Local Cerebral Blood Flow Following Transient Cerebral Ischemia

II. Effect of Arterial P\textsubscript{CO}_2 on Reperfusion Following Global Ischemia


SUMMARY Following 5 minutes of global ischemia, local cerebral blood flow (LCBF) was shown to have an initial reactive hyperemia that was followed, within the first hour, by persistent hypoperfusion (Part I). Intracranial pressure (ICP) was never elevated during the period of poor reperfusion. These experiments attempted to reverse the state of subnormal LCBF by inducing hypercarbia or hypocarbia or maintaining normocarbia. Although hypocarbia did increase LCBF at several electrode sites, neither the intracerebral steal syndrome nor the "squeeze" syndrome are a dominant consequence of hypercarbia in this model of global ischemia. Hypercarbia was consistently more effective in elevating LCBFs and in recovery of the electrocorticogram. It appears that, in the absence of raised ICP, hypercarbia may be preferred to normal or low P\textsubscript{ACO}_2. Even though hypercarbia was superior to normocarbia or hypocarbia, hypercarbia was not a completely satisfactory regimen for reversing the state of poor reperfusion.

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IN THE PREVIOUS PAPER the impaired perfusion that ultimately follows 5 minutes of global ischemia was described. The present report extends these studies and is concerned with the effect of arterial P\textsubscript{CO}_2 on post-ischemic local cerebral blood flow (LCBF) and the recovery of the electrocorticogram (ECoG) during the period of impaired perfusion. Because nerve cells might survive a brief ischemic insult only to ultimately die because of prolonged oxygen debt, considerable motivation exists to develop methods that fully restore the microcirculation. Much attention has been placed on the effects of carbon dioxide on the cerebral vasculature. Studies in humans, dogs, and other mammals have shown that an increase in arterial P\textsubscript{CO}_2 causes an increase in total, regional, and local cerebral blood flow.\textsuperscript{1,2} Although CO\textsubscript{2} is a potent vasodilator, there is evidence that it may, in fact, cause a decrease in blood flow to severely locally infarcted areas of brain.\textsuperscript{3,4} This paradox occurring during focal ischemia is explained by shunting of blood from unreactive ischemic tissue to surrounding tissue that is still vasoactive and has been termed the "intracerebral steal."\textsuperscript{5} Associated complications of raised intracranial pressure and edema resulting in reduced blood flow during hypercarbia have been termed "intracerebral squeeze."\textsuperscript{6} Some have suggested that an "inverse steal" may be produced by hyperventilating with the consequent lowering of the P\textsubscript{CO}_2.\textsuperscript{7} This reduction in P\textsubscript{ACO}_2 is thought to cause vasoconstriction in healthy areas which helps shunt blood toward ischemic regions. Soloway et al.\textsuperscript{8} reported that hyperventilation reduced the area of infarcted tissue. Hyperventilation also assists in lowering the intracranial pressure by reducing the brain blood volume and may aid in restoration of autoregulation.\textsuperscript{9} Other investigators have concluded that neither hypercarbia nor hypocarbia appear to be superior to normocarbia in preventing neurological deficit.\textsuperscript{10,11}

Although the steal and inverse steal syndromes have been defined for flow abnormalities during focal ischemia, it is possible that steal and inverse steal may be manifest in poorly reperfused areas following global ischemia. The present experiments were designed to determine whether there was evidence of a locally induced hypercarbic steal or hypocarbic inverse steal, and to determine which P\textsubscript{CO}_2 regimen was the most efficacious in elevating depressed local cerebral blood flows after global ischemia. Alterations in the P\textsubscript{ACO}_2 were started after the initial phase of reactive hyperemia (when LCBFs fell to subnormal levels) in an attempt to restore the blood flow to deprived areas.

Methods

Anesthesia was induced in adult, healthy mongrel dogs, 15 to 25 kg, with intravenous thiopentone sodium and maintained with pentobarbitone sodium on demand (as determined by eyelid reflex). When gallamine was used to prevent breathing against the mechanical ventilator (after the addition of CO\textsubscript{2} to inspired gases), care was taken to ensure an adequate anesthetic depth.\textsuperscript{12,13} Animal preparation, methods of estimating local cerebral blood flow and cardiac output, and the technique for producing the total ischemia have been described.\textsuperscript{14} Systolic, diastolic, and mean arterial pressure, heart rate, end-expired CO\textsubscript{2} (Fe\textsubscript{CO}_2), mean rectified voltage of the electrocorticogram, and

\textsuperscript{*}All experiments were performed in accordance with "Code of Practice for Control of Experiments in Animals" issued by the Australian National Health and Medical Research Council.

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the intracranial pressure were continuously recorded. Hypercarbia or hypocarbia was induced by adding CO₂ to inspired gases or by hyperventilation. A Radiometer BMS Mk2 blood microsystem was used for serial measurement of arterial pH and Paco₂. Intravenous sodium bicarbonate (4.2%, at a dose not exceeding 0.05 ml/min/kg) was used for adjusting pH₄. During hypercarbia or hyperventilation, pH₄ was corrected to the appropriate value based on the measured Paco₂ and by assuming a base deficit of 0; the appropriate arterial pH was estimated using the Siggaard-Andersen alignment nomogram.¹¹

Using Sherman bone screws implanted in frontal skull, the epidural ECoG was recorded continuously as the mean rectified voltage (MRV). The MRV was suitable for comparing pre-ischemic and post-ischemic ECoG amplitude increases and decreases over the approximate 12 hour span of each experiment. Recovery of ECoG following ischemia was assessed as the recovery of the MRV to pre-ischemic control levels. Determination of generally depressed ECoG activity did not require power spectral analysis. A depressed MRV was always accompanied by loss of high frequency components in the ECoG (as judged by visual inspection of the ECoG). A depressed MRV always indicated an incomplete return of ECoG activity to control levels.

**Results**

The results consisted of 2 groups of experiments. In Group 1, each animal was tested serially with hypocarbia (20–25 mm Hg Paco₂), moderate hypercarbia (50–55 mm Hg Paco₂), severe hypercarbia (65–70 mm Hg Paco₂) and normocarbia (36–40 mm Hg Paco₂), i.e. each animal was subjected to all 4 Paco₂ regimens at various times after onset of impaired reperfusion.

In Group 2, each animal was tested with only one Paco₂ alteration; after the onset of impaired reperfusion, an animal was maintained with only hypocarbia, normocarbia or hypercarbia for 8 hours or until the ECoG returned to (approximately) control values. At the conclusion of the hypocarbic or hypercarbic period, the animal was returned to a normal Paco₂ for further LCBF measurements. Group 1 and Group 2 will be presented and discussed separately.

**Group 1**

When the post-ischemic LCBFs decreased to subnormal levels pH₄ was gradually corrected with sodium bicarbonate over the subsequent hour; the animal was normocarbic during this period. The animal was then treated with hypocarbia or hypercarbia. As reported, correction of pH₄ never resulted in any significant change in LCBFs. Before inducing hypocarbia, moderate hypercarbia, or severe hypercarbia, the animal was always returned to a normal Paco₂ for at least 20 minutes during which LCBF measurements were continued, i.e. between every elevation or depression of Paco₂, the animals were normocarbic prior to the next change in Paco₂. The periods of hypocarbia or hypercarbia were typically 30 to 40 minutes.

Table 1 shows the increases and decreases in LCBF caused by severe hypercarbia, moderate hypercarbia or hypocarbia after a period of normocarbia. Hypercarbia (either severe or moderate) generally caused an increase in LCBF compared to normocarbia, and hypocarbia generally caused a decrease in LCBF compared to normocarbia. During hypocarbia, 9 sites recorded an increase in flow (fig. 1). This observation supports previous research which indicates hyperventilation can cause an increase in blood flow through infarcted tissue.⁸⁻⁻¹ centre. If “cerebral steal” or “squeeze” were significant effects, it would be expected that these 9 sites would exhibit reduced flows during hypercarbia. A reduction in LCBF was seen at 2 of these 9 sites during severe hypercarbia; the remaining 7 sites showed a flow increase during severe hypercarbia that was equal to or greater than the flow increase seen during hypocarbia. Moderate hypercarbia caused a flow increase at all 9 sites; thus indicating that these were still reactive to increased Paco₂. As shown in figure 1, hypercarbia caused a decrease in LCBF at 6 sites (4 sites during severe hypercarbia and 2 sites during moderate hypercarbia). If this change was due to “cerebral steal” or “squeeze,” hyperventilation should have increased blood flow at these sites. Of these 6 sites, hypocarbia caused a flow increase at 2 and did not alter flow at the remaining 4 suggesting that “cerebral steal” or “squeeze” were in evidence at a minority of electrode sites during hypercarbia.

Hypercarbia resulted in a generally increased local CBF, although some flows remained significantly subnormal (fig. 2).

**Group 2**

After the post ischemic decrease in LCBFs, each dog in Group 2 was maintained for the duration of the experiment on hypocarbia, hypercarbia or normocarbia. There were 5 dogs in each CO₂ regimen. Subsequent to ischemia, the ECoG and LCBFs were monitored for about 8 hours or until the ECoG mean rectified voltage (MRV) returned to approximately 100% of the control value.

| TABLE 1 Group 1 Effect of Increased or Decreased Paco₂ on Post-ischemic LCBF Relative to Normocarbia |
|---------------------------------------------------|---------------------------------------------------|---------------------------------------------------|
| | % of sites* Increased LCBF | % of sites Decreased LCBF | % of sites Unresponsive |
| Severe hypercarbia | (Paco₂ 70 mm Hg) | 83% | 10% | 7% |
| Moderate hypercarbia | (Paco₂ 55 mm Hg) | 81% | 5% | 15% |
| Hypocarbia | (Paco₂ 25 mm Hg) | 22% | 56% | 22% |

*Total of 41 electrode sites.
For the hypercarbia experiments, the initial \( P_{a\text{CO}_2} \)
of 70 mm Hg was gradually reduced over a period of
hours until the \( P_{a\text{CO}_2} \) was normal at the conclusion of
the experiments. LCBF estimates were made at each
\( P_{a\text{CO}_2} \). Figure 3 shows a typical hypercarbia experi-
ment. Hypercarbia initially caused a significant in-
crease in local blood flows (fig. 4), but as the \( P_{a\text{CO}_2} \)
was gradually reduced, the LCBFs generally began to
diminish. Even at the initial high \( P_{a\text{CO}_2} \), only 12 of 27
flows increased to control levels.

The \( P_{a\text{CO}_2} \) was maintained at 20–25 mm Hg during
the hypocarbia experiments. At the end of the hypo-
carbia period, tidal volume was gradually reduced to
allow a normal \( P_{a\text{CO}_2} \), and further LCBF mea-
surements were made. At most electrode sites, hypo-
carbia caused an initial LCBF decrease that persisted
for the duration of the experiment (fig. 4). Two elec-
trode sites demonstrated increased flows during hypo-
carbia. When the \( P_{a\text{CO}_2} \) was returned to normal at the
end of the experiment, all of the flows increased or
remained unchanged; at this point, none of the LCBFs
demonstrated any decrease.

The initial high flows during hypercarbia might
promote improved flow when the animals were
returned to normal \( P_{a\text{CO}_2} \), but table 2 shows that there
is no difference in LCBFs among the 3 regimen at the
conclusion of the experiments.

In previous experiments from this laboratory,\(^1\)
hypercarbia (65–70 mm H g \( P_{a\text{CO}_2} \)) in control dogs
always caused an increase in LCBF; this increase
averaged \( 168\% \pm 38\% \) (s.d.) of control with a range of
\( 128\% \) to \( 260\% \). Hypocarbia (20–25 mm H g \( P_{a\text{CO}_2} \))
caused a decrease in LCBF to \( 81\% \pm 15\% \) (s.d.) of
control, with a range from 69\% to 95\%; at 3 of 12 elec-
trode sites, hypocarbia caused an increase to about
125\% of control. It is proposed\(^1\) that these parado-

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**FIGURE 1.** Paradoxical increases in LCBF during hyperventilation (that may reflect reverse steal) and decreases in LCBF during hypercarbia (that may reflect steal) in Group I animals.

**FIGURE 2.** Effect of \( P_{a\text{CO}_2} \) on post-ischemic LCBFs in Group I animals during the period of impaired recirculation. Arterial pH was adjusted to yield a base deficit of approximately 0.
EFFECT OF HYPERCARBIA ON LOCAL CBF FOLLOWING ISCHEMIA

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FIGURE 3. An example of the hypercarbia regimen from a Group 2 dog. The addition of CO₂ resulted in a significant increase in LCBFs. Inspired CO₂ was gradually decreased until Paco₂ was normal near the end of the experiment.

TABLE 2 Group 2 Ultimate Recovery of LCBFs to Pre-ischemic Control Level About 8 Hours Following Ischemia. All Animals at 38 mm Hg Paco₂ and 7.4 pH.

<table>
<thead>
<tr>
<th>Regimen</th>
<th>Normal LCBF</th>
<th>Low LCBF</th>
<th>Total number of electrode sites</th>
<th>No. of dogs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hypercarbic</td>
<td>9</td>
<td>20</td>
<td>29</td>
<td>5</td>
</tr>
<tr>
<td>Normocarbic</td>
<td>9</td>
<td>20</td>
<td>29</td>
<td>5</td>
</tr>
<tr>
<td>Hypocarbic</td>
<td>7</td>
<td>21</td>
<td>28</td>
<td>5</td>
</tr>
</tbody>
</table>

Hypocarbica during hypoperfusion resulted in a flow decrease from 74.6% ± 20.1% (s.d.) to 55.7% ± 23.0% of control; this represents a 19% decrease which is the same percentage decrease seen in hypocarbic normal dogs.

The ECoG's consistently recovered more completely with the hypercarbia regimen (table 3).

In the present experiments, hypercarbia during the hypoperfusion period caused an LCBF increase from 49.4% ± 25.3% (s.d.) to 80.0% ± 47.4% of control; this represents a 31% flow increase as compared to an average 68% flow increase seen for hypercarbic normal dogs. This suggests that ischemia causes the vasculature to be less responsive to hypercarbia. Hypocarbica during hypoperfusion resulted in a flow decrease from 74.6% ± 20.1% (s.d.) to 55.7% ± 23.0% of control; this represents a 19% decrease which is the same percentage decrease seen in hypocarbic normal dogs.

The postischemic intracranial pressure (ICP) was normal in all the animals during treatment with hypercarbia, normocarbia or hypocarbia, i.e. between 0 and 7 mm Hg. Although ICP increased sharply during the
restoration of arterial flow and the initial reactive hyperemia the ICP returned to normal within 15 minutes. Subsequent hypercarbia increased ICP by 2 or 3 mm Hg, and hypocarbia either left ICP unchanged or slightly decreased. Blood pressure was normal during this period with a systolic level of at least 120 mm Hg. Cardiac output (CO) measurements in the 3 animals showed that CO was initially increased for about 15 minutes after ischemia and then decreased to approximately normal values within the first hour.

Discussion

These experiments have studied the effects of Pco₂ on depressed local cerebral blood flows after global ischemia to determine whether there was any evidence that hypercarbia could cause a "steal" of blood from poorly perfused areas, and whether hypocarbia could cause an increase of blood flow in low flow areas, presumably by the "reverse steal" mechanism. The evidence for (or against) steal and reverse steal has been obtained after transient global ischemia, and presumably by the "reverse steal" mechanism. The evidence for (or against) steal and reverse steal has been obtained after transient global ischemia, and steal and reverse steal mechanisms have commonly been applied to focal ischemia.

Because of carbon dioxide's potency as a vasodilator and the ease with which the Pco₂ can be controlled, CO₂ has been used in many situations where increased cerebral blood flow is necessary, e.g., during hypothermia to promote even cooling, after focal or global ischemia, or after carotid endarterectomy. Some studies have indicated that an elevated Pco₂ may cause "cerebral steal" and that a low Pco₂ may actually increase blood flow to infarcted, vasoparalytic areas by shunting blood from regions of normal or supranormal blood flow. Hypercarbia has also been shown to promote intracerebral "squeeze," a condition of reduced blood flow during post-ischemic edema and raised intracranial pressure.

Early reports of post-ischemic hyperventilation indicate that low Pco₂ may be an important therapeutic adjunct in promoting "inverse steal" to ischemic regions and restoring autoregulation, but more recent reports do not support this.

The present experiments indicate that hypocarbia is capable of increasing local flow. In Group 1, 9 of 41 electrode sites (in 7 animals) recorded a flow increase during hyperventilation. If "cerebral steal" or "squeeze" was a dominant effect, then these 9 sites should have shown a flow decrease during hypocarbia, but only 2 of the 9 showed a flow decrease during hypocarbia. In Group 2, 2 of 28 electrode sites (5 animals) showed an increase in local blood flow at low Pco₂ (fig. 4); these animals remained hypocarbic for the duration of the experiment and were not tested with high Pco₂.

In Group 1, hypercarbia (severe or moderate) caused a flow decrease at 6 sites. Again, "cerebral steal" or "squeeze," if present, would be evident when the animals were hyperventilated, i.e., these 6 sites should have shown a flow increase during hyperventilation, but only 2 of the 6 had an increased blood flow during hyperventilation. In Group 2 animals, hypercarbia did not result in a flow decrease at any of the electrode sites; hypocarbic animals in Group 2 were not tested with hyperventilation. Thus, a total of 4 sites (all from Group 1) had a positive indication of "cerebral steal" or "squeeze." These results suggest that hypocarbia is capable of increasing some local blood flows following ischemia, and that "cerebral steal" or "squeeze" is not a dominant result of hypercarbia. These results are similar to those of Meyer et al.

Hypocarbia may cause local tissue hypoxia which results in dilatation and a subsequent increased local blood flow. In the 9 sites in Group 1 animals which showed a flow increase during low Pco₂, only 2 showed a flow decrease during hypercarbia and the remaining 7 showed a flow increase during hypercarbia. Because these 7 sites reacted freely to increases or decreases in Pco₂, these 7 areas were not vasoparalytic. It is possible that these areas were vasoconstricted during hypercarbia, and then were vasodilated when local acidosis resulted from the reduced perfusion. Previous experiments from this laboratory and others have shown that hyperventilation may result in a paradoxical LCBF increase. These experiments suggest that following 5 minutes of global ischemia, low Pco₂ may have a greater capacity to cause local hypoxia than to prevent "cerebral steal" or "squeeze."

After 5 minutes of ischemia, the vasculature was still responsive to changes in Pco₂. If no-reflow was a dominant consequence, then alterations in Pco₂ would have had no effect on LCBF in these areas. There were some electrode sites which were unresponsive to hypercarbia or hypocarbia (table 1) but LCBFs at these sites were from 94% to 56% of control. The vascular response to CO₂ was weak in these areas, but these possibly vasoparalytic regions were still receiving blood, which is important because there is then the opportunity for medication to reach the microvasculature either to induce vasodilation or reduce the cerebral mean requirement of oxygen.
Ischemia caused the vasculature to be less responsive to hypercarbia. Hypercarbia in normal dogs (65–70 mm Hg Paco₂) caused an average LCBF increase of 68%; the average increase in LCBF in hypercarbic post-ischemic dogs was 31%. Hypercarbia (20–25 mm Hg Paco₂) caused an average LCBF decrease of 19% in both normal or post-ischemic dogs.

The general reactivity of the cerebral vasculature to CO₂ supports the hypothesis that impaired recirculation is caused primarily by an increase in cerebral vascular resistance (CVR) rather than by blockages due to microemboli, "blebs," venous congestion, increased blood viscosity or other factors. An increased microvascular tonus has been suggested by other investigators.

A Paco₂ of 70 mm Hg in Group 2 was effective in elevating post-ischemic flows (fig. 4), but when the Paco₂ was gradually returned to normal over a period of hours, LCBFs generally decreased as well. This implies that hypercarbia should be maintained for longer periods until a reduction in Paco₂ does not result in a further decrease of already subnormal flows.

It is evident that neither the hypercarbia, hypocarbia nor normocarbic regimen was effective in ultimately restoring the local blood flows to control levels by the end of the experiment (about 8 hours post-ischemia, table 2). Even though there is no difference among LCBFs in table 2 and figure 5, it appears that the initial LCBF increase induced by hypercarbia was beneficial in restoring the ECoG. The hypercarbia regimen consistently resulted in a more complete return of the ECoG mean rectified voltage (table 3).

The results from Group 1 and Group 2 suggest that, in the absence of raised intracranial pressure, hypercarbia is superior to normocarbia or hypocarbia in elevating local cerebral blood flow and restoring the recovery of local CBF to pre-ischemic control levels.

**Figure 5.** Recovery of LCBF to control values in Group 2 animals that had previously been hypercarbic, normocarbic or hypocarbic. When the final LCBF estimates were recorded (about 8 hours following the ischemia), all animals were normocarbic, with an arterial pH of 7.38–7.4.
Survival Under Hypoxia. Age Dependence and Effect of Cholinergic Drugs

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SUMMARY Survival under hypoxia (5% O2; 95% N2) was tested in mice 1 day to 50-weeks-old. Survival Rate (ratio of number of animals that survived 30 min under 5% O2 to total number of animals exposed) and the time from onset of exposure until the last gasp (Survival Time) were maximum in newborn animals and decreased as a function of age. Survival Rate and Survival Time were strongly influenced by drugs affecting cholinergic transmission. Atropine decreased the high survival under hypoxia of 1-week-old mice in a dose-related manner. Physostigmine increased survival under hypoxia in mice 3 to 50-weeks-old. This effect was not related to a peripheral action of the drug since it was not mimicked by neostigmine, a cholinesterase inhibitor without central actions. Moreover, peripheral cholinergic blockade with glycopyrrolate, a quaternary cholinergic blocker, did not prevent the protective effect of physostigmine.

Since atropine impairs the ability of very young mice to survive hypoxia and physostigmine improves survival at later ages, it is concluded that a central cholinergic mechanism, possibly related to blood flow regulation, plays a significant role in the acute adaptation to hypoxia.

IT IS KNOWN that the brain is particularly vulnerable to reduction of its energy supply but that its ability to withstand a hypoxic or ischemic insult is considerably greater in newborn animals.1-4 Physostigmine, a cholinesterase inhibitor, is able to prolong survival of mice subjected to hypoxic hypoxia,7 a possible indication of a role for cholinergic mechanisms in the adaptation of the brain tissue to this situation. It was of interest to explore the possible involvement of cholinergic mechanisms in the greater ability of young mice to withstand hypoxia. Therefore, the effects on hypoxic survival of cholinergic blockade with atropine and of enhancement of cholinergic transmission by physostigmine were evaluated in mice at different ages. In addition, experiments were performed to determine whether the protective effect of physostigmine relates to its peripheral or central actions.

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