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

O. U. SCREMIN, 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-anesthetized animals also depended on arterial blood pressure, being greatest when pressure was below 70 mm Hg. Intravenous methohexital (Brevital 1B), 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 anesthetized 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 stereotaxic instrument with rabbit adaptor according to Sawyer. A craniootomy 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 X 10⁶ 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
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FIGURE 1. Cortical blood flow as a function of end-tidal CO₂ under three different anesthetics. Correlation coefficients (r), significance (p) and number of observations/number of animals were 1) 1% halothane—60% N₂O—40% O₂, r = .34, p < .05, n = 41/8, 2) 1% halothane—100% O₂, r = .28, p < .05, n = 75/11, 3) 70% N₂O—30% O₂, r = .17, p > .10, n = 46/9. The difference between slopes 1 and 3 was significant (p < .05) while those between 1 and 2 and between 2 and 3 were not.

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The hydrogen current achieved a steady level, H₂ inflow was stopped and the desaturation slope recorded. CoBF was calculated by using the expression 

\[ K = \frac{\ln 2}{T'/2} \]

where 

\[ T'/2 = \text{half time of washout slope in minutes}. \]

Cortical vascular reactivity to CO₂ was determined by changing the inspired concentration of CO₂ in steps between 2 and 6% and recording the concomitant levels of CoBF. Each CoBF (ml/100 g tissue/min) was paired with its corresponding ETCO₂ (volumes per cent). Data from different animals in the same experimental condition were pooled and a regression analysis was performed by the least squares method. The coefficient of the regression equation was taken as the quantitative estimation of cortical vascular reactivity to CO₂. Significance of the difference between coefficients of the slopes was tested by analysis of covariance.

To accomplish precollular decerebration, a rectangular window was made in the skull with a dental drill and rongeur 10 mm from the tip, was lowered through the dura to the base of the skull in a downward-forward direction at an angle of 22° with the vertical plane. A current of 6 mA supplied by a Grass S48 stimulator and continuously monitored by an ammeter was passed for 45–60 seconds. The lesioning procedure was repeated in 13 rabbits at successive 1.5 mm intervals from the midline (complete decerebration: R1.5, 3.0, 4.5, 6.0, L1.5, 3.0, 4.5, 6.0). 5 animals were lesioned as above except for the two points 1.5 mm from midline (lateral lesion: R3.0, 4.5, 6.0, L3.0, 4.5, 6.0) and 5 animals were lesioned only at the points adjacent to the midline (medial lesion: R1.5, L1.5). The brains were removed after completion of the experiments and embedded in 10% buffered formalin. Serial frozen sections at 40 μm were cut with a Model CTD International Harris Cryostat and stained with the Nissl method in order to assess the extent of the lesions.

We have preferred electrolytic decerebration to the surgical procedure because it minimizes bleeding that might lead to an increase of intracranial pressure, an undesirable complication when measuring blood flow. Moreover, it also allows a more accurate determination of the transection plane. Insulation of the electrode, except at the segment traversing the brain stem, avoids unnecessary coagulation of telencephalic structures that might lead to cortical edema.

The following were continuously monitored on a Grass Model 5 Polygraph: a) arterial pressure through the arterial cannula with a Statham P23Db transducer; b) ETCO₂ from the output of the Beckman LB-2 gas analyzer; c) H₂ current recorded as a voltage drop across a 1.5 × 10⁶ ohm resistance monitored with a low level, high impedance preamplifier; d) electrocorticogram recorded between the tip of the platinum electrode and an Ag-AgCl indifferent electrode, with an EEG amplifier (time constant 0.1 sec; ½ amplitude high frequency cut off, 35 cps). The latter was recorded in order to monitor the adequacy of surgical anesthesia. Body temperature was measured with a rectal thermometer and adjusted with a heat lamp to about 38°C.

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);

<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>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 (controls of decerebration)</td>
<td>41/8</td>
<td>67.88</td>
<td>Y = 2.01 X + 59.63</td>
<td>.151</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 > .05). N = number of observations/number of animals.
hence the changes in CoBF associated with alterations in ETCO₂ reflected changes in vascular resistance.

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 (\( \bar{x} = 70.88; \) \( SD = 8.13 \)), the data were recalculated for 2 subgroups, those with high (≥ 70 mm Hg) or low (< 70 mm Hg) MABP. This analysis indicated a statistically significant, 6-times higher reactivity to CO₂ at low than at high MABP. After atropine treatment, however, which itself induced no change in MABP (\( \bar{x} = 69.13; \) \( SD = 10.50 \)), the reactivity to CO₂ 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 N₂O

In the experiments under 70% N₂O-30% O₂, MABP was higher (\( \bar{x} = 86.89; \) \( SD = 9.87 \)) than under 1% halothane-100% O₂; the observed ranges again allowed the separation of reactivities associated with high (≥ 85 mm Hg) and low (< 85 mmHg) values of MABP. The coefficients of the slopes of the regressions of CoBF on ETCO₂ were 1.61 and 2.85 respectively. This tendency to a higher reactivity at a lower pressure was not statistically significant; hence, all the experiments were pooled in a single group. After eserine, the reactivity to CO₂ was greatly increased (figs. 4, 5). MABP in the eserine group (\( \bar{x} = 86.88; \) \( SD = 8.10 \)) was at the same level as that of the untreated controls.

Effect of Precollicular Decerebration

The CO₂ sensitivity of rabbits under 1% halothane-60% N₂O-40% O₂ was considerably reduced from the pre-lesion control level after precollicular decerebration (fig. 6); the

\[
\begin{align*}
\text{MABP} < 70 \text{ mmHg} & : Y = 24.5822X - 34.27 \\
\text{MABP} ≥ 70 \text{ mmHg} & : Y = 4.7058X + 37.55
\end{align*}
\]

\[
\begin{align*}
\text{MABP} < 70 \text{ mmHg} & : Y = 4.7768X + 2087 \\
\text{MABP} ≥ 70 \text{ mmHg} & : Y = 6.0973X + 8.84
\end{align*}
\]
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70% NITROUS OXIDE
30% OXYGEN

Eserine

Control

Y = 10.062X + 15.6560
Y = 1.9969X + 27.9634

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).

decerebration 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 decerebration, 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 decerebration 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 desynchronization 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 decerebrated 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.¹¹⁻¹⁵ Fragmentary reports indicate that CBF and reactivity to CO₂ are higher under halothane than in the awake state.¹¹⁻¹⁵

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 = CoBF (ml/100g/min); X = ETCO₂ (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 = 12.03X - 7.52, r = .40, p < .05, n = 24/5;
3) Medial lesion: Y = 6.86X - 1.52, r = .63, p < .01, n = 17/5;

The difference between slopes 1) and 4) was significant (p < .05) while those between 1) and 2) and between 1) and 3) were not.
The effect of precollicular decerebration in considerably decreasing the cortical vascular reactivity to \( CO_2 \) further supports the interpretation that the action of \( CO_2 \) 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 brainstem and sparing only midline structures, had no discernible effect on the \( CO_2 \) 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 \( CO_2 \) 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 \( CO_2 \) was still high in the normal controls with synchronized cortex. The animals with partial (lateral) transections, in which the EEG remained synchronized, showed a \( CO_2 \) 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 \( CO_2 \) was independent of its effect on the EEG.

The increase in reactivity to \( CO_2 \) at low MABP observed in halothane-anesthetized animals was an unexpected finding. Previous evidence had indicated a decrease\(^9\) or no significant change\(^8\) in \( CO_2 \) 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 vasoconstricting effect of an increase in blood pressure would oppose the vasodilating effect of \( CO_2 \) 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 \( CO_2 \) 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 \( CO_2 \) brought about through stimulation or facilitation of this central cholinergic mechanism.

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Metabolic Profiles of Canine Cerebrovascular Tree: A Histochemical Study

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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 energy metabolism of the arteriolar wall, a comparative histochemical analysis of cerebral microvessels, both pial and parenchymal, 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.

SINCE THE DIRECT observations of the pial circulation by Donders1 in the 19th century and by Forbes2 in the early 20th century, the responses of the pial vessels have served as a useful model for the whole brain microcirculatory unit. Rosenblum and Kontos3 have, however, recently raised the intriguing question of whether the pial vessels do indeed mimic the responses of parenchymal vessels. While pial vessels have been shown to respond in a proper manner to those humoral substances (CO2, H+) generally accepted to be responsible for cerebral autoregulation, other studies6 have indicated that parenchymal vessels behave in a manner which would tend to compensate for alterations in pial vascular calibre. In addition to neurogenic and parenchymal (via vasodilator release) factors, vascular tone is, to some extent, a function of the metabolic properties of the arteriolar wall which mediate the contractile process through production of adenosine triphosphate. Since previous studies in this laboratory have indicated that a metabolic heterogeneity between vessels of different calibre within a vascular bed7 and between vessels of similar calibre from organ to organ8 may exist, the topohistochemical approach utilized in these studies has been applied to the cerebral microvasculature in order to ascertain whether the vessels of the pia are metabolically similar to parenchymal vessels.

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

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