Effect of Non-Respiratory Alkalosis on Brain Tissue and Cerebral Blood Flow in Rats with Damaged Blood-Brain Barrier

J. L. Pannier, M.D., J. Weyne, M.D., G. Demeester, M.S., and I. Leusen, M.D.

SUMMARY Acute alterations in plasma bicarbonate concentration have minimal effects on intracerebral pH and cerebral blood flow, perhaps due to blood-brain barrier mechanisms. To test this hypothesis, the consequences of an acute rise in the plasma bicarbonate concentration were studied in anesthetized rats previously subjected to an acute pressure pulse in the carotid system with unilateral damage to the blood-brain barrier.

In rats subjected to a "heavy" hypertensive insult, the hemisphere on the side of the lesion showed a lactic acidosis, edema, and a depression of cerebral blood flow. An increase in the plasma bicarbonate concentration of 15-20 mEq/l during 35 minutes provoked a marked rise in the total CO2 content of this hemisphere, and a further increase in the lactate concentration, but did not alter the brain edema or affect further the already very low cerebral blood flow.

An increase in the lactate concentration and a decrease of cerebral blood flow in the "reference" hemisphere indicated that the lesion was not completely unilateral.

In rats subjected to a "moderate" hypertensive insult the changes were less pronounced and statistically not significant for all the parameters.

The results illustrate the importance of an intact blood-brain barrier for the maintenance of intracerebral pH in the face of acute alterations in plasma [HCO3-]. The impaired cerebral blood flow after an acute hypertensive insult did not appear to be influenced by the intracerebral [HCO3-].

THE CONSEQUENCES of acute changes in plasma bicarbonate concentration ([HCO3-]) on brain acid-base equilibrium and cerebral metabolism have been studied in animals and man. Both the bicarbonate concentration (and pH) in brain tissue and cerebral blood flow (CBF) have been shown to be minimally affected in these circumstances, at least when concomitant changes in arterial PCO2 (Paco2) are avoided or accounted for. These phenomena were ascribed to the effect of special blood-brain barrier (BBB) mechanisms regulating the [HCO3-] in brain tissue and cerebrospinal fluid (CSF) (review: Leusen1). On the other hand, experiments on cats have shown that CBF can be affected by changes in plasma [HCO3-] when the BBB is damaged.2

The aim of the present experiments was to study the effect of changes in the plasma [HCO3-] on brain tissue [HCO3-] in the presence of a lesion of the BBB, and to relate CBF to these conditions to changes in the brain tissue. The experiments were performed on rats, to allow for tissue analysis. In addition, brain [HCO3-], lactate and water content were also measured.

Methods

1. Preparation of Animals — Experimental Groups

Experiments were carried out on 94 white male rats weighing 280–300 g, of the inbred laboratory colony. The rats were anesthetized with pentobarbital (Nembutal, 50 mg/kg, i.p.), tracheotomized, and small polyethylene tubes were inserted into various blood vessels (femoral artery, carotid artery, caudal artery, and femoral or jugular vein) according to the needs of the particular experiments (see below). After the preparation all the rats were curarized (Flaxedil®, 10 mg i.v.) and artificially ventilated with a rodent ventilation pump (Palmer, London) using a gas mixture containing 30% O2 in nitrogen.

Ventilation of the animals was regulated so as to maintain a constant arterial PCO2 level of approximately 35 mm Hg during the experiment (40 minutes).

Experimental animals were divided into 3 major groups. The "control" animals (group I; n = 21) were subjected to a unilateral ligature of the common carotid artery during the preparation. The second group (group II; n = 40) was subjected to an acute hypertensive pulse in the carotid artery on the side of the occlusion at the end of the preparation. A third group (group III; n = 33) was subjected to the same insult, and received an intravenous infusion of Na2CO3 (0.9 N) solution during the following 4 minutes. The infusion rate was regulated so as to produce an increase in the plasma bicarbonate concentration of 15-20 mEq/l in the first 10 minutes of the infusion, and to maintain a constant (within 10% plasma level for the following 30 minutes). The total fluid volume infused amounted to approximately 2 ml, and the solutions were warmed to 38°C before the start of the infusion.

Each group of rats was further subdivided into several subgroups for the determination of acid-base parameters, water content, and blood content of brain tissue, permeability of blood-brain barrier for Evar blue, and cerebral blood flow. Blood flow and water content were determined on the same animals except in group I (table I). Total CO2 and lactate were also measured on the same animals, except in group I with "heavy insult" (table 2, right panel).

Body temperature was kept constant with a heating pad and arterial blood pressure was continuous recorded from the femoral arterial catheter.

The rats were sacrificed by freezing the head in liquid nitrogen.
aid nitrogen when chemical analysis had to be performed on the brain, or by i.v. injection of saturated KCl in the other experiments.

2. Damage to Blood-Brain Barrier

After the insertion of a short (6 cm) PE-50 polyethylene catheter into one carotid artery with its tip directed toward the head, blood was withdrawn from a femoral arterial catheter, and injected very rapidly into the carotid artery which was occluded by the iatrogen around the catheter. A syringe with the slunger driven by a spring was used to inject 0.10 ml blood in a 0.15 sec time interval ("moderate insult"), or 0.25 ml blood in 0.30 sec ("heavy insult").

The procedure was analogous to the method previously described to damage the blood-brain barrier in dogs, cats, and rats by several authors24 and results in an abrupt and transient pressure increase in the carotid artery.

To study the permeability of the blood-brain barrier, 0.75 ml of Evans blue (2% solution made sonotic by the addition of NaCl) was administered at the end of the experiment and the rats were sacrificed 10 min later. The head of each animal was perfused separately via the 2 carotid arteries, first with 0.9% NaCl, then with 10% formalin, and the brain was removed and inspected macroscopically for blue staining on the brain surface and on coronal sections.

1. Measurement of Cerebral Blood Flow

Cerebral blood flow was measured at the end of the experiment with the microsphere method, using 141Ce or 85Sr labeled carbonized plastic microspheres (3 M) with a diameter of 15 ± 5 μ (2 sd), as previously described. About 40,000 microspheres were injected n 10 seconds into the left ventricular cavity through a PE 50 polyethylene tube introduced via the right carotid artery. A reference blood sample was collected at a constant rate of 0.393 ml/min with a Harvard withdrawal pump from a polyethylene tube inserted in the caudal artery. The withdrawal was started a few seconds before and was stopped 60 seconds after the injection of the microspheres.

After sacrificing the rat, the radioactivity was measured in the brain hemispheres (not in the cerebellum) and in the reference blood sample. Cerebral blood flow (ml/100 g·min) can be calculated according to the equation

\[
BF = \frac{\text{radioactivity in brain/100 g}}{\text{radioactivity in reference sample}} \times 0.393
\]

The amount of microspheres injected was sufficient to obtain at least 400 microspheres in the reference hemisphere in each rat.

Analytical

Analytical methods were previously described by Veyne et al. and will only be briefly summarized here. Arterial blood (0.30 ml) was sampled anaerobically in a heparinized syringe and stored at 0°C before the determination of acid-base parameters. Blood pH and Pco2 were determined with microelectrodes at 38°C (Radiometer, BMS, 3 Copenhagen).

The brain was frozen in situ by dipping the animal's head in liquid nitrogen, and then quickly removed from the skull in the frozen state. Lactate was measured in brain tissue with an enzymatic method and total carbon dioxide content (Tco2) with the Van Slyke and Neill manometric apparatus. Blood content in brain was calculated from measurements of hemoglobin content in tissue and in diluted blood samples. The bicarbonate concentration in brain tissue was calculated from total CO2 content with correction for brain blood as previously described. The results were expressed as the concentration in total brain tissue (mmol/kg wet weight). Brain H2O (%) was determined by weighing before and after drying the brain tissue at 105–110°C to constant weight.

Differences between values obtained for each hemisphere within a group of rats, were analyzed using Student's t-test for paired data.

Results

1. Carotid Occlusion

Total CO2 content, lactate concentration, water content and cerebral blood flow were measured in the 2 hemispheres in 3 groups of rats 40 minutes after unilateral occlusion of a common carotid artery (table 1).

Values for arterial Pco2, plasma bicarbonate concentration, and arterial blood pressure were not consistently different in the 3 groups of rats, and the mean values for all the rats are presented in the table.

The unilateral occlusion of a common carotid artery did not induce statistically significant differences between the 2 hemispheres in any of the measured parameters, except for cerebral blood flow, which was somewhat lower on the side of the occlusion.

The permeability of the blood-brain barrier for Evans blue was investigated in 4 rats with a unilateral occlusion of the carotid artery. The brains of these animals were not colored after the administration of Evans blue.

2. Damage to Blood-Brain Barrier

Damage to the blood-brain barrier was induced in 2 series of rats either with the "moderate" or with the "heavy" insult as described above. The permeability of the blood-brain barrier for Evans blue studied on 4 rats in each series was clearly modified. Macroscopic inspection of the brain showed areas of Evans blue extravasation in the cerebral cortex and in deep gray nuclei localized on the side of the autotransfusion. Occasionally a few blue spots could also be observed in the reference hemisphere.

Total CO2 content, lactate concentration, water content, blood content and cerebral blood flow were measured 40 minutes after the insult in the 2
Table 1  Unilateral Carotid Occlusion (Group I). Mean Values and SEM for Different Parameters in the Cerebral Hemispheres and in Blood Plasma, 40 Minutes After Unilateral Carotid Occlusion

<table>
<thead>
<tr>
<th>Brain</th>
<th>Mean</th>
<th>SEM</th>
<th>n</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>$T_{CO_2}$ (mmol/kg)</td>
<td>reference 15.6</td>
<td>0.68</td>
<td>7</td>
<td>NS</td>
</tr>
<tr>
<td>experim. 15.9</td>
<td>0.83</td>
<td>7</td>
<td></td>
<td></td>
</tr>
<tr>
<td>$HCO_3^-$ (mmol/kg)</td>
<td>reference 14.3</td>
<td>0.65</td>
<td>7</td>
<td>NS</td>
</tr>
<tr>
<td>experim. 14.8</td>
<td>0.88</td>
<td>7</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lactate (mmol/kg)</td>
<td>reference 1.67</td>
<td>0.14</td>
<td>7</td>
<td>NS</td>
</tr>
<tr>
<td>experim. 1.68</td>
<td>0.24</td>
<td>7</td>
<td></td>
<td></td>
</tr>
<tr>
<td>$H_2O$ content (%)</td>
<td>reference 77.90</td>
<td>0.19</td>
<td>7</td>
<td>NS</td>
</tr>
<tr>
<td>experim. 77.77</td>
<td>0.18</td>
<td>7</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Blood flow (ml/100 g.min)</td>
<td>reference 97</td>
<td>14</td>
<td>7</td>
<td></td>
</tr>
<tr>
<td>experim. 81</td>
<td>9</td>
<td>7</td>
<td>0.05</td>
<td></td>
</tr>
</tbody>
</table>

Plasma

| $P_{ACO_2}$ (mm Hg) | 35.6 | 1.5 | 21 | |
| $HCO_3^-$ (mEq/l) | 23.1 | 0.6 | 21 | |

Blood Pressure (cm Hg)

<table>
<thead>
<tr>
<th>Mean</th>
<th>SEM</th>
<th>n</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>$T_{CO_2}$ (mmol/kg)</td>
<td>14.5</td>
<td>0.57</td>
<td>6</td>
</tr>
<tr>
<td>experim. 13.1</td>
<td>0.53</td>
<td>6</td>
<td>0.05</td>
</tr>
<tr>
<td>$HCO_3^-$ (mmol/kg)</td>
<td>13.2</td>
<td>0.64</td>
<td>6</td>
</tr>
<tr>
<td>experim. 11.8</td>
<td>0.55</td>
<td>6</td>
<td>0.02</td>
</tr>
<tr>
<td>Lactate (mmol/kg)</td>
<td>4.8</td>
<td>1.06</td>
<td>6</td>
</tr>
<tr>
<td>experim. 9.0</td>
<td>0.48</td>
<td>6</td>
<td>0.05</td>
</tr>
<tr>
<td>$H_2O$ content (%)</td>
<td>78.64</td>
<td>0.09</td>
<td>7</td>
</tr>
<tr>
<td>experim. 79.32</td>
<td>0.23</td>
<td>7</td>
<td>0.01</td>
</tr>
<tr>
<td>Blood content (ml/100 g)</td>
<td>91</td>
<td>20</td>
<td>7</td>
</tr>
<tr>
<td>experim. 47</td>
<td>12</td>
<td>7</td>
<td>0.01</td>
</tr>
</tbody>
</table>

Damage to the Blood-Brain Barrier and $Na_2CO_3$ Infusion

Damage to the blood-brain barrier was induced in series of rats either with the “moderate” or with the “heavy” insult and evaluated with the Evans blue test in some animals. The same results were obtained wit
the Evans blue test as in rats without infusion of Na₂CO₃ (see section 2). The administration of the same Na₂CO₃ solution with the same infusion protocol to "control" rats (one carotid artery occluded) did not produce an extravasation of Evans blue (4 rats).

Forty minutes after the insult, the same parameters were measured as in the previous section in 2 groups of rats with a moderate insult and in 3 groups of rats with a heavy insult (table 3). During this time interval, the animals were subjected to an infusion of Na₂CO₃ solutions in order to increase the plasma bicarbonate concentration as described above. Mean values for arterial Pco₂, plasma bicarbonate concentration and arterial blood pressure obtained at the end of the experiment in each series are presented in the table.

When the experimental hemisphere is compared to the reference hemisphere, the animals subjected to a "heavy" insult show an increase in total CO₂ content, lactate concentration and water content on the side of the lesion. Blood content is not significantly changed between the 2 hemispheres for Tco₂ content. When the experimental hemisphere is compared to the reference hemisphere, the results show an increase in Tco₂ content.

In the reference hemisphere, the values for lactate and cerebral blood flow are decreased. In the animals with a "moderate" insult, the changes induced in these parameters are qualitatively similar but quantitatively less pronounced and statistically not significant for Tco₂ content.

In the reference hemisphere, the values for lactate are higher than in the rats without infusion of Na₂CO₃ (table 2).

Discussion

The present experiments were performed on rats subjected to a unilateral lesion of the blood-brain barrier. This allows comparison between the affected hemisphere and the reference hemisphere in each rat, and avoids difficulties inherent in the comparison of different groups of animals.

However, the production of such a lesion in rats requires the occlusion of 1 carotid artery, therefore the effects of a unilateral carotid ligation on the brain parameters had to be studied separately. This procedure did not induce statistically significant differences between the 2 hemispheres for Tco₂ content, lactate concentration, or water content, but cerebral blood flow was lower on the side of the occlusion (table 1). That the lower blood flow on the side of the occlusion does not affect the lactate concentration is in line with other observations.8

A rapid pronounced increase of blood pressure can produce a forced arteriolar dilatation with increased blood flow ("break through" of autoregulation), and dysfunction of the blood-brain barrier.9, 10

The extravasation of protein-bound Evans blue in the brain of rats previously subjected to an acute hypertensive pulse in the carotid system indicates an increased cerebrovascular permeability to proteins and confirms the findings of Håggestad and Johansson,9 Pannier and Leusen1 and Rapoport4 in analogous experimental conditions. The opening of the BBB in these conditions is clearly associated with increased intraluminal pressure and over-distention of the capillary wall and may be due to widening of endothelial tight junctions, as proposed by Rapoport.11

When the experimental hemisphere is compared to the reference hemisphere, the results show an increase in the water content, an increased lactate and decreased CO₂ content, and a depression of cerebral blood flow on the side of the lesion both in the "heavy" and in the "moderate" insult animals. The change in Tco₂ is, however, significant only in the animals subjected to a heavy insult (table 2).

The cerebral edema can result from leakage of plasma proteins and fluid through the damaged blood-

### Table 3

| Brain                  | Moderate insult | Heavy insult | |
|------------------------|-----------------|--------------|
| **Tco₂ (mmol/kg)**     | reference 15.4  | 16.7         | 7 |
|                       | experim. 16.8   | 20.0         | <0.01 |
| **HCO₃⁻ (mmol/kg)**    | reference 13.8  | 15.4         | 7 |
|                       | experim. 15.3   | 18.8         | <0.02 |
| **Lactate (mmol/kg)**  | reference 7.4   | 7.5          | 7 |
|                       | experim. 13.7   | 13.5         | <0.01 |
| **H₂O content (%)**    | reference 78.52 | 78.76        | 6 |
|                       | experim. 79.38  | 81.80        | <0.001 |
| **Blood content (ml/100 g)** | reference 1.66 | 1.06        | 7 |
|                       | experim. 1.32   | 1.32         | NS |
| **Blood flow (ml/100 g.min)** | reference 96 | 46          | 6 |
|                       | experim. 67     | 5            | <0.01 |

### Plasma

| **Paco₂ (mm Hg)** | reference 41.5 | 33.8 | 20 |
|                  | experim. 41.7  | 37.3 | 20 |
| **HCO₃⁻ (mEq/L)** | reference 41.7 | 37.3 | 20 |
|                  | experim. 13.5  | 13.8 | 20 |
| **Blood Pressure (cm Hg)** | reference 13.5 | 12.5 | 20 |
brain barrier (vasogenic edema), and/or from cell damage (cytotoxic edema). The changes in the brain are probably complex and cannot be attributed solely to alterations in the permeability of the BBB. The higher lactate concentration (and lower total CO₂ content) can be explained by cellular damage or by ischemia. The lower cerebral blood flow on the side of the lesion, despite tissue acidosis, is probably related to the occurrence of cerebral edema. Studies on focal and generalized brain edema have shown that the normal regulatory mechanisms of CBF through alteration in tissue pH are no longer dominant in edematous tissue areas, the decrease in CBF in such areas being closely correlated to the increase in the tissue water content.12, 13

When the reference hemisphere is compared to the hemispheres of “control” animals (table 1), values for lactate and for H₂O content are higher, and in the animals subjected to a “heavy” insult, CBF is markedly depressed. These phenomena are probably also related to the hypertensive insult and suggest that the insult is not completely unilateral but must also affect the “reference” hemisphere. The blue spots occasionally observed in this hemisphere after the administration of Evans blue point in the same direction.

Although the permeability of the BBB to proteins has been extensively studied during the application of various noxious stimuli, the permeability of the barrier to other endogenous substances with their possible metabolic consequences is less documented.11 An increased permeability for plasma proteins does not necessarily imply an increased exchange of bicarbonate ions through the barrier, since the BBB is viewed as a complex system composed of different more or less specific mechanisms, and any “lesion” does not indiscriminately affect the different barrier functions.14 The blood-brain barrier to HCO₃⁻ was studied previously by Rapoport14 by following pH changes on the brain surface during intravenous injections of NaHCO₃. With this technique the barrier to bicarbonate was shown to be quite resistant to hypoxia and to metabolic inhibitors, but easily damaged by application of hypertonic solutions.

In the present experiments, the permeability of the BBB to bicarbonate was investigated by measuring the total CO₂ content and calculating the bicarbonate concentration in brain tissue of rats previously subjected to a hypertensive insult during infusions of Na₂CO₃ (table 3). It is not possible to know if the administration of hypertonic solutions has exaggerated the lesion of the BBB in these experiments. Although identical infusions did not produce an extravasation of Evans blue in “control” rats (with one carotid artery occluded), the effect of this procedure in animals with an already damaged BBB by the hypertensive insult, cannot be evaluated separately.

In the rats subjected to a “heavy” insult, a rise in the plasma [HCO₃⁻] by 15–20 mmol/l for 35 minutes increased the [HCO₃⁻] in the experimental hemisphere by about 2.0 mmol/kg; (table 2) it can be concluded that the Na₂CO₃ infusion increased the [HCO₃⁻] in the experimental hemisphere by approximately 5.5 mmol/kg, which should represent about 25–35% of the increase in blood plasma. A the extracellular space occupies 15–20% of the brain’s total CO₂ content and does not allow relating CBF to the changes in the extracellular space as these are probably complex and cannot be attributed solely to alterations in BBB permeability.15

The lactate rise in the experimental hemisphere relative to the reference hemisphere is more marked in the Na₂CO₃ infused than in the non-infused rats. The increase in tissue lactate is probably of complex origin in these circumstances since besides tissue hypoxia a consequence of the lowered CBF, the increase [HCO₃⁻] and pH in the experimental hemisphere could also contribute to this phenomenon as it is known that alkalosis stimulates lactate production in brain tissue.19

In the Na₂CO₃ infused animals the lactate concentration in the reference hemisphere is also high compared with the levels observed in rats with a hypertensive insult but with normal plasma bicarbonate level (table 2). A lactate increase in brain tissue during metabolic alkalosis was also observed by Granholm and Siejsjö20 in rats with an intact BBB, and attributed to an increased oxygen affinity of hemoglobin (“Boh shift”) reducing oxygen delivery to the brain.

The infusion of hypertonic carbonate solutions did not influence the occurrence nor the degree of cerebral edema since the values obtained for water content in experiments (bypassing the BBB) indeed indicate rapid penetration of HCO₃⁻ in brain cells.17, 18

In the Na₂CO₃ infused animals the lactate concentration in the reference hemisphere is also high compared with the levels observed in rats with a hypertensive insult but with normal plasma bicarbonate level (table 2). A lactate increase in brain tissue during metabolic alkalosis was also observed by Granholm and Siejsjö in rats with an intact BBB, and attributed to an increased oxygen affinity of hemoglobin (“Boh shift”) reducing oxygen delivery to the brain.

The infusion of hypertonic carbonate solutions did not influence the occurrence nor the degree of cerebral edema since the values obtained for water content are similar to those of the non-infused animals.

The increased bicarbonate concentration (and pH) in the brain tissue on the side of the lesion would be expected to decrease cerebral blood flow in view of the well-known pH dependency of CBF.21, 22 To investigate this point, one can compare directly the CBF values of the experimental hemisphere in animals with (table 3) and without (table 2) carbonate infusion since the values found in the reference hemisphere can be considered to be equal. The CBF in animals with carbonate infusions is somewhat higher in the rat subjected to a moderate insult, and lower in those subjected to a heavy insult, but these changes are not statistically not significant (P > 0.2). For these comparisons the nonparametric Mann-Whitney test was used. Consequently, the results of these experiments do not allow relating CBF to the changes in the [HCO₃⁻] which occur in the brain tissue of rats with damaged BBB during infusions of bicarbonate. This could be related to the occurrence of cerebral edema (cf. supra), but it should also be indicated that in the heavy insult animals the CBF is so low that the number of microspheres trapped in the experiments...
hemisphere is very small (less than 100 microspheres in most animals) so that large errors in the flow determination can be expected.24

In conclusion, the results lend further support to the hypothesis that blood-brain barrier mechanisms are important for the maintenance of intracerebral pH in the face of acute alterations in the plasma [HCO₃⁻]. The changes in cerebral blood flow after an acute hypertensive insult, however, did not appear to be influenced by the intracerebral [HCO₃⁻].

References

15. Rapoport SI: The cortical acidic response to intravenous NaHCO₃ and the nature of blood-brain barrier damage. Int J Neurosci 2: 1-6, 1971
Effect of non-respiratory alkalosis on brain tissue and cerebral blood flow in rats with damaged blood-brain barrier.
J L Pannier, J Weyne, G Demeester and I Leusen

Stroke. 1978;9:354-359
doi: 10.1161/01.STR.9.4.354

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://stroke.ahajournals.org/content/9/4/354

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Stroke can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Stroke is online at:
http://stroke.ahajournals.org/subscriptions/