Cerebrovascular CO₂ Reactivity: Role of a Cholinergic Mechanism Modulated by Anesthesia

O. U. Scrimin, M.D., E. H. Rubinstein, M.D., Ph.D., and R. R. Sonnenschein, M.D., Ph.D.

SUMMARY Cerebral cortical blood flow was measured in rabbits with the hydrogen clearance technique. The reactivity to CO₂ tested by changing the end tidal CO₂ (ETCO₂) in steps from 2 to 6 volumes %, was highly dependent on the kind of anesthesia, being greatest under halothane and least under nitrous oxide. Reactivity to CO₂ in halothane-aneschzetized animals also depended on arterial blood pressure, being greatest when pressure was below 70 mm Hg. Intravenous atropine blocked the increase in reactivity in halothane-aneschzetized animals at low blood pressures. Conversely, intravenous eserine (physostigmine) greatly increased the reactivity to CO₂ in nitrous oxide-aneschzetized animals. Precollucular deecerebration considerably decreased CO₂ reactivity of halothane-aneschzetized rabbits, while partial brain stem lesions that spared midline structures had no effect on CO₂ reactivity. It is concluded that a central neurogenic mechanism with a cholinergic link may be responsible, at least in part, for the cerebrovascular effect of CO₂. Moreover, the cerebrovascular effects of halothane may result from stimulation of the same system.

INCREASING EVIDENCE suggests that the cerebrovascular effect of carbon dioxide, commonly believed to result from a direct action of CO₂ on the smooth muscle of the cerebral vessels, may depend on 1 or more neurogenic mechanisms, as the effect can be altered by lesions of the brain stem or denervation of peripheral chemoreceptors. The involvement of a cholinergic link is suggested by the blockade by atropine and potentiation by a cholinesterase inhibitor of the cortical vasodilatation associated with hypercapnia in the rat, rabbit, and baboon.

Cerebrovascular reactivity to CO₂ is also known to vary widely during general anesthesia, depending on the type of agent used. It is of particular interest that the anesthetic halothane increases cerebral blood flow during normocapnia above that of awake or N₂O-aneschzetized controls while decreasing cerebral oxygen consumption. These observations lead to the following questions: 1) To what extent is the cerebral cortical blood flow (CoBF) response to CO₂ in the rabbit dependent on a cholinergic mechanism? 2) In turn, may an altered reactivity to CO₂, which may involve the putative cholinergic mechanism, be the basis in part for the differing levels of CBF occurring under halothane and N₂O anesthesia? 3) May a neural system originating in the brain stem participate in the CoBF changes induced by CO₂? To answer the first two questions, atropine was administered to halothane-aneschzetized animals (high CoBF condition) and eserine (physostigmine), a cholinesterase inhibitor, to N₂O-aneschzetized animals (low CoBF condition) to test their ability to modify the corresponding sensitivities of CoBF to CO₂. In a number of animals, the effect of precollucular deecerebration on sensitivity of CoBF to CO₂ was determined, in order to test the third hypothesis.

Method

Determinations of 326 CoBF were performed on 44 male rabbits weighing 2.5–3.5 kg. The animals were anesthesized by mask with 1% halothane-60% N₂O after induction with methohexital (Brevital®), 4–5 mg/kg, injected through an ear vein. The femoral artery and vein were cannulated for recording of arterial pressure and injection of drugs, respectively. End-tidal CO₂ (ETCO₂) was continuously monitored with a Beckman LB-2 gas analyzer. The animals were tracheostomized, paralyzed by a continuous intravenous infusion of pancuronium bromide (Pavulon®) at 0.6 mg/hr, placed on a Model 607 Harvard respirator, and ventilated so as to achieve an ETCO₂ concentration of 4% until the completion of the preparative procedures. In anticipation of the CoBF determinations the animals were placed on a T system for ventilation that initially produced a low ETCO₂ (around 2.5–3%). From this low value, ETCO₂ was set at the desired level by adding CO₂ to the inspired gas mixture.

After the initial preparation, one of three experimental anesthetic mixtures was administered, starting at least 60 minutes before experimental procedures were commenced, and was continued throughout the experiment. Twelve rabbits were anesthesized with 1% halothane in 100% O₂, 9 with 70% N₂O-30% O₂, 23 with 1% halothane-60% N₂O-40% O₂. The gases were delivered by a Foregger anesthesia machine with a Copper Kettle vaporizer. Animals were injected intravenously, when specified, with atropine sulphate (Merck), 3 mg/kg or eserine (physostigmine sulfate, Merck), 0.15 mg/kg.

CoBF in the parietal cortex was measured with the hydrogen clearance technique. Prior to surgery the animals were placed in a Labtronics stereotactic instrument with rabbit adaptor according to Sawyer. A craniotomy was performed, and the parietal cortex was exposed after careful reflection of the dura mater. A platinum electrode, 75 µm in diameter, insulated with Teflon except at the tip, was inserted 1 mm below the cortical surface by means of a Narishige micromanipulator, under observation through a Carl Zeiss stereoscopic dissecting microscope, in order to avoid damage to pial vessels. The exposed cortex surrounding the electrode was covered with pieces of Gelfoam that were continuously wetted by the cerebrospinal fluid flowing out from the subarachnoid space. An indifferent Ag-AgCl electrode was placed subcutaneously in the neck. The current flowing in the circuit was monitored as the voltage drop across a 1.5 × 10⁷ ohm resistance. Tissue H₂ saturation was achieved by adding 5% H₂ to the inspired gases in the T system. Total gas flow was kept at about 5 liters/min in order to insure more rapid clearance from the lungs. When
the hydrogen current achieved a steady level, H₂ inflow was stopped and the desaturation slope recorded. CoBF was calculated by using the expression \( K = \frac{\ln 2}{T \frac{1}{2}} \) where \( T \frac{1}{2} \) = half time of washout slope in minutes. Blood flow values were finally expressed as ml/100 g tissue/min.

Cortical vascular reactivity to CO₂ was determined by changes in mean arterial blood pressure (MABP) (table 1); these pooled values, it was greatest under 1% halothane-60% N₂O-40% O₂ and least with 70% N₂O-30% O₂. Under any of the experimental conditions, the induced changes in ETCO₂ were unaccompanied by consistent or statistically significant changes in mean arterial blood pressure (MABP) (table 1);

Y = .26 X + 67.45 .0057

Y = 2.10 X + 78.45 .275

Y = 0.4 X + 70.54 .0057

Y = 1.31 X + 81.18 .229

Y = 0.4 X + 70.54 .0057

Y = 2.41 X + 59.17 .151

Regressions for the control values are given in the table 1.

**TABLE 1**

<table>
<thead>
<tr>
<th>Anesthesia</th>
<th>N</th>
<th>Average MABP (mm Hg)</th>
<th>Regression equation</th>
<th>r</th>
</tr>
</thead>
<tbody>
<tr>
<td>1% Halothane-100% O₂</td>
<td>75/11</td>
<td>70.88</td>
<td>Y = 0.4 X + 70.54</td>
<td>.0057</td>
</tr>
<tr>
<td>1% Halothane-100% O₂</td>
<td>47/10</td>
<td>69.13</td>
<td>Y = 2.41 X + 59.17</td>
<td>.151</td>
</tr>
<tr>
<td>(atropine)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>70% N₂O-30% O₂</td>
<td>46/9</td>
<td>86.89</td>
<td>Y = 2.10 X + 78.45</td>
<td>.275</td>
</tr>
<tr>
<td>70% N₂O-30% O₂</td>
<td>40/9</td>
<td>86.88</td>
<td>Y = 1.31 X + 81.18</td>
<td>.229</td>
</tr>
<tr>
<td>(atropine)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1% Halothane-60% N₂O—40% O₂</td>
<td>41/8</td>
<td>67.88</td>
<td>Y = 2.01 X + 59.63</td>
<td>.151</td>
</tr>
<tr>
<td>(controls of decerebration)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1% Halothane-60% N₂O—40% O₂ (decerebration)</td>
<td>53/13</td>
<td>68.53</td>
<td>Y = .26 X + 67.45</td>
<td>.032</td>
</tr>
</tbody>
</table>

*All correlation coefficients (r) were statistically non-significant (p >0.05). N = number of observations/number of animals.

**Results**

As can be seen in figure 1, cerebrovascular sensitivity to CO₂ was different under each of the anesthetic mixtures; for these pooled values, it was greatest under 1% halothane-60% N₂O-40% O₂ and least with 70% N₂O-30% O₂. Under any of the experimental conditions, the induced changes in ETCO₂ were unaccompanied by consistent or statistically significant changes in mean arterial blood pressure (MABP) (table 1);
Effect of Atropine on Reactivity under Halothane

The effect of atropine on cerebrovascular reactivity was related to the level of MABP. Since, under halothane anesthesia, MABP values were scattered ($\bar{x} = 70.88; \text{SD} = 8.13$), the data were recalculated for 2 subgroups, those with high ($\geq 70$ mm Hg) or low ($< 70$ mm Hg) MABP. This analysis indicated a statistically significant, 6-times higher reactivity to $CO_2$ at low than at high MABP. After atropine treatment, however, which itself induced no change in MABP ($\bar{x} = 69.13; \text{SD} = 10.50$), the reactivity to $CO_2$ at high MABP was unchanged, while that at low MABP decreased to the level seen at high MABP (figs. 2, 3).

Effect of Eserine on Reactivity under $N_2O$

In the experiments under 70% $N_2O-30% O_2$, MABP was higher ($\bar{x} = 86.89; \text{SD} = 9.87$) than under 1% halothane-

Effect of Precollicular Decerebration

The $CO_2$ sensitivity of rabbits under 1% halothane-60% $N_2O-40% O_2$ was considerably reduced from the pre-lesion control level after precollicular decerebration (fig. 6); the

FIGURE 2. The effect of atropine (3 mg/kg, i.v.) in blocking the cortical vascular response to $CO_2$ in a single experiment.

FIGURE 3. The effect of atropine (3 mg/kg, i.v.) in blocking the high cortical vascular reactivity to $CO_2$ at low MABP. Correlation coefficients ($r$), significance ($p$) and number of observations/number of animals were 1) low MABP, before atropine: $r = .70, p < .001, n = 30/7; 2) low MABP, after atropine: $r = .10, p > .10, n = 29/7; 3) high MABP, before atropine: $r = .31, p < .05, n = 47/10; 4) high MABP, after atropine: $r = .60, p < .01, n = 22/8$. The difference between slopes 1) and 2) was significant ($p < .05$).

FIGURE 4. Marked increase in cortical vascular reactivity to $CO_2$ brought about by eserine (0.15 mg/kg, i.v.) in a single experiment.
CHOLINERGIC MECHANISM IN CO₂ REACTIVITY/Scremin et al.

70% NITROUS OXIDE
30% OXYGEN

Eserine

\[ Y = 1.9969X + 27.8634 \]

Control

**Figure 5.** The increase in cortical vascular reactivity to CO₂ brought about by eserine (0.15 mg/kg, i.v.) in N₂O-anesthetized rabbits. Correlation coefficients (r), significance (p) and number of observations/number of animals were: 1) control: r = .17, p > .10, n = 46/9; 2) after eserine: r = .44, p < .01, n = 40/9. The difference between slopes was significant (p < .05).

decebration itself induced no change in MABP (table 1). No edema was observed in the cerebral cortex after the procedure, except for one experiment in which CoBF nearly stopped shortly after the electrolytic lesion and in which gross swelling of the cerebral cortex and an intraventricular hemorrhage were seen. This particular experiment was discarded. However, to rule out a possible nonspecific effect of the brain lesion, a number of animals were subjected to partial brainstem transections that spared only 2 mm of brain tissue at each side of the midline; transection was 80% complete. In these animals cerebrovascular sensitivity to CO₂ did not differ from that in control animals without brainstem lesions (fig. 6). In contrast, small (20%) of complete transection) medial lesions showed a tendency to reduce the sensitivity to CO₂.

The EEG activity under halothane anesthesia alternated between stages of high voltage, slow waves (synchronization) and low voltage, faster waves (desynchronization). After precollicular decebration, however, the EEG remained permanently in the synchronized stage. The CoBF-ETCO₂ relation of the control animals (no CNS lesions) shown in figure 6 is derived from determinations in which the cortex was synchronized or desynchronized in approximately equal numbers of instances; in the total decebration and the partial (both lateral and medial) transection groups, the cortex was synchronized in all the determinations. It was then considered of interest to determine any possible relation between EEG stage and cerebrovascular reactivity to CO₂ in the absence of any brain stem lesion. A reinvestigation of cerebrovascular reactivity to CO₂ in intact animals, taking into account the EEG stage, showed that reactivity was greater and that CoBF at normocapnia was lower during synchronization than during desynchronization (fig. 7). The slope of the CoBF-ETCO₂ relation of the animals with lateral lesions (synchronized cortex) could be superimposed on that of the controls with synchronized cortex, but that of the decebrated animals was significantly lower (fig. 7).

**Discussion**

It is now well established that cerebral blood flow (CBF) is higher during halothane than during N₂O anesthesia. Fragmentary reports indicate that CBF and reactivity to CO₂ are higher under halothane than in the awake state.11-13

![Figure 6. Effect of brain stem lesions (shaded areas) on cortical vascular reactivity to CO₂ in rabbits under 1% halothane — 60% N₂O — 40% O₂. The regression equations \( Y = \text{CoBF (ml/100g/min); } X = \text{ETCO}_2 \text{(vol. %)} \), correlation coefficients (r), significance (p), number of observations/number of animals (n) were:

1) No lesion: \( Y = 14.43X + 2.59, r = .34, p < .05, n = 41/8; \)
2) Lateral lesion: \( Y = 12.02X - 7.52, r = .40, p < .05, n = 24/5; \)
3) Medial lesion: \( Y = 6.86X - 1.52, r = .63, p < .01, n = 17/5; \)
4) Complete decebration: \( Y = 1.18 + 21.67, r = .08, p > .10, n = 53/13. \)

The difference between slopes 1) and 4) was significant (p < .05) while those between 1) and 2) and between 1) and 3) were not.
Halothane is known to induce a decrease in cerebral O₂ consumption, thus its action in increasing CBF can not be accounted for on the basis of a local metabolic vasodilatation.

In the present experiments, we have confirmed the existence of an increase in CBF and cerebrovascular reactivity to CO₂ under halothane compared to N₂O anesthesia. We would suggest, rather, that the observed increase in CBF induced by halothane might be related to its effect of increasing the cerebrovascular sensitivity to CO₂, i.e. that at any given level of arterial Pco₂, CBF is higher under halothane. The fact that eserine increases the sensitivity to CO₂ under N₂O anesthesia to the levels seen in halothane-anesthetized animals suggests a cholinergic mechanism underlying the CO₂ response that is either inhibited by N₂O or facilitated by halothane. The decrease in CO₂ sensitivity observed after atropine at low MABP in halothane-anesthetized rabbits can be interpreted in the same sense.

These results are in line with previous observations in the rat and baboon and confirm in the rabbit the existence of a cholinergic step in the neurogenic component of CO₂ action.

The effect of precollricular decerebration in considerably decreasing the cortical vascular reactivity to CO₂ further supports the interpretation that the action of CO₂ on cortical blood vessels is dependent, at least in part, on a central neurogenic mechanism.

The fact that nearly complete transections, destroying about 80% of the brainstern and sparing only midline structures, had no discernible effect on the CO₂ response indicates that the trauma of the electrolytic decerebration, whether through production of cerebral edema or otherwise, was of no consequence in the present experiments. There is another alternative interpretation, however. After decerebration and also after atropine administration, the EEG remains synchronized most of the time. The threshold for desynchronization in response to hypercapnia is also considerably elevated. It might be argued, then, that the change to cortical synchronization after decerebration or atropine might by itself induce a "nonspecific" change in sensitivity to CO₂ due to the depression of metabolism associated with this stage, or that the absence of the cortical desynchronization normally associated with hypercapnia might tend to give lower CBF values.

However, it was found that the reactivity to CO₂ was still high in the normal controls with synchronized cortex. The animals with partial (lateral) transections, in which the EEG remained synchronized, showed a CO₂ reactivity indistinguishable from that of the controls with synchronized cortex, but the decerebrated animals, also with a synchronized cortex, had a reactivity significantly lower than the 2 above mentioned groups. It can be concluded, then, that the ability of decerebration or atropine to decrease the cerebrovascular reactivity to CO₂ was independent of its effect on the EEG.

The increase in reactivity to CO₂ at low MABP observed in halothane-anesthetized animals was an unexpected finding. Previous evidence had indicated a decrease or no significant change in CO₂ responsiveness with a decrease in blood pressure in barbiturate-anesthetized dogs. One possibility is that the difference between those results and our findings may lie in the use of different anesthetics.

Decreases in blood pressure are normally associated with decreases in cerebrovascular resistance that tend to keep the blood flow constant, i.e. autoregulation. The reverse operates when the blood pressure rises. It may be argued then that the cerebral vasocostricting effect of an increase in blood pressure would oppose the vasodilating effect of CO₂ and vice versa. The phenomenon could thus be explained on the basis of direct effects (if the myogenic theory of autoregulation is accepted) on vascular smooth muscle. However, the fact that atropine blocks the increase in CO₂ responsiveness of CBF during hypotension does not fit in with this interpretation and suggests that a cholinergic mechanism might be involved, as atropine has no known direct effects on vascular smooth muscle and on the other hand it does not change cerebral blood flow at normocapnia.

In conclusion, we postulate the existence of a central neurogenic mechanism with an important cholinergic link which may be responsible, at least in part, for the vasodilator effect of hypercapnia. Moreover, the increase in CBF produced by halothane appears to result from an increase in cerebrovascular reactivity to CO₂ brought about through stimulation or facilitation of this central cholinergic mechanism.

References

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Metabolic Profiles of Canine Cerebrovascular Tree: A Histochemical Study

B. H. COOK, M.D., PH.D., H. J. GRANGER, PH.D., D. N. GRANGER, PH.D., A. E. TAYLOR, PH.D., AND E. E. SMITH, PH.D.

SUMMARY Intriguing questions have recently been raised regarding the applicability of direct observations of the pial microcirculation to the behavior of the total cerebral microcirculation. Operating under the assumption that arteriolar tone and, thus, cerebrovascular resistance is, to some extent, directly related to the intrinsic metabolic properties of the arteriolar wall, a comparative histochemical analysis of cerebral microvessels was undertaken. Reactions were chosen on the bases of representation of substrate and of enzymes of glycolysis, the hexose monophosphate shunt, β-oxidation of fat, Krebs cycle, cytochrome system and ATP hydrolysis. Three metabolically distinct segments of the cerebral microvasculature were delineated with the pial vessels showing strong capacities for glycolysis, β-oxidation of fats and utilization of glucose through the hexose monophosphate shunt.

Monovessels of the gray matter have a qualitatively similar metabolic profile but the capacities of each pathway are lower when compared to pial arterioles. Arterioles of the white matter demonstrate the weakest energy-yielding capacities.

SINCE THE DIRECT observations of the pial circulation by Donders in the 19th century and by Forbes in the early 20th century, the responses of the pial vessels have served as a useful model for the whole brain microcirculatory unit. Operating under the assumption that arteriolar tone and, thus, cerebrovascular resistance is, to some extent, directly related to the intrinsic metabolic properties of the arteriolar wall, a comparative histochemical analysis of cerebral microvessels, both pial and pachymembral, was undertaken. Reactions were chosen on the bases of representation of substrate and of enzymes of glycolysis, the hexose monophosphate shunt, β-oxidation of fat, Krebs cycle, cytochrome system and ATP hydrolysis. Three metabolically distinct segments of the cerebral microvasculature were delineated with the pial vessels showing strong capacities for glycolysis, β-oxidation of fats and utilization of glucose through the hexose monophosphate shunt. Microvessels of the gray matter have a qualitatively similar metabolic profile but the capacities of each pathway are lower when compared to pial arterioles. Arterioles of the white matter demonstrate the weakest energy-yielding capacities.

Methods

Ten mongrel dogs were anesthetized with sodium pentobarbital. Skin and scalp muscles overlying the skull were dissected away before a rectangular opening was made in the fronto-parietal bones for biopsy of underlying cerebral cortex. Biopsied samples were immediately frozen at -170°C in isopentane precooled in liquid nitrogen. Ten micron sections were cut on an Ames Lab-Tek cryostat and were not allowed to thaw prior to the application of incubating medium. Standard histochemical reactions were performed for representative enzymes of each of the major metabolic pathways.
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