**The CO₂ Response in Focal Cerebral Ischemia — Sequential Changes Following Recirculation**

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and **Jiro Suzuki, M.D.**

**SUMMARY** The study was undertaken to determine the effects of various levels of ischemia on the CO₂ response and on the development of infarction using the canine thalamic infarction model. Three groups were studied: those with severe ischemia (rCBF below 40% of the pre-occlusion levels), moderate ischemia (between 40% and 70%) and mild ischemia (greater than 70%). The CO₂ response was measured after 30 minutes, 1, 2 and 6 hours of occlusion and then for 4 hours after recirculation.

The CO₂ response recovered after 30 minutes of occlusion in the severely ischemic animals, but in 8 of the 9 animals with 1 or more hours of occlusion, an impaired CO₂ reactivity was found during occlusion and during recirculation. Among moderately ischemic animals, many showed impaired CO₂ reactivity during occlusion, but following recirculation no single trend was observed. Among mildly ischemic animals, almost no abnormalities in the CO₂ response were seen either during 6 hours of occlusion or thereafter. Among the 11 animals from all 3 groups which showed impaired CO₂ reactivity, 10 developed infarction, while among the 10 animals which showed no impaired CO₂ reactivity, in 9 infarction did not arise.

**Experimental Methods**

Twenty one adult mongrel dogs, weighing approximately 10 kg each, were used. Under intravenous administration of sodium pentobarbital (35 mg/kg), an endotracheal tube was inserted and right temporal craniotomy was performed. After securing the dog’s head in a stereotaxic apparatus, an electrode was stereotaxically implanted in the anterior portion of the nucleus ventralis of the thalamus following the canine brain atlas of Lim et al. The electrode to be used for the CO₂ response, effects on the CO₂ response due to recirculation, following ischemia or of histological changes have been reported.

In the present study we have investigated the relationship between impaired CO₂ reactivity and the severity and duration of experimentally induced cerebral ischemia, using the canine thalamic infarction model. We have also studied the sequential changes in the CO₂ response, effects on the CO₂ response due to recirculation, and the relationship between the development of infarction and changes of the CO₂ response. Sequential measurements of the CO₂ response were made during vascular occlusion and following recirculation at the center of the foci and, finally, histological study of the autopsied brain was performed.

IT IS WELL KNOWN that the regulation of cerebral circulation is disturbed in ischemic brain disease and a variety of experimental studies concerning the relationship between ischemia and the CO₂ response have been reported. However, it is noteworthy that no studies of sequential changes in the CO₂ response to recirculation following ischemia or of histological changes have been reported.

The CO₂ response in each animal was determined during occlusion and placed on the forehead. The animals were immobilized with pancuronium bromide (0.04 mg/kg/hr) and respiration regulated with a Harvard respirator. Simultaneously, venous drip of sodium pentobarbital (2.5 mg/kg/hr) was instituted to maintain a state of adequate anesthesia throughout the experiment. Arterial blood was sampled periodically and pH, PaCO₂, and PaO₂ were analyzed using an arterial blood gas analyzer. These parameters were kept within physiological limits and, simultaneously, a catheter was inserted into the abdominal aorta to monitor systemic blood pressure. The rectal temperature was also monitored and kept within a physiological range.

Next, using a surgical microscope, the right temporal dura mater was cut and 4 trunk arteries (the internal carotid artery, anterior cerebral artery, middle cerebral artery and posterior communicating artery) were dissected. After making a measurement of CO₂ response, all 4 intracranial arteries were occluded using Scoville clips. Occlusion was maintained for 30 minutes, 1 hour, 2 hours or 6 hours and depending upon the degree of reduction in rCBF caused by the occlusion, the animals were placed in one of three groups: severe, moderate or mild ischemia. The severe ischemia group included all animals with a post-occlusion rCBF level which was less than 40% of the pre-occlusion level; moderate ischemia included those with rCBF levels between 40% and 70%; and mild ischemia included those with rCBF levels which were 70% or greater. The CO₂ response in each animal was determined during occlusion and the 4 hours of recirculation (table 1). Brains were removed 6 hours after release of the vascular occlusion and placed in a 10% formalin solution.
The formation of foci of infarction was judged by the shrinkage or the swelling of nerve cells and severe spongiosis of the neuropile.\textsuperscript{13,14} rCBF measurements were made following inspiration of 5–10% H\textsubscript{2} gas for 3 minutes, and the clearance curve was calculated using the initial slope method.\textsuperscript{10} The CO\textsubscript{2} response was determined by PaCO\textsubscript{2} and rCBF measurements made during loading of 5–10% CO\textsubscript{2} gas, as compared to pre- and post-loading values for PaCO\textsubscript{2} and rCBF.

Values for CO\textsubscript{2} responsiveness were expressed as the changes in rCBF due to increases in PaCO\textsubscript{2} (% \(\Delta\) rCBF/\(\Delta\) PaCO\textsubscript{2}). Disturbance of the CO\textsubscript{2} response was judged as any value below 1% \(\Delta\) rCBF/\(\Delta\) PaCO\textsubscript{2}.

### Experimental Results

The pre-occlusion rCBF in the 21 dogs ranged from 21.0 to 55.5 ml/100g/min — averaging 35.5 ± 10.5 ml/100g/min. Following vascular occlusion, there were 11 dogs in the severe ischemia group, 8 dogs in the moderate ischemia group and 2 dogs in the mild ischemia group (table 2).

The values for the pre-occlusion CO\textsubscript{2} responsiveness in the 21 dogs were between 2.2 and 6.2% \(\Delta\) rCBF/\(\Delta\) PaCO\textsubscript{2} — averaging 3.5 ± 1.0% \(\Delta\) rCBF/\(\Delta\) PaCO\textsubscript{2}.

#### A. Severe Ischemia Group

1. 30 minutes of occlusion (2 dogs): Approximately 30 minutes after recirculation, both showed disturbance in the CO\textsubscript{2} response, but measurements made after 1–4 hours of recirculation indicated recovery to pre-occlusion values (Fig. 1-a). Histologically, neither dog showed signs of infarction.

2. 1 hour occlusion (4 dogs): In 3 dogs, the CO\textsubscript{2} response was found to be disturbed after recirculation. The other dog showed disturbance of the CO\textsubscript{2} response after one hour of recirculation with subsequent recovery 3 hours after recirculation (fig. 1-b). Histologically, the 3 dogs which showed impaired CO\textsubscript{2} responses had infarction in the anterior thalamus, whereas the one dog which showed recovery of the response after 3 hours of recirculation did not.

3. 2 hour occlusion (3 dogs): The CO\textsubscript{2} response was disturbed in one animal during occlusion. After release of the occlusion, all 3 animals showed a lasting disturbance in this response (fig. 1-c). Histologically, as well, all 3 animals showed signs of infarction.

4. 6 hour occlusion (2 dogs): Both showed impaired reactivity after 2 hours of occlusion and this disturbance persisted throughout the period of occlusion and recirculation (Fig. 1-d). Both dogs showed signs of cerebral infarction which, moreover, was clearly hemorrhagic.

#### B. Moderate Ischemia Group

1. 1 hour occlusion (3 dogs): One animal showed an impaired CO\textsubscript{2} reactivity during occlusion, but this had recovered after 1 hour of recirculation. This animal demonstrated no signs of infarction. The other two dogs showed an impaired CO\textsubscript{2} reactivity after 2 hours of recirculation, but this recovered to some extent (fig. 2-a). In these animals, histologically, one had infarction, while the other had no infarction.

2. 2 hour occlusion (3 dogs): 2 dogs showed impaired CO\textsubscript{2} reactivity for a time during occlusion, but the response recovered after 2 hours of recirculation. The other dog showed an impaired response throughout the period of occlusion and recirculation (Fig. 2-b). Histologically, both of the dogs which showed recovery of the CO\textsubscript{2} response had no foci of infarction, whereas the dog which showed no recovery did have infarction.

3. 6 hour occlusion (2 dogs): One dog showed impaired CO\textsubscript{2} reactivity for a time during occlusion, but the response recovered after 1 hour of recirculation. The other animal showed an impaired response after 1 hour of occlusion, and this did not improve even following recirculation (fig. 2-c). Histologically, the dog which did not show improvement in the CO\textsubscript{2} response had infarction, whereas the dog which showed recovery had no infarction.

### Table 1 A Summary of Occlusion Times and Severity of Ischemia in the 21 Dogs

<table>
<thead>
<tr>
<th>Degree of ischemia</th>
<th>Duration of vascular occlusion</th>
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<tbody>
<tr>
<td></td>
<td>30 min</td>
</tr>
<tr>
<td>Severe ischemia</td>
<td>2</td>
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<tr>
<td>Moderate ischemia</td>
<td>3</td>
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<td>Mild ischemia</td>
<td>2</td>
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### Table 2 rCBF Changes Following Vascular Occlusion in the 21 Dogs

<table>
<thead>
<tr>
<th>Degree of ischemia</th>
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<tbody>
<tr>
<td></td>
<td>30 min</td>
</tr>
<tr>
<td>Severe ischemia</td>
<td>47.5±13.0</td>
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<tr>
<td></td>
<td>25.0±5.0</td>
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<tr>
<td>Moderate ischemia</td>
<td>27.5±13.0</td>
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<td></td>
<td>22.5±11.5</td>
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<tr>
<td></td>
<td>21.0±9.0</td>
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<tr>
<td>Mild ischemia</td>
<td>43.5±35.5</td>
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29.5±22.5
CO, RESPONSE IN FOCAL CEREBRAL ISCHEMIA/Seki et al

FIGURE 1. Sequential changes in the CO, response in severe ischemic group.

a. 30 min occlusion  b. 1 hour occlusion  c. 2 hour occlusion  d. 6 hour occlusion

C. Mild Ischemia Group

1. 6 hour occlusion (2 dogs): Both maintained a CO, response close to the pre-occlusion level during occlusion and during recirculation except for one measuring point (fig. 3). Neither dog showed histological signs of infarction.

Discussion

Some studies in the past have dealt with the CO, response during brain ischemia. Using an experimental model of middle cerebral artery occlusion in the baboon, Symon et al5 found a disturbed CO, response in the vicinity of the ischemia focus produced by the occlusion. They observed that in regions of severe ischemia, CO, loading produced a paradoxical response, where rCBF actually decreases. Waltz7 measured the CO, response used an experimental model involving ligation of the middle cerebral artery in the cat, and showed the responses in the ischemic cortex were disturbed or paradoxical. Kogure et al14 ligated the middle cerebral artery of dogs and found an increase in rCBF due to CO, loading — i.e., no paradoxical response.

Thus far, most reports on the CO, response following recirculation have been concerned with models in which "global ischemia" is produced. Hossmann et al6 produced global ischemia of 1 hour duration in cats and Nemoto et al3 produced ischemic foci of 15 min duration in dogs. Both groups found disturbance in the CO, response measured over a few hours following recirculation.

However, there have been no studies of sequential changes in the CO, response after different grades of ischemia. One of the major reasons appears to be that appropriate experimental models have not been used. The canine thalamic infarction model,8 which was used in the present experiments, is suitable for research on these changes in the CO, response.8,12 Since the ischemic focus in this model is confined to the anterior half of the thalamus, it is possible to monitor easily the hemodynamics at the center of the focus. Furthermore, since the size of the focus is relatively small the model has various advantages: almost no brain edema is observed during vascular occlusion or recirculation, the focus of cerebral infarction can be produced without tampering with blood pressure, and the period of vascular occlusion can be regulated at will.

In the present study, we divided the dogs into 3 groups according to the severity of the ischemia: severe ischemia (where rCBF fell to less than 40% of the pre-occlusion level), moderate ischemia (where rCBF fell to 40%-70%), and mild ischemia (where rCBF fell to 70% or more of the pre-occlusion level). Classification of the ischemia into these 3 groups was based upon the known correlation between changes in brain electrical activity and changes in rCBF following occlusion.16

In order to express the CO, response quantitatively, the %ΔrCBF/ΔPaCO, value is widely used.6,7,17 In the present study we have used this usual method and taken all values below 1.0%ΔrCBF/ΔPaCO, as indicative of a disturbed CO, response.

Our experimental results in the severe ischemia group during occlusion showed disturbed CO, re-
responses at all measurements except one. In contrast, in the mild ischemia group, all measurements except one showed no disturbance of the CO₂ response. In the moderate ischemia group, however, no consistent trend was apparent. These findings are thought to indicate that, to a limited extent, there is a correlation between the severity of the ischemia and the disturbance of the CO₂ response.

In order to investigate the effects of recirculation on the CO₂ response, the response prior to the release of occlusion and the response following 4 hours of recirculation were compared. In the severe ischemia group, none of the dogs in which CO₂ response measurements were made during occlusion, (1 dog undergoing 1 hour of occlusion, 1 dog undergoing 2 hours of occlusion, and 2 dogs undergoing 6 hours of occlusion), showed recovery of the response after recirculation. In contrast, in the case of even severe ischemia during 30 minutes of occlusion, although measurement of the CO₂ response is not possible because of the brief period of occlusion, transient disturbances in the response are noted following recirculation, but these soon recover. These findings are also in agreement with our previously published results on the EEG findings and histological study.

The relationship between findings of the CO₂ response immediately prior to autopsy and the histological findings were investigated (fig. 4). The average value of the CO₂ response of the animals with infarction was $-0.3 \pm 0.7\% \Delta \text{rCBF}/\Delta \text{PaCO}_2$, whereas that of the animals with no infarction was $3.0 \pm 1.4\% \Delta \text{rCBF}/\Delta \text{PaCO}_2$. This difference is statistically significant at the $p < 0.01$ level. It is therefore apparent

FIGURE 2. Sequential changes in the CO₂ response in moderate ischemic group
   a: 1 hour occlusion  b: 2 hour occlusion  c: 6 hour occlusion

FIGURE 3. Sequential changes in the CO₂ response in mild ischemic group.
   6 hour occlusion
CO₂ RESPONSE IN FOCAL CEREBRAL ISCHEMIA/Seki et al

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<td>Moderate ischemia</td>
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<td>Mild ischemia</td>
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- Deficit of CO₂ response
- No deficit of CO₂ response

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- Infarction
- No infarction

Figure 4. Summary of the results
a. the CO₂ response after 4 hours of recirculation
b. the histopathological findings at autopsy

that by measuring the CO₂ response after 4 hours of recirculation, it can be determined whether or not the ischemic focus has resulted in infarction after recirculation.

In our experiments, the mechanism of the CO₂ disturbance4 18-21 is thought to be as follows: Vascular occlusion causes acidosis within the ischemic focus and a normal vascular response to CO₂ loading cannot then occur. Recirculation results in gradual improvement in the tissue acidosis and CO₂ response then recovers. Such changes of the CO₂ response are in good agreement with the following data. As we have previously reported,10,12 in cases where foci of infarction do not develop, although there is the transient appearance of hyperperfusion after recirculation, rCBF values similar to those found prior to occlusion are soon obtained. In contrast, in 10 of the 11 dogs in the present study which developed infarction, recovery of the CO₂ response was not found either during occlusion or recirculation. One of the causes of this phenomenon is thought to be tissue acidosis, but more importantly the tissues including the cerebral vessels are thought to be histologically damaged. We have previously reported13,14 on electron microscopic observations on the sequential changes in small vessels made following the production of severely ischemic foci. There, after 1 or 2 hours of vascular occlusion, swelling of glial cells and pericytes in the vicinity of blood vessels were found. In contrast, following recirculation, the swelling of the endothelial cells of capillaries and pericytes becomes even more remarkable and stenosis of the vascular lumen could be observed. As time elapses such small vessels begin to show signs of necrosis. When 6 hours of occlusion is performed,22,23 these changes as well as bleb formation and opening of tight junctions13 can be seen. Following recirculation, there is the appearance of diapedesis.22,23 In this type of case in which recovery of the CO₂ response is not seen in the acute period of recirculation, irreversible histological changes are thought to have occurred already.

References
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14. Watanabe T, Suzuki M, Yoshimoto T, Suzuki J: Recirculation in ischemic focus in acute stage — electron microscopic examination — Brain Nerve (Tokyo) in press (Eng Abst)
FOCAL CEREBRAL ISCHAEMIA is frequently the result of occlusion of a major cerebral artery. On the other hand, cerebral ischaemia may develop when incomplete arterial obstruction is combined with a degree of perfusion failure. This may manifest clinically as a transient ischaemic attack (TIA) or a reversible ischaemic neurological deficit (RIND) depending on the duration. The concept of a haemodynamic cerebral ischaemic event is controversial. Experimentally, carotid occlusion alone has been shown to be without effect on cerebral blood flow (CBF) in rats and baboons unless there is an added insult such as hypoxia or hypotension.

We have set out to examine the distribution of the changes in CBF and their topographical relationship to the ischaemic brain damage which develops following unilateral carotid occlusion and hypotension at the lower limit of autoregulation.

### Materials and Methods

Thirty male Sprague-Dawley rats (weighing 300–500 g) were used. Anaesthesia was induced in a perspex box with halothane (5%) and maintained with (0.5–1.0%) halothane and a nitrous oxide/oxygen mixture (70%:30%). A tracheostomy was performed and the animals' ventilation controlled by a small animal respirator. Both femoral arteries were catheterised, the one for continuous blood pressure measurements and the other for direct intra-arterial withdrawal and re-infusion of blood to maintain an exactly uniform blood pressure. A femoral vein was catheterised for infusion of radio-isotope. Arterial blood samples were withdrawn for measurement of blood gases, haematocrit and blood glucose (Corning blood gas analyser and Beckman glucose meter). Rectal temperature was maintained at 37°C with an automated heating box. After a 30 minute stabilisation period the right carotid bifurcation was exposed and the external carotid artery and all its branches ligated. The common carotid artery was isolated between ligatures, and at the start of the experiment it was ligated after an 0.8 mm outside diameter catheter had been inserted distally to monitor internal carotid artery stump pressure, and another inserted proximally to permit the sampling of arterial blood during the measurement of local CBF. Systemic
The CO2 response in focal cerebral ischemia--sequential changes following recirculation.
H Seki, T Yoshimoto, A Ogawa and J Suzuki

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