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Spinal cord transection inhibits HR reduction in anesthetized rats immersed in an artificial CO2-hot spring bath

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Spinal cord transection inhibits HR reduction in anesthetized rats immersed in an artificial CO_2-hot spring bath

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Running head: Afferent pathway in CO_2-water immersion evokes HR reduction

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Abstract

Like humans, the heart rate (HR) of anesthetized rats immersed in CO_2-water is lower than that when immersed in tap water at the same temperature. To investigate the afferent signal pathway in the mechanism of HR reduction, Wistar rats were anesthetized with urethane and then the spinal cord was transected between T_4 and T_5. The animals were immersed up to the axilla in a bathtub of tap-water (CO_2 contents: 10-20 $mg \cdot L^{-1}$) or of CO_2-water (965-1400 $mg \cdot L^{-1}$) at 35°C while recording HR, arterial blood pressure, and arterial blood gas parameters ($PaCO_2$, PaO_2, pH). Arterial blood gas parameters did not change during immersion irrespective of CO_2 concentration of the bath water, whereas the HR was reduced in the CO_2-water bath. The inhalation of CO_2-mixed gas (5 % CO_2, 20% O_2, 75 % N_2) resulted in increased levels of blood gases and an increased HR during immersion in all types of water tested. The HR reduction observed in sham transected control animals immersed in CO_2-water disappeared after subsequent spinal cord transection. These results show that the dominant afferent signal pathway to the brain, which is involved in inducing the reduced HR during immersion in CO_2-water, is located in the neuronal route and not in the bloodstream.

Key words: blood gas, HR reduction, cardiovascular modulation, CO_2 balneotherapy, neuronal afferent pathway

Introduction

Balneotherapy and spa therapy emerged as important European treatment modalities during the 1800s (Matz *et al.* 2003) that are still practiced in many countries today. Carbon dioxide (CO_2) balneotherapy using hot springs containing free CO_2 at concentrations above 1 $g \cdot L^{-1}$ (ppm) (CO_2-hot spring) has long been clinically applied to improve cardiovascular symptoms (Savin *et al.* 1995; Toriyama *et al.* 2002). The effects of percutaneously applied CO_2-hot spring water on humans have been documented (Diji 1959; Schnizer *et al.* 1985), and the list of effects now includes heart rate (HR) reduction, a slight decrease in blood pressure, and increased blood flow in the skin (Dorrance 1940; McClellan and Doulin 1944). Although CO_2-hot springs contain various mineral ingredients depending on geographical location, the effects of CO_2-hot spring baths appear to be attributable to the high-CO_2 concentrations. Accordingly, artificial bath water containing high CO_2 concentrations (CO_2-water) produces similar effects on physiological functions, such as increased blood flow to the skin (Nishimura *et al.* 2002; Toriyama *et al.* 2002).

Changes in human physiological functions while bathing in CO_2-hot springs have also been clinically investigated (Stein 1942; McClellan 1963; Hartmann *et al.* 1997; Hartmann *et al.* 1997; Toriyama *et al.* 2002). However, the detailed mechanism of action of percutaneously applied CO_2 on physiological functions remains to be elucidated because of ethical restrictions on such research. In addition, the

difficulties associated with promptly generating sufficient quantities of CO_2-water to fill a bathtub and maintaining high-CO_2 concentrations during the experiments also seem to have obstructed research progress in laboratories located far from natural springs. However, such difficulties can now be overcome using a novel apparatus to produce artificial CO_2-water (Hashimoto and Yamamoto 2004). The CO_2-water used in our previous study increased blood flow to immersed rat skin in a similar manner to that of CO_2-hot spring baths on human skin, suggesting that the effects of the artificial CO_2-water are comparable to those of natural CO_2-hot spring water. Although the skin of rats does not become red, immersion in CO_2-water does result in increased blood flow to the skin and decreased vascular resistance compared with immersion in tap-water (Hashimoto and Yamamoto 2004).

We previously reported that HR of rats was lower during immersion in CO_2-water than in tap-water, and the lowered HR is probably achieved through the inhibition of sympathetic nerve activity rather to the facilitation of parasympathetic nerve activity (Hashimoto and Yamamoto 2004). Compared with efferent mechanisms, the afferent signal route that evokes a lowered HR remains unclear. To lower the HR, information about percutaneous invasion by CO_2 (Komoto *et al.* 1986) such as an increase in CO_2 gas and/or neuronal signals evoked by CO_2, must be transported to the brain. We postulated that signals regarding cutaneous CO_2 levels are produced in the skin and then transported to the brain through the neuronal route.

Thus, the present study investigates the afferent pathway involved in the induction of a lowered HR during immersion in CO_2-hot-springs, using laboratory animals and artificial CO_2-water.

Materials and Methods

The Committee for Animal Experiments at Asahikawa Medical University approved the experimental protocol, 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 (body weight, 269-345 g; n=18) were separated into 3 groups for a blood gas analysis experiment (n=6), for an immersion after spinal cord transection experiment (n=6) and for an evaluation of the transection experiment (n=6). Animal preparation in detail was described elsewhere (Hashimoto and Yamamoto 2004). Briefly, the animals were anesthetized with urethane (1.39-1.60 $g \cdot kg^{-1}$ body weight, i.p.) throughout the experiments, and prepared for measurement of the HR, arterial blood pressure (BP), skin blood flow (BF_{skin}), colon and skin temperatures and immersed in bath water. All wounds immersed in the water were sealed with acrylic resin adhesive to prevent water infiltration. Accomplishment of the wound sealing was confirmed after the experiment.

Immersion

Water containing a high CO_2 concentration (965-1400 ppm, 35°C) was generated using an MRE-Spa

(Hashimoto and Yamamoto 2004). The animals were loosely fixed to plastic lattice-plates in the head-up position of about 30° to the horizontal in a polycarbonate bathtub (30 x 20 x 15 cm). The lower half of the rat body was immersed into CO_2-water or tap-water (control) for 30 minutes at 35°C. Thereafter, the water was quickly siphoned off and replaced with that containing a different CO_2 concentration at the same temperature. Fresh air was constantly supplied to the face of the animals to minimize the inhalation of CO_2 that diffused from the bath water.

Blood gas analysis during immersion

While bathing in CO_2-hot springs, about 100-fold more CO_2 (about 30 $mL \cdot min^{-1} \cdot m^{-2}$) is absorbed through the skin surface than in regular water (Pratzel *et al.* 1984). Moreover, CO_2 that diffuses from the surface of CO_2-hot spring water might be inhaled. We therefore analyzed blood gases to clarify whether CO_2-water immersion affects blood gas contents, and how the inhalation of CO_2 during immersion affects cardiovascular functions.

We initially confirmed that the HR was lower during immersion in CO_2-water than in tap-water while breathing fresh air, and then measured hemodynamic parameters and blood gases. We also investigated the effect of hypercapnia on the cardiovascular functions of anesthetized rats under a hydrostatic pressure load by immersion up to the axilla. CO_2-mixed gas (5 % CO_2, 20% O_2, 75 % N_2) instead of fresh air was supplied to the area around the face and then the same parameters were measured. The blood was drawn

(about 100 µl) through a polyethylene catheter in the right femoral artery by using a heparinized glass capillary pipet at the end of each immersion period, and the blood gas parameters (pH, oxygen partial pressure; PaO_2 and carbon dioxide partial pressure; $PaCO_2$) during immersion in both tap-water and CO_2-water were monitored using a blood gas analyzer (Rapidlab 850, Bayer, Leverkusen, Germany). The total amount of the blood sampled from each animal did not exceed 0.8 ml.

Responses to immersion in CO_2-water after spinal cord transection

We transected the spinal cord to preserve a major cardiac branch of the sympathetic nervous system and to disconnect the input neuronal route from the immersed skin. A midline incision (about 2 cm) was cut on the dorsal skin over the thoracic vertebrae (T_2-T_6), and the dura matter was exposed between T_4 and T_5 in 6 animals with all probes attached. After the laminectomy, the skin incisions were temporarily closed with small Bulldog-type clamps positioned above the bath water level. The animals were then immersed in the bathtub and recordings were taken. After the HR reduction during immersion in CO_2-water was ascertained, the plastic plate with the animals was removed from the bath water and placed horizontally over the bathtub. Sham spinal cord transection then proceeded as follows.

The clamps closing the skin wounds were removed, the incision was reopened, the spinal cord between T_4 and T_5 was exposed but not transected, and then the skin was closed with the same clamps. The animals were then challenged using the immersion protocol described above. Thereafter, control

measurements were taken, the animals were removed from the water again, and the spinal cord between T_4 and T_5 was transected using a surgical blade (No.11, Feather Inc. Tokyo, Japan). The wounds were closed, the animals were again immersed in the bathtub and the effects of the spinal cord transection (SCT) on the HR were examined. The experiments started about 30 min after SCT when the HR stabilized. Successful SCT was determined as the absence of a reaction to pain (BP increase) evoked by pinching the skin below the transection with toothed forceps. After the experiment, the site and range of the transection in the spinal cord were anatomically confirmed. All experiments proceeded in an air-conditioned room (26 ± 1 °C, 65 ± 10 %).

Figure 1 summarizes the schedules of the water exchanges in the bathtub and experimental procedures.

Evaluation of effect of SCT on cardiac sympathetic nerve activity

To evaluate the influence of SCT on cardiac sympathetic nerve activity, we examined baroreflex modulation of cardiac functions and the effects of the autonomic nervous system blockade in a group of 6 male Wistar rats. A femoral vein was cannulated with polyethylene tubing for drug administrations, and the common carotid arteries of both sides were exposed and loosely tied with a silk suture (size 5). Bilateral carotid artery occlusion (CAO) was established by pulling both ends of the sutures through a short polyethylene tube for 15 sec. Following a bolus intravenous (i.v.) injection of atropine sulfate (parasympathetic blocker, Sigma, 1 $mg \cdot ml^{-1} \cdot kg^{-1}$ body weight), an additional dose was infused (30 μg·77

$\mu l^{-1} \cdot kg^{-1} \cdot h^{-1}$, i.v.) using a pump (IP-21, Nikkiso, Tokyo, Japan) until the end of the experiment. When all recorded parameters were stabilized, the first baroreflex responses were induced by a 15 s-CAO. After BP and HR recovered from the first CAO, control sham-transection was performed as described above, and the CAO was repeated. Thereafter, SCT proceeded as described for the immersion experiments, and then the baroreflex responses to BP and HR was examined. Following these procedures, atenolol (heart-selective sympathetic blocker, 100 $\mu g \cdot kg^{-1}$; 100 µl) was intravenously infused for 1 min, and the contribution of the cardiac sympathetic nerve to the baroreflex in HR was evaluated. The doses of these autonomic nerve antagonists were selected based on published dose-response relationships to induce maximal inhibition (atenolol) or facilitation (atropine) of HR in the rats (Merrick *et al.* 1979; Tabrizchi *et al.* 1988; Hashimoto and Yamamoto 2004). At the end of the experiment, a bolus injection of atropine sulfate (1 $mg \cdot kg^{-1}$, i.v.) was administered to evaluate whether cardiac parasympathetic activities had been negligible throughout the experiment. The colon temperature of the anesthetized animals was maintained within the range of 35-36°C throughout the experiment using a heat-pad (BWT-100, Bio Research Center Co. Ltd., Nagoya, Japan) and a blanket.

Data analysis

All signals obtained during the immersion experiments were captured and stored every second using a personal computer (PC9801, NEC, Tokyo, Japan) and simultaneously recorded using an R-66 multi-pen

recorder (Rika Denki, Tokyo, Japan) on chart paper. To evaluate the effect of CO_2-water immersion on the skin vascular system, the BP divided by BF_{skin} was determined as an index of vascular resistance (VR_{skin}). For statistical analysis, data stored for 20 min starting from 10 min after the bath water exchanges were averaged to establish representative values during bath immersion. When CO_2-water immersion or tap-water immersion was repeatedly performed, a representative value of each parameter recorded during CO_2-water immersion or tap-water immersion was calculated from more stable recordings. Data were statistically analyzed by two-way ANOVA with repeated measures followed by pair-wise contrasts using the Newman-Keuls post-hoc test. Differences (means ± SEM) were considered significant at $P < 0.05$.

Results

The temperatures of the colon and skin of all animals before the first immersion were 35.9 ± 0.3°C (n=18) and 33.1 ± 1.1°C, respectively. The HR was 364 ± 8 beats·min^{-1}, BF_{skin} was 4.8 ± 0.5 ml·min^{-1}·100 g^{-1} and the BP was 83 ± 6 mmHg. The pH, PaO_2 and $PaCO_2$ (n=6) before the first immersion were 7.34 ± 0.03, 92.3 ± 2.30 mmHg and 48.9 ± 0.74 mmHg, respectively.

Compared with immersion in tap-water, the BF_{skin} significantly increased (28.2 ± 5.0%), and the HR significantly decreased by 4.3 ± 1.1% while the BP did not change during immersion in CO_2-water. A large fluctuation recorded for some parameters after each water exchange disappeared within 10 min. The

temperatures of the colon and skin during CO_2-water immersion were 37.1 ± 0.2°C and 35.7 ± 0.2°C, respectively, and were identical to the values obtained during immersion in tap-water.

Blood gas analysis during immersion

Table 1 summarizes the results of the blood gas analysis experiments. Neither the pH nor the blood gases (PaO_2 and $PaCO_2$) were affected by immersion in either tap-water or CO_2-water when the animals inhaled fresh air.

Respiratory frequency and depth seemed to increase after changing from fresh air to CO_2 mixed-air inhalation in the immersed rats both in tap-water and in CO_2-water. While breathing 5%-CO_2 for 5 min, blood gases of the animals immersed in tap-water significantly increased by 29.9 ± 4.3% (PaO_2) and 36.8 ± 3.9% ($PaCO_2$). Similarly, the PaO_2 and $PaCO_2$ of the animals immersed in CO_2-water and breathing CO_2 increased by 29.0 ± 2.5% and 27.1 ± 3.0%, respectively. The blood pH, on the other hand, significantly decreased during immersion in both tap-water and CO_2-water. Compared with fresh air inhalation, the HR of animals breathing CO_2 mixed-air increased by 4.4 ± 0.8% (tap-water) and 6.7 ± 0.2% (CO_2-water), but these differences did not reach statistical significance.

Responses to immersion after SCT

Figure 2 shows representative recordings of HR, BP and BF_{skin}. No sudden temporal movements, such as hind leg extension, occurred before (the sham operation) or after SCT. Before and after sham SCT,

the rat HR in CO_2-water was larger than that in tap-water in all animals. Though an increase in BF_{skin} was not always observed by changing bath-water from tap-water to CO_2-water, VR_{skin} was always smaller in CO_2-water than in tap-water. After the SCT, the basal level (during tap water immersion) of BF_{skin} increased and VR_{skin} decreased, and the effect of CO_2-water immersion on these parameters disappeared as well as on HR.

Figure 3 summarizes the effects of SCT on the changes in the HR and BP induced by immersion in CO_2-water. The HR before SCT during CO_2-water immersion was significantly smaller (2.7 ± 0.7%) than that during tap-water immersion (Fig. 3A), but this difference disappeared after SCT. Base-line level of mean BP remained constant during immersion in both tap-water and CO_2-water before and after SCT (Fig. 3B).

Before SCT, the values of BF_{skin} were 8.7 ± 2.0 and 10.0 ± 2.2 $ml \cdot min^{-1} \cdot 100g^{-1}$ during immersion in tap-water and in CO_2-water, respectively. The BF_{skin} during immersion in tap-water significantly increased after SCT (by 19.1 ± 5.1%), and the increased BF_{skin} caused by immersion in CO_2-water disappeared after SCT. These changes in BF_{skin} were reflected in the VR_{skin} (Fig. 3C) immersed in tap-water that was significantly decreased by SCT (14.7 ± 4.4%). The reduced VR_{skin} (9.5 ± 2.1%) of the control animals induced by immersion in CO_2-water disappeared after SCT.

Evaluation of the influence of SCT on cardiac sympathetic nerve activity

The representative recording in Figure 4 shows that baroreflex increases in BP and HR in response to common carotid artery occlusion (CAO) occurred in all animals. Table 2 summarizes the influence of SCT on the changes of BP and HR in response to an autonomic blockade and CAO. The first intravenous administration of atropine in the experiment produced a statistically significant HR increase of about 6%, while BP did not change. However, atenolol administration decreased HR by about 17% ($p<0.05$) without any significant BP changes. Neither HR nor BP was influenced by SCT under these experimental conditions. Figure 5 summarizes the HR and BP responses to CAO. Significant increases in HR were evoked by CAO before SCT (11.8%, $p<0.05$) as well as after SCT (10.2%, $p<0.05$), but the differences were not statistically significant. Figure 5 shows that atenolol suppressed the baroreflex-induced HR increase that was significantly sustained after SCT ($p<0.01$). The degree of the baroreflex BP increase induced by CAO did not statistically differ among experiments irrespective of SCT and autonomic blockade.

Discussion

Immersion did not affect arterial blood gases or pH in animals inhaling fresh air, irrespective of the CO_2 concentration in the water, whereas HR was reduced in rats immersed in CO_2-water (Table. 1). When the rats breathed air containing 5%-CO_2, the HR and $PaCO_2$ of those immersed in tap-water significantly increased. Irrespective of the consciousness state of the rats, response to HR either decreases

or does not change in response to CO_2 inhalation ranging from mild (3-6%) to severe (12%) hypercapnic levels under general room conditions (Walker 1987; Greenberg *et al.* 1999; Hirakawa and Hayashida 2002; Krohn *et al.* 2003). In contrast to these findings, we induced an increase in the HR of anesthetized rats during water immersion. On the other hand, many studies have shown that water immersion in a thermoneutral environment induces a reduced HR in both humans (Perini *et al.* 1998; Pump *et al.* 2001) and other animals (Yoshino *et al.* 1988; Cornish *et al.* 1999). Although the mechanism remains ambiguous, when taking this circumstantial evidence into consideration, CO_2 inhalation combined with immersion either in tap-water or CO_2 water might have induced the HR increase. Except for the variety of HR responses, one coincidence among the different experimental conditions was that the $PaCO_2$ indicated hypercapnea in all the animals. However, we found that the $PaCO_2$ level was not significantly elevated in rats immersed in CO_2-water while breathing fresh air. Furthermore, considering that the HR was lower in rats immersed in CO_2-water than in tap-water under 5%-CO_2 inhalation, the HR decrease induced by CO_2-water immersion in rats breathing fresh air was not apparently induced by these blood parameters.

To intercept possible afferent signals conducting information about increased CO_2 invasion in the skin, we performed SCT. The HR reduction observed during CO_2-water immersion disappeared after SCT at T_4-T_5 (Fig. 3). These results imply that stimulation by CO_2-water immersion is received by a mechanism in the skin. This information is conducted to the brain through the spinal cord, which results in

a reduction of cardiac sympathetic activity (Hashimoto and Yamamoto 2004). Usually, SCT at this level does not directly affect cardiac functions because the dominant preganglionic sympathetic nerve to the heart passes at a higher level of the spinal cord in rats (Pardini *et al.* 1989; Baldridge *et al.* 2002). Baldridge and co-workers (2002) observed that cardiac function was facilitated after SCT at the same level in anesthetized rats. However, they postulated that the HR increase is not a primary response to the SCT but a secondary baroreflex cardiac response induced by a decrease in the blood pressure caused by vascular atonia after SCT. If SCT indeed increases the HR and compensates for the HR reduction induced by immersion in CO_2-water, then an HR decrease after SCT and immersion in CO_2-water might have been undetectable under our conditions.

In the last series of experiments that evaluated the influence of SCT on the cardiac sympathetic nervous system, a parasympathetic antagonist (atropine) was continuously infused throughout the experiment. The inhibitory effect of atropine upon the cardiac parasympathetic nerves is thought to have been functionally maintained throughout the experiment, since a bolus injection of atropine administered at the end of the experiment did not increase the HR. Though a baroreflex HR increase can be achieved not only by sympathetic nerve activation but also by cardiac parasympathetic nerve inhibition (Brezenoff *et al.* 1982; Ferrari *et al.* 1991), our results indicated that the CAO-evoked HR increase was controlled by the sympathetic nervous system under our conditions. Even if the HR did not change after SCT it would not

mean that the cardiac sympathetic nerve was unaffected by the transection, because the parasympathetic effects could compensate for the HR changes. This is unlikely however, since a parasympathetic blockade was maintained throughout the experiment. The finding of an unchanged HR after SCT agrees with the notion that SCT does not inhibit cardiac sympathetic nerve activity.

The HR decrease induced by atenolol in animals after SCT suggests that SCT did not damage the tonic activity of the cardiac sympathetic nerve. The fact that the HR response to CAO was not influenced by the spinal cord transection also supports this notion. In addition, the inhibition of the baroreflex response to HR by atenolol showed that the sympathetic nervous system through which cardiac functions are modulated, was functionally unaffected by SCT. All the results of this additional experiment showed that the regulatory function of the cardiac sympathetic nervous system is maintained after SCT.

All of the present results indicate that neuronal signaling through the spinal cord is the dominant source of the decreased HR of rats immersed in CO_2-water, rather than humoral factors in the bloodstream. Information about CO_2 levels in the skin is probably generated in and/or around the skin, and then transported to the brain through the spinal cord. Electrophysiological observations show that cold nerve activity in cat skin is inhibited by CO_2 (Dodt 1956) and that warm neurons in the preoptic area of the rat hypothalamus are facilitated by CO_2 (Tamaki *et al.* 1989). Cold exposure generally induces a metabolic increase that results in an increased HR (Fisher *et al.* 1985), and also suggests that inhibition of the cold

sensation would reduce HR. Our findings also imply that thermosensitive neurons in the skin are candidates for the input signal generator. However, whether neurons and receptor mechanisms actually generate information regarding CO_2 invasion into the skin tissue from bath water containing high concentrations of CO_2 remains to be addressed in future studies.

The present results also suggest that bathing in CO_2-hot springs when patients have cardiovascular diseases and spinal cord damage is a valid therapy. To standardize the clinical effects of bathing in CO_2-hot springs, the basic mechanism of action should be further analyzed.

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References

Baldridge BR, Burgess DE, Zimmerman EE, Carroll JJ, Sprinkle AG, Speakman RO, Li SG, Brown DR (2002) Heart rate-arterial blood pressure relationship in conscious rat before vs. after spinal cord transection. Am J Physiol Regul Integr Comp Physiol 283(3):R748-756.

Brezenoff HE, Carney K, Buccafusco JJ (1982) Potentiation of the carotid artery occlusion reflex by a cholinergic system in the posterior hypothalamic nucleus. Life Sci 30: 391-400.

Cornish KG, Hughes K, Dreessen A, Olguin M (1999) Head-out immersion in the non-human primate: a model of cardiovascular deconditioning during microgravity. Aviat Space Environ Med 70: 773-779.

Diji A (1959) Local vasodilator action of carbon dioxide on blood vessels of the hand. J Appl Physiol 14: 414-416.

Dodt E (1956) Die Aktivität der Thermoreceptoren bei nicht-thermischen Reizen bekannter thermoregulatorischer Wirkung. Pflügers Arch 263: 188-200.

Dorrance C, McClellan WS (1940) Effect of natural carbonated baths on rate and amplitude of pulse and blood pressure. Archives of Physical Therapy 21: 133-140.

Ferrari AU, Daffonchio A, Albergati F, Mancia G (1991) Differential effects of aging on the heart rate and blood pressure influences of arterial baroreceptors in awake rats. J Hypertens 9: 615-621.

Fisher LA, Cave CR, Brown MR (1985) Central nervous system effects of bombesin on the cardiovascular

response to cold exposure. Brain Res 341: 261-268.

Greenberg HE, Sica A, Batson D, Scharf SM (1999) Chronic intermittent hypoxia increases sympathetic responsiveness to hypoxia and hypercapnia. J Appl Physiol 86: 298-305.

Hartmann BR, Bassenge E, Hartmann M (1997) Effects of serial percutaneous application of carbon dioxide in intermittent claudication: results of a controlled trial. Angiology 48: 957-963.

Hartmann BR, Bassenge E, Pittler M (1997) Effect of carbon dioxide-enriched water and fresh water on the cutaneous microcirculation and oxygen tension in the skin of the foot. Angiology 48: 337-343.

Hashimoto M, Yamamoto N (2004) Decrease in heart rates by artificial CO_2 hot spring bathing is inhibited by beta1-adrenoceptor blockade in anesthetized rats. J Appl Physiol 96: 226-232.

Hirakawa H, Hayashida Y (2002) Responses of autonomic cardiovascular systems induced by hypoxia: rebound phenomenon and species difference. J Uoeh 24: 117-129.

Komoto Y, Komoto T, Sunakawa M, Eguchi Y, Yorozu H, Kubo Y (1986) Dermal and subcutaneous tissue perfusion with a CO_2 -bathing. Z Physiother Jg 38: 103-112.

Krohn TC, Hansen AK, Dragsted N (2003) The impact of low levels of carbon dioxide on rats. Lab Anim 37: 94-99.

Matz H, Orion E, Wolf R (2003) Balneotherapy in dermatology. Dermatol Ther 16: 132-140.

McClellan W (1963) Carbon Dioxide Baths. In: Lights S (ed) Physical Medicine Library New Haven,

Elizabeth Licht, pp 311-320.

McClellan WLM, Doulin AT (1944) Physiologic effects of carbon dioxide water baths on alveolar carbon dioxide tension, skin temperature, and respiratory metabolism. American Heart Journal 29: 44-61.

Merrick A, Hadley WM, Holcslaw TL (1979) The effect of large doses of atropine sulfate on heart rate and blood pressure in rats. Res Commun Chem Pathol Pharmacol 25: 13-22.

Nishimura N, Sugenoya J, Matsumoto T, Kato M, Sakakibara H, Nishiyama T, Inukai Y, Okagawa T, Ogata A (2002) Effects of repeated carbon dioxide-rich water bathing on core temperature, cutaneous blood flow and thermal sensation. Eur J Appl Physiol 87: 337-342.

Pardini BJ, Lund DD, Schmid PG (1989) Organization of the sympathetic postganglionic innervation of the rat heart. J Auton Nerv Syst 28: 193-201.

Perini R, Milesi S, Biancardi L, Pendergast DR, Veicsteinas A (1998) Heart rate variability in exercising humans: effect of water immersion. Eur J Appl Physiol 77: 326-332.

Pratzel H. Aufnahme, Abgabe Stoffwechsel von (1984) CO_2 beim Kohlensäurebad. Z phys Med Blan Med Klim 13: 25-32.

Pump B, Shiraishi M, Gabrielsen A, Bie P, Christensen NJ, Norsk P (2001) Cardiovascular effects of static carotid baroreceptor stimulation during water immersion in humans. Am J Physiol Heart Circ Physiol 280: H2607-H2615.

Savin E, Bailliart O, Bonnin P, Bedu M, Cheynel J, Coudert J, Martineaud JP (1995) Vasomotor effects of transcutaneous CO_2 in stage II peripheral occlusive arterial disease. Angiology 46: 785-791.

Schnizer W, Erdl R, Schops P, Seichert N (1985) The effects of external CO_2 application on human skin microcirculation investigated by laser Doppler flowmetry. Int J Microcirc Clin Exp 4: 343-350.

Stein ID, Weinstein I (1942) The value of carbon dioxide baths in the treatment of peripheral vascular disease and allied conditions. Am Heart J 23: 349-361.

Tabrizchi R, King KA, Pang CC (1988) Pressor response to beta 1- and beta 2-blockers in conscious rats treated with phentolamine. Pharmacology 37: 385-393.

Tamaki Y, Nakayama T, Kanosue K (1989) Effects of peripheral chemo- and baro-receptor denervation on responses of preoptic thermosensitive neurons to inspired CO_2. Pflügers Arch 414: 495-499.

Toriyama T, Kumada Y, Matsubara T, Murata A, Ogino A, Hayashi H, Nakashima H, Takahashi H, Matsuo H, Kawahara H (2002) Effect of artificial carbon dioxide foot bathing on critical limb ischemia (Fontaine IV) in peripheral arterial disease patients. Int Angiol 21: 367-373.

Walker BR (1987) Cardiovascular effect of V1 vasopressinergic blockade during acute hypercapnia in conscious rats. Am J Physiol Regul Integr Comp Physiol 252: R127-R133.

Yoshino H, Curran-Everett DC, Hong SK, Krasney JA (1988) Altered heart rate-arterial pressure relation during head-out water immersion in conscious dog. Am J Physiol Regul Integr Comp Physiol 254:

R595-R601.

Table 1. Effect of fresh air and CO_2 mixed gas inhalation on hemodynamic parameters and blood gas of anesthetized rats immersed in tap- and CO_2-water.

	BP [mmHg]	**HR [bpm]**	**BF_{skin} [mL·min^{-1}· 100g^{-1}]**	**VR_{skin} [mmHg·min· 100g·mL^{-1}]**	**pH**	**PaO_2 [mmHg]**	**$PaCO_2$ [mmHg]**
Fresh air							
Tap-water (Ft)	**104±9**	**390±15**	**3.9±09**	**24.7±2.2**	**7.38±0.01**	**87.9±7.8**	**43.7±1.9**
CO_2-water (Fc)	**97±9**	**374±14[a]**	**5.0±09[a]**	**19.2±2.9[a]**	**7.35±0.02**	**90.4±8.0**	**44.0±2.8**
Air containing 5 % CO_2							
Tap-water (Ct)	**114±5**	**407±18[b]**	**4.0±0.8**	**25.4±2.8[b]**	**7.24±0.04[b]**	**114.2±4.0[b]**	**59.8±3.6[b]**
CO_2-water (Cc)	**107±9**	**399±15[c]**	**4.5±1.0**	**23.4±4.2[c]**	**7.25±0.03[c]**	**116.6±5.7[c]**	**55.9±1.4[c]**

Values are means ± SEM from 2 groups of 6 rats each. BP, mean arterial blood pressure; HR, heart rate; BF_{skin}, immersed skin tissue blood flow; VR_{skin}, vascular resistance of immersed skin; pH, arterial blood pH; PaO_2, arterial blood partial pressure of oxygen; $PaCO_2$, arterial blood partial pressure of carbon dioxide. Statistical significance ($p < 0.05$); [a], Fc compared with Ft; [b], Ct compared with Ft; [c], Cc compared with Fc.

Table 2. Heart rate (HR) and mean arterial blood pressure (BP) changes in response to bilateral carotid artery occlusion for 15s (CAO) before and after spinal card transection (SCT) and i.v administration of autonomic antagonist (atropine, atenolol).

Treatment		HR〔bpm〕	BP〔mmHg〕
Atropine	Before	372 ± 10	57 ± 1
	After	394 ± 8*	56 ± 2
CAO	Before	391 ± 9	58 ± 1
	At peak	437 ± 10*	80 ± 3*
SCT	Before	391 ± 9	58 ± 1
	After	392 ± 10	60 ± 2
CAO (after SCT)	Before	392 ± 10	60 ± 2
	At peak	432 ± 13*	79 ± 4*
Atenolol (after SCT)	Before	391 ± 10	59 ± 2
	After	334 ± 7*	59 ± 3
CAO after Atenolol (after SCT)	Before	334 ± 7	58 ± 2
	At peak	347 ± 8	72 ± 3*
Atropine at the end (after SCT)	Before	342 ± 13	59 ± 2
	After	349 ± 15	53 ± 1

Values are means ± SEM (n=6). *, statistically significant difference ($p < 0.05$) from previous value at each treatment.

Figures (with Legends)

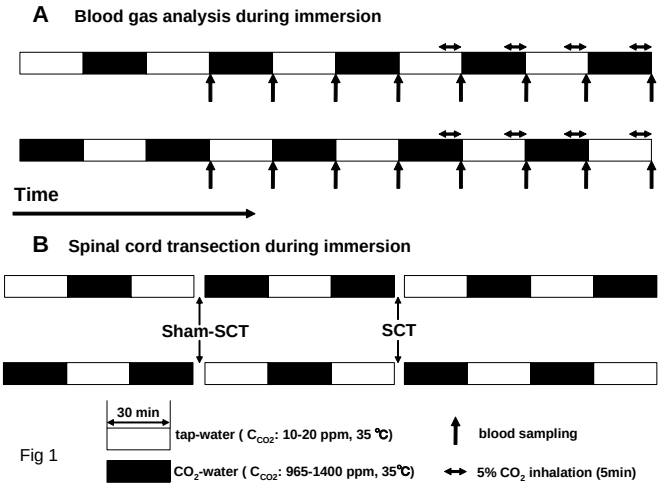

Fig 1

Fig. 1. Schedule of water immersion, blood gas analysis (A) and spinal cord transection (SCT, B). To avoid effect s of this order, the starting bathtub water was randomly tap- or CO_2-water in each experiment.

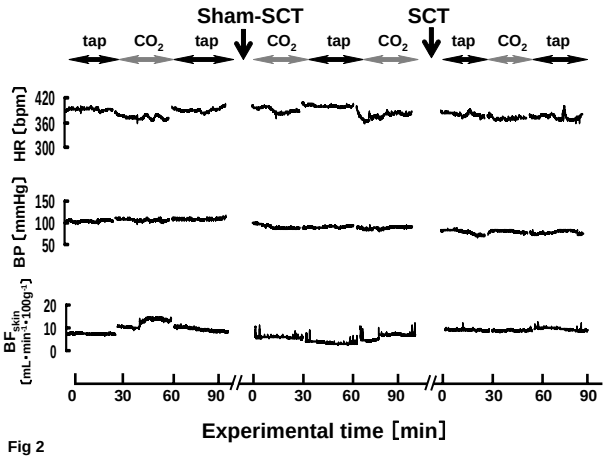

Fig. 2. Representative recordings of heart rate (HR), mean blood pressure (BP) and skin tissue blood flow in the skin (BF_{skin}) from anesthetized rat during spinal cord transection. Bathtub water temperature was maintained at 35°C throughout. Arrows show time of sham (Sham SCT) and spinal cord (SCT) transections. CO_2, water containing 965～1400 ppm CO_2; tap, normal tap-water containing ～20 ppm CO_2.

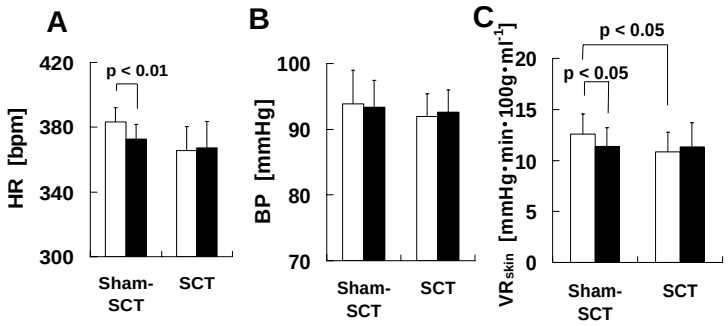

Fig 3

Fig. 3. Effect of spinal cord transection (SCT) on heart rate reduction during immersion in CO_2–water. Effects of SCT on heart rate (HR), mean blood pressure (BP) and resistance index of blood vessels in immersed skin (VR_{skin}) before (Sham-SCT) and after (SCT) transection. Values are means ± SEM from same group of 6 animals during immersion in tap-water (open columns) and in CO_2-water (closed columns).

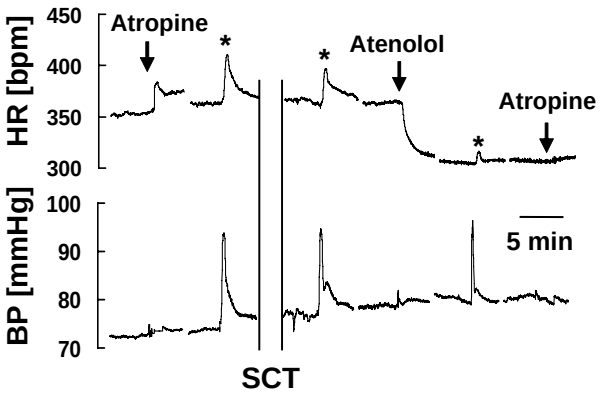

Fig. 4. Representative recordings of baroreflex in heart rate (HR) and mean blood pressure (BP) in response to bilateral common carotid artery occlusion for 15 s (CAO) before and after spinal cord transection (SCT).

Arrows show timing of drug injection to block cardiac sympathetic nerves (Atenolol) or parasympathetic nerves (Atropine). Asterisks indicate CAO. After BP and HR recovery from first 15s-CAO responses, SCT was performed.

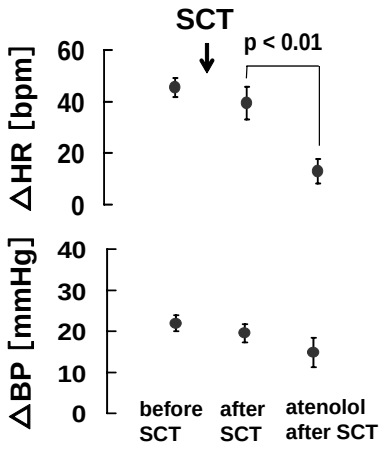

Fig 5

Fig. 5. Effects of spinal cord transection (SCT) and administration of heart-selective sympathetic antagonist (atenolol) on baroreflex cardiac activation by bilateral common carotid artery occlusion.

Mean increases in heart rate (ΔHR) and in mean blood pressure (ΔBP) with SEM of same group of 6 rats (atropinized).