J Med Sci 2013;33(3):147-154
http://jms.ndmctsgh.edu.tw/3303147.pdf
DOI:10.6136/JMS.2013.33(3).147

The Buffering Power in Human Monocytes

Chung-Yi Lee[2], Yi-Tin Tsai[2], Chao-Chin Chung[1], Hsueh-Ju Lin[3], Chung-Yi Chang[4], Chien-Sung Tsai[2], and Shih-Hurng Loh[1]

[1]Department of Pharmacology; [3]Graduate Institute of Aerospace and Undersea Medicine, National Defense Medical Center (NDMC), Taipei;
[2]Division of Cardiovascular Surgery, Tri-Service General Hospital, National Defense Medical Center (NDMC), Taipei;
[4]Department of General Surgery, Cheng-Hsin General Hospital (CYC), Taipei, Taiwan, Republic of China

Intracellular pH (pH_i) plays a vital role in the regulation of many cell functions. Apart from active transmembrane pH_i regulators, passive intracellular buffering power acts in the first line to attenuate the impact of pH_i changes. Moreover, the quantification of the total intracellular buffering power (β_{tot}) is essential for calculating transmembrane acid-equivalent fluxes from pH_i recordings. The β_{tot} has two components: intrinsic buffering power (β_i) and CO_2-dependent buffering power (β_{CO2}). By microspectrofluorimetry with a fluorescence probe BCECF (2',7'-bis(carboxyethyl)-5(6)-carboxyfluorescein), we calculated the buffering power in human monocytes.

Experiments were performed under conditions free of Na^+, Cl^- and high K^+ to prevent the operation of active transmembrane pH regulators. Small stepwise reductions of external NH_4Cl (from 30 to 0 mM) resulted in stepwise reductions of pH_i. Similar procedures were performed either in the CO_2/HCO_3^-- or the HEPES-buffered solution. The results showed in the pH_i ranges of 6.9~7.5, under the CO_2/HCO_3^--buffered condition, the values of β_{tot} can be described as $\beta_{tot}=1403.1[pH_i]^2-19169.7[pH_i]+65538$ ($R^2=0.81$). Under HEPES-buffered condition, the values of β_i can be described as $\beta_i = -1293.2[pH_i]^2-18539.6[pH_i]+66519.9$ ($R^2=0.64$). Note, the factor of β_{tot} becomes more important while in the alkaline direction. In addition, the magnitude of intracellular β_{CO2}, derived from $\beta_{tot}-\beta_i$, has been described as $\beta_{CO_2}=745.7[pH_i]^2-9832.1[pH_i]+32306.3$ ($R^2=0.99$). This demonstrated the CO_2-dependent buffering power in the human monocytes was not consistent with a fully open cell-system for CO_2, i.e. β_{CO2} is not equal to $2.3\times[HCO_3^-]$. In other words, CO_2-permeation and –hydration/dehydration reaction are not rapid enough to behave as an open system. In conclusion, our present study, for the first time, quantifies the buffering power in human monocytes.

Key words: intracellular buffering power, human monocytes, intracellular pH, microspectrofluorimetry, fluorescence probe-BCECF

INTRODUCTION

The regulation of intracellular pH (pH_i) is important because many cellular processes are sensitive to pH_i changes. These pH-sensitive changes include enzyme activities, transporters/channels conformational states, signal transduction and regulation of cellular growth and differentiation.[1-5] In general, it has been demonstrated the pH_i in mammalian cells is kept within a narrow range (7.2 ± 0.1) through the combined operation of active transmembrane pH_i transporters and the intracellular buffering power (β_{tot}).[6-7] Buffering occurs within a rapid time course but will not restore pH_i to its original valued following an acid-base perturbation. The total restoration requires operation of active transporters with a slower time course (mins).[8] Therefore, the ability to maintain optimal pH_i is an essential requirement for all cells.

Given the considerable role of β_{tot} in minimizing pH_i changes, the quantification of β_{tot} is essential for calculating sarcolemmal acid equivalent fluxes from pH_i-recordings. The total intracellular buffering power (β_{tot}) has two components: the intrinsic buffering power of the cell (β_i) and the CO_2-dependent buffering power (β_{CO2}) caused by intracellular CO_2/HCO_3^-.[9] The β_i is principally

Received: February 6, 2013; Revised: April 2, 2013; Accepted: April 9, 2013
*Corresponding author: Shih-Hurng Loh, Department of Pharmacology, National Defense Medical Center, Neihu 114, P. O. Box 90048-504, Taipei, Taiwan, Republic of China. Tel (Fax): +886-2-87924861; E-mail: shloh@ndmctsgh.edu.tw

due to physicochemical buffers, such as the weak acid/base moieties of cytoplasmic proteins and phosphate. The human atrial myocardium cell β_i has been shown to vary approximately linearly with pH_i in accordance with the equation:

$\beta_i = -212.1\ pH_i + 1931.9$ (R^2=0.026) in the pH_i ranges of 7.1~7.5. Note, there is not a significant correlation between β_i and pH_i.[10]

In the absence of CO_2, β_{tot} is simply equal to the β_i. In the presence of CO_2/HCO_3^-, β_{tot} equals the sum of β_i and β_{CO2}. CO_2-dependent buffering power occurs through the chemical reactions:

$$CO_2+H_2O \Leftrightarrow H_2CO_3 \Leftrightarrow H^+ + HCO_3^-$$

Therefore, in mammalian systems, the magnitude of intracellular β_{CO2} depends on whether a cell behaves as an open or a closed system to CO_2. Koppel & Spiro initially suggested cellular CO_2/HCO_3^- could serve as a buffer in a closed CO_2- system.[11] This would mean all reactions occur within a single compartment with no exchange of the buffer species (CO_2) with the surroundings. In such a system, the total buffer concentration always remains constant and exerts its maximum buffering power at a pH equal in value to the pK_a of the buffer. In an open system, it is assumed two compartments exist, with the outer compartment acting as a reservoir of uncharged buffer species (CO_2) that freely enters and equilibrates with the inner compartment. Van Slyke demonstrated the contribution of CO_2/HCO_3^- to the buffering of blood tends to operate as an open CO_2 system.[12] In previous study, the results indicated applying and removing CO_2/HCO_3^- have effects on steady-state pH_i in anoxia. For instance, acute anoxia elicited a small pH_i decrease of ~ 0.06 in mouse CA1 hippocampal neurons bathed in CO_2/HCO_3^-, but a considerably larger pH_i increase of 0.46 in the neurons bathed in the absence of CO_2/HCO_3^-.[13-15] Our previous study in human atrial myocardium cells showed, in the CO_2/HCO_3^--condition, the values of β_{tot} can be described as $\beta_{tot} = -1633.3\ pH_i + 12544.9$ (R^2=0.86) in the pH_i ranges of 7.1~7.5. This implies the buffering of blood tends to operate as an open CO_2 system.[10]

In human THP-1 monocytes, systematic measurements of intracellular β_{tot} have not so far been made. Knowledge of β_{tot} is essential to calculate transporter-mediated membrane fluxes of acid-equivalents, apart from the indispensable basic database of the physiological parameters of cells. Therefore, in the present study, our aim is to estimate β_{tot}, β_{CO2} and β_i in human monocytes.

MATERIALS AND METHODS

Culture of human monocytes (THP-1) cell lines

The human monocytes cell line (THP-1) was obtained from the European Collection of Cell Cultures (Salisbury, UK). The cells were cultured in RPMI-1640 medium (Sigma, Poole, UK), supplemented with10% FCS, 4mM l-glutamine, 50 U/ml penicillin, 50 μg /ml streptomycin in a humidified atmosphere of 95% air with 5% CO_2 at 37 ^oC (Sigma).[16]

Measuring pH_i

The pH_i of human THP-1 monocytes were measured using a pH sensitive dye BCECF and as described in our previous reports.[17-18] In brief, the monocytes were loaded with BCECF for 30 min by incubating in the standard HEPES-buffered solution containing 10 μM of the cell permeant acetoxymethylester (-AM) form of the dye, BCECF. The cells were then secured in a flow-through chamber, which is in a temperature-controlled situation. The chamber was mounted in the excitation light path of the spectrofluorometer. The emitted light filtered at 510 nm from 490-nm excitation is pH sensitive, whereas that from 440-nm excitation is relatively pH insensitive. The ratio of the 510 nm emission at 490 nm and 440 nm excitation (F490/F440) is calculated and converted to a linear pH using the following equation:

$$pH_i = pK_a + \log [(R_{max} - R) / (R - R_{min})] + \log (F440_{min} / F440_{max})$$

where R is the 510 nm emission at 490 nm excitation/510 emission at 440 nm excitation ratio, Rmax and Rmin are, respectively, the maximum and minimum ratio values from the calibration curve (pH_i 5.5~8.5; data not shown), and pK is the dissociation constant for the dye, taken as 7.05. F440min/F440max is the ratio of the fluorescence measured at 440 nm of Rmin and Rmax. The overall sampling rate for the recorded fluorescent ratio (440nm /490 nm) is 0.5 Hz in the experiment. Thus, the fluorescence–ratio (F490/F440) is predominately a function of pH. Throughout the whole experiment, the change in resting pH_i induced by the tested drug was compared at the steady-state after treating the drug, unless otherwise stated.

Calibration of the BCECF fluorescence ratio signals

Normalized ratios were converted to pH_i values using the in situ calibration curves.[10,17] Intracellular pH was estimated as an E490/E440 ratio of fluorescence and calibrated as follows: Human THP-1 monocytes were

exposed to high K^+-nigericin calibration solutions, which equilibrate the pH_i with the known extracellular pH. The E490/E440 ratios were obtained during perfusion with five pH standard solutions (pH 5.5, pH 6.5, pH7.0, pH 7.5 and pH 8.5). Because the response ratio was linear in the pH range from 7.5 to 6.5, a simple transformation was performed to obtain the corresponding pH_i values with the linear range. All experiments were performed at 37 ^oC.

Method for Determining β

In the present work, pre-pulsing a cell with a permeant weak base NH_4Cl was used to induce an intracellular acid load. The magnitude of the intracellular acid load, in combination with the size of the pH_i-change was used to compute intracellular buffering power. A detailed description of utilizing weak acids in estimating β was given by Roos and Boron.[9] In brief, β can be defined as:

$$\beta\,(mM)=[H^+]_i / \triangle pH_i \qquad (e.1)$$

where $[H^+]_i$ is the concentration of acid introduced to the cell and $\triangle pH_i$ is the resulting change in pH_i. For experiments with the NH_4Cl prepulse technique, the application of NH_4Cl externally induces an intracellular alkalosis. This is due to the rapid diffusion of NH_3 into the cell and its subsequent hydration to form NH_4^+. Upon removal of extracellular NH_4Cl, NH_4^+ exits the cell as an uncharged NH_3, leaving behind an equal concentration of H^+ and causing an intracellular acidosis. If $[H^+]_i$ is assumed to equal the intracellular concentration of NH_4^+ at the moment of their removal from the external solution, then equation 1 can be expressed as:

$$\beta\,(mM)=[NH_4^+]_i / \triangle pH_i \qquad (e.2)$$

According to the Henderson-Hasselbalch equation, the relationship between internal and external NH_4^+ concentration is as follows:

$$pH_o\text{-}pH_i = \log ([NH_4^+]_i / [NH_4^+]_o) \qquad (e.3)$$

Equation 3 can then be re-arranged:

$$[NH_4^+]_i = [NH_4^+]_o \times 10^{(pHo\text{-}pHi)} \qquad (e.4)$$

In the extracellular solution, $pH_o = pK_a + \log ([NH_3]_o/[NH_4^+]_o)$ (Henderson-Hasselbalch equation). Therefore, re-arranging:

$$[NH_4^+]_o = C/(10^{(pHo\text{-}pK)} +1) \qquad (e.5)$$

where C is the total extracellular concentration of the NH_4^+ and pK is the dissociation constant of the NH_4Cl [9].

Combining equations 4 and 5, we can derive $[NH_4^+]_i$ at a given pH_i:

$$[NH_4^+]_i = [C/(10^{(pHo\text{-}pK)} +1)] \times 10^{(pHo\text{-}pHi)} \qquad (e.6)$$

In an open system, the theoretical $\beta_{CO2} = 2.3 \times [HCO_3^-]_i$ (e.7)

Similar to the calculation procedures above for NH_4^+ and, the $[HCO_3^-]_i$

can then be calculated as

$$[HCO_3^-]_i = [C/(10^{(pK\text{-}pHo)} +1)] \times 10^{(pHi\text{-}pHo)} \qquad (e.8)$$

Experimental Protocol

The addition of NH_4Cl induces a rise in pH_i due to the entry of the membrane-permeant form of the weak base NH_3, which then associates with H^+ thus raising pH_i. Removing NH_4Cl induces a fall of pH_i as $[NH_4^+]_i$ rapidly dissociated into H^+ and NH_3, the latter diffusing out of the cell.[18] In the study, Na^+-free, Cl^--free, and high K^+ conditions are used to prevent the effects of pH_i regulators. It is desirable to prevent the effects of pH_i regulators when quantifying intracellular buffering power.[20] Procedures are performed in a stepwise and in two kinds of solutions which are buffered with the CO_2/HCO_3^-- condition or the HEPES- condition, respectively.

Solutions

Standard HEPES-buffered Tyrode solution (air equilibrated) contained (mM): NaCl, 140; KCl, 4.5; $MgCl_2$, 1; $CaCl_2$ 2.5; glucose, 11; HEPES, 20; pH adjusted to 7.4 with 4N NaOH. Unless otherwise stated, pH adjustments of all HEPES-buffered solutions (including those where ionic-substitutions are made; see below) are performed at 37 ^oC. Standard bicarbonate-buffered Tyrode solution (equilibrated with 5%CO_2/22 mM HCO_3^-) is the same as above, except the NaCl concentration is reduced to 117 mM, and 22 mM $NaHCO_3$ is added instead of HEPES (pH 7.40 at 37 ^oC).

Ion-substituted solutions: In Na^+-free, HEPES-buffered Tyrode solution, NaCl is replaced with 140 mM N-methyl-D-glucamine (NMDG), and the pH adjusted to 7.4 with HCl. Cl^--free, CO_2/HCO_3^--buffered Tyrode solution contained (mM): sodium gluconate, 117; potassium gluconate, 4.5; calcium gluconate, 12; $NaHCO_3$, 22; $MgSO_4$, 1; glucose, 11. When 10 mM ammonium chloride is used, it is added directly as solid to solution without osmotic compensation.

Nigericin calibration solutions contained (mM): KCl, 140; $MgCl_2$, 1; 10 μM nigericin; buffered with one of the following organic buffers: 20 mM 2-(N-morpholino) ethanesulphonic acid (MES, pH 5.5), 20 mM HEPES

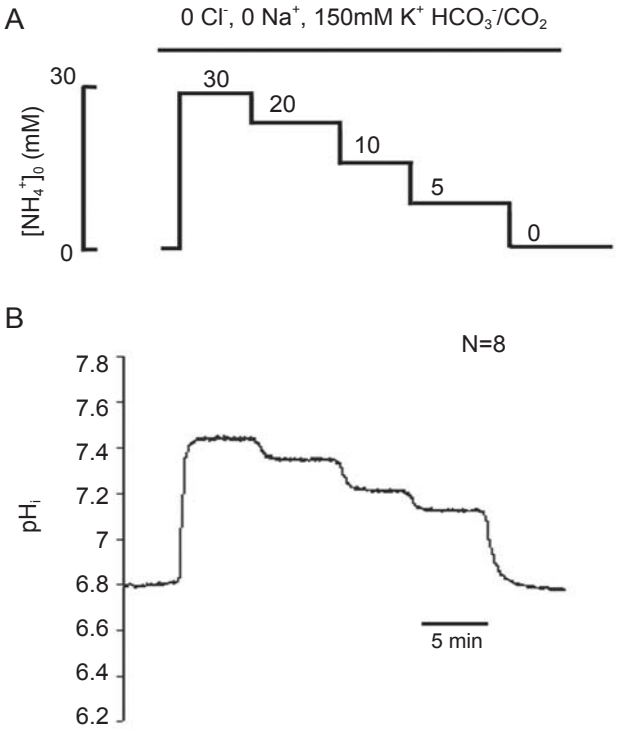

Fig. 1 Experiment to determine pH_i dependence of β_{tot} using NH_4Cl removal in 0 Cl^-, 0 Na^+, 150 mM K^+, bicarbonate-buffered Tyrode solution. A: External NH_4Cl is added and then removed stepwise as indicated. B: The trace shows the changes in intracellular pH (pH_i).

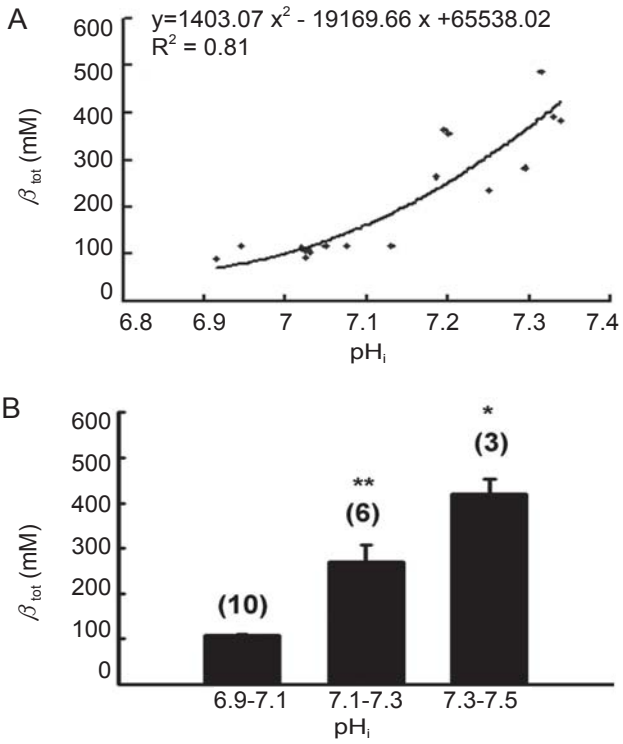

Fig. 2 β_{tot} increases as pH_i becomes more alkaline. A: Each value of β_{tot} is plotted versus pH_i. (pH_i is taken as the mid-point of each stepwise acid load followed by NH_4Cl reduction; see Fig. 1). Line is fitted by linear regression and drawn according to the equation: $\beta_{tot} = 1403.07\ [pH_i]^2 - 19169.66[pH_i] + 65538.02$ ($R^2 = 0.81$). B: Histograms show the total intracellular buffering power (β_{tot}) averaged over three groups of pH_i (6.9~7.1; 7.1~7.3; 7.3~7.5). **: $p < 0.01$ vs. pH_i 6.9~7.1.

(pH 7.5) or 20 mM 3-(cyclohexylamino)-2-hydroxy-1-propane-sulphonic acid (CAPSO, pH 9.5), and is adjusted (37 °C) to the correct pH with 4N NaOH. All drugs mentioned above are ordered from Sigma-Aldrich (United States).

Statistics

Data are reported as mean±standard error of the mean (SEM), where n refers to the number of experiments. Significance in means was assessed using either the paired or unpaired Student's t-test, and $P < 0.05$ is considered significant. Univariate linear regression is carried out to explore the determinants of variability in β value as the dependent pH_i variable.

RESULTS

Determination of βtot by ammonium removal

To determine total buffering power (β_{tot}), we used CO_2/HCO_3^--buffered Tyrode solution in the present experiments. Moreover, to rule out the possible affection of pHi recovery from all active pH_i regulators following an internal acid-load, acid extrusion is inhibited by superfusing a Na^+-free, Cl^--free, solution (Na^+ replaced isosmotically with N-methyl-D-glucamine) and high potassium solution (150 mM K^+) as shown in the top bar of the figure 1A.[20] Figure 1A shows the protocol of small stepwise reductions of external NH_4Cl from 30 to 0 mM (*the mechanism please see material and method for details*).[18] The stepwise reductions of pH_i caused by small stepwise reductions in external NH_4Cl in CO_2/HCO_3^--buffered are shown in figure 1B.

Figure 2A shows the calculated values of β_{tot} plotted *versus* pH_i (*See Material and methods for detail of the calculation*). The relationship is fitted an exponential equation and can be described as:

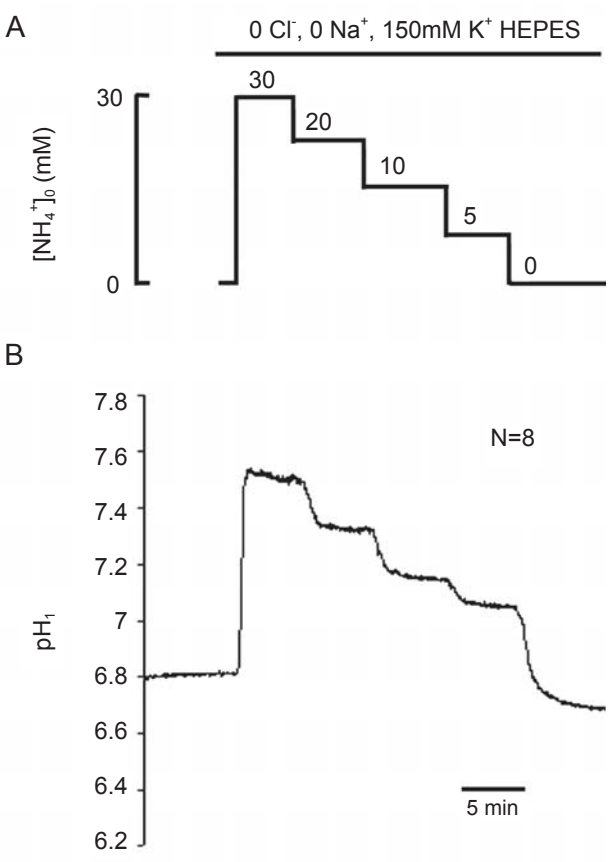

Fig. 3 Experiment to determine pH_i dependence of β_i using NH_4Cl removal in 0 Cl^-, 0 Na^+, 150 mM K^+, HEPES-buffered Tyrode solution. A: External NH_4Cl is added and then removed as indicated. B: The trace shows the changes in intracellular pH (pH_i).

$\beta_{tot} = 1403.07\ [pH_i]^2 - 19169.66[pH_i] + 65538.02$ (correlation coefficient $R^2 = 0.81$).

This means the values of β_{tot} increase as pH_i rising. Figure 2B shows the comparisons of β_{tot} in three different pH_i ranges of 6.9~7.1, 7.1~7.3 and 7.3~7.5. The former pH_i range from 6.9 to 7.1 represents the acid intracellular condition. The pH_i range from 7.1 to 7.3 represents the normal physiological resting condition and the latter pH_i range from 7.3 to 7.5 represents the slight alkali condition. From the statistics bar of figure 2B, we demonstrated an averaged β_{tot} in a mean of 263.5±20.8 mM (n=6) in the normal physiological resting condition which was elevated to 425.6±35.5 mM (n=3) when resting pH_i shifted to a slightly alkaline condition (~0.2 pH unit). On the contrary, averaged β_{tot} was decreased to 108±7.3 mM (n=10) when resting pH_i shift to a slightly acidic condition (~0.2 pH unit).

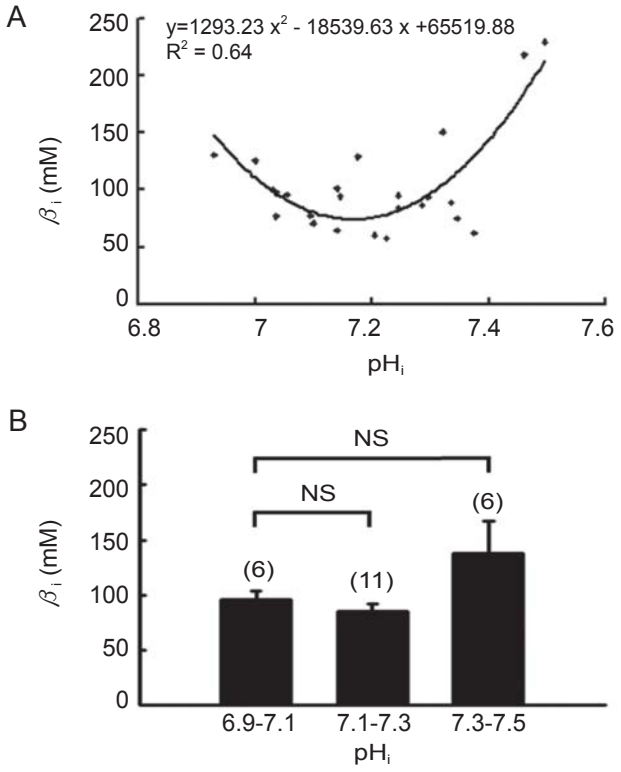

Fig. 4 β_i increases as pH_i becomes more alkaline. A: Each value of β_i is plotted versus pH_i. (pH_i is taken as the mid-point of each stepwise acid load following NH_4Cl reduction.) Line is not a good linear regression and drawn according to the equation: $\beta_i = 1293.23[pH_i]^2 - 18539.63[pH_i] + 66519.88$ (R^2=0.64). B: Histograms show the intrinsic buffering power (β_i) averaged over three groups of pH_i (6.9~7.1; 7.1~7.3; 7.3~7.5). **: $p < 0.01$ vs. pH_i 6.9~7.

Determination of β_i by ammonium removal

To estimate β_i, the present experiment is derived from the pH_i-fall and followed by stepwise of NH_4Cl reduction from 30 mM to zero in the superfusate of HEPES-buffered Tyrode solution. Each fall of pH_i then can be used to estimate β_i, as the active acid extrusion is inhibited by superfusing a Na^+-free, Cl^--free, solution (Na^+ replaced isosmotically with N-methyl-D-glucamine) and high potassium solution (150 mM K^+) HEPES-buffered -buffered solution, as shown in the top bar of the figure 3A. Figure 3A shows the protocol of the experiment for determination of β_i in human monocyte. Figure 3B shows an original pHi record of 8 similar experiments. Figure 4A shows the calculated values of β_i that plotted *versus* pH_i. The relationship is not linear and

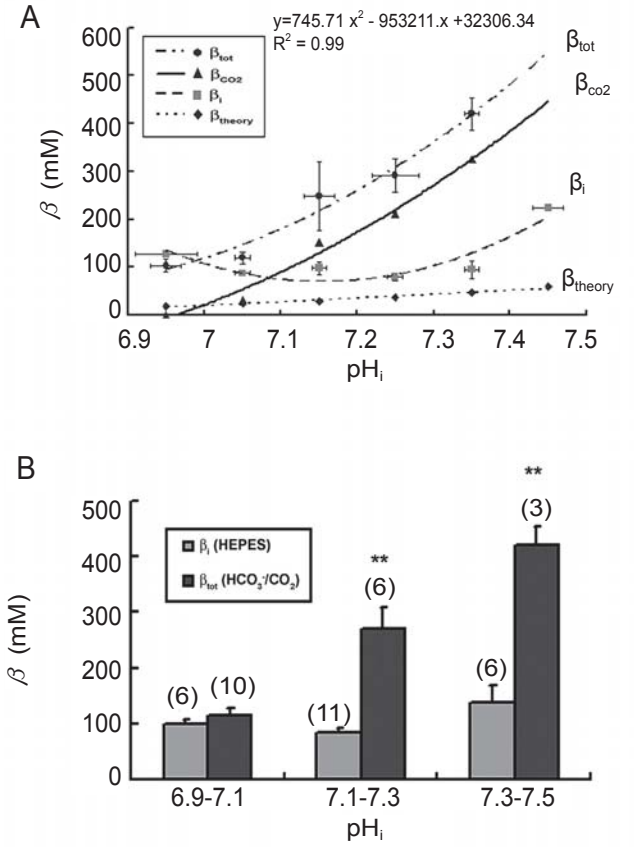

Fig. 5 βCO_2 increases as pH_i becomes more acidic. A: Each value of β_{C02} is the result of the mean β_{tot} mean β_i over four ranges of pH_i. Line is fitted by linear regression and drawn according to the equation: $\beta_{CO2} = 745.71[pH_i]^2 - 9832.11\ [pH_i] + 32306.34$ (R^2=0.99) B: Histograms show the significant difference between β_i and β_{tot} in the pH_i ranges of (7.1~7.3, 7.3~7.5). **: $p < 0.01$.

can be described by the equation: $\beta_i = 1293.23[pH_i]^2 - 18539.63[pH_i] + 66519.88$ (linear regression, correlation coefficient R^2=0.064). It shows the tendency of a bipolar increase of β_i in both the acid and alkali direction. Figure 4B shows comparisons of three groups of β_i in pH_i ranges of 6.9~7.1, 7.1~7.3, and 7.3~7.5. The means of β_i are 98.2±9.8, 85.3±11.4 and 135.1±28.5, respectively ($p>0.05$). The mean of β_i elevated as pH_i becomes more alkali *versus* the acid group (pH_i 6.9~7.1), but this result is not significantly different, though the tendency has been well observed.

Characterization of β_{CO2}

To characterize β_{CO2}, we can simply derive from the value of β_{tot} and β_i shown in Figure 2A and Figure 4A, respectively. As prediction, the pH_i changes in each steps associated with the addition and removal of NH_4Cl are considerably larger in HEPES-buffered conditions than in CO_2/HCO_3^--buffered conditions, as shown in Figure 1B and Figure 3B, respectively. These results indicate the intracellular buffering capacity is considerably increased in the presence of a CO_2/HCO_3^- buffer system. In Figure 5A, the β_{CO2} (filled triangle) is derived as (β_{tot}-β_i) (filled circle and filled rectangle). Note, the theory β_{CO2} has also been plotted in Figure 5A (filled diamond). Figure 5A pools the results of β_{CO2} *versus* pH_i. The relationship is good linear and can be described empirically by the equation: $\beta_{CO2} = 745.71[pH_i]^2 - 9832.11\ [pH_i] + 32306.34$ (linear regression, correlation coefficient R^2=0.99). For comparisons of the values of β_{tot} and β_i, two kinds of β are grouped into three pH_i ranges of 6.9~7.1, 7.1~7.3 and 7.3~7.5 as shown in Figure 5B. There is a significant difference in the pH_i ranges of 7.1~7.3 ($p < 0.01$) and 7.3~7.5 ($p < 0.01$). However, in the pH_i ranges of 6.9~7.1, there is no significant difference ($p > 0.05$). This means, in the alkali condition, β_{CO2} becomes more important in the intracellular buffering power of human THP-1 monocytes.

DISCUSSION

In our study, for the first time, we quantify the buffering power in human THP-1 monocytes and present an important tendency of pH_i-dependent buffering power in human monocytes. Moreover, our result shows CO_2-permeation and CO_2-hydration/dehydration reaction are sufficiently rapid to behave as an open system for CO_2 in human monocytes, i.e. β_{CO2} is not fit to the equation of $2.3\times[HCO_3^-]_i$ (*see below for more details*).

The clinical implication of pH dependent values of buffering power

The pH_i in mammalian cells is kept within a narrow range (7.0-7.2) through the combined operation of active sarcolemmal transporters and passive intracellular buffering power.[6-7] The membrane transporters can be divided into two main categories: acid extrusion carriers and acid loading carriers. Acid extrusion carriers such as Na^+/H^+ exchanger (NHE) and Na^+/HCO_3^- cotransporter (NBC) can be activated when cells are in an acidic condition (pH_i < 7.1).[17-18] On contrast, when the cell was in an alkalized direction (pH_i > 7.2), the acid loaders such as Cl^-/OH^- exchanger (CHE) and Cl^-/HCO_3^- exchanger (AE) will be triggered.[21]

The function of human monocytes is subject to regu-

lation by pH_i. This is not only because the diameter of vessel is very sensitive to the change in phi which further affects the blood flow and related functions of monocytes, but also due to the changes in pH_i influence monocyte migration and adhesion.[22] Kaloyianni et al found the activation of NHE-1 in high glucose solution stimulated an increase in the expression of CD36 receptors on the surface of monocytes[22]. The adhesion of circulating monocytes to endothelial cells and smooth muscle cells are thought to be early events in the development of atherosclerosis.[23]

The present study demonstrated the buffering power, either β_i or β_{CO2}, is pH_i dependent (Fig. 5A and Fig. 5B), larger in the alkaline direction while smaller in the acid direction. In other words, it implies the human monocyte has more protection in alkaline conditions. Whether this is related to its patho-physiological function waits for further experiments. Intrinsic, CO_2/HCO_3^--independent, buffering power is non-liner and bipolar increased in the acid and alkali direction.

As the intrinsic buffering power plays a major and direct role in the impact of pH_i change through moieties of cytoplasmic phosphate and protein, therefore the characterization of it is very important for clinical therapy as well as basic understanding. We have found, in human monocytes, the relationship is not linear and shows the tendency of bipolar increase of β_i in both the acid and alkali direction ($\beta_i = 1293.23[pH_i]^2 - 18539.63[pH_i] + 66519.88$; R^2=0.064), as shown in figure 4B. In other words, our results imply human monocytes possess higher intrinsic buffering power when cells meet the server conditions, i.e., at more alkali or more acid surroundings, such as under inflammatory or infection challenge.

CO_2/HCO_3^--dependent buffering dose is not consistent with an open CO_2-system

Apart from intrinsic buffering power (β_i), the main component of the value of β_{tot} is the contribution of CO_2/HCO_3^- to cell-buffering, i.e. β_{tot}. In an open system, it is assumed CO_2 freely enters and equilibrates with the inner compartment. If one considers the intracellular & extracellular spaces as the two compartments and assumes the cell is open to highly membrane-permeant CO_2, then the intracellular concentration of CO_2 should remain constant, even during changes in pH_i. In such a system, intracellular buffering power is increased, even when the pH values are very different from the apparent pK. In an open system, providing CO_2-permeation and CO_2 hydration/dehydration reaction are sufficiently rapid, $\beta_{CO2} = 2.3 \times [HCO_3^-]_i$.[24]

As shown in the figure 5A, the equation of β_{CO2} we derived in human monocyte is $745.71[pH_i]^2 - 9832.11[pH_i] + 32306.34$ (linear regression, correlation coefficient $R^2 = 0.99$). It strongly suggests β_{CO2} significantly differs from that of theoretical $\beta_{CO2} = 2.3 \times [HCO_3^-]_i$. In other words, the present result shows the model of CO_2-dependent buffering power is not consistent with an open CO_2- system fully. We find as pH_i increased, β_{CO2} decreased. This means the factor influencing $[HCO_3^-]_i$ may not simply come from free CO_2-permeation. The activity of carbonic anhydrase may be involved. This result is the same as what we found in our previous study in human atrial myocardium cells[10], while differing from studies in early sheep Purkinje fiber[25], human smooth muscle cells[9] and rabbit single pulmonary vascular smooth muscle cells.[26] For example, in the sheep cardiac Purkinje fiber[16], a component of β_{tot} due to CO_2/HCO_3^- buffer was measured experimentally and was very similar to that predicted for an open, fully equilibrated system for CO_2. In short, our results demonstrate, in human monocytes, extracellular CO_2 can influence β_{CO2} through hydration and further equilibration of H_2CO_3/HCO_3^- in the same way as a non-open (fully) equilibrated system.

In conclusion, our study suggests, in human monocytes, (1) CO_2/HCO_3^- makes a clear contribution to intracellular buffering power, especially in the intracellular alkaline condition, (2) the human monocyte behaving as a buffered compartment does not fully open to CO_2.

ACKNOWLEDGEMENTS

This study was supported by grants from the National Science Council (NSC 96-2320-B-016-015-MY3; 97-2321-B-016-001-MY3), National Defense Medical Bureau (DOD100-I-30; DOD 101-15-6), and Tri-Service General Hospital (TSGH-C98-24; TSGH-C100-088), Taipei, Taiwan, Republic of China to SH Loh and JY Lee. The authors also acknowledge Dr. Daniel Steve Villarreal and Dr. Adonis Wu for reading and editing the manuscript.

REFERENCES

1. Goossens JF, Henichart JP, Dassonneville L, Facompre M, and Bailly C. Relation between intracellular acidification and camptothecin-induced apoptosis in leukemia cells. Eur J Pharm Sci 2000;10:125-131.
2. Grinstein S, Rotin D, and Mason MJ. Na+/H+ exchange and growth factor-induced cytosolic pH changes. Role in cellular proliferation. Biochim Bio-

phys Acta 1989;988:73-97.
3. Grinstein S, Woodside M, Sardet C, Pouyssegur J, and Rotin D. Activation of the Na+/H+ antiporter during cell volume regulation. Evidence for a phosphorylation-independent mechanism. J Biol Chem 1992;267:23823-23828.
4. Jeremy RW, Koretsune Y, Marban E, and Becker LC. Relation between glycolysis and calcium homeostasis in postischemic myocardium. Circ Res 1992;70:1180-1190.
5. Kiss L, and Korn SJ. Modulation of N-type Ca2+ channels by intracellular pH in chick sensory neurons. J Neurophysiol 1999;81:1839-1847.
6. Leem CH, Vaughan-Johns RD. Chloride-hydroxyl exchange in the guinea-pig ventricular myocyte: no role for bicarbonate. J Mol Cell Cardiol 1997;29:2483-2489.
7. Vaughan-Jones RD. Regulation of intracellular pH in cardiac muscle. In: Bock, G. & Marsh, J. eds. Proton Passage across Cell Membranes. Ciba Foundation Symposium 1988;139:23-46.
8. Leem CH, Lagadic-Gossmann D, and Vaughan-Jones RD. Characterization of intracellular pH regulation in the guinea-pig ventricular myocyte. J Physiol 1999;517:159-180.
9. Roos A, Boron WF. Intracellular pH. Physiol Rev 1981;61:296-434.
10. Tsai CS, Tsai YT, Lee CY, Chang CY, Tsai KL, Shih YJ, Yang YZ and Loh SH. The Buffering Power in the Human Atrial Myocardium. J Med Sci 2010;30:085-092, doi: 10.6136/JMS.2010.30(3).085.
11. Koppel M, Spiro K. Uber die Eirkung von Moderatoren (Puffern) bei der Verschiebung des Saure-Basengleichgewichtes in biologischen Flussigkeiten. Biochemische Zeitschrift 1914;65:409-439.
12. Van Slyke DD. On the measurement of buffer values and on the relationship of buffer value to the dissociation constant of the buffer and the concentration and the reaction of the buffer solution. J Biol Chem 1922;52:525-570.
13. Bevensee MO, Boron WF. Effects of acute hypoxia on intracellular-pH regulation in astrocytes cultured from rat hippocampus. Brain Res 2008;1193:143-152, doi: 10.1016/j.brainres.2007.12.002.
14. Bevensee MO, Cummins TR, Haddad GG, Boron WF, Boyarsky G. pH regulation in single CA1 neurons acutely isolated from the hippocampi of immature and mature rats. J Physiol 1996;494:315-328.
15. Bevensee MO, Boron WF. Effects of acute hypoxia on intracellular-pH regulation in astrocytes cultured from rat hippocampus. Brain Res 2008;1193:143-152, doi: 10.1016/j.brainres.2007.12.002.
16. Foster N, Cheetham J, Taylor JJ, Preshaw PM. VIP Inhibits Porphyromonas gingivalis LPS-induced immune responses in human monocytes. J Dent Res 2005;84:999-1004.
17. Loh SH, Chen WH, Chiang CH, Tsai CS, Lee GC, Jin JS, Cheng TH, and Chen JJ. Intracellular pH regulatory mechanism in human atrial myocardium: functional evidence for Na(+)/H(+) exchanger and Na(+)/HCO(3)(-) symporter. J Biomed Sci 2002;9:198-205.
18. Loh SH, Jin JS, Tsai CS, Chao CM, Chiung CS, Chen WH, Lin CI, Chuang CC, and Wei J. Functional evidence for intracellular acid extruders in human ventricular myocardium. Jpn J Physiol 2002;52:277-284.
19. Bountra C, Powell T, Vaughan-Jones RD. Comparison of intracellular pH transients in single ventricular myocytes and isolated ventricular muscle of guinea-pig. J Physiol 1990;424:343-365.
20. Lagadic-Gossmann D, Buckler KJ, Vaughan-Jones RD. Role of bicarbonate in pH recovery from intracellular acidosis in the guinea-pig ventricular myocyte. J Physiol 1992;458:361-384 .
21. Sun B, Leem CH, and Vaughan-Jones RD. Novel chloride-dependent acid loader in the guinea-pig ventricular myocyte: part of a dual acid-loading mechanism. J Physiol 1996;49565-82.
22. Kaloyianni M, Zolota Z, Paletas K, Tsapas A, Koliakos G. Cariporide counteracts atherosclerosis-related functions in monocytes from obese and normal individuals. Obes Res 2005;13:1588-1595.
23. Ross R. The pathogenesis of atherosclerosis: a perspective for the 1990s. Nature 1993;362:801-809.
24. Woodbury JW. Regulation of pH. In: Ruch TC, Patton HD. eds. Physiology and Biophysics. Philadelphia: Saunders, 1965, p899-934.
25. Dart C. Bicarbonate transport in mammalian cardiac muscle. D. Phil. Thesis, University of Oxford, U.K.; 1991.
26. Stevens K. Factors influencing intracellular pH and calcium in pulmonary vascular smooth muscle. D. Phil. Thesis, University of Oxford, U.K. 1997.