Effects of Carbon Dioxide Inhalation on Cerebral Blood Flow and Oxygen Tissue Level in Spontaneously Hypertensive Rabbits

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Background and Purpose: Because previous studies have yielded conflicting results, this study was designed to investigate the efficiency of cerebrovascular reactivity to carbon dioxide in hypertension associated with moderate diffuse cerebral ischemic lesions.

Methods: The effects of carbon dioxide inhalation on mean arterial blood pressure, heart and respiration rates, cerebral cortical blood flow, polarographically detected oxygen currents (oxygen availability), and cerebral electrical activity were compared in 14 spontaneously hypertensive and 16 normotensive rabbits anesthetized with urethane and α-chloralose. Blood flow was measured with the hydrogen clearance and thermal clearance methods.

Results: In the resting state the frequency of electrical activity shifted to slower components, the levels of oxygen availability and cerebral blood flow were lower (p<0.01), and the ratio of the two latter parameters was greater (p<0.01) in hypertensive rabbits than in normotensive animals. Carbon dioxide inhalation induced more marked increases in cerebral blood flow, respiration rate, and oxygen availability in hypertensive (p<0.01) than in normotensive (p<0.05) rabbits. The ratio of oxygen availability to cerebral blood flow decreased (p<0.01) in the former and did not change significantly in the latter group. The carbon dioxide–induced rise in blood flow was also slower and more protracted in hypertensive rabbits (p<0.01). Histological investigation revealed groups of neurons with ischemic changes in the cortex of the hypertensive rabbits.

Conclusions: We suggest that in hypertensive rabbits the mild multiple ischemic lesions are the basis of functional disturbances, including reduced resting cerebral blood flow, greater oxygen tissue level, slower response to carbon dioxide, and greater vasodilatory capacity. (Stroke 1992;23:569–575)

Although cerebral vascular reactivity to changes in arterial CO₂ tension has been investigated in hypertensive humans² and in animals,¹ observations have been controversial so far. Spontaneous cerebrovascular diseases occur rarely in animals, and until recently a spontaneously hypertensive strain had been found only among rats.³

Previously, we described a strain of hypertensive rabbits characterized by adventitial melanosis of the cortical blood vessels, hypertrophy of the arteriolar wall, and multiple foci of moderate cortical ischemic lesion.³ This strain of animals seemed to be suitable for investigating the efficiency of cerebrovascular reactivity to CO₂ in hypertension associated with moderate diffuse cerebral ischemic lesions.

Materials and Methods

Experiments were performed on 14 hypertensive (HT) and 16 normotensive (NT) rabbits of either sex weighing 2.5–3.0 kg. Each animal was anesthetized with 0.5 g/kg i.p. urethane and 70 mg/kg i.p. α-chloralose.

Both femoral arteries were cannulated to record the systemic blood pressure and to draw blood samples. The animal breathed spontaneously via a trachea cannula and was placed in a stereotaxic frame. Body temperature was maintained at 37°C. The pressure points and the wounds were infiltrated with lidocaine. The skull was exposed, and holes were drilled on both sides for recording electrodes and thermistors.

Mean arterial blood pressure (MABP) and heart rate were measured with a Statham pressure transducer (Hellige, Freiburg, FRG), and the frequency of respiration was recorded by means of a thermistor inserted into the trachea cannula.

Baseline cerebral blood flow (CBF) was estimated by the H₂ clearance method.⁴ One platinum-iridium (90%-10%) electrode (insulated in the manufacturing process) 85 μm in diameter with a 1.0 mm exposed tip was placed obliquely 1.5 mm deep in the parietal cortex. The electrode was polarized with 300 mV against a silver-silver chloride reference electrode positioned in the temporal muscle on the same side. Hydrogen gas was administered until the H₂ polarographic current between the cortical electrode and the reference electrode reached a plateau sustained for 3 minutes. The first 30 seconds of the subsequent H₂ clearance curve was ignored, and CBF (as milliliters per minute per 100 g) was calculated from the next 90 seconds of the curve.
using the initial slope index method. Three or four baseline CBFs were measured at intervals of 15–20 minutes, at both the beginning and the end of each experiment. In addition, CBF was continuously registered by a heat clearance method. A heated thermistor was placed in the region adjacent to the platinum-iridium electrode, and a reference (unheated) thermistor was placed on the contralateral parietal cortex. This method is based on the continuous recording of the electrical power required to maintain the temperature difference between the heated and reference thermistors. The thermistors form two arms of a Wheatstone bridge. The temperature difference (1°C) is maintained by a negative feedback system. Zero flow conductivity was obtained after killing the animal. The circuitry and calibration of the thermistors were as described by other authors. No significant differences in brain temperature were found between the two hemispheres as measured by the calibrated thermistors.

Because of their strong linear relation, the combined use of these two clearance methods makes possible the continuous quantitative measurement of local CBF. For registration of the O2 reduction current a polarographic method was used. According to the literature, it is more accurate to describe the observed O2 current as O2 availability (O2a) rather than O2 tension. A gold electrode was chosen because it could also be used for recording the electroencephalogram (EEG) after stopping the polarizing voltage. The electrode with a 1–1.5 mm exposed tip was constructed of gold wire 75 µm in diameter. A polarizing voltage of –600 mV was applied between the parietal gold electrode and a separate silver–silver chloride reference electrode placed into the neck muscles.

In a preliminary study using eight rabbits anesthetized as in the present study, the gold electrodes were calibrated by continuously increasing the amounts of inhaled O2 and N2. The O2a values were related to changes of Paco2 measured in the femoral artery. In the case of the inhalation of pure O2, increases in cortical O2a of 1.9 µA and a rise of Paco2 in the femoral artery to 500–520 mm Hg were induced. A 1.5-minute inhalation proved sufficient to saturate the brain tissue with O2. The inhalation of pure N2 for 0.5 minute induced a decrease in cortical O2a of a similar magnitude, while Paco2 in the femoral artery fell to 10–15 mm Hg. Therefore, the inhalation of pure N2 for 1.5 minutes was determined to be sufficient to establish the baseline level of O2a in further experiments. In NT rabbits, the resting values for O2a were found to be in the range observed in gerbils and cats.

The major factors determining O2a are local blood flow, systemic Paco2, local metabolic consumption of O2, and temperature. Because there were no significant differences between the NT and HT animals in either Paco2 or brain temperature, the ratio of O2a to CBF indicates those changes of tissue O2 level that are independent of changes in CBF. Correlation analysis excluded any relation between the changes of O2a and those of DC potentials registered with the EEG electrodes (r2<0.07) adjacent to the O2 electrode.

The EEG was recorded with four gold ball electrodes on each side, placed on the dura in sagittal (frontoocipital) order. The reference electrode was put into the nasal bone in the midline. The registration was started 3 hours after insertion of the electrodes and thermistors. The data were digitized by an analog-to-digital converter (sampling frequency 80 Hz, integration every 3 seconds), stored on magnetic tape, and later analyzed off-line by an IBM AT personal computer (software program developed by Department of Experimental Physics, Technical University, Budapest). Throughout the course of the experiments, the baseline values and changes of parameters could also be followed on-line, and this enabled us to judge the stability of the baselines and to monitor the development of the steady state during H2 or O2 inhalations. The baselines were stable during the course of the 5–7-hour experiments. After digitizing the EEG signals from two monopolar derivations, the total power spectrum (µV2·sec), the classical frequency bands, and their percentile participations in the total power spectrum were calculated by the computer.

The effect of 5% CO2 (in room air) inhalation for 1.5 minutes was investigated three times at intervals of 30–40 minutes. Samples of arterial blood were drawn before, during, and after CO2 inhalation to check blood gases (Paco2, Paco2, pH, hematocrit, and the glucose concentration).

The data of both groups of rabbits were divided into three subgroups: period A, consisting of data (mean values) collected during the 3 minutes prior to CO2 inhalation; period B, containing data obtained from the top of the CO2 response curves of various parameters; and period C, comprising data from the newly developed steady state. Data are presented as mean±SEM and were analyzed by one-way analysis of variance (ANOVA). Statistical significance was assessed by multiple range tests including Scheffé's S test, the least significant difference test, and Tukey's honestly significant difference test. Statistical significance in these post hoc tests was at least p<0.05.

For histological examination, the brains of all rabbits were fixed in 10% formalin solution and cut into five coronal sections. Then, 7-µm-thick sections were stained with hematoxylin and eosin and with the Spielberg method for myelin.

**Results**

The initial values of the parameters are summarized in Table 1. In the HT group MABP was higher, CBF and O2a were lower, and the EEG was shifted to slower components than in the NT group. The ratio of baseline O2a to resting CBF was significantly greater in HT rabbits (ANOVA: p<0.003, multiple range tests: p<0.01).

Under the effect of CO2 inhalation, Paco2 increased significantly (ANOVA: p<0.0001, multiple range tests: NT and HT p<0.01) in both groups (by 9.74±2.14 mm Hg in the NT group and by 8.92±1.44 mm Hg in the HT group). In both groups, CO2 inhalation induced significant increases in both CBF (ANOVA: p<0.00001, multiple range tests: NT p<0.05 and HT p<0.01) (Figure 1) and O2a (ANOVA: p<0.0001, multiple range tests: NT p<0.05 and HT p<0.01) (Figure 2). In response to CO2 inhalation, the ratio of O2a to CBF decreased nonsignificantly in NT rabbits (Figure 3); in HT animals the decrease of this ratio was significant.
TABLE 1. Resting Values of Various Parameters in Normotensive and Hypertensive Rabbits

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Normotensive</th>
<th>Hypertensive</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean arterial blood pressure (mm Hg)</td>
<td>101.43±6.07</td>
<td>144.55±3.64</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Heart rate (min⁻¹)</td>
<td>274.50±8.92</td>
<td>278.58±10.07</td>
<td>NS</td>
</tr>
<tr>
<td>Respiration frequency (min⁻¹)</td>
<td>41.75±3.89</td>
<td>48.41±3.03</td>
<td>NS</td>
</tr>
<tr>
<td>Cerebral blood flow (mV)</td>
<td>39.82±2.1</td>
<td>24.14±0.95</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>(ml/100 g/min)</td>
<td>81.25±3.42</td>
<td>50.83±3.13</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Oxygen availability (μA)</td>
<td>1.92±0.12</td>
<td>1.41±0.08</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Electroencephalogram</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Power spectrum (μV²·sec)</td>
<td>1,465.34±76.79</td>
<td>1,192.47±107.39</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Delta (%)</td>
<td>54.86±3.63</td>
<td>70.79±4.99</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Theta (%)</td>
<td>41.43±3.43</td>
<td>25.98±4.67</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Alpha (%)</td>
<td>2.56±0.38</td>
<td>2.16±0.49</td>
<td>NS</td>
</tr>
<tr>
<td>Beta (%)</td>
<td>0.81±0.13</td>
<td>1.05±0.43</td>
<td>NS</td>
</tr>
<tr>
<td>Hematocrit</td>
<td>39.24±2.79</td>
<td>41.33±3.26</td>
<td>NS</td>
</tr>
<tr>
<td>Glucose concentration (mM/l)</td>
<td>12.28±0.96</td>
<td>12.44±0.50</td>
<td>NS</td>
</tr>
<tr>
<td>pH</td>
<td>7.43±0.02</td>
<td>7.42±0.01</td>
<td>NS</td>
</tr>
<tr>
<td>Pao₂ (mm Hg)</td>
<td>101.66±5.47</td>
<td>98.72±4.18</td>
<td>NS</td>
</tr>
<tr>
<td>Paco₂ (mm Hg)</td>
<td>30.23±2.27</td>
<td>30.14±1.04</td>
<td>NS</td>
</tr>
</tbody>
</table>

Hematocrit, glucose concentration, pH, Paco₂, and Pao₂ were measured in blood drawn from femoral artery. NS, not significant. Values are mean±SEM.

(ANOVA: p<0.002, multiple range tests: p<0.01) and occurred after a transient increase (Figure 3). In both groups there were no significant changes in MABP, heart rate, and EEG power spectrum, even in its frequency bands, in response to CO₂ inhalation (data not shown). The respiration frequency increased significantly in both groups (by 27±2 per minute in the NT group and by 34±3 per minute in the HT group; ANOVA: p<0.0008, multiple range tests: NT p<0.05 and HT p<0.01).

In HT rabbits, CBF began to increase in response to CO₂ inhalation 40 seconds later than in NT animals (ANOVA: p<0.0001, multiple range tests: p<0.01). The beginning of the changes in O₂a and respiration frequency was very similar in both groups. In NT rabbits,

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**Figure 1.** Bar graph of effect of CO₂ inhalation on cerebral blood flow (CBF) in normotensive (NT) and hypertensive (HT) rabbits. Y axis on left shows CBF values in mV and on right in ml/100 g/min. Filled bars, period A (mean values collected during 3 minutes prior to CO₂ inhalation); open bars, period B (data recorded at top of response curve); shaded bars, period C (data from newly developed steady state). *p<0.05, **p<0.01 different from initial level using multiple range tests.

**Figure 2.** Bar graph of effect of CO₂ inhalation on available oxygen in normotensive (NT) and hypertensive (HT) rabbits. Filled bars, period A (mean values collected during 3 minutes prior to CO₂ inhalation); open bars, period B (data recorded at top of response curve); shaded bars, period C (data from newly developed steady state). *p<0.05, **p<0.01 different from initial level using multiple range tests.
there was no significant difference between the onset of the change in CBF and that in O$_2$a. In the HT group, the CBF increase reached its maximum significantly later than in NT rabbits (ANOVA: \(p<0.0003\), multiple range tests: \(p<0.01\)). Although respiration frequency started to return to baseline after a similar time in the two groups, the plateau of CBF and O$_2$a increases did not last as long in HT rabbits (ANOVA: \(p<0.0002\), multiple range tests: CBF \(p<0.05\) and O$_2$a \(p<0.01\)). In HT animals, the parameters returned to their initial levels markedly later than in NT rabbits (ANOVA: \(p<0.00002\); multiple range tests: respiration frequency \(p<0.05\), O$_2$a \(p<0.05\), and CBF \(p<0.01\)).

The increased baseline tissue O$_2$ level observed in the HT rabbits may be related to either reduced O$_2$ consumption or increased cortical vascularization.\(^23\)\(^{-}24\) On microscopic investigation of the brains of HT rabbits, no increase in cortical vascularization was observed. Any differences in vascularization would, however, influence both the tissue O$_2$ level and CBF in the same manner. Therefore, changes of the O$_2$a/CBF ratio must reflect mainly the changes in O$_2$ extraction. In HT animals, the greater ratio of baseline O$_2$a to resting CBF and the increased percentage in the delta frequency band, also indicating depressed cerebral O$_2$ consumption (CMRO$_2$),\(^25\) may be the consequence of ischemic cerebral damage.\(^15\)\(^,25\) Our histological investigation demonstrates that this always develops in HT rabbits. It is known that decreased cerebral metabolism leads to the reduction of resting CBF.\(^26\)\(^{-}28\) The more severe the cerebrovascular disease (i.e., the more reduced CMRO$_2$), the more resting CBF is reduced.\(^27\)\(^,29\) Moreover, the possibility exists that the ischemic lesions of the brain stem, even if not as pronounced as those of the cortex, may contribute to the reduction of resting CBF.\(^28\)\(^,30\)^{,}\(^31\) in HT rabbits.

**Discussion**

Several authors suggest that resting CBF in chronic hypertension does not differ from that observed in normotension, in either humans or animals.\(^2\)\(^,3\)\(^,17\)\(^,18\) In contrast, Yamori and Horie\(^19\) observed that the resting CBF of aged stroke-prone spontaneously hypertensive rats is significantly lower than that of normotensive and stroke-resistant spontaneously hypertensive animals. We also observed a significantly lower resting CBF in the HT than in the NT rabbits. The resting CBF values of the latter were very similar to those reported by other authors, who had also used the H$_2$ clearance technique in rabbits, but with other anesthetic agents.\(^20\)\(^,21\)

The observations of Skolasinska and Kostrzewska\(^22\) indicate that anesthesia induced by either urethane or chloralose does not affect the resting CBF of spontaneously hypertensive animals. Furthermore, since the values of Paco$_2$, PaO$_2$, and hematocrit were similar in the two groups, these parameters cannot be responsible for the lower resting CBF measured in HT rabbits.

The increased baseline tissue O$_2$ level observed in the HT rabbits may be related to either reduced O$_2$ consumption or increased cortical vascularization.\(^23\)\(^,24\) On microscopic investigation of the brains of HT rabbits, no increase in cortical vascularization was observed. Any differences in vascularization would, however, influence both the tissue O$_2$ level and CBF in the same manner. Therefore, changes of the O$_2$a/CBF ratio must reflect mainly the changes in O$_2$ extraction. In HT animals, the greater ratio of baseline O$_2$a to resting CBF and the increased percentage in the delta frequency band, also indicating depressed cerebral O$_2$ consumption (CMRO$_2$), may be the consequence of ischemic cerebral damage.\(^15\)\(^,25\) Our histological investigation demonstrates that this always develops in HT rabbits. It is known that decreased cerebral metabolism leads to the reduction of resting CBF.\(^26\)\(^^{-}28\) The more severe the cerebrovascular disease (i.e., the more reduced CMRO$_2$), the more resting CBF is reduced.\(^27\)\(^,29\) Moreover, the possibility exists that the ischemic lesions of the brain stem, even if not as pronounced as those of the cortex, may contribute to the reduction of resting CBF in HT rabbits.
In agreement with some authors,\textsuperscript{19,32} we believe that in hypertension the resting CBF decreases only in those cases in which cerebral ischemic damage also develops. However, discussion of the pathomechanism of ischemic alterations accompanying hypertension is beyond the scope of this paper.

The observed cerebrovascular responses of NT rabbits to CO\textsubscript{2} inhalation fall within the range of previous studies.\textsuperscript{33,34} However, cerebrovascular reactivity to CO\textsubscript{2} can vary considerably depending on the magnitude of Paco\textsubscript{2} elevation and the type and dose of anesthetic agent used.\textsuperscript{35} We chose the parameters of CO\textsubscript{2} inhalation intentionally so that the values of Paco\textsubscript{2} remained within the normal range, thus making investigation of the physiological effect of CO\textsubscript{2} possible.

The CO\textsubscript{2} responsiveness in chronic hypertension is also reported to be variable; it can remain normal,\textsuperscript{2} it may decrease,\textsuperscript{3} or it may increase.\textsuperscript{1} Our observation that the absolute value of the CBF increase in response to CO\textsubscript{2} inhalation is markedly larger in LIT than in NT rabbits is surprising because CO\textsubscript{2} reactivity is generally found to be decreased when the brain is damaged.\textsuperscript{11,30,35,36,37}

It has been observed that the vessels of spontaneously hypertensive rats are narrower than those of normotensive animals.\textsuperscript{38} Reduction of CMRO\textsubscript{2} may also evoke vascular constriction because of the decreased tissue PCO\textsubscript{2} levels,\textsuperscript{27,28,30} thus contributing to the narrowing of cerebral blood vessels. Therefore, a possible explanation may be that the constricted vessels have a greater vasodilatory capacity.\textsuperscript{27} The observations of Scremin et al\textsuperscript{35} also demonstrate that decreased cerebral metabolism must be associated with greater CBF responsiveness. The larger CBF increase can also be related to the arteriolar structural changes induced by chronic hypertension since distensibility of the vessel wall generally increases in the advanced stages of hypertension.\textsuperscript{39}

The increase in O\textsubscript{2a} in response to CO\textsubscript{2} inhalation is generally held to be a consequence of a CBF increase.\textsuperscript{11,13} Under normal circumstances, CMRO\textsubscript{2} is reported to be either unchanged\textsuperscript{40} or increased\textsuperscript{41} in response to CO\textsubscript{2} inhalation. Our findings in NT animals that the ratio of O\textsubscript{2a} to CBF did not change significantly under the influence of CO\textsubscript{2} inhalation confirm this observation.

Dyken et al\textsuperscript{32} observed that under pathological conditions, CMRO\textsubscript{2} may either decrease or increase in response to CO\textsubscript{2} inhalation. They found that during CO\textsubscript{2} inhalation CMRO\textsubscript{2} decreased in patients with complete carotid occlusion, whereas in patients with incomplete carotid occlusion CMRO\textsubscript{2} increased. Our findings that...
the changes in the ratio of O$_2$ to CBF in response to CO$_2$ inhalation depend inversely on the level of ischemia agree with these observations.

It seems obvious that in moderate diffuse chronic ischemia there are both dead cells and viable but nonfunctioning cells in the cortex, resembling the phenomenon designated as ischemic penumbra (transmission failure). Thus, it is understandable that parallel with the CBF increase the functional capacity of these viable but inactive cells recovers or even increases, which results in the enhancement of CMRO$_2$.

Hypercapnia generally causes an arousal pattern. The nonsignificant EEG changes in response to CO$_2$ inhalation may be explained by the fact that we did not reach hypercapnia. In agreement with Scrimin et al., our results also indicate that the effect of CO$_2$ inhalation on CBF and O$_2$ may develop without affecting the electrical activity of the brain. The cerebrovascular CO$_2$ response has both rapid and slow components. The rapid component occurs about 10–30 seconds after the start of CO$_2$ inhalation and depends on the value of PaCO$_2$. The slow component develops about 50–60 seconds later, in relation to the increase in the tissue Pco$_2$ level. Under normal circumstances the rapid component predominates, and neural mechanisms are believed to be responsible for its development. The slow component can most probably be explained by the direct effect of tissue Pco$_2$ on the vessel wall. In NT animals the beginning of the CBF increase corresponds to the time of the rapid component, whereas in HT animals it coincides with the time of the slow component.

According to the observations of Tuteur et al. decreased cerebral metabolism may influence the time course of the CBF increase induced by CO$_2$ inhalation. Those authors found that in stroke patients the slower the CO$_2$-induced CBF increase, the lower the value of CMRO$_2$. One possible explanation is that the development of the slow component may be related to disturbance of neural regulation on the basis of transmission failure. In addition, brain stem damage may also lead to the disturbance of neural regulation.

In summary, the explanation for the pathological features described in our HT rabbits does not seem to lie in the hypertension itself, but more probably the features are consequences of the development of multiple cerebral ischemic lesions. These lesions, containing viable but inactive cells as well as entirely damaged cells, may then lead to a reduction in the resting CBF and a loss of the fast component of CO$_2$ reactivity. In addition, the inactive cells remain capable of increasing their $O_2$ metabolism when CBF increases.

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