Initial effect of sodium bicarbonate on intracellular pH depends on the extracellular nonbicarbonate buffering capacity

Jacques Levraut, MD; Carine Giunti, MD; Jean-Pierre Ciebiera, MD; Georges de Sousa, PhD; Roger Ramhani, PhD; Patrick Payan, PhD; Dominique Grimaud, MD

Objective: The effect of sodium bicarbonate on intracellular pH under conditions close to those in vivo, with both bicarbonate and nonbicarbonate buffering systems, is unknown. We postulated that this effect depends on the nonbicarbonate buffering capacity because the alkali-induced back-titration of these buffers results in a concentration-dependent release of CO₂ in the extracellular space, leading to a decrease in intracellular pH.

Design: The study was conducted in two stages. First, human hepatocytes were perfused with pH 7 bicarbonate-buffered medium (5 mM HCO₃⁻, 20 torr P CO₂) containing no nonbicarbonate buffer or small amounts (5 mM 4-[2-hydroxyethyl]-1-piperazineethanesulfonic acid [HEPES]) or large amounts (20 mM HEPES) of nonbicarbonate buffer. Second, the changes in intracellular pH of hepatocytes placed in acidic human blood (pH 7, 5 mM HCO₃⁻, 20 torr PCO₂) at three hematocrits (40%, 20%, and 5%) were measured.

Setting: Research laboratory at a medical university.

Subjects: Cryopreserved human hepatocytes thawed the day before the experiments.

Interventions: Sodium bicarbonate was infused for 10 mins to increase the HCO₃⁻ concentration from 5 to 30 mM. In the second part, 20 mM sodium bicarbonate was added directly to the blood bathing the cells.

Measurements and Main Results: The intracellular pH was measured with the pH-sensitive fluorescent dye bis-carboxyethyl carboxy-fluorescein in its esterified form, acetoxy-methyl ester, by using a single-cell imaging technique. Gas analyses were performed before and during the sodium bicarbonate load. Sodium bicarbonate caused a decrease in intracellular pH with all media except the artificial medium containing no HEPES. This decrease was small in media with low nonbicarbonate buffering capacity (5 mM HEPES and 5% hematocrit blood) and large in media with high nonbicarbonate buffering capacity (20 mM HEPES and 40% hematocrit blood). The change in intracellular pH was linked closely to the change in PCO₂ caused by the sodium bicarbonate.

Conclusions: The effect of sodium bicarbonate on intracellular pH depends on changes in PCO₂ in the medium bathing the cells. The increase in PCO₂ is correlated with the extracellular nonbicarbonate buffering capacity because of the release of H⁺ ions coming from the back-titration of these buffers. We conclude that sodium bicarbonate may exacerbate cell acidosis under buffering conditions close to those in vivo and that the initial changes in cell pH caused by sodium bicarbonate depend on the extracellular nonbicarbonate buffering capacity. (Crit Care Med 2001; 29:1033–1039)

Key Words: metabolic acidosis; intracellular pH; sodium bicarbonate; hepatocyte; cell culture; carbon dioxide; buffers; hemoglobin; albumin; 4-[2-hydroxyethyl]-1-piperazineethanesulfonic acid
with an artificial HCO$_3^-$/CO$_2$ buffered medium containing no nonbicarbonate buffers as well as low or high concentrations of nonbicarbonate buffers and in cells bathed in variously diluted human blood.

**MATERIALS AND METHODS**

**Cell Preparation**

Our Institutional Review Board, which waived the need for informed consent, approved the study. Human hepatocytes were prepared as previously described (13, 14). Briefly, hepatocytes were isolated from liver fragments resected for secondary tumors by using two-step perfusion with 0.05% collagenase (Boehringer-Mannheim, Meylan, France). Isolated cells were suspended in Williams’ medium E containing 10% fetal calf serum, 50 IU/mL penicillin, 50 μg/mL streptomycin, 50 μg/mL netilmicin, and 0.1 IU/mL human insulin (medium I) and incubated at 37°C in a humidified 5% CO$_2$ atmosphere incubator for 10–12 hrs. The cells then were suspended in Leibovitz L15 medium at 20 x 10$^6$ cells/mL in freezing vials and were stored in liquid nitrogen.

The day before an experiment, the hepatocytes were thawed in a 37°C water bath, washed with L15 medium, and purified on Percoll colloidal silica solutions. Viable cells were removed from the interface of the centriuge gradient, washed, and suspended in medium I. The cell suspension was distributed among 3-ML Petri dishes each containing a collagen-coated coverglass to which the cells adhered. The dishes were placed in the 5% CO$_2$ incubator at 37°C, and hepatocytes formed typical monolayers within 24 hrs.

**Measurement of pHi**

The pHi was measured by using the pH-sensitive fluorescent dye bis-carboxyethyl carboxyfluorescein in its esterified form, acetoxymethyl ester (BCECF/AM), the fluorescence intensity of which was measured by using a microspectrofluorometric imaging technique (15). The coverslip bearing the hepatocytes was placed under the base of a 3-ML perfusion chamber specially made for pHi measurement. Cells incubated in the appropriate medium (see subsequent description) were loaded with 5 μM BCECF/AM (Sigma-Aldrich, Saint-Quentin Falavier, France) for 20 mins at 37°C in a 2.5% CO$_2$ incubator. The chamber was then placed on the stage of an inverted fluorescence microscope (IMT2, Olympus, Paris, France), and fluorescence emission intensity at 530 nm was quantified after alternative excitation at 440 (pH insensitive) and 490 nm (pH sensitive) wavelengths by using a 100W xenon light source. The rapid shift from one wavelength to the other was obtained with a filter wheel and a shutter (Lambda 10-2; Sutter Instrument, Novato, CA). The movement of which was piloted by a computer with an Argus 50 image analysis system (Hamamatsu, Massy, France). The fluorescence images were captured via a charge coupled device camera and a photomultiplier (C2400, Hamamatsu), and the 490/440 ratios were transmitted directly to the personal computer. The fluorescence images were analyzed by using the Hamamatsu Argus software by defining regions of interest among the fluorescence field (the selected cells). The system was calibrated at the end of each series of experiments by measuring the fluorescence ratio of intensity of cells incubated in medium containing 140 mM KCl, 10 mM 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES), and 10 μM nigericin at pH 6.4, 6.8, 7.2, and 7.8. Because it was not possible to do the calibration on the same cells as used to test the effect of sodium bicarbonate, only the changes in pHi (A$pHi$) were taken into consideration.

**Media**

**Artificial Media.** The three perfusion media (H0, H5, H20) used to evaluate the effect of sodium bicarbonate on pH had the same bicarbonate buffering capacity but different nonbicarbonate buffering capacities (Table 1). The media were prepared by adding the HEPES, warming to 37°C, bringing the pH to 7 with NaOH, and then adding the bicarbonate and bubbling with 2.5% CO$_2$. Gas analysis (ABL30; Radiometer, Paris, France) was used to check the PCO$_2$ and the calculated bicarbonate concentration. Adjustments were made if necessary.

**Human Blood.** Human blood was regenerated from a mixture of frozen fresh plasma and packed red blood cells (French blood bank) that could not be used for transfusion. Fresh plasma was added to the packed red blood cells to obtain a final hematocrit of 40%. This blood mixture was warmed to 37°C and dialedized through a hemofilter irrigated with the H0 medium in a closed circuit to remove the citrate from the conserved blood and to equilibrate the regenerated blood to the desired bicarbonate concentration and PCO$_2$ (5 mM and 20 torr, respectively). The PCO$_2$ and the HCO$_3^-$ concentrations were checked by blood gas analysis, and the regenerated blood was diluted with a 50% mixture of hydroxethyl starch and 0.9% NaCl containing 5 mM HCO$_3^-$ and 20 torr PCO$_2$. This provided blood samples of hematocrit 5%, 20%, and 40%, all with 5 mM HCO$_3^-$, 20 torr PCO$_2$, and pH 7.

**Experimental Protocol**

**Artificial Media.** The effect of sodium bicarbonate on pH of a single batch of cells was studied for H0, H5, and H20 media. The six possible orders (H0–H5–H20; H0–H20–H5; H5–H0–H20; H5–H20–H0; H20–H0–H5; H20–H5–H0) were tested in six experiments on hepatocytes from the same cell preparation, to check that the sequence did not influence the response.

The chamber was rinsed three times with the first medium, and the cells were incubated with 2 mL of this medium with the chamber in a 2.5% CO$_2$ incubator at 37°C for 15 mins. The cells then were loaded with 5 μM BCECF/AM for the next 20 mins. Last, the chamber was hermetically sealed and placed under the inverted microscope. The cells were perfused continuously with the same medium warmed at 37°C at 10 mL/min. Cells with a typical morphology of good viability (adherent and confluent cells with a visible nucleus) were observed by using a 40× objective (5–10 cells per field). The cells then were observed under fluorescence light, and the photomultiplier intensity was adjusted to obtain sufficient cell brightness for each wavelength. The pH was measured every 20 secs. Once the pH was stable, 0.5 M NaHCO$_3$ was infused in the circuit upstream to the chamber by using a motor-driven syringe, which increased the HCO$_3^-$ concentration from 5 to 30 mM/L in <1 min. The NaHCO$_3$ infusion was continued for 10 mins. The cells then were rinsed with the second medium for 5–10 mins until the pH was stable again, and the same procedure was repeated. The same protocol was used for the third medium. Samples of perfusion medium were taken right at the exit of the chamber before and during NaHCO$_3$ infusion for gas analysis.

**Human Blood.** A coverslip carrying hepatocytes was placed below the chamber, and the cells were loaded with 5 μM BCECF/AM in H0 medium for 20 mins at 37°C in a 2.5% CO$_2$ incubator. The chamber then was rinsed three times with H0 medium, kept open, filled with 3 mL of H0 medium, and placed under the inverted microscope. When the fluorescence ratio measured at 15-sec intervals was stable under H0 conditions, the H0 medium was removed and replaced by 3 mL of 5% hematocrit blood. This procedure did not alter the cellular fluorescence intensity but made it impossible to observe the hepatocytes with the optical light any-

**Table 1.** Composition of the media perfusing the cells used to test the effect of sodium bicarbonate on intracellular pH

<table>
<thead>
<tr>
<th>Medium</th>
<th>H0</th>
<th>H5</th>
<th>H20</th>
</tr>
</thead>
<tbody>
<tr>
<td>NaCl (mM/L)</td>
<td>130</td>
<td>130</td>
<td>130</td>
</tr>
<tr>
<td>KCl (mM/L)</td>
<td>3.5</td>
<td>3.5</td>
<td>3.5</td>
</tr>
<tr>
<td>CaCl$_2$ (mM/L)</td>
<td>1.2</td>
<td>1.2</td>
<td>1.2</td>
</tr>
<tr>
<td>KH$_2$PO$_4$ (mM/L)</td>
<td>0.8</td>
<td>0.8</td>
<td>0.8</td>
</tr>
<tr>
<td>NaHCO$_3$ (mM/L)</td>
<td>5.0</td>
<td>5.0</td>
<td>5.0</td>
</tr>
<tr>
<td>MgCl$_2$ (mM/L)</td>
<td>1.5</td>
<td>1.5</td>
<td>1.5</td>
</tr>
<tr>
<td>Glucose (g/L)</td>
<td>1.5</td>
<td>1.5</td>
<td>1.5</td>
</tr>
<tr>
<td>HEPES (mM/L)</td>
<td>0</td>
<td>5</td>
<td>20</td>
</tr>
<tr>
<td>PCO$_2$ (mm Hg)</td>
<td>20</td>
<td>20</td>
<td>20</td>
</tr>
<tr>
<td>pH (pH units)</td>
<td>7.00</td>
<td>7.00</td>
<td>7.00</td>
</tr>
</tbody>
</table>
more. When the pH was stable, 20 mM sodium bicarbonate was added to the chamber. The pH was measured at 15-sec intervals, and 1 mL of blood was sampled 15 secs after the bicarbonate load for gas analysis. Three minutes later, the blood was removed and replaced by 3 mL of 20% hematocrit blood. The protocol was repeated with the 40% hematocrit blood.

Data Analysis

Five cells were selected for analysis in each experiment. The mean change in pH during the bicarbonate infusion (load) was calculated by dividing the area under the ΔpHi/time curve by the infusion (load) time. The effect of sodium bicarbonate on ΔpHi and the mean change in pH were analyzed by using a balanced analysis of variance, applied to a model with three factors: liver, medium, and rank of measure. All the changes in PCO2, bicarbonate concentration, and external pH (pHe) were compared by a two-way analysis of variance. The relationships between the ΔPCO2 or ΔpHe and ΔpHi were tested by linear regression (least square method). The multiple regression revealed the effect of ΔpHe on ΔpHi for a constant ΔPCO2. A p value of .05 was considered to be significant.

RESULTS

Artificial Media. Eighteen experiments were conducted on 90 cells from three livers. The effect of sodium bicarbonate on pH was closely correlated with the type of medium used to perfuse the cells (Figs. 1 and 2, Table 2). Neither the order used to test the medium nor the type of cell preparation significantly affected the results. The decrease in pH caused by sodium bicarbonate occurred only in cells perfused with the media containing the nonbicarbonate buffer. This decrease was greatest with the H20 medium, which contained the most nonbicarbonate buffer (Table 2).

The initial change in pH closely followed the change in PCO2 caused by sodium bicarbonate (Table 2, Fig. 3), which itself was linked closely to the extracellular nonbicarbonate buffering capacity (Table 2). The effect of sodium bicarbonate on extracellular pH was three times greater in the H0 medium than with the H20 medium (Table 2). There was a strong correlation between the changes in pHe and pH (r = .70, p < .0001). However, the multiple regression analysis showed that this relationship was no more significant for a given change in PCO2 (p = .69), whereas the relationship between changes in pH and changes in PCO2 remained highly significant for a given change in pHe (p < .005).

From the third minute after the beginning of sodium bicarbonate infusion (i.e., after the decrease of pH with the H5 and H20 media), the pH increased in a similar way in all three media (Fig. 2). The mean change in pH during the bicarbonate infusion was positive with the H0 medium, zero with H5, and negative with H20 (Table 2).

Human Blood. Similar results were obtained when the hepatocytes were placed in blood (Table 3, Fig. 4). The sodium bicarbonate caused a decrease in pH that was greatest when the cells were placed in high hematocrit blood. This change in pH varied with change in PCO2 (Fig. 5). Sodium bicarbonate caused a dramatic increase in PCO2 when it was added to the 40% hematocrit blood, whereas the change in PCO2 was less pro-
Hg, and 5 mmol/L [HCO₃⁻] before the sodium bicarbonate infusion. The change in pHi is calculated as the area under the curve.

**Figure 2.** Effect of sodium bicarbonate on the mean (±SD) change in intracellular pH (pHi) of human hepatocytes (n = 90) perfused with pH 7 bicarbonate-buffered medium containing none (squares), 5 mM (circles), and 20 mM (diamonds) 4-[2-hydroxyethyl]-1-piperazineethanesulfonic.

**Table 2.** Effect of a continuous infusion of sodium bicarbonate on the changes in external pH, P CO₂, and intracellular pH 2 mins after the infusion and mean change in intracellular pH (over the 10 mins infusion).

<table>
<thead>
<tr>
<th></th>
<th>HEPES-Free</th>
<th>HEPE (5 mmol/L)</th>
<th>HEPE (20 mmol/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of experiments</td>
<td>18</td>
<td>18</td>
<td>18</td>
</tr>
<tr>
<td>No. of cells</td>
<td>90</td>
<td>90</td>
<td>90</td>
</tr>
<tr>
<td>ΔHCO₃ (mmol/L)</td>
<td>26.9 ± 3.4</td>
<td>25.9 ± 5.9</td>
<td>25.2 ± 5.2</td>
</tr>
<tr>
<td>ΔpHe (pH units)</td>
<td>0.61 ± 0.06ᵇᶜ</td>
<td>0.38 ± 0.12ᵇᶜ</td>
<td>0.20 ± 0.06ᵇ</td>
</tr>
<tr>
<td>ΔPco₂ (torr)</td>
<td>8.3 ± 2.9ᵇᶜ</td>
<td>26.8 ± 4.4ᵇᶜ</td>
<td>54.6 ± 7.2ᵇᶜ</td>
</tr>
<tr>
<td>ΔpHi2mins (pH units)</td>
<td>0.04 ± 0.10ᵇᶜ</td>
<td>-0.15 ± 0.12ᵇᶜ</td>
<td>-0.40 ± 0.21ᵇᶜ</td>
</tr>
<tr>
<td>ΔpHimem (pH units)</td>
<td>0.19 ± 0.12ᵇᶜ</td>
<td>0.01 ± 0.13ᵇᶜ</td>
<td>-0.24 ± 0.17ᵇᶜ</td>
</tr>
</tbody>
</table>

HEPES, 4-(2-Hydroxyethyl)-1-piperazineethanesulfonic acid; Δ, change; pHe, external pH; pHi, intracellular pH.

*p < .001 vs. HEPES-free; b p < .001 vs. HEPES 5 mM; c p < .001 vs. HEPES 20 mM. Mean change in pH is calculated as the area under the curve ΔpHi vs. time (mins) during the sodium bicarbonate infusion divided by the duration of the infusion. All three media were at pH 7, Pco₂ 20 mM Hg, and 5 mmol/L [HCO₃⁻] before the sodium bicarbonate infusion.

**Figure 3.** Relationship between the changes in intracellular pH (pHi) and the changes in Pco₂ caused by sodium bicarbonate infusion over human hepatocytes perfused by pH 7 bicarbonate-buffered medium containing none (H0), 5 mM (H5), and 20 mM (H20) 4-[2-hydroxyethyl]-1-piperazineethanesulfonic. Each point represents the mean change in pHi of five cells 1 min after the beginning of the infusion.

**DISCUSSION**

This study was conducted to determine the initial effect of sodium bicarbonate on the pH of human hepatocytes under both bicarbonate and nonbicarbonate buffering conditions. We previously showed that sodium bicarbonate exacerbates acidosis in rat hepatocytes only under nonbicarbonate buffering conditions (12). Sodium bicarbonate produces a net decrease in pH when cells are bathed in a pure nonbicarbonate-buffered medium and a frank intracellular alkalization when cells are placed in a pure bicarbonate-buffered medium. In the present study, we show that sodium bicarbonate enhances cell acidosis when cells are bathed in a mixed-buffered medium, which is closer to the physiological conditions, in a way that is proportional to the concentration of the nonbicarbonate buffers. For a given bicarbonate load, the higher the concentration of nonbicarbonate buffers in the extracellular space, the greater the expected decrease in cell pH. This result can be obtained by varying the nonbicarbonate buffering capacity in an artificial medium or by changing it in human blood by hemodilution.

These results can be explained by the interactions occurring between the bicarbonate- and the nonbicarbonate-buffers (NBB), which may be understood by looking at their respective chemical equations:

\[
\text{HCO}_3^- + \text{H}^+ &\rightleftharpoons \text{CO}_2 + \text{H}_2\text{O} \quad [1] \\
\text{NBB}^- + \text{H}^+ &\rightleftharpoons \text{NBBH} \quad [2]
\]

When HCO₃⁻ is added, it shifts the equilibrium of Equation 1 to the right. The CO₂ that is released during this first step is minimal because the free H⁺ (i.e., those that are not bound to the NBB) concentration is very small, even at very low pH. For example, at pH 6.7 where the H⁺ concentration is 200 nmol/L (1/10⁶), a buffering of all the free protons would increase the Pco₂ by <0.1 mm Hg. However, in the simultaneous presence of NBB, the abrupt decrease of the H⁺ concentration induced by the HCO₃⁻ load acts on Equation 2 and shifts it to the left (so-called back-titration of the buffer). This process releases a lot of new H⁺ ions.
that react with the HCO₃⁻ to generate a lot of CO₂ (Eq. 1). Thus, the increase in PCO₂ induced by sodium bicarbonate needs the presence of NBB to be significant. Because the CO₂ but not the HCO₃⁻ enters readily into the cells, this will shift Equation 1 to the left in the intracellular compartment, thus decreasing pH. This decrease in pH will locally shift Equation 2 to the right, which means that the presence of NBB in the intracellular compartment probably minimizes the CO₂-induced decrease in pH.

The initial effect of sodium bicarbonate on pH was followed by a parallel increase in pH, whatever the nonbicarbonate capacity of the medium. This led to a recovery of the initial pH after several minutes except in high nonbicarbonate media such as the H20. This was probably due to bicarbonate entering the cell through specific channels (the electrogenic Na⁺/HCO₃⁻ cotransporter and the Cl⁻/HCO₃⁻ antiporter systems) and protons leaving the cell through the Na⁺/H⁺ antiporter system activated by both increasing osmolarity (16) and decreasing cell pH (17). However, other studies that use specific blockers, such as stilbenes to block the bicarbonate entry and amiloride to block the Na⁺/H⁺ activation, are needed to confirm this.

To our knowledge, this is the first study to evaluate the effect of sodium bicarbonate on pH in human hepatocytes and the first to evaluate the pH in blood-bathed cells by a fluorescence technique. However, this study has several limitations.

First, it was performed in a closed system, preventing the escape of the generated CO₂, which considerably amplified the increase in PCO₂ and thus the decrease in cell pH. Moreover, we used a high flow rate that made the bicarbonate concentration increase in the medium in <1 min. Sodium bicarbonate used under clinical conditions is injected intravenously and most slowly, allowing most of the generated CO₂ to escape during the first pulmonary passage. This leads to an increase in PₐCO₂ that is rarely >15 torr, whereas we observed increases in PCO₂ >70 torr.

The second limitation is that the increase in pH triggered by a given sodium bicarbonate load depends on the buffering capacity of the medium. Therefore, there are greater increases in pH when bicarbonate is added to a medium containing little nonbicarbonate buffer (blood with a low hematocrit, for example) than when it is added to a medium containing concentrated nonbicarbonate buffers (high hematocrit). This makes it difficult to compare changes in pH when pH does not change to the same extent. However, statistical analysis showed that the change in pH was not a function of the change in pHe for a given change in PCO₂, whereas the change in pH was closely linked to the change in PCO₂ for a given change in pHe.

The third limitation is that, physiologically, hepatocytes are not in direct contact with the blood because the interstitial fluid separates them. However, the experiments were performed directly in the blood, or in an artificial media that contained HEPES (artificial nonbicarbonate buffer of pH 7.5 to simulate the buffering provided by hemoglobin and albumin). We could separate the cells from the blood by a semipermeable membrane, the cells being bathed by a medium simulating the interstitial fluid (i.e., NBB-free and only bicarbonate buffered). However, we do not believe that this procedure would change our results, because the CO₂ generated in the blood then would diffuse rapidly and readily across the membrane and the interstitial fluid before entering the cells. Under clinical conditions, sodium bicarbonate is most often used by intravenous route during acute metabolic acidosis, and thus the CO₂ coming from its administration depends on the buffering system of the

<table>
<thead>
<tr>
<th>Hematocrit (5%)</th>
<th>Hematocrit (20%)</th>
<th>Hematocrit (40%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of experiments</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>No. of cells</td>
<td>25</td>
<td>25</td>
</tr>
<tr>
<td>ΔHCO₃ (mmol/L)</td>
<td>25.6 ± 1.8</td>
<td>25.3 ± 2.8</td>
</tr>
<tr>
<td>ΔpH (pH units)</td>
<td>0.57 ± 0.08 (b,c)</td>
<td>0.31 ± 0.12 (b,c)</td>
</tr>
<tr>
<td>ΔPCO₂ (torr)</td>
<td>15.9 ± 7.5 (c)</td>
<td>44.2 ± 5.9 (c)</td>
</tr>
<tr>
<td>ΔpHe₀₃₅ (pH units)</td>
<td>−0.14 ± 0.07 (c)</td>
<td>−0.28 ± 0.10 (c)</td>
</tr>
</tbody>
</table>

Δ, change; pHe, external pH; pH, intracellular pH; Hct, hematocrit.

*p < .001 vs. Hct 5%; †p < .001 vs. Hct 20%; ‡p < .001 vs. Hct 40%. The blood was at pH 7, PCO₂ 20 mm Hg, and [HCO₃] 5 mmol/L before the sodium bicarbonate load.

**This study shows that the initial effect of sodium bicarbonate on intracellular pH depends on the change in PCO₂ of the medium bathing the cells.**
blood and not on that of the interstitial fluid.

The limitations of this study do not allow us to assert that sodium bicarbonate causes paradoxical intracellular acidosis in vivo. However, it shows that, if this phenomenon exists in vivo, it is more likely to occur in patients with high hematocrit. Several other reports are directly or indirectly in accordance with this hypothesis. First, we recently reported that the bicarbonate-induced release of CO₂ in critically ill patients is linked to their hemoglobin and albumin concentration (18). Another animal study reported a significant correlation between the increase in expired CO₂ caused by a sodium bicarbonate load and the hemoglobin concentration (19). Moreover, all the experimental studies that have reported side effects after bicarbonate infusion were performed on hypoxic animals with high hemoglobin concentrations (5–7, 20–22). The studies on hypovolemic animals with low hemoglobin concentrations generally failed to demonstrate such side effects (23–25).

Finally, an experimental study on dogs showed that the hemodynamic side effects caused by a sodium bicarbonate load during metabolic acidosis were attenuated greatly by hemodilution (26).

During the low flow states such as cardiogenic shock or resuscitation of cardiac arrest, several factors theoretically are able to alter the bicarbonate- induced increase in PvCO₂. Indeed, a decrease in cardiac output is able to affect both the tissue CO₂ clearance and the increase in CO₂ production induced by a bicarbonate administration. The tissue CO₂ clearance mostly depends on venous return and thus on cardiac output. Accordingly, for a given bicarbonate-induced increase in tissue CO₂ production, the resulting increase in PvCO₂ will tend to be higher in case of low cardiac output. On the other hand, a decrease in oxygen delivery will modify the pH of hemoglobin, which will alter the CO₂ production from bicarbonate. The closer the pH is to the pH of a medium, the better the buffer minimizes the change in pH during an alkaline load because greater amounts of H⁺ ions are released from the buffer. As a consequence, greater amounts of CO₂ will be released from bicarbonate when blood pH is close to the pH of the nonbicarbonate buffers present in the blood. The pH of hemoglobin depends on its oxygen saturation. Oxyhemoglobin has a pH of about 6.5, and reduced hemoglobin has a pH of about 7.7. In case of severe acidosis, the venous blood pH is therefore close to the pH of oxyhemoglobin, which makes the CO₂ production from a bicarbonate administration probably smaller when SvO₂ is low than when SvO₂ is high. Accordingly, in case of low cardiac output responsible for a low oxygen delivery, the resulting decrease in SvO₂ should lower the bicarbonate-induced CO₂ production. So, during low flow states, on one hand the PvCO₂ will tend to be higher because of the impaired tissue CO₂ clearance, but on the other hand the low SvO₂ will tend to attenuate the CO₂ production from bicarbonate. These opposite effects make it difficult to predict the final increase in PvCO₂ induced by a bicarbonate administration under such conditions.

In conclusion, this study shows that the initial effect of sodium bicarbonate on pH depends on the change in PCO₂ of the medium bathing the cells. The increase in PCO₂ is linked closely to the extracellular nonbicarbonate buffering capacity, because of the back-titration of these buffers caused by the alkali. The consequence is that sodium bicarbonate causes only a small transient decrease in pH when it is added to a medium containing little nonbicarbonate buffer (such as in blood with a low hemoglobin and albumin), whereas it can considerably worsen intracellular acidosis under conditions of high nonbicarbonate buffering capacity.

ACKNOWLEDGMENT

We thank Dr. Pascal Staccini from the Departement d’Information Medicale, University of Nice Hospitals, for reviewing the statistical analysis.

REFERENCES


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