

Topical Application of CO₂ Increases Skin Blood Flow

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The topical application of carbon dioxide water to the rat hindpaw produced a concentration-dependent increase of skin blood flow as measured by a laser Doppler flowmeter. About a 100% increase of skin blood flow occurred in response to CO₂ when the bath temperature was at 23°C or 34°C, but there was no significant effect of CO₂ at 41°C.

Carbon dioxide (CO₂) applied externally to the skin is well known to produce an erythematous or hyperemic reaction in the human and CO₂ baths have been used for the treatment of peripheral vascular disorders [1-3]. However, not much basic knowledge has been accumulated about the quantitative effect of CO₂ or the mechanism of the effect of CO₂ on the skin vasculature, partly because of lack of suitable animal models. Recently, laser Doppler flowmetry has been established as a useful technique to demonstrate cutaneous blood flow changes [4-9] and has been used to quantitate the vascular effect of CO₂ in the human and rabbits [10,11].

In the present study we have examined the effect of CO₂ exposure on cutaneous blood flow using the rat hindpaw as a model for hairless skin and reported that CO₂ bathing increases the skin blood flow in a concentration related manner at the bathing temperature of 23°C or 34°C and that this effect of CO₂ does not differ among the control, acutely denervated, and chronically denervated animals, indicating no involvement of neuronal mechanism in the vascular effect of CO₂.

MATERIALS AND METHODS

Male Sprague-Dawley rats (300-400 g) were anesthetized with pentobarbital and chloral hydrate (40 and 200 mg/kg, i.p., respectively) and were maintained under artificial respiration with room air after tracheal intubation. Cutaneous blood flow was monitored by laser Doppler flowmeters (models Pf2 and Pf3, Perimed, Sweden) from the digital pad of each hindpaw. The flowmeters were calibrated by adjusting the gain to the same reading in millivolts by use of Pf 100 motility standard (Perimed, Piscataway, NJ) and they were used randomly to measure the blood flow either from the control site or CO₂-bathed sites. To eliminate muscle movement and to obtain stable recordings the animals were paralyzed with gallamine triethiodide (2-10 mg/kg, i.v.), administered through a cannula placed into a jugular vein. A carotid artery also was cannulated to monitor the mean blood pressure by means of a pressure

transducer (Statham P23Dd). The measured skin blood flow and blood pressure were recorded on a Grass 7B polygraph. A bath chamber made of plastic was specially designed with two sections so that both hindpaws of each rat could be lowered into the water individually, allowing one hindpaw to be used as a control for each experiment. Each section of the chamber was filled with 400 ml of distilled water buffered 1:10 with CO₂ buffer solution (Orion Research Inc., Cambridge, MA, 95-02-10). The buffered solution is not only required for use of the CO₂ electrode (see below) but also served to maintain a stable pH (5.25) during the introduction of CO₂ into the solution. The pH did not change when CO₂ was bubbled into the solution. One chamber was bubbled with CO₂ gas at a rate of 50 ml/min through an air stone, whereas the other one remained without bubbling as a control.

Whenever the skin blood flow of the control monitoring site became unstable the experiment was stopped and redone from the beginning after rinsing the chamber and replacing the solutions. The chamber was put on a thermostatically controlled heating unit to keep the water temperature stable at a set temperature [23 ± 0.2°C (low), 34 ± 0.3°C (middle), or 41°C ± 0.6°C (high)] during CO₂ bubbling.

Carbon dioxide concentration was measured using a CO₂ electrode (Ionanalyzer, Model 95-02-00, Orion) with a microprocessor ionanalyzer (Model-901, Orion). Table I shows the relationship between the time of bubbling with CO₂ (50 ml/min) and the measured CO₂ concentration at the three temperatures used. The effect of temperature on skin blood flow was studied by increasing water temperature continuously from room temperature (22°C-23°C) to 42°C over approximately 70-80 min.

Acute denervation was performed by section of the sciatic and femoral nerves of one hindpaw and the skin blood flow was measured after a 30-45-min period of stabilization. In the chronic denervation experiments, the surgery was performed 5-7 d before the experiment.

Five to 9 rats were used for each experimental group. Results are represented as the means ± SEM. Statistical analysis was performed by Student's *t*-test for unpaired observations.

RESULTS

The rat hindlimb digital pad showed reasonably stable skin blood flows when the hindlimb was immersed in distilled water at room temperature (22°C-23°C) and flows remained at about the same level up to a temperature of 25°C. At temperatures above 25°C, the skin blood flow increased markedly and the magnitude of increased flow was correlated with the increase of the temperature of the bath up to 42°C (Fig 1). This effect of increasing temperature on skin

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Table I. Relationship of Bubbling Time and CO₂ Concentration in the Bathing Water at Various Temperatures

| Time (min) | Water Temperature | | |
|------------|-------------------|------------|-----------|
| | 23°C | 34°C | 41°C |
| 3 | 366 ± 32 | 367 ± 28 | 365 ± 39 |
| 6 | 740 ± 56 | 704 ± 79 | 705 ± 64 |
| 9 | 1165 ± 84 | 932 ± 103 | 894 ± 61 |
| 12 | 1483 ± 77 | 1149 ± 119 | 1102 ± 64 |
| 15 | 1808 ± 75 | 1326 ± 89 | 1276 ± 60 |
| 20 | 2133 ± 79 | 1498 ± 86 | 1450 ± 48 |

Values represent mean ± SEM of CO₂ concentration in ppm. n = 5 for each value.

blood flow was not significantly different among the control, acutely denervated, or chronically denervated groups. The maximal effect of temperature on skin blood flow appeared to increase flow by approximately 600% or more (Fig 2). Basal skin blood flow measurements (laser Doppler flowmeter readings in mV) before heating at 24°C were 164.4 ± 23.7 in the control, 129.0 ± 10.7 in the acutely denervated, and 198.0 ± 45.1 in the chronically denervated animals and after heating at 42°C flows were 1107.8 ± 155.4, 1134.5 ± 169.2, and 1294.0 ± 176.9, respectively. When comparing denervated groups with the controls, no significant difference was observed. There was, however, a significant difference between the acutely and chronically denervated animals at 24°C ($p < 0.05$).

Carbon dioxide gas bubbled into the bath water produced an increase in the cutaneous blood flow. A minimal effect was observed after about 3 min of CO₂ bubbling and the maximal response occurred in about 20 min while the skin blood flow of the control remained unchanged (Fig 3). Figure 4 illustrates typical skin blood flow changes in the acutely denervated paw at three different temperatures. As can be seen, CO₂ elicited about a 100% increase of the skin blood flow at the low and middle temperatures, but did not show a significant effect at the high temperature. As shown in Figures 5 and 6, this effect of CO₂ to increase skin blood flow at the low or middle temperature was not significantly different among control, acutely denervated, and chronically denervated animals. In these groups, CO₂ did not appreciably increase skin blood flow at the high temperature. Composite results from the three experimental groups of actual laser Doppler flowmeter readings before and 20 min after CO₂ bubbling are shown in Table II. Bubbling with room air up to a rate of 200 ml/min did not alter skin blood flow.

DISCUSSION

Carbon dioxide bathing has an old history, especially in Europe, and is thought to be effective in the treatment of peripheral vascular diseases, hypertension, and heart disease [1-3,12-14]. The effect of CO₂ bathing to increase cutaneous blood flow has been investigated mainly in humans and there have not been many experimental studies to determine the mechanism(s) of the CO₂ effect. It has been

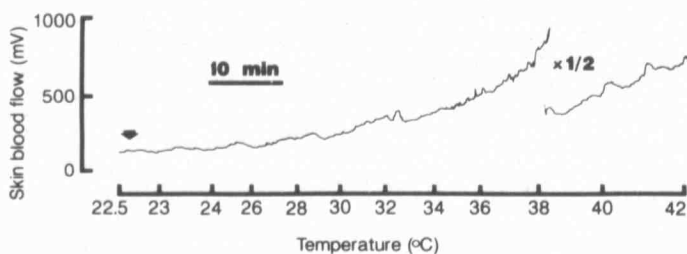


Figure 1. Effect of increasing bathing water temperature on the skin blood flow of a rat hindlimb digital pad as measured by a laser Doppler flowmeter (in mV, ordinate). Innervation to the vasculature was intact in this preparation. Note the recording sensitivity change at 38°C.

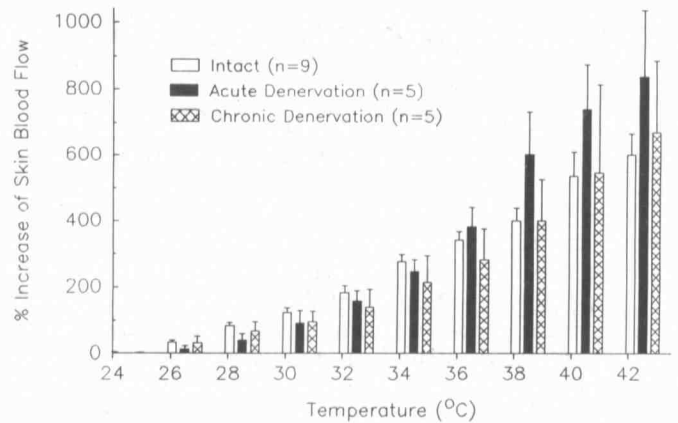


Figure 2. Composite representation of the effect of water temperature on the cutaneous blood flow of the rat hindlimb digital pad. The ordinate represents percentage of increase of laser Doppler flowmeter readings from the control level before heating. Values represent mean ± SEM. There were no significant differences between the three experimental groups.

reported that CO₂ bathing might exhibit a diuretic effect by decreasing sympathetic tone in humans [15]. In contrast, Bühring et al [16] recently observed that CO₂ has an antidiuretic effect in temperature-matched bathing experiments in humans. These investigators proposed as a possible mechanism that blood pooling in the skin, produced by the vasodilating effect of CO₂, reduces renal circulation to cause less urine formation [16]. The present results did not show a significant difference of the effect of CO₂ to increase cutaneous blood flow among the intact, acutely, or chronically denervated groups of animals. This would appear to indicate that there is no involvement of a neural mechanism, at least in the local effect of CO₂, on skin blood flow. This finding is in agreement with the theory proposed by Jordan [17] that CO₂ is acting as a first messenger directly on the vascular smooth muscle cells or that the effect of CO₂ on the skeletal muscle is to reduce the tension through decreasing intracellular pH [18].

It is well known that increase of Pco₂ or decrease of Po₂ in blood can also dilate blood vessels. The Pco₂ in mixed venous blood and the amount of CO₂ exhaled can be increased when CO₂ water is applied to the whole body of humans [19]. In the present study, however, we did not see any increase of the blood flow on the control paw. This observation suggests that the change of systemic

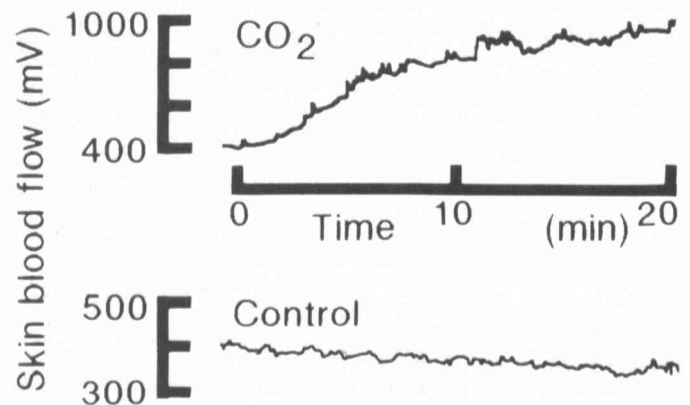


Figure 3. Effect of CO₂ on the cutaneous blood flow of a rat hindlimb digital pad with intact innervation. The CO₂ was bubbled into the bathing water using an air stone (50 ml/min). Time from the commencement of bubbling is shown in minutes (upper panel). Lower panel represents the control skin blood flow from the other hindpaw immersed into distilled water without CO₂ bubbling. Both paws were innervated. Water temperature was held constant at 34°C.

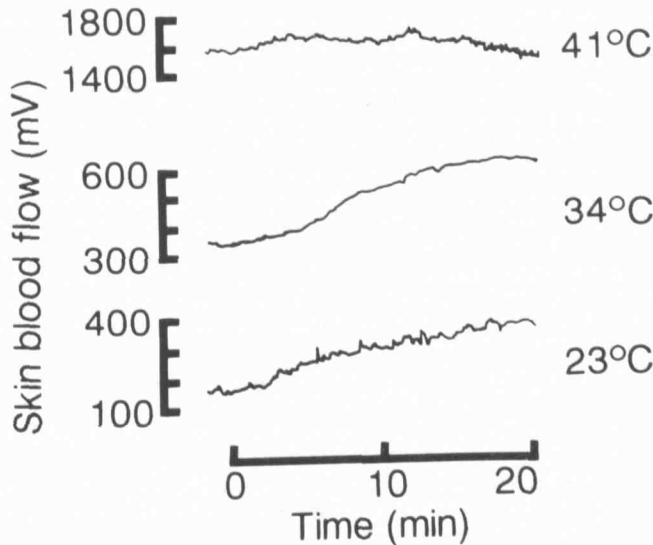


Figure 4. Effect of CO₂ on the cutaneous blood flow of an acutely denervated hindlimb digital pad of a rat at different bathing water temperatures (23°C, 34°C, and 41°C). The abscissa represents the time from the beginning of CO₂ bubbling into the bathing water (50 ml/min).

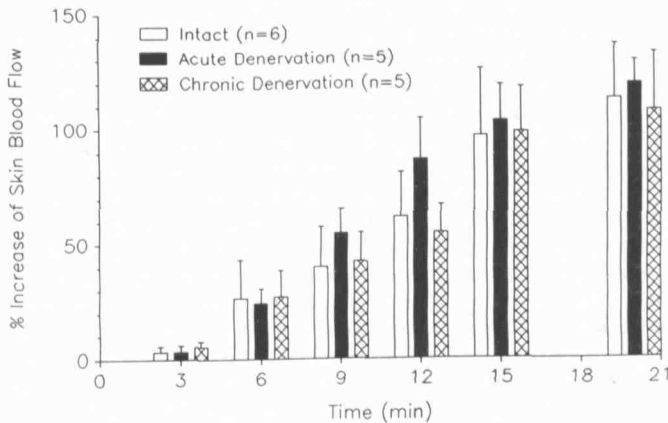


Figure 5. Effect of CO₂ on the cutaneous blood flow measured at 23°C. The abscissa represents time of CO₂ bubbling. The final set of bars represents measurements taken at 20 min. No significant differences were seen between three experimental groups at each time of measurement. Values represent mean \pm SEM.

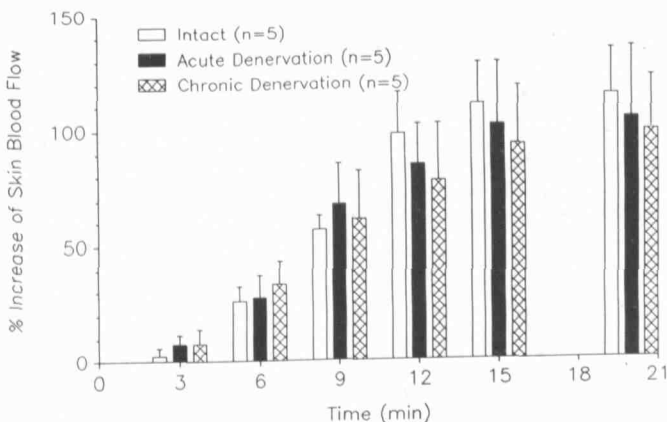


Figure 6. Effect of CO₂ on the cutaneous blood flow measured at 34°C. The final set of bars represents measurements taken at 20 min. No significant differences were seen between three experimental groups at each time of measurement. Values represent mean \pm SEM.

Table II. Effect of CO₂ on Skin Blood Flow of the Rat Hindlimb Digital Pad (Laser Doppler Flowmeter Readings in mV)

| Temperature | | Intact Nerves | Acutely Denervated | Chronically Denervated |
|-------------|----------------|----------------|--------------------|------------------------|
| 23°C | ^a B | 147 \pm 41 | 205 \pm 52 | 140 \pm 17 |
| | ^b A | 304 \pm 53 | 454 \pm 128 | 280 \pm 14 |
| 34°C | B | 344 \pm 50 | 472 \pm 142 | 606 \pm 217 |
| | A | 758 \pm 167 | 864 \pm 199 | 1050 \pm 292 |
| 41°C | B | 1784 \pm 365 | 1362 \pm 143 | 1498 \pm 293 |
| | A | 1874 \pm 311 | 1412 \pm 104 | 1410 \pm 241 |

^a Before and ^b after 20 min of CO₂ bubbling (50 ml/min). Values represent readings of laser Doppler flowmeter in mV with mean \pm SEM. Five or 6 animals per group.

PCO₂ or PO₂ is not a causative factor in the skin blood flow response to CO₂ bathing in our experiments.

Another point of interest in the present study is the relationship of the effect of temperature to that of CO₂ on cutaneous blood flow. Our results showed that CO₂ increases skin blood flow about 100% above basal flow at both 23°C and 34°C, but laser Doppler flowmeter readings reveal that the absolute amplitude of the increase in cutaneous blood flow at 34°C is more than two times of that at 23°C (Table II). The lack of CO₂ effect on skin blood flow at high temperature (41°C) might be explained by the more apparent effect of high temperature itself. If the maximum increase of blood flow was attained by high temperature alone, CO₂ would be unable to increase flow further. We have no information concerning the mechanism of the interaction between temperature change and CO₂ effects, but there might be a certain temperature where CO₂ can produce the greatest effect on the cutaneous blood flow.

From the clinical aspect the present observations provide experimental support for the benefit of CO₂ bathing in the treatment of skin circulatory disorders. Although the effect of high temperature on skin blood flow is more obvious than that of CO₂ itself, possibly the effectiveness of CO₂ at the temperature of 34°C, which is close to the neutral temperature, may help the healing of skin wounds and chronic skin ulcers. This might be particularly true in cases like livedo reticularis with summer ulceration, where increased temperature is suspected to cause skin ulceration by increasing tissue O₂ demand [20]. The increase of blood flow in the denervated skin by CO₂ bathing also would be advantageous for wound healing in patients with nerve disturbances of the skin as neural mechanisms are not needed for the effect of CO₂ to be manifest.

Carbon dioxide bathing is thought to manifest its effect on degenerative or tropic diseases partly through increasing tissue O₂ tension or tissue metabolism [10]. In this sense, CO₂ bathing might have an effect similar to other methods (e.g., hyperbaric, ultrasound, or microwave). With its action to increase skin blood flow and to improve tissue metabolism, CO₂ bathing might be a useful clinical method to treat skin wounds and ulcers or skin circulatory disorders with trophic changes.

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