Brain pH Responses to Acetazolamide and Hypercapnia in Cats

Kiyotaka KOHSHI*,**, Yoshimasa KINOSHITA*, and Koichi FUKATA***

Departments of *Neurosurgery and **Hyperbaric Medicine, School of Medicine, and ***Division of Clinical Examination, School of Medical Technology, University of Occupational and Environmental Health, Kitakyushu, Fukuoka

Abstract

The involvement of increased brain tissue CO₂ tension in acetazolamide-induced brain acidosis was investigated by comparing the brain pH response to acetazolamide with that to hypercapnia. CO₂ and pH sensors were placed bilaterally into cerebral white matter to 15 mm depth in cats. Group I cats (n = 9) breathed spontaneously, and in situ brain tissue Pₐ CO₂ and pH (Pₐ CO₂ and pHb) were measured after intravenous acetazolamide administration (20 mg/kg). Group 2 cats (n = 9) were paralyzed and ventilated mechanically, and the changes of pHb were investigated by adjusting the ventilation to maintain the same Pₐ CO₂ values as in the acetazolamide-treated group. Pₐ CO₂ changes were not significantly different between the two groups. However, pHb responses were quite different: the fall in pHb was progressive in Group 1 but transient in Group 2. Brain acidosis after acetazolamide administration is not due to the rise in brain tissue CO₂ tension.

Key words: carbonic anhydrase, acetazolamide, pH regulation, cerebrovascular diseases

Introduction

Measurements of regional cerebral blood flow (CBF) have been used to evaluate the hemodynamic significance of ischemic cerebrovascular disease. Assessment of the capacity to increase CBF in response to vasodilators such as CO₂ or acetazolamide is another way of measuring cerebral circulatory reserve.

Acetazolamide, a selective inhibitor of carbonic anhydrase, is more easily administered to patients with cerebrovascular disease than CO₂, because it has little influence on the cardiopulmonary system. Acetazolamide increases the CBF remarkably and induces an increase in both brain tissue Pₐ CO₂ (Pₐ CO₂) and carbonic acidosis, but the mechanisms for these effects have remained obscure. Moreover, there is a dissociation between the changes in Pₐ CO₂ and brain tissue pH (pHb); the fall in pHb is large despite the rise in Pₐ CO₂ being only slight after acetazolamide administration. The effects of acetazolamide on the acid-base balance might occur via a direct inhibition of carbonic anhydrase localized on brain cells. However, the effects may be due to carbonic anhydrase inhibition in red blood cells (RBCs), because acetazolamide injected intravenously crosses the blood-brain barrier (BBB) slowly whereas both Pₐ CO₂ and pHb change rapidly immediately after intravenous administration of acetazolamide. If these effects are caused only by disturbance of the CO₂-carrying capacity of RBCs, the pH responses to both acetazolamide and hypercapnia should be similar if the time course of Pₐ CO₂ is similar.

The recent development of the ion-sensitive field-effect transistor (ISFET) allows the measurement of Pₐ CO₂ and pH in situ. In animal and clinical experiments, catheter-tip CO₂ and pH sensors based on ISFET technology have been successfully used to monitor continuously in situ Pₐ CO₂ and pH in blood and tissues. The present study investigated whether acetazolamide-induced brain acidosis is due to the concomitant rise in brain tissue CO₂ tension.

Materials and Methods

Twenty-two cats of both sexes (body weight 3.4 ± 0.3 kg, mean ± SD) were sedated with an in-
traperitoneal injection of 120 mg ketamine. Anesthesia was maintained by continuous infusion of ketamine (200 mg in 10 ml physiological saline) delivered at 2 ml/hr via a femoral venous catheter. The animals had miotic eyes and salivation during the measurements. Rectal temperature was monitored and kept at 38°C by means of a heating table. Arterial and venous catheters were inserted so that arterial P\textsubscript{CO}\textsubscript{2} (P\textsubscript{A}\textsubscript{CO}\textsubscript{2}) could be monitored continuously and drugs could be infused. The animals were fixed to a stereotactic cradle, and a midline scalp incision was made after local anesthesia with 1% lidocaine. After the scalp was retracted, two holes of 0.5 cm diameter were drilled bilaterally through the cranium with a burr drill. Hemostasis was achieved with cauterization and bone wax. The centers of the holes were 1.0 cm anterior from the ears and 1.0 cm lateral from the midline. The exposed dura was coagulated and incised.

CO\textsubscript{2} and pH sensors (CO\textsubscript{2} sensor, CO-1035; pH sensor, pH-2135; Nihon Kohden, Tokyo) used catheter-tip electrodes which contained the pH-ISFET. The CO\textsubscript{2} sensor was coated with silicone, and the pH sensor was encapsulated in the end of a nylon tube. These CO\textsubscript{2} and pH sensors can measure continuously in situ P\textsubscript{CO}\textsubscript{2} and pH, respectively, in blood and other tissues.\textsuperscript{18,23} The pH sensor reveals rapid responses to acidosis and alkalosis, and the response time of the CO\textsubscript{2} sensor is 2 minutes for a 90% change in CO\textsubscript{2} tension.\textsuperscript{28} The CO\textsubscript{2} sensor was calibrated at 37°C with a standard phosphate buffer of pH 7.27, and the CO\textsubscript{2} sensor was calibrated at 37°C with sterile P\textsubscript{CO}\textsubscript{2} standard solutions of 36 and 86 mmHg. The baseline drift of the CO\textsubscript{2} sensor was less than 2 mmHg during 5 hours of measurements.\textsuperscript{18} The CO\textsubscript{2} and pH sensors were inserted bilaterally to 15 mm depth from the cortical surface in the center of the holes. Another CO\textsubscript{2} sensor was inserted via a femoral arterial catheter to measure P\textsubscript{A}\textsubscript{CO}\textsubscript{2} continuously. pH/P\textsubscript{CO}\textsubscript{2} monitors (KR-5000; Nihon Kohden) were used to record the output of the CO\textsubscript{2} and pH sensors. Respiratory rate was monitored by the impedance method and averaged every 10 seconds, and heart rate was determined from the electrocardiogram.

Two groups of cats were studied. The first (Group 1) was designed to study the effects of acetazolamide and the second (Group 2) to study those of hypercapnia. Group 1 animals (n = 9) were allowed to breath spontaneously for at least 30 minutes to let all parameters stabilize. At time 0 (control), after determination of all steady-state values, they received a bolus injection of 20 mg/kg acetazolamide dissolved in physiological saline (at a concentration of 100 mg/ml) via a femoral catheter. During the next 60 minutes, the animals remained in the cradle to monitor the parameters, and in situ P\textsubscript{B}\textsubscript{CO}\textsubscript{2} and pH\textsubscript{b} were measured continuously. Group 2 animals (n = 9) underwent tracheotomy and muscle paralysis was achieved with intravenous pancuronium (1.0 mg/kg/hr). They were mechanically ventilated with air with a Harvard respirator (model 665d; Harvard Apparatus, South Natick, Mass., U.S.A.). P\textsubscript{B}\textsubscript{CO}\textsubscript{2} was controlled by mechanical ventilation to maintain the mean P\textsubscript{A}\textsubscript{CO}\textsubscript{2} value at that measured during the pre-acetazolamide period in Group 1 animals. Then, by reducing the rate and depth of respiration, P\textsubscript{A}\textsubscript{CO}\textsubscript{2} was elevated to the mean P\textsubscript{B}\textsubscript{CO}\textsubscript{2} value measured after acetazolamide injection within 10 minutes to reflect the P\textsubscript{B}\textsubscript{CO}\textsubscript{2} change, because hypoxemia is not induced by hypercapnia\textsuperscript{20,33} and the P\textsubscript{CO}\textsubscript{2} gradient between the brain and arterial blood is constant.\textsuperscript{16} The mechanically induced P\textsubscript{B}\textsubscript{CO}\textsubscript{2} elevation was maintained for more than 60 minutes. All parameters were recorded every 10 seconds by a programmable data logger (7V07; NEC San-Ei, Tokyo).

The obtained data were averaged over 1 minute (6 points) in each animal and analyzed using the Statview statistical package (Abacus Concepts). All values are reported as means ± SE. Within each group, significant differences between time points were determined by one-way analysis of variance followed by Fisher’s least significance test. For inter-group comparison of P\textsubscript{B}\textsubscript{CO}\textsubscript{2} values, two-tailed unpaired t tests were applied. Statistical significance was taken as a p value of <0.05.

### Results

Satisfactory recordings of P\textsubscript{A}\textsubscript{CO}\textsubscript{2}, P\textsubscript{B}\textsubscript{CO}\textsubscript{2}, and pH\textsubscript{b} were obtained in 18 of 22 cats. The results in other animals, which developed bleeding at some point in the study, were discarded. The effects of acetazolamide and hypercapnia on P\textsubscript{A}\textsubscript{CO}\textsubscript{2}, P\textsubscript{B}\textsubscript{CO}\textsubscript{2}, and pH\textsubscript{b} are summarized in Table 1.

The changes in P\textsubscript{A}\textsubscript{CO}\textsubscript{2}, P\textsubscript{B}\textsubscript{CO}\textsubscript{2}, and pH\textsubscript{b} are illustrated by the representative electrode tracings from a Group 1 animal (Fig. 1). Acetazolamide administration was always followed by a rapid rise in P\textsubscript{B}\textsubscript{CO}\textsubscript{2}, as well as by a concomitant fall in pH\textsubscript{b}. Such P\textsubscript{B}\textsubscript{CO}\textsubscript{2} changes always reached a steady-state value by 20 minutes after drug administration, and always persisted for at least 40 minutes. The mean P\textsubscript{B}\textsubscript{CO}\textsubscript{2} value increased by 25.3 ± 5.6 mmHg at 60 minutes after acetazolamide administration. In contrast, the fall in pH\textsubscript{b} gradually progressed during the measurements. pH\textsubscript{b} decreased by 0.20 ± 0.04 pH in 60 minutes. P\textsubscript{A}\textsubscript{CO}\textsubscript{2} showed a transient slight rise immediately after acetazolamide administration and then decreased gradually, reaching a steady state at 10 minutes after

---

K. Kohshi et al.

Neurol Med Chir (Tokyo) 37, April, 1997
drug infusion. The fall in Paco2 was maintained from 10 to 40 minutes. After this plateau phase, Paco2 increased gradually and reached a new steady-state value, which was not statistically different from the control value. No significant changes were found in respiratory and heart rates at any point.

Figure 2 shows representative tracings for Paco2, Pbco2, and pHb in a Group 2 animal. The Paco2 was maintained at 35 ± 1 mmHg to match the Pbco2 to the control Pbco2 values in Group 1. Paco2 was increased by hyperventilation sufficient to increase Pbco2 by 25 mmHg, i.e., the increase caused by acetazolamide administration. pHb decreased from the baseline to the lowest value of 6.93 pH after 10-minute exposure to hypercapnia, and thereafter showed a significant rise from 40 minutes (p < 0.05). Heart rate decreased transiently after changing the ventilation and soon returned to the baseline. The baseline values in Pbco2 and pHb in the two groups were not significantly different. Reduction in the rate and depth of respiration changed Pbco2 values similarly to those of the acetazolamide-treated animals. However, the time courses of pHb

Table 1 Effects of acetazolamide and hypercapnia on Pbco2 and pHb

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>Acetazolamide</th>
<th>Hypercapnia</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pbco2 (mmHg)</td>
<td>pHb</td>
</tr>
<tr>
<td>-30</td>
<td>66.5 ± 2.7</td>
<td>7.03 ± 0.03</td>
</tr>
<tr>
<td>0</td>
<td>66.8 ± 2.6</td>
<td>7.03 ± 0.03</td>
</tr>
<tr>
<td>5</td>
<td>77.8 ± 3.5</td>
<td>7.00 ± 0.04</td>
</tr>
<tr>
<td>10</td>
<td>87.4 ± 5.1</td>
<td>6.97 ± 0.04</td>
</tr>
<tr>
<td>15</td>
<td>90.6 ± 4.9</td>
<td>6.95 ± 0.03</td>
</tr>
<tr>
<td>20</td>
<td>91.9 ± 5.2</td>
<td>6.94 ± 0.03</td>
</tr>
<tr>
<td>25</td>
<td>92.1 ± 4.9</td>
<td>6.92 ± 0.02</td>
</tr>
<tr>
<td>30</td>
<td>91.7 ± 4.7</td>
<td>6.91 ± 0.02</td>
</tr>
<tr>
<td>40</td>
<td>91.7 ± 4.6</td>
<td>6.88 ± 0.03</td>
</tr>
<tr>
<td>50</td>
<td>91.4 ± 4.5</td>
<td>6.86 ± 0.04</td>
</tr>
<tr>
<td>60</td>
<td>92.0 ± 4.5</td>
<td>6.84 ± 0.04</td>
</tr>
</tbody>
</table>

Values are means ± SE. PaCO2 and PbCO2: arterial and brain tissue Pco2; pHb: brain tissue pH. Time 0 min indicates acetazolamide (20 mg/kg, i.v.) administration and reducing respiration. p and Fisher values were determined by analysis of variance.

Fig. 1 Representative PaCO2, PbCO2, and pHb changes in a Group 1 cat. Arrow indicates the time of intravenous administration of 20 mg/kg of acetazolamide.

Fig. 2 Representative PaCO2, PbCO2, and pHb changes in a Group 2 cat. Arrow indicates the time at which hypercapnia was induced.

Neurol Med Chir (Tokyo) 37, April, 1997
Discussion

This study found differences in both the trend and the magnitude of brain acidosis due to treatment with acetazolamide and hypercapnia despite maintenance of P_{bCO2} at similar levels.

The time course of the mean percentage changes in P_{bCO2} and pHb were quite different in animals treated with acetazolamide (Fig. 3), although the response time of the CO2 sensor is slower than that of the pH sensor. This finding is in contrast to previous observations of the same mean percentage changes in P_{CO2} and pH at the medullary surface.28) However, measurement of P_{CO2} and pH at the brain surface found a fall in pH persisted whereas P_{CO2} was reduced to the control values before treatment through changes in ventilation.9) The reaction (CO2 + H2O = H2CO3 = H+ + HCO3-) requires 20 seconds to reach 90% change of the total change in plasma.8) The same time is estimated to be required to reach equilibrium in brain extracellular fluid because carbonic anhydrase is not present there. If both P_{CO2} and pH are regulated together by this equilibrium reaction in brain extracellular fluid, then such a large difference in the mean percentage change would not occur even if the hydration reaction of CO2 is uncatalyzed. Therefore, the previous and our present findings indicate that both brain H+ and CO2 can increase independently in response to acetazolamide.

The exact mechanisms by which acetazolamide induces a rise in P_{bCO2} and brain acidification have not been clarified. The importance of carbonic anhydrase in brain tissue has been emphasized in the rise in P_{bCO2},4) but carbonic anhydrase inhibition in RBCs causes both decreased CO2-carrying capacity of blood and greater P_{CO2} gradient between tissue and blood.5,21,22,31) because acetazolamide injected intravenously penetrates the BBB slowly and may take several hours before the physiological effect is maximum.21,23,29) The overall pattern of carbonic anhydrase inhibition by acetazolamide injected intravenously is simply due to the greatly increased gradient of P_{CO2} from tissue to alveolus.21) Membrane-associated carbonic anhydrase is widely present on the luminal surface of endothelial cells of brain capillaries.6) This type of carbonic anhydrase is present in the lungs23) and is involved in CO2 exclusion from the lungs.8) The carbonic anhydrase in brain capillaries is probably important for CO2 transport from the brain to blood and inhibition may cause CO2 retention in the brain.3,10) Therefore, there are at least two sources for the rise in P_{bCO2} after acetazolamide administration: inhibition of carbonic anhydrase located either in RBCs or in brain capillary endothelium.

Brain carbonic acidosis might occur via direct inhibition of carbonic anhydrase located in brain tissue, but the slow movement of acetazolamide across the BBB suggests that the rapid pHb change after drug administration is not caused by this mechanism. The excess rise in P_{bCO2} after acetazolamide administration may cause the formation of carbonic acid in brain tissue.22,28) According to this hypothesis, pHb responses to both acetazolamide and hypercapnia should be similar if the time course of P_{bCO2} is the same. However, our study found the fall in pHb was progressive after acetazolamide administration, but transient in hypercapnia.

The pHb response to hypercapnia shows the phenomenon of pH buffering in brain extracellular fluid due to carbonic anhydrase in RBCs and brain tissue. Such brain pH buffering capacity is well known to be activated under hypercapnia and hypocapnia.1,2) Our result that the pHb buffering is inhibited by acetazolamide administration suggests that one of the pHb regulating mechanisms is carbonic anhydrase-dependent. Brain pH is regulated mainly by transmembrane ion transport in both the brain capillary wall and the brain cell membrane.14,24) Especially, Na+/H+ and Cl-/HCO3− exchanges are important in brain pH regulation.3,12,14) In the cerebrospinal fluid, Na+/H+ and Cl−/HCO3− exchanges may be dependent on carbonic anhydrase located in the cells of the choroid plexus and glia.15,20) Our result that acetazolamide inhibited pHb regulation is similar to the observation that in-
traperitoneal acetazolamide interfered with carbonic anhydrase-dependent ion transport in the brain cell membrane. However, we believe that this phenomenon cannot be explained by this site of inhibition because of the slowness of acetazolamide penetration across the BBB. Intra- and extracellular pH changes are interdependent in brain tissue. Thus, the inhibition of intracellular pH regulation might be greatly affected by the extracellular pH change. Although our data support the hypothesis that brain capillary endothelial carbonic anhydrase interferes with pH regulation in brain tissue, how intravenously injected acetazolamide affects the pH regulatory mechanism is not shown.

The pHb responses to acetazolamide and hypercapnia are quite different from each other when the values of PbCO₂ are not significantly different at any point during the measurement. Acetazolamide-induced brain carbonic acidosis is not due to the concomitant rise in brain tissue CO₂ tension.

References


Neurol Med Chir (Tokyo) 37, April, 1997
Commentary

Kohshi et al. have provided important insight into the mechanism of induction of brain acidosis by acetazolamide. In their well-designed and creative set of experiments, these investigators have shown clear differences in the temporal profile and magnitude of brain acidosis due to acetazolamide and hypercapnia. By adjusting for \( \text{PbCO}_2 \) in the two experimental groups, they have shown that the mechanism for acetazolamide-induced brain acidosis is not simply the result of a rise in brain \( \text{CO}_2 \) tension induced by the pharmacologic agent.

As with most good experiments, this study answers an important question and also raises some new unanswered questions that require further investigation. The authors' scholarly discussion applies their novel finding to existing hypotheses on mechanisms by which acetazolamide induces a rise in \( \text{PbCO}_2 \) and brain acidification. The results strongly suggest that brain capillary endothelial carbonic anhydrase interferes with \( \text{pH} \) regulation in brain tissue. Further work is indicated to determine the precise mechanism by which acetazolamide affects the \( \text{pH} \) regulatory mechanism.

Daniel L. Barrow, M.D.
Department of Neurosurgery
The Emory Clinic

Dr. Kohshi and his colleagues report interesting data which disclose striking differences in brain tissue \( \text{pH} \) responses between acetazolamide administration and hypercapnia. They demonstrate that, when acetazolamide is administered intravenously, the brain tissue \( \text{pH} \) decreases slowly, whereas the increase in \( \text{CO}_2 \) tension of the brain tissue reaches its maximum level far more rapidly. In contrast, hypercapnia causes a decrease in \( \text{pH} \) in parallel with an increase in \( \text{CO}_2 \) tension within the brain tissue. This difference in immediate responses of the brain tissue \( \text{pH} \) should be taken into account when cerebrovascular responses are analyzed by these procedures. They also demonstrate that, while the decrease in brain tissue \( \text{pH} \) during hypercapnia is transient, acetazolamide causes the brain tissue \( \text{pH} \) to decrease progressively. As mentioned by the authors, the slowly progressive decrease in brain tissue \( \text{pH} \) caused by acetazolamide may reflect disturbances in the mechanisms of \( \text{pH} \) regulation. The authors suggest that inhibition of carbonic anhydrase located on the capillary endothelial cells of the brain could play a major role in producing such disturbances of \( \text{pH} \) regulation within the brain tissue. This is another important factor which should be borne in mind when acetazolamide is used clinically.

Yoichi Katayama, M.D.
Department of Neurological Surgery
Nihon University School of Medicine
Tokyo, Japan

Kohshi et al. have used \( \text{CO}_2 \) and \( \text{pH} \) sensors, which facilitate continuous monitoring of brain tissue \( \text{CO}_2 \) tension (\( \text{PbCO}_2 \)) and \( \text{pH} \) (\( \text{pHb} \)), and studied the effects of acetazolamide and hypercapnia on \( \text{PbCO}_2 \) and \( \text{pHb} \). It is well-known that acetazolamide is a potent cerebrovascular dilator and it is used to examine cerebrovascular reserve capacity in stroke patients. However, the mechanism by which acetazolamide causes tissue acidosis is not completely understood. The authors demonstrated clear \( \text{PaCO}_2 \), \( \text{PbCO}_2 \), and \( \text{pHb} \) changes in acetazolamide and hypercapnia groups. Acetazolamide-treated animals showed progressive and persistent falls in \( \text{pHb} \) associated with \( \text{PbCO}_2 \) rise. Hypercapnia animals showed the same \( \text{PbCO}_2 \) rise as seen in acetazolamide-treated animals, but the fall in \( \text{pHb} \) was transient. Thus, the \( \text{pHb} \) responses to acetazolamide and hypercapnia are quite different when the values of \( \text{PbCO}_2 \) are not significantly different at any point during the measurement. They concluded that acetazolamide-induced brain carbonic acidosis was not due to the concomitant rise in brain tissue \( \text{CO}_2 \) tension. This conclu-
sion rises another question about the discrepancy between the rapidity of the brain acidosis after acetazolamide administration and the slowness of acetazolamide penetration across the BBB. Further new information about the biochemical effects of acetazolamide on cerebral pH regulation are necessa-ry to answer these complex problems.

Minoru MAEDA, M.D.
Department of Neurosurgery
Juntendo University Izunagaoka Hospital
Shizuoka, Japan