Effects of Hypercapnia on Enhancement of Decreased Perfusion Flow in Non-Infarcted Brain Tissues

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**SUMMARY** The effects of hypercapnia on enhancement of reduced cerebral perfusion were re-evaluated in areas of ischemia produced by occlusion of the canine middle cerebral artery. Perfusion was measured by $^{85}$Kr ($\beta$-ray) and $^{133}$Xe ($\gamma$-ray) clearances, fluorescein angiography and diameter measurement of arteries. Between 45 and 55 mm Hg of Paco$_2$ rCBF measured with both isotopes increased significantly. When Paco$_2$ was elevated above 55 mm Hg, there was a remarkable dissociation in the rCBF measured by both isotopes. Cortical blood flow measured by $^{85}$Kr clearance decreased and, conversely, rCBF measured by $^{133}$Xe continued to increase. Arteries of less than 50 $\mu$m in diameter in areas of ischemia dilated significantly during hypercapnia. At Paco$_2$ above 65 mm Hg, progressive sub-pial hemorrhage and extravasation of dye were observed as side effects of hypercapnia. The use of mannitol combined with hypercapnia appeared to be harmful. A Paco$_2$ level between 45 and 55 mm Hg increases perfusion in areas of mildly reduced rCBF.

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IN THE ACUTE STAGE of cerebrovascular occlusive disease, it is essential to prevent irreversible neuronal changes caused by ischemia by restoring decreased cerebral blood flow.1-3 Carbon dioxide, a potent cerebral vasodilator which does not reduce systemic blood pressure in the intact brain, has been considered a non-surgical method for enhancement of perfusion. In spite of numerous investigations in the last decade, the effect of CO$_2$ on improving CBF in ischemic brain still remains controversial.5-13 Inconsistency of the results is probably due to different methods or isotopes used to measure CBF and also due to differences in the degree of ischemia in animals or patients.14 The effect of hypercapnia on restoration of reduced CBF was re-evaluated with beta-emitting $^{85}$Kr and gamma emitting $^{133}$Xe clearance techniques.14-17 In addition, the combined use of mannitol injection and CO$_2$ inhalation was tested in the same model.18

**Material and Methods**

**Procedure**

Twenty-nine mongrel dogs, unselected as to age and sex, and weighing 19–24 kg were initially anesthetized with intravenous pentobarbital, 25 mg/kg. Additional pentobarbital, 25–50 mg, was given through a femoral vein cannula as necessary. The femoral artery was cannulated for continuous recording of blood pressure and sampling for blood gas analysis (I.L. Model 113 gas analyzer). After tracheal intubation, respiratory rate and volume were controlled and Flaxedil was given as needed. A small polyethylene catheter was introduced into the left lingual artery so that the tip of the catheter was at the junction with the external carotid artery. With the head of the dog fixed, a large
cranectomy exposed the left cerebral hemisphere and allowed easier access to the proximal portion of the left middle cerebral artery. After the dura was reflected, the surface of the brain was protected with transparent polymer film (Saran) and by frequent irrigation with Elliot's solution maintained at 36°C.

Arterial Occlusion

In 23 dogs, the proximal portion of the left middle cerebral artery was exposed in the anterior part of the Sylvian fissure and occluded with a Mayfield clip supplemented by another one or 2 clips when the site of trifurcation was located too proximal to allow safe access to the main artery. Arterial occlusion was confirmed with fluorescein angiography and postmortem observation. The sites of occlusion were approximately 1.3 cm lateral to the origin of the left middle cerebral artery with preservation of its small orbital branch.

The hypercapnic state was maintained by regulation of respiratory rate and volume with gas containing CO₂ of 5%, 10%, or 25%.

In 15 dogs, an intravenous infusion of 20% mannitol solution was started at the same time as 5% CO₂ was inhaled, and 100 cc was injected during the first 20 minutes. Thereafter, the infusion rate was reduced and 3 hours later, when sequential flow studies were completed, the mean dosage injected was 240 cc (2.2 g/kg body weight). During the mannitol infusion systemic blood pressure was repeatedly checked and remained within normal limits.

Fluorescein Angiography of the Brain

Sodium fluorescein solution (1.6 cc of 1%) was injected into the left common carotid artery as rapidly through a low-power microscope (X 100) with a X 10 objective and a X 10 ocular, which allowed easier access to the proximal portion of the left middle cerebral artery. After the dura was reflected, the surface of the brain was protected with transparent polymer film (Saran) and by frequent irrigation with Elliot's solution maintained at 36°C.

Diameter Measurement of Epicerebral Arteries

Using a motor-driven Nikkon F₂ camera with fixed focus 200 mm Medical Nikkon lens, photographs of the cortical surface were taken on Kodachrome II color film at one time magnification. Five to 9 areas were chosen from each photograph and 4–9 arteries of 30 to 560 µ in diameter from each area were measured. Diameters were measured by viewing the vessel through a low-power microscope (X 100) with a X 10 objective and a X 10 ocular to which a micrometer was fitted.

The appearance of sub-pial hemorrhage and of red veins in the epicerebrum was also examined in each photograph through a lower-power microscope (X 35).

Measurement of rCBF

Four semiconductor detectors with a disc of lithium-drifted silicon (1.8 mm in diameter and 1 mm thick) were placed on the transparent polymer film covering the exposed cerebral cortex to measure cortical blood flow (cortical BF) with ⁸⁵Kr and rCBF with ¹³³Xe at normocapnia before, and at normocapnia, and various hypercapnic states after occlusion of the middle cerebral artery.

With four detectors over the polymer film covering the cortex, 8 to 13 µCi of ⁸⁵Kr or ¹³³Xe, dissolved in 3 cc of saline solution, were rapidly injected through the catheter placed in the left carotid artery. The recording from each detector was monitored through an on line computer system (PDP-12) and cortical BF and rCBF values were calculated from each clearance curve by a modification, developed by Yamamoto.¹⁰ of the stochastic analysis by Zierler.²¹ The maximum range of the detecting field for the 610 KeV beta radiation of ⁸⁵Kr is 2.5 mm in brain tissue. This is the appropriate range for measurement of cortical BF in the dog brain.

The depth sensitivity of our semiconductor detector system was determined from point source of 81 KeV gamma emitting ¹³³Xe in water, with a lower energy discrimination level of 50 KeV. The depth sensitivity is represented by a 10% isoresponse curve, extending to a depth of 8 mm from the surface and 7 mm along the surface from the center of the detector, which indicates ¹³³Xe clearance technique tends to give misleading blood flow determinations in the ischemic area.

Postmortem Examination

The autopsied brain was investigated macroscopically and microscopically. H-E stain was used for light microscopic examination.

Results

A) Degree of Reduced rCBF in Model

Following occlusion of the left middle cerebral artery, rCBF measured with ⁸⁵Kr and ¹³³Xe were significantly reduced by 42% from 1.346 ± 0.110 (se) (n = 32) to 0.763 ± 0.072 (n = 29) F/λ (p < 0.01) and by 19% from 0.414 ± 0.008 (se) (n = 32) to 0.330 ± 0.010 (n = 32) F/λ (p < 0.001) respectively (F = Flow, λ = Partition Coefficient). The meaning of the unit F/λ has been documented in detail previously.⁹

B) Change of rCBF under Hypercapnia

Following the control study after arterial occlusion at normocapnia, CO₂ inhalation was started. Figure
HYPERCAPNIA TO RESTORE rCBF/Nakagawa et al.

1-a shows the actual blood flow rate (F/λ) from one representative dog. Cortical blood flow measured by \( ^{85} \text{Kr} \) significantly increased by 75%, exceeding the values at the pre-clipped state, at 50 mm Hg. However, these values decreased at 75 mm Hg and were lower than the values at the pre-clipped state by 9%.

rCBF measured by \(^{133} \text{Xe} \) increased gradually, nearly linearly, as \( \text{Paco}_2 \) was elevated. Figure 1-b shows the fluorescein angiogram taken at the same level of \( \text{Paco}_2 \) as in figure 1-a, indicating that filling of dye was definitely more increased at 50 mm Hg (C) than at 40 or 75 mm Hg (B or D).

rCBF values expressed as percent change compared to normocapnia after arterial occlusion are summarized in figure 2. This illustration clearly indicates that rCBF values measured with both isotopes were significantly increased at \( \text{Paco}_2 \) level of 45 and 55 mm Hg. However, after \( \text{Paco}_2 \) was elevated above this level, rCBF measured by the 2 isotopes took a different course.

\( \text{CO}_2 \) inhalation combined with mannitol administration resulted in a very similar alteration of rCBF as compared to \( \text{CO}_2 \) inhalation alone. However, a minimum difference was observed in that reduction of cortical blood flow tended to appear at a lower \( \text{Paco}_2 \) level in the combined therapy group.

Statistical comparisons of cortical blood flow are shown in figure 3. Figures 4 and 5 illustrate typical response patterns of cortical blood flow to hypercapnia in normocapnic conditions. These figures show the response patterns of cortical blood flow to hypercapnia in normocapnic conditions.

**Figure 1-a:** Dog 75-56. Graph showing rCBF measured with \( ^{85} \text{Kr} \) and \( ^{133} \text{Xe} \) clearance during increase of \( \text{Paco}_2 \). Cortical blood flow measured with \( ^{85} \text{Kr} \) significantly increased at \( \text{Paco}_2 \) of 50 mm Hg.

**Figure 1-b:** Dog 75-56. Fluorescein angiogram at the same level of \( \text{Paco}_2 \) as figure 1-a. Dye was injected through a catheter placed in the lingual artery. Numbers marked in the right lower corner of each photograph represent the time interval, expressed in seconds, from injection of dye. A: Angiogram before occlusion of the middle cerebral artery (\( \text{Paco}_2 = 40 \) mm Hg). Normal filling of dye in the epicerebral vessels is illustrated. B: Angiogram 30 min after occlusion. Area of lesser filling is clearly demarcated on fluorescein angiogram (\( \text{Paco}_2 = 40 \) mm Hg). C: At 2 hours 10 min after occlusion with 5% inhalation (\( \text{Paco}_2 = 50 \) mm Hg). Filling of dye improved definitely. D: At 3 hours 40 min after occlusion with 10% \( \text{CO}_2 \) inhalation (\( \text{Paco}_2 = 75 \) mm Hg). There is less filling of dye in the whole area as compared to C.
FIGURE 2. Summary of change of rCBF values expressed as percent change compared to normocapnia after arterial occlusion. This figure clearly indicates that rCBF values measured with both isotopes were significantly increased at PaCO₂ level of 45 and 55 mm Hg. However, after PaCO₂ was elevated above this level, rCBF measured by 2 isotopes took a different course.

measured by the ⁸¹Kr clearance technique are summarized in figure 3. At PaCO₂ 50 mm Hg, rCBF values in both groups increased significantly without a significant difference between the 2 values. At PaCO₂ range between 65 and 100 mm Hg, the values differed significantly from each other, the control values increased gradually and there was a steady decline in the dogs with clipped middle cerebral arteries.

A similar comparison of rCBF measured by the ¹³³Xe clearance technique is summarized in figure 4, indicating that values of both groups increased in proportion, to the elevation of PaCO₂. It is interesting that this increase was more marked without arterial occlusion at each level of PaCO₂.

C) Change in Diameters of Epicerebral Arteries under Hypercapnia

In total, 407 portions of arteries ranging from 30 to 560μm in diameter were measured at each level of PaCO₂. A total of 1519 measurements of arterial diameter were made so that the arteries measured were fairly distributed over the entire area of reduced rCBF.

The change of diameter of pial arteries expressed as percent change is shown in figure 5. Following arterial occlusion, arteries less than 50μm in diameter dilated by 18 ± 2(SE)% (p < 0.001) with dilation of 19 ± 3% in arteries between 50 and 100μm (p < 0.001), 8 ± 2% in arteries between 100 and 200μm (p < 0.001) and 7 ± 2% in arteries more than 200μm in diameter (p < 0.001).

The change in artery diameter following arterial occlusion differed characteristically according to artery size. Arteries of less than 50μm in diameter dilated gradually and significantly as PaCO₂ increased, while the diameter of arteries between 50 and 100μm remained nearly the same as in the normocapnic state. Arteries of more than 100μm in diameter initially decreased in diameter by over 10% at PaCO₂ of 50 to 60 mm Hg and then gradually dilated to the same size as in the normocapnic state at a PaCO₂ level of 65 mm Hg. Hypercapnia combined with mannitol ad-
ministration produced a very similar response in pial arteries when compared to changes produced by hypercapnia alone.

D) Circulation Time of Fluorescein Dye with Hypercapnia

A change in circulation time of the dye in the pial vessel is shown in figure 6. The time interval between injection of the dye and appearance of the dye in arteries of 100 to 400μ in diameter became shorter as PaCO₂ increased, with the shortest period of time observed at PaCO₂ of 50 and 70 mm Hg. This phenomenon was similar to maximum capillary filling time. The time interval from injection of the dye until disappearance of the dye in veins of 100 to 200μ in diameter tended to be gradually prolonged, particularly after PaCO₂ was elevated above 80 mm Hg.

The time interval until maximum filling of dye in capillaries through pial arteries was nearly the same in both groups. However, with hypercapnia and mannitol infusion, disturbance of venous circulation became more pronounced than with hypercapnia alone.

E) Occurrence of Sub-Pial Hemorrhage, Extravasation of Dye and Red Veins

The appearance of sub-pial hemorrhage in both groups is summarized in table 1. In an occurrence of sub-pial hemorrhage, grade "mild" represents a sub-

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**Table 1** Frequency of Appearance of Sub-Pial Hemorrhage.

<table>
<thead>
<tr>
<th>Grade</th>
<th>Mean PaCO₂</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>40 torr</td>
</tr>
<tr>
<td>Absent</td>
<td>8</td>
</tr>
<tr>
<td>Mild</td>
<td>1</td>
</tr>
<tr>
<td>Moderate</td>
<td></td>
</tr>
<tr>
<td>Marked</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>8</td>
</tr>
</tbody>
</table>

M = with Mannitol

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**Figure 4.** Statistical comparison of rCBF measured with 133Xe clearance technique. rCBF values of both groups increase steadily in response to elevation of PaCO₂ with significantly higher response in control group at each level of PaCO₂.

**Figure 5.** Graph showing change in diameter of arteries, during hypercapnia, following the occlusion.
and severity after this level, particularly with mannitol infusion.

The extravasation of dye frequency is shown in Table 2, indicating that extravasation of dye was seen more frequently at a PaCO₂ level of 50 mm Hg in the group with mannitol infusion. At PaCO₂ above 60 mm Hg, the frequency of extravasation increased in proportion to elevation of PaCO₂ in both groups, particularly with mannitol infusion. Sub-pial hemorrhages and extravasation of dye were observed mainly around the capillaries and small veins, close to the superior sagittal sinus. There was no evidence of red veins at PaCO₂ below 70 mm Hg, with hypercapnia or with hypercapnia with mannitol infusion.

**Table 2 Frequency of Extravasation of Dye.**

<table>
<thead>
<tr>
<th>Grade</th>
<th>Absent</th>
<th>Mild</th>
<th>Moderate</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean PaCO₂ (torr)</td>
<td>40</td>
<td>50</td>
<td>60</td>
<td>72</td>
</tr>
<tr>
<td>M</td>
<td>M</td>
<td>M</td>
<td>M</td>
<td>M</td>
</tr>
<tr>
<td>Absent</td>
<td>8</td>
<td>8</td>
<td>13</td>
<td>4</td>
</tr>
<tr>
<td>Mild</td>
<td>6</td>
<td>1</td>
<td>1</td>
<td>3</td>
</tr>
<tr>
<td>Moderate</td>
<td>1</td>
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<tr>
<td>Total</td>
<td>8</td>
<td>5</td>
<td>8</td>
<td>13</td>
</tr>
</tbody>
</table>

**Discussion**

rCBF measured with ¹⁵³Xe clearance method does not always reflect blood flow only in an ischemic area because of counts contributed from adjacent normal brain tissue. Therefore, beta-emitting ⁸⁸Kr is more suitable for experimental studies of focal cerebral ischemia since it has a discrete detecting field of 2.5 mm. Geiger-Müller counters have been used for detecting the beta rays of ⁸⁸Kr in animal models of focal cerebral ischemia.

Using a Geiger-Müller counter, Waltz reported that in ischemic cortex, one day or less after occlusion of the cat's middle cerebral artery, cortical blood flow measured with ⁸⁸Kr did not increase with hypercapnia. However, cortical BF increased significantly in response to an increase in PaCO₂ 5 days or more after clipping. Recently, Hanson et al. stated that in the squirrel monkey there was no increase of cortical blood flow measured with ⁸⁸Kr clearance with hypercapnia between 40 and 60 mm Hg, in spite of the fact that rCBF determined by ¹⁵³Xe increased steadily. Their measuring points after clipping the middle cerebral artery were PaCO₂ levels of 24, 40 and 57 mm Hg. Since a Geiger-Müller counter has a long resolving time of 200 to 500 μsec, it is not suitable for a dynamic study such as ⁸⁸Kr clearance technique because there is considerable count loss in the initial portion of the clearance curve, resulting in low flow values.

The semiconductor detectors used in this study have a very short resolving time of 0.5 to 1.0 μsec, making this system a better beta-detecting device than a Geiger-Müller counter. Using these semiconductor detectors, the cortical BF measured by the ⁸⁸Kr clearance technique increased significantly at PaCO₂ levels between 45 and 55 mm Hg (Figs. 1-a and 2). This was confirmed both by an increase of retrograde blood flow on the fluorescein angiogram (Fig. 1-b) and by significant dilation of smaller epicerebral arteries on diameter study (Fig. 5). The most probable reason why cortical BF measured with ⁸⁸Kr was increased during...
mild hypercapnia, is that the reduced rCBF in our study was mild and at the acute stage. The response of cerebral arteries during hypercapnia depends on degree and stage of ischemia.

However, as PaCO₂ was elevated above 55 mm Hg, a remarkable dissociation in cortical BF values (measured by ⁸¹Kr) and rCBF (measured by ¹³³Xe) was observed. Cortical BF started to decrease and, conversely, rCBF kept on increasing steadily (fig. 2). No significant increase in arterial blood pressure was observed during hypercapnia. The cause of the steady reduction in the cortical BF at PaCO₂ levels above 60 mm Hg, is still unknown despite the fact that smaller arteries between 30 to 50μ in diameter were steadily and significantly dilated up to a PaCO₂ level of 80 mm Hg (fig. 5). A significant increase in the frequency of perivascular exudation of fluorescein dye and hemorrhage from the capillaries and small veins (tables 1, 2), with prolongation of venous circulation time at a PaCO₂ level above 60 mm Hg (fig. 6), may be an important factor in the steady reduction of cortical BF with higher hypercapnia.

In contrast to the sub-pial hemorrhages, there was no evidence of intracerebral hemorrhage, macroscopic or microscopic, even in dogs which showed sub-pial hemorrhage. This may be due to the intracerebral vessel wall being protected by the surrounding brain tissue.

The use of mannitol in addition to hypercapnia was expected to permit a further increase in cortical BF in areas of reduced rCBF. ²⁻⁹ However, regardless of a presumably sufficient dosage of mannitol, response of cortical BF measured by ⁸¹Kr and rCBF by ¹³³Xe was very similar to that obtained with hypercapnia alone. ²⁻¹⁰ With simultaneous use of carbon dioxide and mannitol, sub-pial hemorrhages and extravasation of dye were more marked (table 1, 2) and fluorescein dye circulated more slowly in the venous phase, as compared to hypercapnia alone (fig. 6).

It has been demonstrated that capillary permeability is reversibly increased by injection of small amounts of such hypertonic solutions as 2 M urea or 5% NaCl, ²¹⁻²² which is likely due to opening of the tight junctions between endothelial cells by osmotic shrinkage. ²¹⁻²² Moderate to marked elevations of PaCO₂ are known to increase barrier permeability. ²³⁻²⁵ These 2 factors, hypercapnia and hyperosmolality in serum, may have promoted the occurrence of sub-pial hemorrhage and extravasation of dye, possibly resulting in marked disturbance of epicerebral venous circulation (fig. 6). In addition, the frequent appearance of extravasation of dye at PaCO₂ levels of 50 mm Hg must be closely related to increased serum osmolarity achieved by rapid infusion of mannitol at this stage.

It is concluded that hypercapnia between 45 and 55 mm Hg was definitely effective in enhancement of decreased perfusion flow in the areas of mildly reduced rCBF and, also, that mannitol administration combined with hypercapnia did not produce any additional benefit in restoration of reduced flow.
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