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Decrease in heart rates by artificial CO2 hot spring bathing is inhibited by beta(1)-adrenoceptor blockade in anesthetized rats

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Decrease in heart rates by artificial CO_2 hot spring bathing is inhibited by β_1-adrenoceptor blockade in anesthetized rats

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Hashimoto, Masaaki, and Noriyuki Yamamoto. Decrease in heart rates by artificial CO_2 hot spring bathing is inhibited by β_1-adrenoceptor blockade in anesthetized rats. *J Appl Physiol* 96: 226–232, 2004. First published August 29, 2003; 10.1152/japplphysiol.00812.2003.—To investigate the effects of carbon dioxide (CO_2) hot spring baths on physiological functions, head-out immersion of urethane-anesthetized, fur-sheared male Wistar rats was performed. Animals were immersed in water (30 or 35°C) with high-CO_2 content (~1,000 parts/million; CO_2-water). CO_2-water for bathing was made by using an artificial spa maker with normal tap water and high-pressure CO_2 from a gas cylinder. When a human foot was immersed for 10 min in the CO_2-water at 35°C, the immersed skin reddened, whereas skin color did not change in normal tap water at the same temperature. Arterial blood pressure, heart rate (HR), underwater skin tissue blood flow, and temperatures of the colon and immersed skin were continuously measured while animals were immersed in a bathtub of water for ~30 min at room temperature (26°C). Immersed skin vascular resistance, computed from blood pressure and tissue blood flow, was significantly lower in the CO_2-water bath than in tap water at 30°C, but no differences were apparent at 35°C. HR of rats in CO_2-water was significantly slower than in tap water at 35°C. Decreased HR in CO_2-water was inhibited by infusion of atenolol (β_1-adrenoceptor blocker), but it was unaffected by atropine (muscarinic cholinoceptor blocker). Theses results suggest that bradycardia in CO_2 hot spring bathing is caused by inhibition of the cardiac sympathetic innervation. This CO_2-water maker should prove a useful device for acquiring physiological evidence of balneotherapy.

carbon dioxide balneotherapy; bradycardia; artificial carbon dioxide hot spring maker; head-out immersion

CO_2 BALNEOTHERAPY USING HOT springs containing a high concentration [≥1,000 parts/million (ppm)] of free carbon dioxide (CO_2-hot spring) has long been applied clinically to improve cardiovascular symptoms in European countries. Records of clinical observations about the effects of CO_2-hot spring baths on human subjects have been accumulated, and the list of effects now includes bradycardia, slight changes in blood pressure, and hyperemia of skin exposed to the spring water (6, 21). Although CO_2-hot springs contain many kinds of mineral ingredients, depending on geographical location, the effects of the above-mentioned CO_2-hot spring bath appear to be attributable to the high-CO_2 concentrations. Accordingly, an artificially made bath containing high concentrations of CO_2 (CO_2-water) could be expected to display similar effects on physiological functions, such as increased skin blood flow, as the natural CO_2-hot spring bath water (24, 33).

Physiological changes during bathing in CO_2-hot spring baths have also been clinically investigated (11, 12, 20, 33). However, details of the actions of percutaneously applied CO_2 on various physiological functions remain unknown because of ethical restrictions on clinical research. In addition, the difficulties associated with promptly making CO_2-water in sufficient quantities for bathtub water and maintaining high-CO_2 concentrations during the experiments seem to have obstructed the progress of research in laboratories located far from natural springs. Despite such circumstances, experimental analyses performed in subjects and laboratory animals have revealed that increased skin blood flow in CO_2-water baths results from the action of percutaneous CO_2, with skin blood vessels becoming dilated at comparatively low water temperatures (5, 18, 24, 29). Areas of skin immersed in bath water redden when human subjects bathe in CO_2-hot springs, and the boundaries with nonimmersed parts are clear (20). Although this is also observed in pure water baths at comparatively higher water temperatures, the color change is commonly observed in CO_2-water even at relatively low water temperatures, i.e., <35°C (20, 24). Changes in skin color seem to be based on hyperemia in the capillary bed caused by vasodilatation (30). Furthermore, not only does increased preload in the heart develop due to hydrostatic pressure but also reduced afterload and bradycardia are known to occur (20).

Cardiac function is well known to be under the twin influences of the sympathetic and parasympathetic nervous systems. Although bradycardia during CO_2-hot spring bathing appears to be generated by activity in the parasympathetic nervous system and/or a fall in activity of the sympathetic nervous system, which system predominates is unclear.

The present study was performed by using laboratory animals and bath water produced with newly developed equipment with a water temperature regulator, allowing the manufacture of water with a high concentration of free CO_2 (artificial CO_2-hot spring). This allowed investigation of whether this artificial CO_2-hot spring yields physiological results equivalent to those of natural CO_2-hot springs and whether the phenomena (skin vasodilatation and bradycardia) reported in human subjects can be reproduced in rat models. In addition, the involvement of sympathetic and parasympathetic nervous systems in the generation of bradycardia during CO_2-hot spring bathing was examined where such phenomena were observed.

MATERIALS AND METHODS

Animals. Male Wistar rats (body weight, 192–409 g; $n = 22$) were used in this experiment. Animals were anesthetized with urethane (1–1.5 g/kg body wt ip, ethyl carbamate; Tokyo Chemical Industry,

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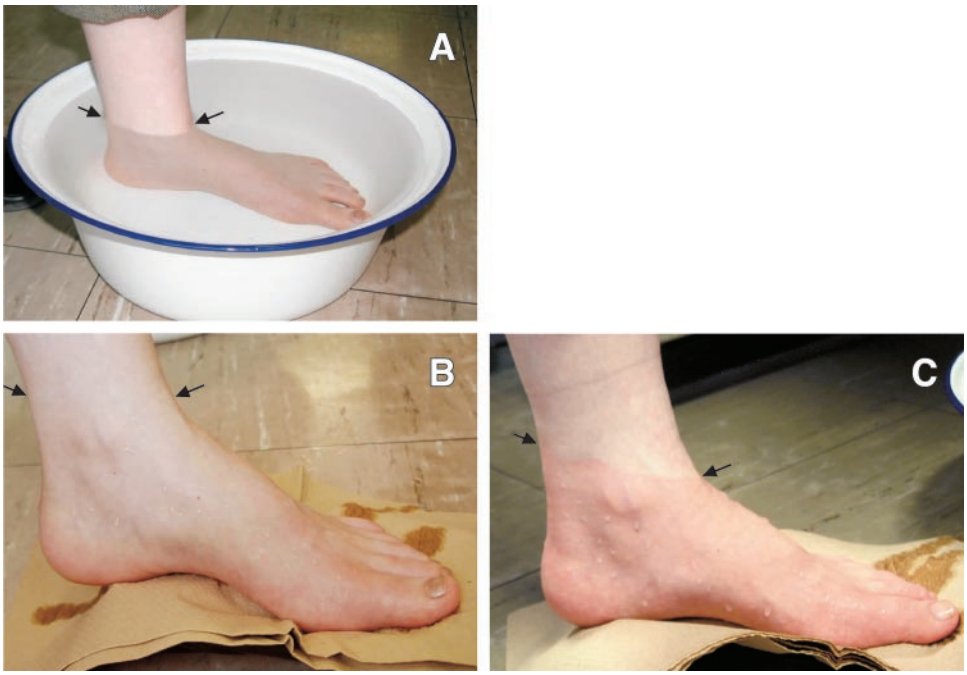

Fig. 1. Immersion in CO_2-water used in this experiment induced reddening of foot skin in human subjects. *A*: a healthy woman (29 yr old) immersed her foot for 10 min in tap water or CO_2-water at 34.5°C. Photographs were taken just after removal from normal tap water (*B*) and CO_2-water [CO_2 concentration (C_{CO_2}): 1,000–1,100 parts/million (ppm)] made using the MRE-Spa (*C*), at a room temperature of 29°C. Arrows show the border between immersed and nonimmersed skin.

Tokyo, Japan), and the fur on the lower half of the body was sheared to the axillary level. An area of abdominal side skin of ~1 cm^2 was shaved for attachment of a disk-type flowmetry probe (type C, Advance, Tokyo, Japan). The probe was attached to the skin surface by using a small spacer to allow bath water into a gap between probe and skin and was connected with a laser-Doppler flowmeter (ALF-21N, Advance) to measure skin tissue blood flow. A 27-G Surflo tube (Terumo, Tokyo, Japan) was inserted into the femoral artery and connected with a transducer (Life-kit DX360, Nihonkoden, Tokyo, Japan) to measure blood pressure. Heart rate (HR) was calculated from pulsatile changes in blood pressure by using a polygraph system (Nihonkoden). The femoral vein was cannulated by using polyethylene tubing for drug administration. All incisions in the skin were closed and sealed by using acrylic adhesive (Alon Alpha, Toa Chemical, Tokyo, Japan) to prevent water infiltration. A copper-constantan thermocouple was attached to shaved skin to be immersed, with another inserted into the colon to measure changes in body temperature.

Immersion. Bath water containing high concentrations of CO_2 (700–1,300 ppm, 30 and 35°C) was made from high-pressure CO_2 in a cylinder and tap water by using an MRE-Spa (Mitsubishi Rayon Engineering, Tokyo, Japan). When the feet of healthy human subjects were immersed in this CO_2-water, the color of the immersed skin changed to red within 10 min, whereas immersion in tap water at the same temperature resulted in no obvious color change (Fig. 1).

Animals were loosely fixed to plastic lattice plates by using adhesive tape, and the plates were set in a head-up position of ~30° to horizontal in a Plexiglas animal cage (30 × 20 × 15 cm) used as a bathtub (Fig. 2). Temperature of the bathtub water was maintained at 30 or 35°C throughout the experiment by immersing the bathtub into a water bath incubator (BT-25, Yamato Scientific, Tokyo, Japan). CO_2 concentration in bathtub water was measured by using either a CO_2 probe with a pH-ion meter (model 290A, Orion Research) or pH of the water converted into CO_2 concentration. Animals were immersed into one sample of water for 30 min. At the end of this period, bathtub water was quickly siphoned off and replaced by another sample with a different CO_2 concentration at the same temperature (except for temperature changing, Fig. 3).

Infusion of autonomic blockers. To investigate which innervation is dominant for changes to HR in CO_2-water bathing, sympathetic or parasympathetic, autonomic nervous system blockade was performed by using heart-specific sympathetic (atenolol) and parasympathetic (atropine) blockers. Atenolol (1 mg/ml, β_1-adrenoceptor antagonist; Sigma) was injected at a dose of 1 mg/kg body wt ip and then infused (30 $\mu g \cdot 60\ \mu l^{-1} \cdot h^{-1}$ iv) after the initial dose. Atropine sulfate (1 mg/ml, muscarinic cholinoceptor antagonist; Sigma) was infused at a dose of 60 μg/h, or vehicle sterile saline was infused through the femoral vein catheter at a rate of 60 μl/h by using an IP-21 infusion pump (Nikkiso, Tokyo, Japan). Doses of autonomic nerve antagonists were chosen on the basis of dose-response relationships shown in previous studies to induce maximum inhibition (atenolol) or facilitation (atropine) of HR in rats (22, 31). Schedules for water exchanges and drug infusion are summarized in Fig. 3.

All signals were recorded with an R-66 multipen recorder (Rikadennki, Tokyo, Japan) on chart paper, with data simultaneously captured and stored every 1 s by using a personal computer system

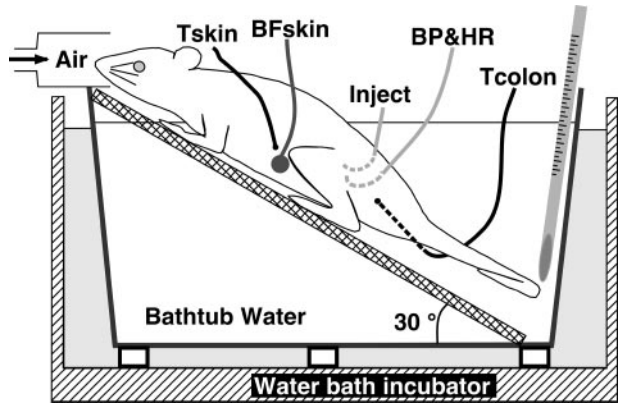

Fig. 2. Bathing rat with probes. Two thermocouples were used for measuring underwater skin surface (T_{skin}) and colon temperatures (T_{colon}); disk-type laser-Doppler flowmetry probe was used for measuring immersed skin tissue blood flow (BF_{skin}); femoral vein catheter was used for drug injection (Inject); and femoral artery catheter was used for blood pressure (BP) and heart rate (HR) measurements. Bath water temperature was maintained by controlling water bath incubator in which the bathtub was immersed.

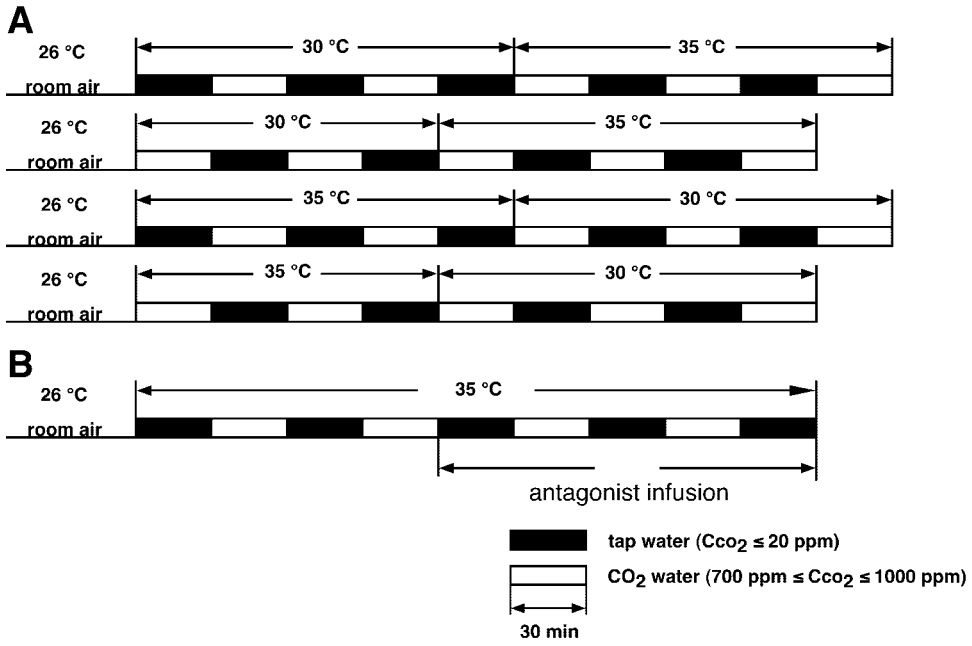

Fig. 3. Schedule for water immersion and drug infusion experiments. *A*: schedule for observation of the effects of C_{CO_2} and temperature of bath water. Initial temperature and C_{CO_2} of bathtub water were randomly selected to avoid the order effect. *B*: schedule for autonomic blockade experiments.

(PC9801, NEC, Tokyo, Japan). For statistical analysis, data stored for 20 min from 10 min after the exchange of bath water were averaged and taken as representative values for bath immersion. Statistical significance of changes in calculated values was evaluated by using Student's *t*-test for paired comparisons. Values of $P < 0.05$ were accepted as statistically significant. The present experiments were performed under the permission (no. 02165) of the Committee for Animal Experiments at Asahikawa Medical University, according to the Guiding Principles for the Care and Use of Animals approved by the Council of the Physiological Society of Japan.

RESULTS

Temperatures of the colon and skin before the first immersion was begun were 35.0 ± 0.2°C (means ± SE; $n = 8$) and 31.8 ± 0.3°C, respectively. Similarly, HRs and skin tissue blood flows were 354 ± 15 beats/min and 3.5 ± 0.3 ml·min^{-1}·100 g^{-1}, respectively. Mean arterial blood pressure was 86 ± 3 mmHg. Figure 4 shows a representative recording of the experiments conducted by using tap water and CO_2-water at two temperatures (30 and 35°C). Although exchange of bathtub water was performed as quickly as possible, changes in some recording parameters persisted after water was changed, such as marked changes in skin temperature. However, the influence of water exchange did not seem to continue beyond 10 min.

Colonic and skin temperatures were influenced more by water temperature than by CO_2 concentration (Fig. 5). Shivering was not observed for rats immersed in water at 30°C under the present experimental conditions. Compared with tap water, immersed skin tissue blood flow in CO_2-water at 30°C was significantly increased (21.8 ± 2.5%). However, such differences were not apparent at 35°C (Fig. 6*A*). To evaluate the effect of CO_2-water immersion on the skin vascular system, mean arterial blood pressure divided by skin tissue blood flow was determined as an index of vascular resistance. Figure 6*B* shows a summary of resistance index changes. CO_2 concentration influenced resistance at 30°C (19.7 ± 1.1% difference), but no significant relationship was apparent at 35°C. Conversely, resistance was significantly larger at 30°C than at 35°C

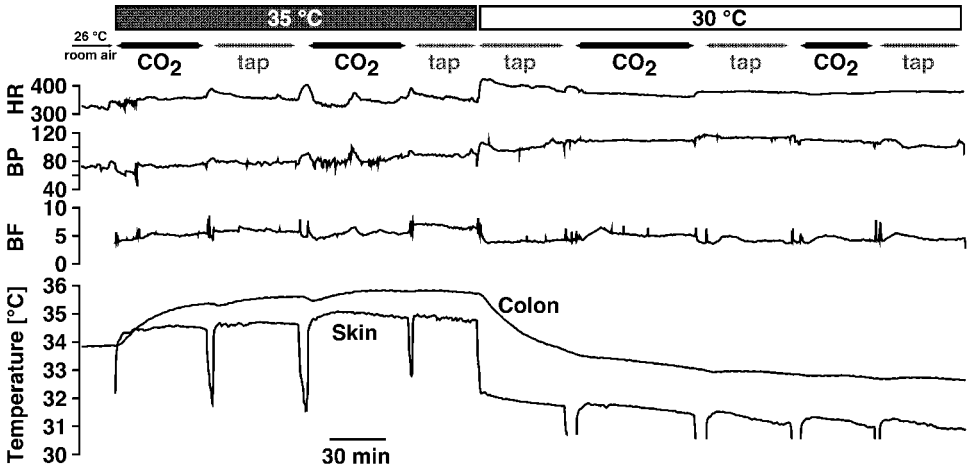

Fig. 4. Representative recordings of HR, mean arterial BP, BF_{skin} (BF; ml·min^{-1}·100 g^{-1}), and temperatures during bathing in an anesthetized rat. Bathtub water temperatures were maintained at 30 or 35°C. CO_2, CO_2-water containing ~1,000 ppm CO_2; tap, normal tap water.

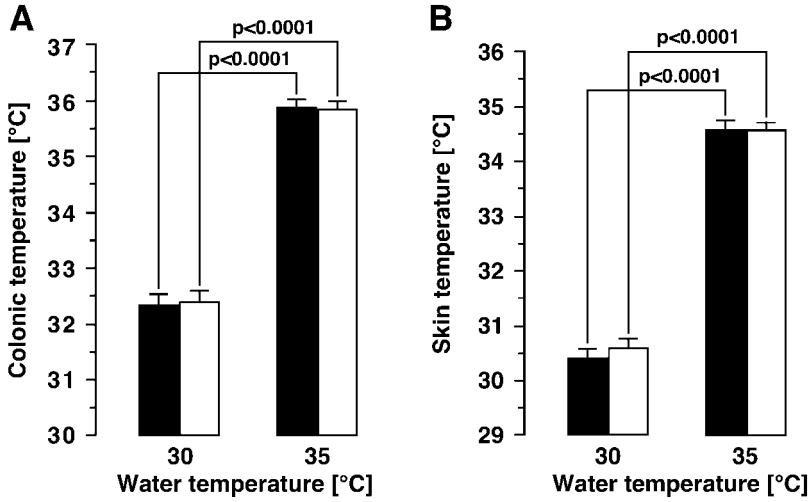

Fig. 5. Effect of C_{CO_2} and temperature of bath water on T_{colon} (*A*) and T_{skin} (*B*) in anesthetized Wistar rats. Values are means ± SE from the same group of 8 animals in tap water (C_{CO_2} < 20 ppm; solid bars) and CO_2-water (700 ≤ CO_2 ≤ 1,300 ppm; open bars).

for both tap water (by 41.4 ± 5.4%) and CO_2-water (by 27.8 ± 8.8%). Mean arterial blood pressure changes displayed negative correlation to water temperature but did not show any significant correlation to CO_2 concentration of water at either temperature (Fig. 7*A*). HRs were significantly lower in CO_2-water than in tap water (by 5.6 ± 0.4%) at 35°C but were roughly equal at 30°C (Fig. 7*B*). Compared with that of rats immersed in tap water at 30°C, HRs at 35°C were increased in 50% of animals but decreased in the remainder, resulting in no overall statistical difference.

Experiments using autonomic antagonists with cardiac selectivity were performed with water temperatures of 35°C, because bradycardia during CO_2-water immersion was observed only at this temperature. Drug administrations were performed in 14 rats during tap water immersion. All recorded parameters in this experiment, except for HR, are summarized in Table 1. In tap water, colon and skin temperatures, skin tissue blood flow, and skin vascular resistance were all unaffected by infusion of atenolol or atropine. Mean arterial blood pressure, however, was significantly increased (by ~5%) on atropine infusion but was unchanged by atenolol. Part of the representative HR data recording is shown in Fig. 8. Atenolol decreased HRs in tap water by 68 ± 6 beats/min (18.5 ± 1%), whereas atropine increased HRs by 34 ± 13 beats/min (10.5 ± 4.1%). Figure 9 summarizes the results of blockade experiments. The decrease in HR of ~5% on CO_2-water immersion observed with saline or atropine infusion rats was inhibited by infusion of atenolol.

DISCUSSION

In human subjects bathing in hot springs containing high concentrations of CO_2, reddening of the immersed skin of bathing subjects is very common at a certain range of bath water temperatures (20, 24). Unlike normal tap water bathing, this reddening is not associated simply with water temperature. The artificial CO_2-hot spring water used in the present experiments resulted in similar changes in human feet (Fig. 1), suggesting that this CO_2-water exerts comparable effects as natural CO_2-hot spring water. Given this, skin reddening seems likely to be caused by a direct or indirect effect of the high concentrations of CO_2 in the water. Skin reddening on bathing in CO_2-water is also reportedly associated with concomitant increases in skin blood flow in human subjects (24). Although skin reddening was not obviously observed in rats, CO_2-water immersion at 30°C results in increased skin blood flow and

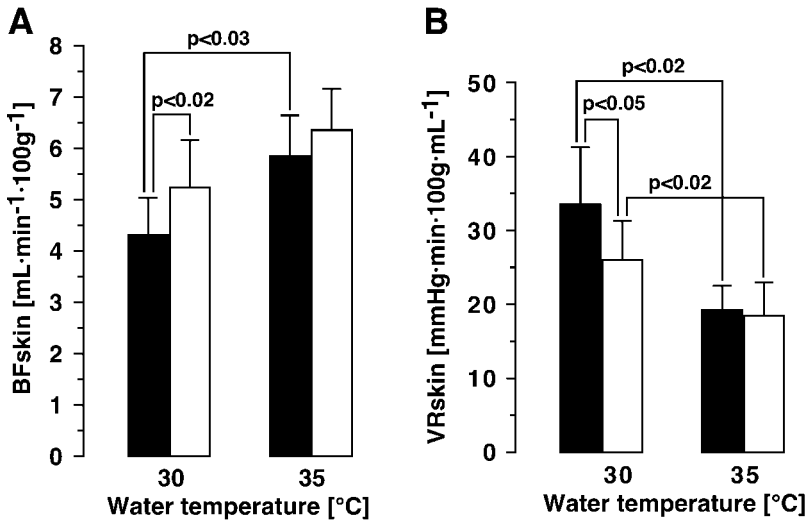

Fig. 6. Effect of C_{CO_2} and temperature of bath water on BF_{skin} (*A*) and index of vascular resistance of immersed skin (VR_{skin}; *B*) in anesthetized Wistar rats. Values are means ± SE from the same group of 8 animals in tap water (C_{CO_2} < 20 ppm; solid bars) and in CO_2-water (700 ≤ CO_2 ≤ 1,300 ppm; open bars).

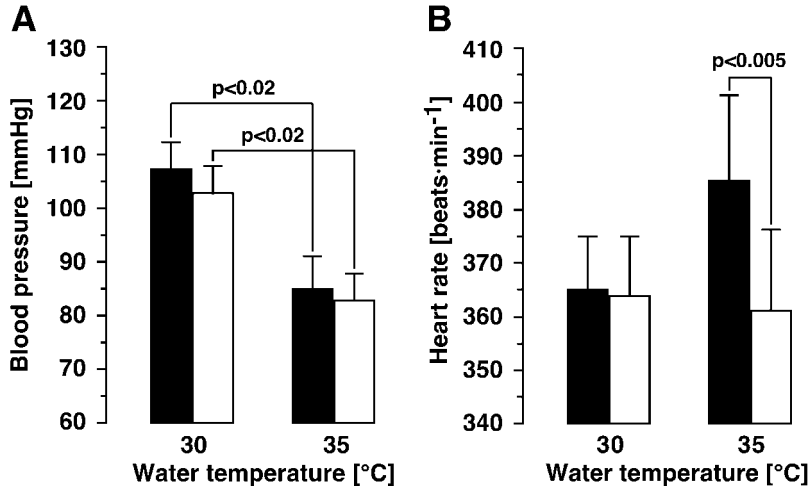

Fig. 7. Effect of C_{CO_2} and temperature of bath water on mean arterial BP (*A*) and HR (*B*) in anesthetized Wistar rats. Values are means ± SE from the same group of 8 animals in tap water ($C_{CO_2} < 20$ ppm; solid bars) and in CO_2-water ($700 \leq CO_2 \leq 1{,}300$ ppm; open bars).

decreased vascular resistance, compared with tap water immersion, as shown in the present results. The unclear visual color changes in rat skin during CO_2-water immersion might be attributable to structural differences between human and rat skin (32). In experimental animals with thick fur, blood flow measurements and calculation of vascular resistance seem to provide better indications of vascular events induced by CO_2-water immersion than observations of color change.

At a water temperature of 30°C, vascular resistance of the immersed skin of rats in CO_2-water was significantly smaller than that in tap water, whereas no such difference was observed at 35°C (Fig. 6*B*). Ito and coworkers (15) observed an increase in digit pad skin blood flow when rat hind legs were immersed in 34°C CO_2-water, but no such changes were noted for immersion in distilled water at the same temperature. As shown by the present results, skin blood flow in hairy skin on the side abdomen tends to increase with CO_2-water immersion, but no significant difference with tap water was observed. Histological observations indicate that a difference exists in vascular bed composition between hairless plantar paw skin (rich in arterioles, venules, and arteriovenous anastomoses) and dorsal hairy skin (rich in capillaries) (27, 28). Moreover, skin tissue blood flow is reportedly larger in the hairless skin than in hairy skin, and blood flow increases in response to warm temperature stimulus are also larger in the hairless skin (26, 28). Differences in blood flow responses to CO_2-water immersion at 35°C between the results of Ito et al. and the present experiment might be due to differences in the composition of the tested vascular beds. If a water temperature of 35°C enhances vasodilatation in abdominal skin, additional dilatory effects from CO_2 might be masked, and thus vascular resistance might not have been affected by CO_2-water immersion at this temperature.

Hypercapnia caused by CO_2 inhalation is known to evoke vasodilatation in most vascular beds in human and laboratory animals, and the mechanisms involved in CO_2-induced vasodilatation have been extensively investigated (2, 7, 14, 23, 34, 35). In the present experiments, animals inhaled fresh air (including inconsequential levels of CO_2) through a facial mask, excluding the possibility of animals inhaling high concentrations of CO_2 diffusing from the surface of bath water. Furthermore, unchanged blood pressure for rats in CO_2-water baths indicates that negligible amounts of CO_2 would have been inhaled, as hypercapnia caused by CO_2 inhalation results in hypertension in urethane-anesthetized rats (8). Because an elevation of proton levels under hypercapnic conditions inhibits its smooth muscle contractility (1), CO_2 permeating percutaneously from bath water may locally influence skin vasodilatation. Conversely, in the visceral and cerebral vascular beds, mediators of vasodilatation caused by hypercapnia include nitric oxide (9, 25), prostanoids (4, 13), and cyclic nucleotides (19). Mediators in the skin vascular bed remain unclear. Skin vasodilation caused by percutaneous influx of CO_2 will be investigated more closely in the future.

During tap-water bathing, water temperature correlated positively with HR and negatively with mean arterial blood pressure. These findings agree with a previous report showing decreased pulse rate and increased blood pressure after reductions in core body temperature (17). Increased mean arterial blood pressure at 30°C suggests that increases in total peripheral vascular resistance at 30°C would exceed the decreased cardiac output accompanying decreased HR. Increased total peripheral vascular resistance is probably attributable to a large decrease in body core temperature (3). HR decreases during CO_2-hot spring bathing at ~35°C have long been reported in human subjects, and we have confirmed that HR of rats in CO_2-water exceeded that in tap water. This effect of CO_2-water bathing on HR changes seems to be achieved via decreased

Table 1. *Effect of atenolol and atropine infusion on the hemodynamic parameters of anesthetized rats in tap water (35°C)*

	β_1-Blockade		Muscarinic Blockade	
	Saline	Atenolol	Saline	Atropine
T_{colon}, °C	35.5±0.1	35.4±0.2	35.9±0.3	35.6±0.3
T_{skin}, °C	34.7±0.2	34.6±0.2	34.9±0.2	34.7±0.1
BP, mmHg	84±6	88±7	86±6	91±6*
BF_{skin}, ml·min^{-1}·100 g^{-1}	9.7±1.0	8.6±1.2	9.8±3.4	11.3±4.3
VR_{skin}, mmHg·ml^{-1}·min·100 g	9.7±1.1	12.7±2.6	17.5±7.1	15.8±5.6

Values are means ± SE acquired from 2 different groups comprising 7 rats each. T_{colon}, colon temperature; T_{skin}, underwater skin temperature; BP, blood pressure; BF_{skin}, immersed skin tissue blood flow; VR_{skin}, vascular resistance of immersed skin. *Statistically significant difference compared with results for control saline infusion, $P < 0.05$.

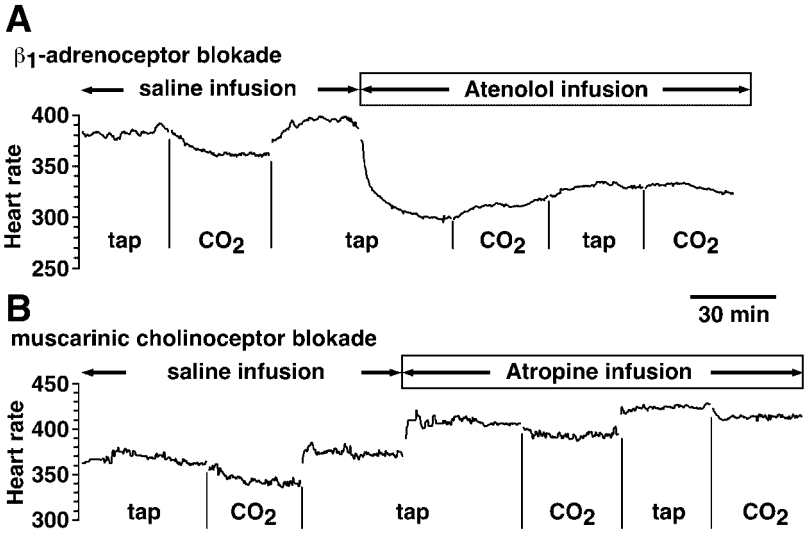

Fig. 8. Effect of sympathetic (β_1; *A*) and parasympathetic (muscarinic; *B*) blocker infusion on CO_2-water immersion-induced bradycardia in an anesthetized Wistar rat. Bath water temperature was maintained at 35°C in normal tap water (tap) and CO_2-water (CO_2; 900 ppm $< CO_2 <$ 1,100 ppm). Lack of recorded data was due to the small size of available computer memory.

sympathetic nerve activity, rather than increased parasympathetic nerve activity, as sympathetic blockade influenced HR more than parasympathetic blockade. This possibility is also supported by clinical observations in human subjects bathing in CO_2-hot springs, showing reduced plasma catecholamine levels that suggest decreased sympathetic activity (10).

Although descriptions have referred to the sedative effect of bathing in CO_2-hot springs on autonomic function in human subjects (16), detailed experimental analyses of the underlying mechanisms do not seem to have been performed. One of the reasons why experiments in laboratories have been restricted is probably the difficulty of maintaining sufficiently high bath water CO_2 concentrations during the experiment, in addition to difficulties obtaining CO_2-water easily, rapidly, and inexpensively. The results of the present study show that the newly developed CO_2-water maker satisfies these demands and is useful for analyzing the physiological effects of CO_2-hot spring bathing in popular experimental animals.

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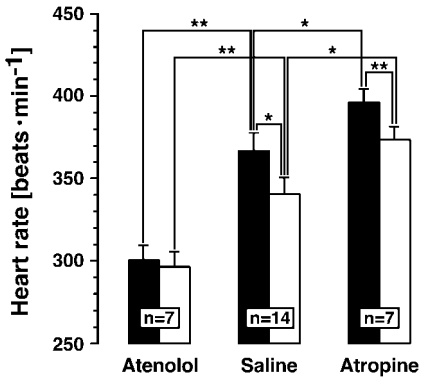

Fig. 9. Effect of sympathetic (atenolol: β_1-adrenoceptor antagonist) and parasympathetic (atropine: muscarinic cholinoceptor antagonist) blocker infusion on HR in anesthetized Wistar rats during tap water ($C_{CO_2} < 20$ ppm; solid bars) and CO_2-water ($700 \leq CO_2 \leq 1{,}200$ ppm; open bars) immersion. Water temperature was maintained at 35°C throughout experiments. Values are means ± SE; n = sample size. $^*P < 0.03$, $^{**}P < 0.001$.

GRANTS

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