Bicarbonate therapy and intracellular acidosis

D. J. A. GOLDSMITH*, L. G. FORNI and P. J. HILTON
Renal Laboratory, St Thomas’ Hospital, London SE1 7EH, U.K.

(Received 24 March/4 August 1997; accepted 8 August 1997)

1. The correction of metabolic acidosis with sodium bicarbonate remains controversial. Experiments in vitro have suggested possible deleterious effects after alkalinization of the extracellular fluid. Dis-equilibrium of carbon dioxide and bicarbonate across cell membranes after alkali administration, leading to the phenomenon of 'paradoxical' intracellular acidosis, has been held responsible for some of these adverse effects.

2. Changes in intracellular pH in suspensions of leucocytes from healthy volunteers were monitored using a fluorescent intracellular dye. The effect in vitro of increasing extracellular pH with sodium bicarbonate was studied at different sodium bicarbonate concentrations. Lactic acid and propionic acid were added to the extracellular buffer to mimic conditions of metabolic acidosis.

3. The addition of a large bolus of sodium bicarbonate caused intracellular acidification as has been observed previously. The extent of the intracellular acidosis was dependent on several factors, being most evident at higher starting intracellular pH. When sodium bicarbonate was added as a series of small boluses the reduction in intracellular pH was small. Under conditions of initial acidosis this was rapidly followed by intracellular alkalinization.

4. Although intracellular acidification occurs after addition of sodium bicarbonate to a suspension of human leucocytes in vitro, the effect is minimal when the conditions approximate those seen in clinical practice. We suggest that the observed small and transient lowering of intracellular pH is insufficient grounds in itself to abandon the use of sodium bicarbonate in human acidosis.

INTRODUCTION

Metabolic acidosis remains a commonly encountered clinical problem with lactic acidosis its most frequent cause [1]. In severe cases profound circulatory collapse develops which in turn reduces oxygen delivery, further compounding the problem [2, 3]. Although therapy should ideally be directed at the primary cause of the acidosis, correction of the arterial pH with sodium bicarbonate and other alkalis has frequently been attempted and has been the subject of much debate [4, 5]. Various arguments have been raised against the use of sodium bicarbonate and it has been suggested that in certain types of metabolic acidosis its use is potentially dangerous [6, 7]. Evidence from both in vivo and in vitro experimental models together with clinical studies have been advanced in support of this claim [8–11]. In particular the observed disequilibrium across cellular membranes between carbon dioxide and bicarbonate ions, leading to increased intracellular acidosis after alkalinization of the extracellular fluid, has been proposed as a major deleterious consequence of bicarbonate therapy. This so-called 'paradoxical' acidosis has been described after the rapid addition of sodium bicarbonate to a suspension of platelets in a bicarbonate-free buffer [12]. In order to examine this phenomenon further we subjected a suspension of leucocytes to alkalinization in a bicarbonate-buffered solution in equilibrium with carbon dioxide. The effect of bicarbonate addition in simulated metabolic acidosis was studied using buffers of different bicarbonate concentrations and pH.

MATERIALS AND METHODS

Subjects

The subjects comprised 20 healthy volunteers, 12 men and 8 women, with an age range of 21 to 50 years.

Cell preparation

Leucocytes were obtained from peripheral blood by dextran sedimentation and osmotic lysis of contaminating erythrocytes as described previously [13]. One donor’s blood yielded a quantity of leucocytes which was then aliquoted into different buffers (see below). Although the intracellular pH (pHi) varied between subjects no significant difference was seen between the pHi of manipulated leucocytes.

Key words: bicarbonate, intracellular pH, sodium/proton exchange.

Abbreviations: BCECF-AM, 2',7'-bis-(2-carboxyethyl)-5(6)-carboxyfluorescein acetylmethoxy ester; pHi, intracellular pH.

*Present address: Trafford Department of Renal Medicine, The Royal Sussex County Hospital, Eastern Road, Brighton BN2 5BE, U.K.

Correspondence: Dr P. J. Hilton.
Ethics committee approval was obtained as appropriate.

Measurement of pH and buffering capacity

Leucocytes were loaded for 30 min at 37°C with 5 mmol/l BCECF-AM [2',7'-bis-(2-carboxyethyl)-5(6)-carboxyfluorescein acetyl-methoxy ester] (Molecular Probes, Eugene, OR, U.S.A.) as described previously [14]. pH was monitored with a Perkin-Elmer luminescence spectrometer (Model LS50). Calibration was performed using established methods [15]. Least-squares linear regression was employed to linearize the fluorescence/pH relationship ($r > 0.995$). After equilibration with BCECF-AM the cells were washed and resuspended in buffer. The effect on pH of several buffering systems was studied (described below). pH measurements were performed after 20 min of equilibration and intracellular buffering capacity was calculated from the change in pH after the addition of 3 mmol/l ammonium chloride to an aliquot of the cell suspension [16]. Extracellular pH was measured with a Whatman pH meter with micro-probe attachment. Samples of buffer were analysed for pH, $P_{CO_2}$ and standard bicarbonate using a Corning 168 pH and blood gas analyser. The effect of addition of sodium bicarbonate sufficient to increase the extracellular bicarbonate concentration by 12 mmol/l was then studied (subsequently termed a large bolus).

Buffering solutions

Leucocytes were exposed to four buffering systems: normal bicarbonate buffer (24 mmol/l), low bicarbonate buffers (6 mmol/l and 12 mmol/l) and Heps buffer (10 mmol/l). In each case, the effect of the addition of sodium bicarbonate was studied. Earle's medium was used as the standard buffer, containing physiological concentrations of sodium (140 mmol/l), potassium (6 mmol/l), calcium (1.8 mmol/l), magnesium (0.8 mmol/l), phosphate (0.8 mmol/l), glucose (5 mmol/l) and sodium bicarbonate (24 mmol/l). After equilibration with 5% CO$_2$ the pH was 7.40. The low bicarbonate buffers were prepared in an identical manner except that the bicarbonate concentration was varied to produce final concentrations of 6 mmol/l and 12 mmol/l, the concentration of chloride being adjusted in a reciprocal fashion. Equilibration of these buffers with 5–10% CO$_2$ resulted in an extracellular pH ranging from 6.90 to 7.40. Heps buffer was also used where the sodium bicarbonate was replaced by 10 mmol/l Heps. The pH of this buffer was titrated to pH 7.40 with sodium hydroxide. Choline bicarbonate (6–24 mmol/l, equilibrated with either 5 or 10% CO$_2$) and choline–Heps buffers were used in order to provide sodium-free conditions where minimizing the effect of Na$^+$/H$^+$ exchange between the leucocytes and the extracellular buffer was required. Acidification of the leucocytes was achieved by addition of lactic acid (10 mmol/l) and propionic acid (5 mmol/l) to the extracellular fluid. All chemicals were supplied by Sigma Chemical Company (Poole, Dorset, U.K.) and used without further purification.

RESULTS

Heps buffer

After equilibration of leucocytes in Heps buffer the pH was 7.32±0.04 and buffering power was calculated as 16.7±2.3 mmol l$^{-1}$ pH unit$^{-1}$ ($n = 16$, where $n$ = number of experimental data points, each being performed in duplicate). On addition of a large bolus of sodium bicarbonate a fall in pH of 0.22±0.15 was observed over 20 s (Fig. 1). This was associated with an increase in buffer $P_{CO_2}$ from <0.1 kPa to 3.5±0.3 kPa. Over the next 120 s an increase in pH of 0.05±0.02 was observed. When choline bicarbonate was substituted for sodium bicarbonate this later rise in pH did not occur, implying that it was due to Na$^+$/H$^+$ exchange.

Normal bicarbonate buffer (24 mmol/l, $P_{CO_2}$ 5.1–5.3 kPa, pH 7.4)

pH averaged 7.24±0.02 and buffering power averaged 33.6±5.2 mmol l$^{-1}$ pH unit$^{-1}$ ($n = 12$). Both were significantly different from the values obtained with the Heps buffer ($P < 0.05$). Addition
of a large bolus of sodium bicarbonate caused a similar fall in pH of 0.09 ± 0.03, which recovered to a variable extent towards baseline values over 90 s.

Low bicarbonate (choline) buffer (6 mmol/l, \( \text{Pco}_2 \) 2.5–3.2 kPa, pH 6.9–7.0)

The pH in sodium-free solutions of choline bicarbonate (6 mmol/l) was 7.09 ± 0.03 (n = 8). In order to induce intracellular acidosis the cells were then incubated with lactic acid (10 mmol/l) and the pH fell to 6.98 ± 0.02. Buffering capacity was calculated as 29.2 ± 2.4 mmol l\(^{-1}\) pH unit\(^{-1}\). No appreciable effect on extracellular pH was observed. Further acidosis was achieved through the addition of 5 mmol/l propionic acid which resulted in a fall in the pH to 6.89 ± 0.04 (Fig. 2). The effects of alkalinization on this acidified system were then studied. Figure 2(A) shows the effect of the addition of a large bolus of sodium bicarbonate at 30 s, resulting in a rapid alkalinization of the extracellular buffer (0.36 pH units) as well as a progressive intracellular alkalinization. No intracellular acidification was observed. Figure 2(B) shows the effect of the addition of equimolar 12 mmol/l sodium chloride (Hepes buffer) at pH 7.0 under identical starting conditions. Some recovery of pH is demonstrated through Na\(^+\)/H\(^+\) exchange. Under these conditions the observed effect on the extracellular buffer pH was less marked than with sodium bicarbonate. Figure 2(C) shows the addition of equimolar 12 mmol/l choline bicarbonate. Under these conditions the buffering system is entirely sodium free and a minimal recovery of pH is seen.

Low bicarbonate buffer (12 mmol/l, \( \text{Pco}_2 \) 8.5–10.0 kPa, pH 6.9–7.0)

The resting pH in sodium-containing buffer was 7.06 ± 0.04 and in low-sodium choline bicarbonate buffer was 6.98 ± 0.05 (n = 6). Buffering capacity was 37.7 ± 4.3 mmol l\(^{-1}\) pH unit\(^{-1}\). Addition of 12 mmol/l sodium bicarbonate caused an average 0.31 ± 0.02 pH unit rise in extracellular buffer pH and a concomitant 0.05 ± 0.01 pH unit fall in pH. However, where the initial pH was 7.0 or below, pH recovered after sodium bicarbonate administration. This only occurred in the presence of extracellular sodium (Fig. 3).

Effect of the rate of sodium bicarbonate addition

The effect of the rate of addition of sodium bicarbonate was studied in both normal bicarbonate buffers and Hepes buffers (n = 4). Sodium bicarbonate was added as eight 1.5 mmol/l boluses at 10 s intervals ('small boluses'). In the normal bicarbonate buffer system (starting pH 7.24 ± 0.08) little change in pH was observed after each addition. The maximum observed total fall in pH after all eight additions was 0.04 (Fig. 4A). In contrast, in
the Hepes buffer the effect of each addition of bicarbonate is clearly seen (Fig. 4B) and the cumulative effect is significantly greater, similar to that observed when the sodium bicarbonate is added as a large bolus (approx. 0.2 pH units). Figure 5 is a superimposition of pH traces obtained from 2 aliquots of cells in normal bicarbonate buffer, to one of which was added a single 12 mmol/l large bolus of sodium bicarbonate (as in Fig. 4A), and to the other was added eight sequential 1.5 mmol/l boluses of sodium bicarbonate.

**DISCUSSION**

These studies using human leucocytes have shown that the effect of adding sodium bicarbonate to a cell suspension depends on the experimental conditions. In the Hepes buffering system intracellular PCO₂ and bicarbonate are depleted and as a consequence the cells' buffering capacity is reduced. Under such conditions the addition of sodium bicarbonate to the suspension produces a marked intracellular acidification (Fig. 1). This reflects the liberation of carbon dioxide in the buffer solution which rapidly enters the cell. These results are in agreement with those reported previously [12, 17]. However, on subjecting cells where the intracellular bicarbonate is close to normal to the same change in extracellular pH, a much smaller fall in pHᵳ was observed, reflecting the increased buffering capacity of the cells (Table 1). Both the above results were obtained in cells of near normal pHᵳ. The extent of the transient fall in pHᵳ is also influenced by the rate of addition of bicarbonate ions to the extracellular fluid. The observed effect is extremely small where they are added as a series of small boluses although

---

**Table 1. Summary of results.**

<table>
<thead>
<tr>
<th>Figure</th>
<th>pHᵳ</th>
<th>Extracellular buffer</th>
<th>Additions</th>
<th>Effect on pHᵳ</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>7.3–7.35</td>
<td>Na-Hepes, 10 mmol/l</td>
<td>12 mmol/l NaHCO₃</td>
<td>Decreased (Δ 0.22)</td>
</tr>
<tr>
<td>2(A)</td>
<td>6.9</td>
<td>Choline HCO₃, 6 mmol/l</td>
<td>12 mmol/l NaHCO₃</td>
<td>Recovery</td>
</tr>
<tr>
<td>2(B)</td>
<td>6.9</td>
<td>Choline HCO₃, 6 mmol/l</td>
<td>12 mmol/l HEPES</td>
<td>Attenuated recovery</td>
</tr>
<tr>
<td>2(C)</td>
<td>6.9</td>
<td>Choline HCO₃, 6 mmol/l</td>
<td>12 mmol/l Choline HCO₃</td>
<td>Nil</td>
</tr>
<tr>
<td>3(A)</td>
<td>7.0–7.1</td>
<td>Choline HCO₃, 12 mmol/l</td>
<td>12 mmol/l NaHCO₃</td>
<td>Recovery</td>
</tr>
<tr>
<td>3(B)</td>
<td>7.0–7.1</td>
<td>Choline HCO₃, 12 mmol/l</td>
<td>12 mmol/l Choline HCO₃</td>
<td>Nil</td>
</tr>
<tr>
<td>4(A)</td>
<td>7.25</td>
<td>NaHCO₃, 24 mmol/l</td>
<td>8 × 1.5 mmol/l NaHCO₃</td>
<td>Minimal decrease (Δ 0.025)</td>
</tr>
<tr>
<td>4(B)</td>
<td>7.35</td>
<td>Na-Hepes, 10 mmol/l</td>
<td>8 × 1.5 mmol/l NaHCO₃</td>
<td>Marked decrease (Δ 0.2)</td>
</tr>
<tr>
<td>5(A)</td>
<td>7.25</td>
<td>NaHCO₃, 24 mmol/l</td>
<td>8 × 1.5 mmol/l NaHCO₃</td>
<td>Minimal decrease (Δ 0.025)</td>
</tr>
<tr>
<td>5(B)</td>
<td>7.25</td>
<td>NaHCO₃, 24 mmol/l</td>
<td>1 × 12 mmol/l NaHCO₃</td>
<td>Modest decrease (Δ 0.1)</td>
</tr>
</tbody>
</table>
in the Hepes buffering system the effect is cumulative (Figs 4 and 5). When the experiments are performed with cells of lower pH in an acidified buffer the effect of addition of sodium bicarbonate is a rapid intracellular alkalinization and is not preceded by any appreciable fall in pH (Fig. 2A). This recovery is maximal in the presence of extracellular sodium ions and is mediated by Na\(^+\)/H\(^+\) exchange, and under conditions of intracellular acidification the cells' buffering capacity increases [18]. The effect is virtually abolished in the absence of extracellular sodium where the slight recovery in pH may reflect slow bicarbonate leakage into the cells (Fig. 2C). Interestingly, where alkalinization of acidified cells was performed using equimolar sodium--Hepes buffer (Fig. 2B), less recovery in pH was noted. Under such conditions the change in extracellular pH was less marked than with sodium bicarbonate. Thus the increased extracellular H\(^+\) inhibits the recovery in pH reflecting the inhibition of Na\(^+\)/H\(^+\) exchange by the lower extracellular pH despite equimolar sodium concentrations. The importance of Na\(^+\)/H\(^+\) exchange is further highlighted by the results shown in Fig. 3. In sodium-free buffer at pH 7.0 the addition of a large bolus of sodium bicarbonate results in an initial modest acidification followed by recovery. Under the same conditions the addition of equimolar choline bicarbonate leads to a considerably slower recovery despite equivalent extracellular pH changes. Previous studies have demonstrated that Na\(^+\)/H\(^+\) exchange is the principal pathway involved in leucocyte pH homeostasis under these conditions [14, 19, 20].

Clearly healthy leucocytes in vitro are not directly comparable to, for example, ischaemic/anoxic (and hence acidotic) myocardium. Under these experimental conditions, leucocytes cannot be kept acidotic unless sodium is removed from the extracellular fluid, but the data show clearly that the 'paradoxical' acidosis on the addition of bicarbonate is very small, even when Na\(^+\)/H\(^+\) exchange is prevented (Fig. 2). Another important consideration is that in most experiments of this type the carbon dioxide/bicarbonate buffering system is effectively 'closed' during the relatively short period of study, with incomplete equilibration between the carbon dioxide in solution and that in the atmosphere. Clearly this is not the case in the patient with acidosis where, through either ventilatory drive or adequate mechanical ventilation, the buffering system is 'open' compensating for any rise in pH.

The use of sodium bicarbonate to correct metabolic acidosis has attracted considerable criticism with two principal charges being levelled at the practice of administering alkali to acidotic patients. The first is that it produces a disequilibrium across cellular membranes with respect to carbon dioxide and bicarbonate ions which leads to intracellular acidosis. The second is that there is no evidence from the clinical trials that have been attempted that bicarbonate administration improves survival, or speeds recovery of metabolic equilibrium. Other theoretical disadvantages of alkalinization include adverse effects on the oxygen-carrying ability of the blood and the development of hypernatraemic hyperosmolar states when hypertonic sodium bicarbonate is used to correct the acidosis [21]. Whether or not a disequilibrium across the cell membrane develops depends fundamentally on the permeability of the membrane to bicarbonate ions as well as specific symptoms if present. There is substantial evidence that in both hepatocytes and vascular smooth muscle cells there exist cell membrane ion transport processes which can rapidly transport bicarbonate into the cell, hence preventing disequilibrium [22, 23]. This inward flux of bicarbonate acts in parallel in some cells with the Na\(^+\)/H\(^+\) exchanger to eliminate acid. This symptom is not present in leucocytes. In keeping with our results, studies on the effect of bicarbonate administration to acidotic rats showed rapid intracellular alkalinization [24, 25]. Similar results have been reported in isolated hearts [26].

We conclude that it is unsafe to extrapolate from the experiments previously performed in vitro to the clinical entity of lactic acidosis, and that fear of inducing 'paradoxical' intracellular acidosis is not sufficient ground to proscribe the use of sodium bicarbonate in patients with acidosis. While administering sodium bicarbonate has attendant risks so do many other potentially beneficial therapeutic manoeuvres. Comparison may be made with the use of intravenous potassium salts in potassium-depleted individuals. When the rate of infusion is carefully controlled, the effects are predictably beneficial. On the other hand, over-rapid administration can be fatal even while the whole-body potassium deficit remains uncorrected. We shall continue the cautious use of sodium bicarbonate in patients with severe acidosis, particularly those with impaired circulation.

REFERENCES