The Effect of Carbon Dioxide Inhalation on Cerebral Blood Flow: A Two-Hour Duration Study in Dogs With Microspheres

BY RICHARD T. JACKSON, PH.D., ALBERT A. CLAIRMONT, M.D., AND RICHARD A. POLLOCK, M.D.

Abstract:
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Dogs breathed one of four gas mixtures (5% CO₂-95% O₂, 5% CO₂-95% air, 10% CO₂-90% O₂, and 10% CO₂-90% air) for as long as two hours. Regional cerebral blood flow as well as flow in nasal, otic, pituitary and skin tissue were measured by means of 15 ± 5 μ radioactively labeled microspheres. The normal values for cerebral blood flow and arterial blood gases were very similar to those of other investigators. Inhalation of CO₂ induced an increase in cerebral blood flow that was significantly higher than is usually reported. Increases varied from 100% (with 5% CO₂-95% air) to 250% (with 10% CO₂-90% O₂). Blood flow in the temporal bone behaved much like that of brain in response to CO₂. In most instances, the pituitary gland blood flow did not increase with inhalation of CO₂.

Additional Key Words
hypercapnia circulation radioactive tracer vasodilation pituitary circulation

Introduction

It is well known that CO₂ is a potent dilator of cerebral blood vessels. We wished to determine if CO₂ inhalation had the same effect on otic blood vessels. The usual problems associated with measuring cerebral blood flow are made more difficult by the size and anatomy of the labyrinth. We had gained some experience with the use of 15 μ radioactively labeled microspheres,1,2 and felt that the method was useful and sound. Although we were primarily interested in labyrinthine blood flow changes, we also measured flow changes in several areas of the brain. We felt that if our values for regional cerebral blood flow were similar to the values of others, more credence could be placed in the responses of labyrinthine blood flow to CO₂.

Some of our results relating to regional brain blood flow are novel (pituitary blood flow and long-term exposure to CO₂ mixtures); others (the magnitude of the response to CO₂) are at variance with the results of some other investigators. It appears relevant to report our results here.

Methods

Sixty-seven mongrel dogs weighing from 10 to 20 kg were anesthetized with either intravenous sodium pentobarbital (Nembutal, 25 mg per kilogram) or alpha chloralose (80 mg per kilogram). The trachea of each dog was intubated with a cuffed endotracheal tube and a Rudolph valve* was attached to the tube with a minimum dead space. During surgical preparation and control periods, the two-way valve remained open to room air; during experimentation, the valve opened to a CO₂ reservoir bag.

A 5 mm catheter was inserted by cutdown procedure through the femoral artery into the distal abdominal aorta and attached to a Physiograph (Narco Biosystems, Houston, Texas) blood pressure transducer. In this manner, continuous measurement of systemic blood pressure was possible, as was removal of blood for determination of arterial pH, Pco₂, Po₂, and percent saturation. The blood was analyzed by the Emory University Pulmonary Function Laboratory using an Instrumentation Laboratories electrometer. A 3 mm catheter was inserted by cutdown procedure into the femoral artery of the opposite side and threaded into the left ventricle.

The retroperitoneal space was entered by flank incision and the renal artery identified. In some instances, the kidney was found to have a dual blood supply (i.e., two renal arteries), requiring a flank incision on the opposite side. Following adequate exposure of the renal artery, an electromagnetic flow probe (Statham) was placed around the artery. Signals from the probe were then read directly on the DMP-4B Physiograph recorder.

The intracardiac catheter served as a conduit for injection of each of four kinds of radioactively labeled microspheres (3M Company, St. Paul, Minnesota). The microspheres are made of carbonized plastic, measure 15 ± 5 μ in diameter, and are labeled with one of four

The changes in blood flow in several brain, nasal and otic tissues due to breathing 5% CO₂-95% O₂ twice for ten minutes in a single animal. In this animal, ten minutes elapsed between the end of each control measurement and the beginning of each gas test. Ten minutes also elapsed between the end of the first gas test and the second control measurement. Not all the tissues that were measured in this animal are shown in the figure. Note the close correspondence of the paired brain samples.

gamma-emitting isotopes having a specific radiation band: I-125, Ce-141, Sr-85, or Sc-46. The isotopes are incorporated into the spheres and do not “wash off” into the suspending medium, 10% dextran. The use of four differently labeled spheres permitted one control measurement and three drug measurements or two control and two drug tests.

Following injection into the left ventricle, the labeled microspheres lodge in the small arteriolar vessels of all tissues. Portions of each tissue to be studied were then removed, weighed, and subsequently counted for radioactivity. The radioactivity for each isotope was determined by a gamma spectrometer equipped with a pulse-height analyzer (a scintillation detector attached to a Packard Model 930 Spectrazoom and a Model 939 Digital Printer); the spectrometer is able to separate counts even when multiple isotopes (in our case four) are present. The activities were calculated according to the methods of Rudolph and Heymann (as cited in reference 3).

Changes in flow — and thus the effect of CO₂ administration — are determined using the kidney as a reference organ, its flow being measured by the electromagnetic flow probe on the renal artery. Knowing the volume of blood (milliliter per minute) flowing through the kidney and being able to determine the number of spheres in ear and kidney, we can then calculate the rate of flow of blood through otic tissue: flow/ear/sphere = flow/kidney/sphere/kidney. Changes in flow due to CO₂ administration (or that of any other drug) are reflected in this proportion equation.

Four gas combinations were administered to the animals via endotracheal tube and Rudolph valve from reservoir bags. The mixtures, all clinically feasible, were: 5% CO₂-95% air, 5% CO₂-95% O₂, 10% CO₂-90% air, 10% CO₂-90% O₂, each combination being administered for periods consisting of 10, 30, 60, and 120 minutes. The extended time intervals, though not clinically feasible, were included to stress the tissue in order to accentuate differences.

A typical experiment would have the following sequence: the catheters and renal flow probe are placed, a 15-minute waiting period ensues for equilibration, a pre-
control blood gas is obtained, and if the value is reasonable, the measurements are begun. A "control" arterial blood sample is obtained. One minute later, a bolus of microspheres (the first) is injected into the left ventricle (between 200,000 and 300,000 spheres) and flushed with 8 ml of Ringer's solution. The renal blood flow at the moment of sphere injection is recorded. After a ten-minute waiting period the gas bag is attached to the Rudolph valve and the dog permitted only to breathe the gas mixture, exhaled gases being expelled into the room air. Ten minutes after attaching the gas bag another arterial blood sample is obtained and a bolus of a second and different microsphere is injected into the ventricle. The dog continues to breathe the gas mixture, the bags being replenished as needed. Twenty-nine to 30 minutes after attaching the bag, blood is withdrawn and the third bolus of spheres is injected. This is continued and repeated at either 60 or 120 minutes. After the fourth microsphere injection, the dog is killed with an anesthetic overdose. Samples of the following tissues are removed for weighing and counting: nasal mucosa and turbinates, kidney, pons, cerebellum (ansiform lobule), cerebral cortex (entolateral gyrus), pituitary gland, foot pad, face and leg skin, mastoid and temporal bone. The latter two bone samples are obtained by drilling a half-inch core from petrosal crest to tympanic bulla and the core is readily broken at the middle ear space, forming two pieces. The inner half (temporal bone), extending from promontory to petrous apex, contains the labyrinth and extraneous bone. The outer, lateral portion of the core contains mastoid air cells and portions of the bulla, and is referred to as the mastoid area.

**Results**

All the tissues did not behave in the same way in response to CO₂. Only the pons, cerebellum, cortex and temporal bone samples consistently showed an increase in blood flow in response to the initial rise in Pco₂. Other tissues showed a variable response, with the majority of the cases occurring in the same direction. The responses to the gas mixtures were fairly rapid. Within two to three minutes, the animal's breathing pattern and blood gases had reached levels very near those obtained at ten minutes. In four experiments, the blood flow measurements were made after only five minutes of inhalation and were about 25% less than the flows at ten minutes. When the gas mixture was removed and the animal allowed to

The percent change in blood flow in five brain samples and the mean of two temporal bone samples after breathing 10% CO₂-90% O₂ for up to two hours. The values represent the mean of 12 animals. Abbreviations the same as in figure 2.

![Figure 3](http://stroke.ahajournals.org/)

![Figure 4](http://stroke.ahajournals.org/)

The percent change in blood flow in five brain samples and the mean of two temporal bone samples after breathing 10% CO₂-90% O₂ for up to two hours. The values represent the mean of 12 animals. Abbreviations the same as in figure 2.
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TABLE 1
Change in Arterial pH and PCO₂ at Four Time Intervals After Breathing Four CO₂ Mixtures

<table>
<thead>
<tr>
<th>Inhalation mixture</th>
<th>10 minutes</th>
<th>20 minutes</th>
<th>30 minutes</th>
<th>40 minutes</th>
<th>50 minutes</th>
<th>60 minutes</th>
<th>70 minutes</th>
<th>80 minutes</th>
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<tr>
<td></td>
<td>pH</td>
<td>PCO₂</td>
<td>pH</td>
<td>PCO₂</td>
<td>pH</td>
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<td>pH</td>
<td>PCO₂</td>
<td>pH</td>
<td>PCO₂</td>
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<tr>
<td>5% CO₂-95% O₂</td>
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<tr>
<td>Control</td>
<td>7.35</td>
<td>37.2</td>
<td>78</td>
<td>93.8</td>
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<td></td>
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<tr>
<td>After 10 minutes</td>
<td>7.25</td>
<td>48.4</td>
<td>453</td>
<td>100</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>After 30 minutes</td>
<td>7.25</td>
<td>49.5</td>
<td>465</td>
<td>100</td>
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<tr>
<td>After 60 minutes</td>
<td>7.23</td>
<td>50.2</td>
<td>472</td>
<td>100</td>
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<tr>
<td>After 120 minutes</td>
<td>7.24</td>
<td>48.3</td>
<td>486</td>
<td>100</td>
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<tr>
<td>5% CO₂-95% air</td>
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<tr>
<td>Control</td>
<td>7.34</td>
<td>41.4</td>
<td>81</td>
<td>94.3</td>
<td></td>
<td></td>
<td></td>
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<td>After 10 minutes</td>
<td>7.16</td>
<td>65.2</td>
<td>418</td>
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<tr>
<td>After 30 minutes</td>
<td>7.13</td>
<td>71.4</td>
<td>437</td>
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<tr>
<td>After 60 minutes</td>
<td>7.13</td>
<td>69.1</td>
<td>451</td>
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<tr>
<td>After 120 minutes</td>
<td>7.15</td>
<td>64.7</td>
<td>455</td>
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</table>

Discussion

The values we obtained for the normal blood gases and regional brain blood flows are quite similar to those of others, the most startling difference being in the degree of change of brain blood flow after breathing CO₂. Many investigators have obtained increases in CBF of 30% to 70%. It is commonly stated that doubling the PCO₂ will double the CBF, or that each mm Hg increase in PCO₂ is associated with an increase in CBF of about 1 ml/100 gm of brain per minute. We commonly recorded increases of 200% in blood flow when the PCO₂ had changed acutely by 10 to 20 mm Hg. If our normal or control values did not correspond so closely to the data of others, we would feel that our techniques were faulty. Since they do correspond, other explanations are sought.

First of all, the data from many investigators do not fit the common statements relating brain flow to CO₂, the blood flow change being higher per unit of change of PCO₂. Also, we feel that some of the discrepancy may lie in the combination of techniques used in this experiment. To our knowledge, none of the other investigators have measured changes in CBF due to CO₂, using 15 µ microspheres with our gas mixtures for as long as 30 minutes.

Part of the discrepancy may be that previous estimates of CO₂ reactivity are based on relatively short-term exposures to the gas. The change in blood flow in relation to changes in PCO₂ may be small in the first five minutes, but perhaps one cannot extrapolate the relationship to longer breathing times. Figures 2 and 3 contain values that are fairly close to the usually quoted changes, i.e., about 100% change at ten minutes. At five minutes, these changes would be near 75%, quite in line with most data.

It is possible that a unique vascular architecture becomes operative during CO₂ inhalation and biases our values toward the higher end. For example, the microsphere method gives faulty values with vasodilators in the measurement of blood flow in the intestinal mucosa. Our series of "on-off" experiments (fig. 1), in which the second control value is like the first, would seem to be good evidence that this is not the problem.

It seems equally possible that the small spheres are measuring something different than the flow being measured by silicon detectors and inert radioactive gases. We have no other ready explanation for our larger increases in blood flow due to CO₂.

The time course of the responses to CO₂ are of
An example of the blood flow and blood gas responses in an animal after breathing 10% CO₂-90% O₂ for 10, 30 and 60 minutes.

Some interest. With 10% CO₂, the change in brain blood flow remains relatively stable. With 5% CO₂, the difference between air and O₂ is exaggerated, adaptation occurring more readily with air. The obser-

A photomicrograph (450 X) of a portion of the posterior pituitary showing a microsphere impacted in a small blood vessel.
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vation that the blood flow change in the pons is relatively higher than that in the cerebellum and cortex may have a relationship to the needs of the respiratory center.

Temporal bone blood flow was found to be about one-tenth that of brain tissue. Following CO₂ inhalation, the increase in blood flow varied from 60% to 170% in the first ten minutes of gas inhalation. With three of the four gas mixtures, the pattern of changes in temporal bone was unlike that in brain tissues. Only with the 5% CO₂-95% O₂ mixture did they coincide.

Pituitary Blood Flow

We did not measure the blood flow in the pituitary initially because we had no special reason for doing so and we felt that the gland was so small it would not give reliable statistical data. It was eventually measured in 15 animals to see if it could be done and to determine if the pituitary responded to CO₂. The mean ± SE of pituitary blood flow in the 15 animals is 241.9 ± 33.7 ml/100 gm per minute. The blood flow in the pons in these same animals is 22.5 ± 2.2 ml/100 gm per minute.

Microscopic examination of the pituitary revealed that the posterior pituitary had a random scattering of microspheres impacted in small blood vessels throughout its substance (fig. 7). The anterior pituitary was devoid of spheres, as they had been trapped in the stalk portal system. The stalk and gland proper had been counted as one unit weighing about 90 mg. We did not attempt to dissect and measure the flows through the separate portions of the gland. Our mean value of 242 ml/100 gm per minute is significantly higher than the 80 ml/100 gm per minute for the anterior pituitary reported by Porter et al.

The direction of the response to CO₂ was our main concern. Only two of the 15 animals had an increased blood flow after CO₂, the change being modest. The other 13 had a decreased blood flow.

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References

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