. 6. Effects of bathwater factors (4.0% NaCl, 20% sucrose, 4.0% KCl) on heart rate (HR), mean arterial blood pressure (BP), skin blood flow (BF_{skin}) and skin vascular resistance (VR_{skin}=BP/ BF_{skin}).

Columns with bars show mean and SEM of each parameter recorded from anesthetized rats during immersion in tap water (open columns) and CO_2-water (closed columns). Numbers in parentheses at the bottom of the figure show numbers of animals used in experiments. *, $P < 0.05$.

Fig. 7. Effects of BP-fluctuations of bath water ingredients (4.0% NaCl, 4.0% and 20%sucrose, 4.0% KCl) on integrated power spectrum density (BP iPSD).

Columns with bars show means and SEM of BP iPSD acquired from same animal groups shown in Fig. 6. Numbers in parentheses below each column pair show numbers of animals used in experiments. Open columns, tap water; closed columns: CO_2-water. *, $P < 0.05$.

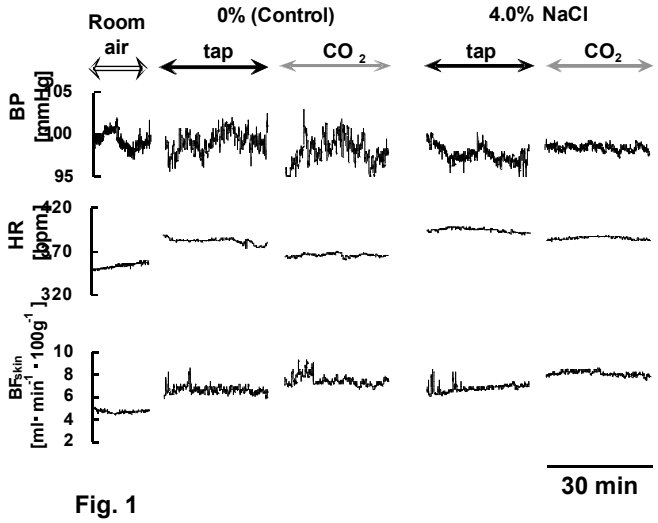
Room air
0% (Control)
tap
CO 2
4.0% NaCl
tap
CO2
BP [mmHg]
105
100
95
HR [bpm]
120
70
20
BFskin [ml · min-1 · 100g-1]
10
8
6
4
2
30 min

Fig. 1

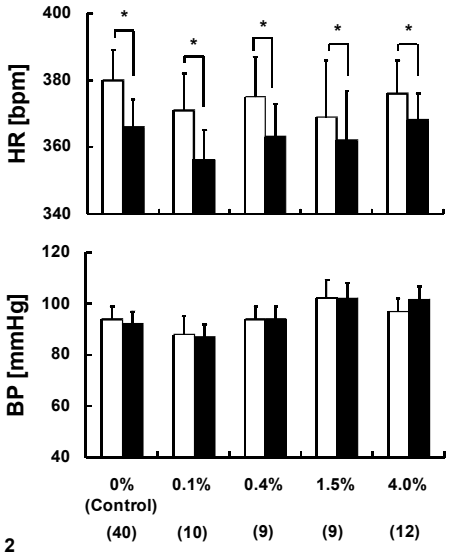
HR [bpm]
400
380
360
340
BP [mmHg]
120
100
80
60
40
*
0%
(Control)
0.1%
0.4%
1.5%
4.0%
(40)
(10)
(9)
(9)
(12)

Fig. 2

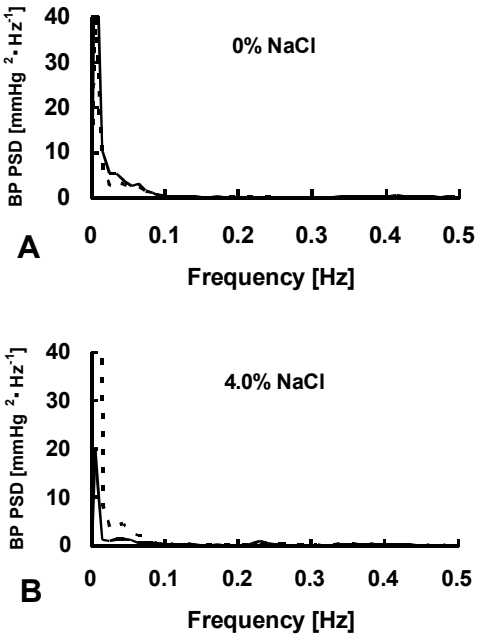
BP PSD [mmHg ².Hz⁻¹]
40
30
20
10
0
0% NaCl
0
0.1
0.2
0.3
0.4
0.5
A
Frequency [Hz]
BP PSD [mmHg ².Hz⁻¹]
40
30
20
10
0
4.0% NaCl
0
0.1
0.2
0.3
0.4
0.5
B
Frequency [Hz]

Fig. 3

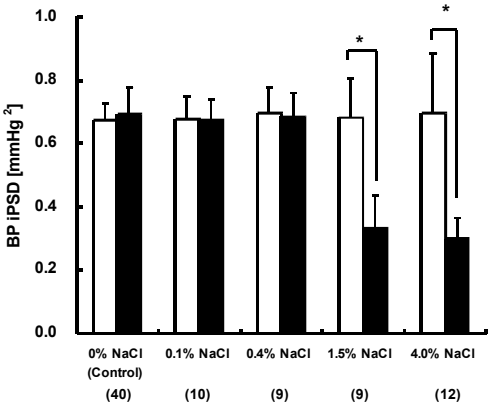
1.0
0.8
0.6
0.4
0.2
0.0
BP iPSD [mmHg 2]
*
*
0% NaCl
(Control)
0.1% NaCl
0.4% NaCl
1.5% NaCl
4.0% NaCl
(40)
(10)
(9)
(9)
(12)

Fig. 4

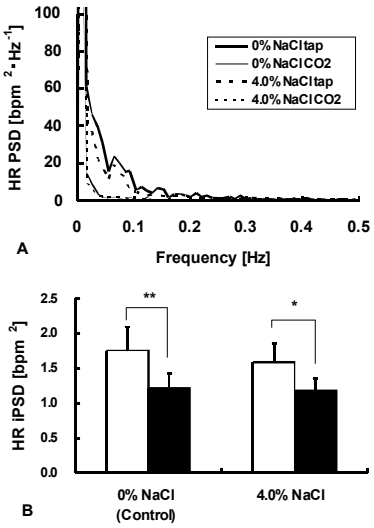
100
80
60
40
20
0
HR PSD [bpm 2 ·Hz -1]
0% NaCl tap
0% NaCl CO2
4.0% NaCl tap
4.0% NaCl CO2
0
0.1
0.2
0.3
0.4
0.5
Frequency [Hz]
A
2.5
2.0
1.5
1.0
0.5
0.0
HR iPSD [bpm 2]
**
*
0% NaCl
(Control)
4.0% NaCl
B

Fig. 5

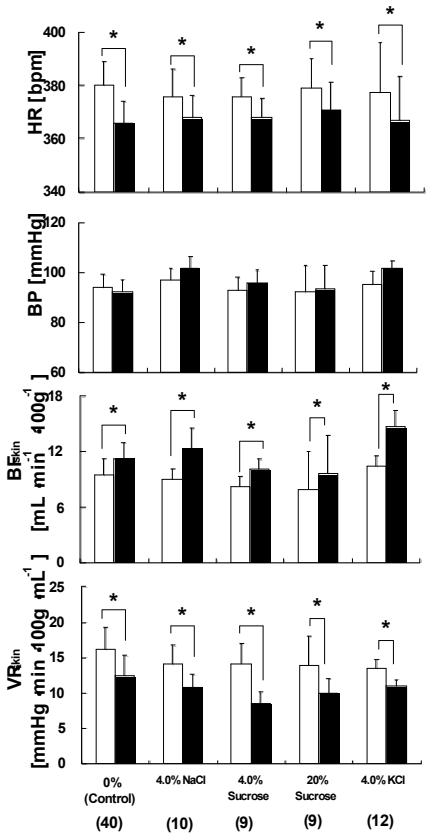
HR [bpm]
400
380
360
340
BP [mmHg]
120
100
80
60
BF_{skin} [mL min^{-1} 400g^{-1}]
18
12
6
0
VR_{skin} [mmHg min 400g mL^{-1}]
25
20
15
10
5
0
0% (Control)
4.0% NaCl
4.0% Sucrose
20% Sucrose
4.0% KCl
(40)
(10)
(9)
(9)
(12)

Fig. 6

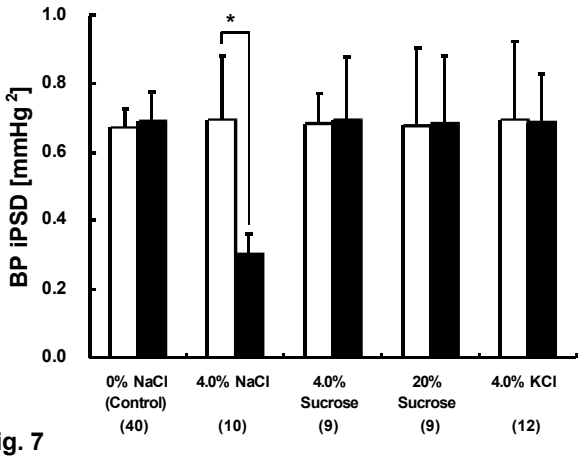
1.0
0.8
0.6
0.4
0.2
0.0
BP iPSD [mmHg 2]
*
0% NaCl
(Control)
(40)
4.0% NaCl
(10)
4.0%
Sucrose
(9)
20%
Sucrose
(9)
4.0% KCl
(12)

Fig. 7