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The Buffering Power in Human Monocytes

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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

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