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Effects of Indomethacin on Intracellular pH and Na^+/H^+ Exchanger in the Human Monocytes

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Abstract

The ability to maintain optimal intracellular pH (pH_i) is an essential requirement for all cells. Na^+-H^+ exchanger (NHE), a ubiquitously expressed transmembrane protein, has been found widely as a major acid extruder in many different cell types, including human monocytes. We therefore investigated the mechanism of the active pH_i recovery from intracellular acidosis (induced by NH_4Cl prepulse) using intracellular 2',7'-bis (2-carboxethyl)-5(6)-carboxyl-fluorescein (BCECF) fluorescence in cultured human monocytes. Indomethacin is a potent, nonselective inhibitor of cyclooxygenases. Due to its toxicity, the clinical use of indomethacin as an analgesic-antipyretic agent is limited. However, it has recently been found that indomethacin can effectively treat many inflammatory/immune disorders. In this study, we further investigated the effect of indomethacin on the pH_i and explored the underlying mechanism. In HEPES (nominally HCO_3^--free) Tyrode solution, a pH_i recovery from induced intracellular acidosis could be blocked completely by 30 μM HOE 694, a specific NHE1 inhibitor, or by removing $[Na^+]_o$. Therefore, in the present study, we provided functional evidence, physiologically and pharmacologically, that the HCO_3^--independent acid extruder was mostly likely the NHE1 which was involved in acid extrusion in the human monocytes. Moreover, indomethacin (1 μM-1 mM) decreased pH_i levels in a concentration-dependent manner and significantly suppressed the activity of the NHE1, suggesting that indomethacin-induced intracellular acidosis is caused both by the inhibition of NHE1 activity and the non-specified NHE1-independent acidifying mechanism.

In conclusion, our present study demonstrates that NHE1 exists functionally in human monocytes, and the indomethacin-induced pH_i decreasing is summation effects on NHE1-dependent and -independent mechanism.

Key Words: fluorescent-BCECF, human monocytes, indomethacin, intracellular pH, microspectrofluorimetry, Na^+/H^+ exchanger (NHE)

Introduction

Cardiovascular diseases comprise a class of diseases that involve heart and systemic blood vessels. Though the multifactorial background makes it difficult to unravel initial pathological events, inflamma-

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tion is considered to play a key role in both disease initiation and progression (27, 28). Monocytes are essential cellular components of the innate immune system during the inflammatory process. Moreover, these cells play a major role in development of atherosclerosis and rupture of atherosclerotic plaques (44).

Many cellular functions are sensitive to changes of intracellular pH (pH_i). These include enzyme catalysts (22), control of cell volume (14, 18), permeability of ion channels (25), the regulation of cell differentiation, growth and apoptosis (3, 15, 17). The pH_i in mammalian cells is kept within a narrow range (7.0-7.2) through the combined operation of active transmembrane transporters and passive intracellular buffering power (31, 50). The membrane transporters can be divided into two main categories: acid extrusion carriers and acid loading carriers. Acid extrusion carriers such as an Na^+/H^+ exchanger (NHE) and Na^+/HCO_3^- cotransporter (NBC) can be activated when cells are in an acidic condition ($pH_i < 7.1$) (20, 29, 31, 50).

Net acid extrusion from mammalian cells is mediated by the NHE and the NBC (20, 29, 31-33). NHE mediates the electroneutral exchange of extracellular Na^+ for intracellular H^+ (1, 16, 20, 31, 32) pH_i recovery in HEPES-buffered media (HCO_3^--free condition) can be inhibited by the removal of extracellular Na^+ or by the addition of amiloride or HOE 694 (3-methylsulfonyl-4-piperidinobenzoyl, guanidine hydrochloride), a compound that inhibits NHE1 activity through its high affinity and selectivity (31-33). In the previous studies, angiotensin II has been found to cause NHE1 activation through pathways involving isoforms of protein kinase C with the participation of superoxide and nitric oxide (38).

In the last years, interest in the cardiovascular effects of the relatively selective inhibitors of cyclooxygenase 2 (COX-2) has been intense. In 2004, rofecoxib was withdrawn from world markets after a randomized placebo-controlled trial found that it increased rates of cardiovascular events in patients with colorectal polyps (6). The prevailing hypothesis that has been put forward to explain increased incidence of cardiovascular events is induction of a prothrombotic state (21). However, lots of nonselective nonsteroidal anti-inflammatory drugs (NSAIDs) are used extensively and some are available in many countries without prescription. Indomethacin, a highly potent nonsteroidal anti-inflammatory drug with nonselective inhibition on cyclooxygenases (5) is known to possess both prominent anti-inflammatory and analgesic-antipyretic properties (12). The clinical use of indomethacin has been effectively used in various clinic conditions, such as several acute and chronic pains (40), rheumatoid arthritis (45) and ocular inflammation (19). Apart from the potent inhibition on cyclooxygenases, the anti-inflammatory and analgesic effects of indomethacin is also achieved through a variety of mechanisms, including regulation of NHE (40) and Cl^-/HCO_3^- ion transporter (48).

There are reports showed that high concentration of protons induce neutrophil activation (34, 49) and results in the production of platelet-activating factor, a strong inflammatory stimulus (37). Monocyte is one of the most important components of flowing blood, which expose onto a wide diversity of physical and chemical stimuli, and pH_i is constantly affected. It is therefore important to understand the pH_i regulations in human monocytes. The aim of the present study is to examine the effect of indomethacin on the pH_i regulation in the monocytes.

Materials and Methods

Preparation of Human Monocytic Cell Line

The human monocytic cell line THP-1 (American Type Culture Collection, Rockville, MD, USA) was cultured in RPMI 1640 medium supplemented with 10% fetal bovine serum, 100 U/mL penicillin, and 100 μg/mL streptomycin at 37°C and 5% CO_2 in a humidified incubator. Cells were pelleted and resuspended with fresh media in 24-well tissue culture plates (Nunc, Naperville, IL, USA) at a concentration of 10^6/mL for 24 h before experimental use.

Measurement and Calibration of the pH_i

Measurement of the pH_i has been described in detail in our previous reports (29, 30). In brief, the pH_i in the human monocyte was measured using the pH-sensitive, dual excitation dual-emission fluorescent dye, 2',7'-bis(2-carboxethyl)-5(6)-carboxy-fluorescein-acetoxymethyl (BCECF-AM) (Molecular Probes). The preparations were loaded with BCECF-AM (5 μM) by incubating them for 30 min at room temperature and exciting them alternately with 490 and 440 nm wavelength light. The BCECF fluorescence emission ratio of the 510 nm emission at 440 nm and 490 nm excitation (440/490) was calibrated using the K^+-nigericin method (29). Briefly, this method consisted of exposing a BCECF-loaded cell to the six nigericin calibration solutions (listed below in the Solution section) that clamps pH_i to the value of pH_o of the calibration solution. Fig. 1A showed the emission ratio changes seen on perfusing human artery smooth muscle cells with calibration solutions with different 5 pH values (5.5~8.5) in the presence of 10 μM nigericin. The emitted ratio 510 nm emission at 440 nm and 490 nm excitations (R; $R = F_{490}/F_{440}$) was increased as the pH value of superfusing solution was increased. R_{max} and R_{min} are, respectively,

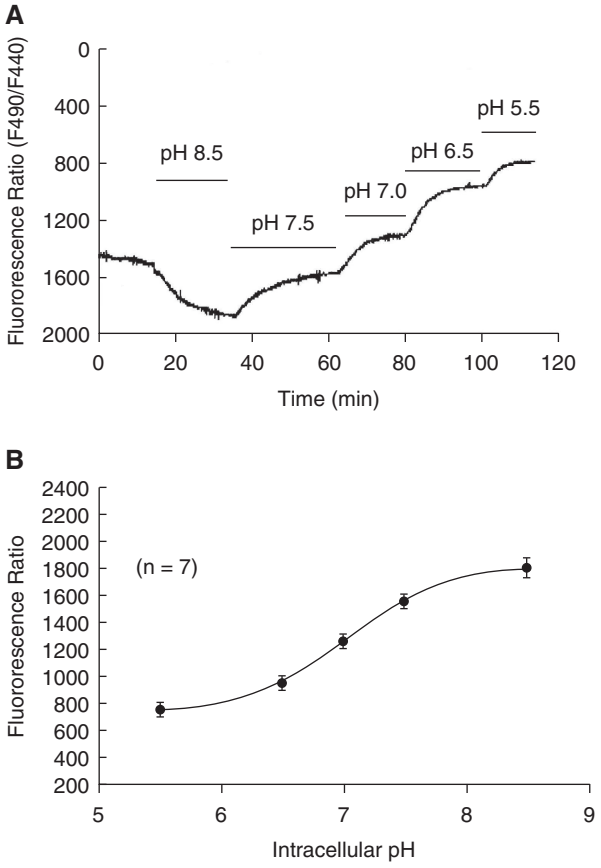

Fig. 1. *In situ* calibration of pH_i in human monocytes. A & B: *In situ* pH_i calibration curve in human monocytes. A: The trace shows the BCECF fluorescence (510 nm emission at 440 nm and 490 nm excitations) in human monocytes. (Please see *Materials and Methods* for details). B: The curve shows data pooled from 7 similar experiments shown in A.

the maximum and minimum ratio values for the data curve. The fluorescence of BCECF at 490 nm to 440 nm is a function of pH_i and the overall sampling rate in the experiment was 0.5 Hz for the recorded fluorescent ratio (440 nm/490 nm). Using the linear regression fit of the data (shown in the Fig. 1B) obtained from 7 calibration experiments similar to that shown in Fig. 1A, the mean apparent dissociation constant (pK_a) at 37°C was found to be 7.22, very close to the value determined by our previous study of the human heart, as well as the value determined by other investigators (4, 29, 46). The following equation (7) was used to convert the fluorescent ratio in to pH_i:

$$pH_i = pK_a + \log [(R_{max} - R) / (R - R_{min})] + \log (F_{440min}/F_{440max})$$

where R is the ratio of the 510 nm fluorescence at 440 nm and 490 nm excitation, R_{max} and R_{min} are, respectively, the maximum and minimum ratio values from the data curve and the pK_a (-log of dissociation constant) is 7.22. F_{490max}/F_{440max} is the ratio of fluorescence measured at 440 nm of R_{min} and R_{max}, respectively.

Experimental of NH_4Cl Pre-Pulse Technique

NH_4Cl pre-pulse techniques were used in the present work to induce acute acid loading (4, 41). NH_4Cl pre-pulses were achieved with (7~10 min) extracellular exposures to 20 mM NH_4Cl. Briefly, the mechanism of the NH_4Cl prepulse technique relies upon the characteristic of incomplete dissociation. Although both the charged and uncharged species of a weak base exist at the same time in solution, the uncharged species is lipid soluble and therefore able to permeate the lipid bi-layer of the cell membrane. In contrast, the charged species permeates relatively slowly, through various membrane protein routes. The details of trace change of pH_i please see the result section of Fig. 2. Throughout the whole experiment, the change of pH_i induced by the tested drug was compared around the 3rd min after treating the drug, unless otherwise stated. The background fluorescence and auto-fluorescence were small (< 5%) and haves been ignored.

Chemicals and 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 were performed at 37°C (these adjustments included those where ionic-substitutions were made, see below).

In a Na^+-free, HEPES-buffered Tyrode solution, NaCl was replaced with 140 mM N-methyl-D-glucamine (NMDG)-Cl, and the pH was adjusted to 7.4 with HCl. When 20 mM ammonium chloride was used, it was added directly as a solid to solution without osmotic compensation. HOE 694 (3-methylsulfonyl-4- piperidinobenzoyl, guanidine hydrochloride) was added, as solid, to solutions shortly before use.

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 (pH 7.5) or 20 mM 3-(cyclohexylamino)-2-hydroxy-1-propane-sulphonic acid (CAPSO, pH 8.5), and were adjusted (37°C) to the correct pH with 4N NaOH.

Indomethacin was first dissolved in dimethyl sulphoxide (DMSO) as 100 mM stock solution and brought to the final concentrations with HEPES solution for experiment. Before experiment, the indometha-

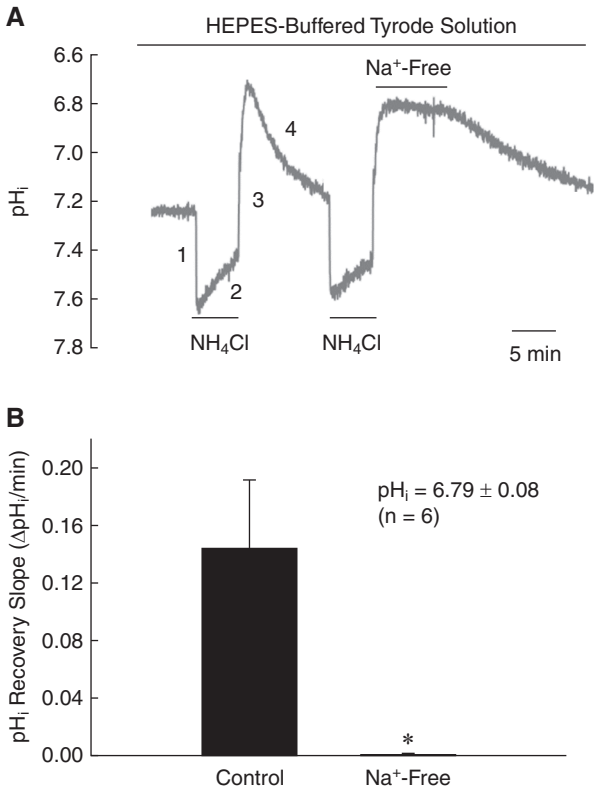

Fig. 2. Effect of Na^+-free on pH_i recovery from induced acidosis (evidence of Na^+-H^+ exchanger) in human monocytes superfused with HEPES-buffered Tyrode solution. A: Top bar shows buffer system used in the superfusate. The periods of application of NH_4Cl and Na^+-free solution are indicated with bars above or below the trace. The left part of traces A show a typical recovery of pH_i-recovery from an intracellular acidosis induced by a 10 min NH_4Cl (20 mM) pre-pulse in HEPES-buffered Tyrode solution (pH_o = 7.4, 37°C) in human monocytes. For details of the mechanism of the pre-pulse technique, please see the *Materials and Methods* section. The right parts of traces A represents experiments showing the effect of Na^+-free on pH_i recovery in human monocytes. B: Histograms, showing the pH_i recovery slope of acid extrusion after NH_4Cl-induced intracellular acidosis averaged for 6 experiments (measured at $pH_i = 6.79 \pm 0.08$) similar to those shown in A. *: $P < 0.005$ *vs.* control.

cin contained HEPES solution will be neutralised with 1 N NaOH to pH 7.4. Note that the background effect of DMSO on pH_i/NHE activity were small and haves been ignored (data not shown). HOE 694 was kindly provided by Hoechst Aktiengesellshaft (Frankfurt, Germany). All other chemicals were from Sigma (Darmstadt, UK) and Merck (Darmstadt, Germany).

Statistics

All data are expressed as the mean ± the standard error of the mean (SEM) for N preparations. Statistical analysis was performed using one-way analysis of variance (one-way ANOVA) with Scheffe's posterior comparison. A P value smaller than 0.01 was regarded as significant. The asterisk (*) denotes P value smaller than 0.01.

Results

The Functional Existence of a NHE

To examine whether an acid-extrusion mechanism exists in the human monocytes, the experiments were first performed in HEPES-buffered superfusate (nominally free of CO_2/HCO_3^-). The steady-state pH_i value for the human monocytes was found to be 7.25 ± 0.04 (n = 19) in HEPES-buffered solution. The steady-state pH_i value of human monocytes is similar to 7.2, which is the value that was reported previously for mature mammalian cells of both animal and human models (26, 29, 31).

As shown in the left part of Fig. 2A, the pH_i recovered completely from intracellular acidosis that was induced by using an NH_4Cl pre-pulse technique. It can be explained in terms of four phases as shown in Fig. 2. Phase 1 (rapid entry; see left part of Fig. 2A): the application of 10 mM NH_4Cl to the superfusate induces an initial rapid alkalosis. This is due to the passive entry of NH_3 and its subsequent protonation ($NH_3 + H^+ \Leftrightarrow NH_4^+$). Phase 2 (slow recovery; see left part of Fig. 2A): NH_4^+, the charged species, slowly enters, partly *via* K^+ channels. On entry it dissociates to NH_3 and H^+ by the principle of mass action. This causes a decrease in pH_i. With the slow but continuous influx of NH_4^+, the intracellular NH_3 concentration ($[NH_3]_i$) will soon exceed extracellular NH_3 concentration ($[NH_3]_o$). Thus excess NH_3 diffuses out of the cell, allowing more H^+ to be formed the dissociation of NH_4^+ ions inside the cell. This is the main reason for the slow pH_i-recovery from initial alkalosis. A second reason is due to the activation of the two acid loading transporters following intracellular alkalosis (Cl^--HCO_3^- exchange and Cl^--OH^- exchange). Phase 3 (rapid exit; see left part of Fig. 2A): once the external NH_4Cl is removed, the permeant intracellular NH_3 passively diffuses out of the cell. This further induces a rapid dissociation of intracellular NH_4^+ into NH_3 and H^+. The NH_3 again diffuses out, leaving behind H^+ ions, therefore, causing a rapid and large intracellular acidosis. The magnitude of the induced acid load is dependent upon pH_i at NH_4Cl-removal. The lower the pH_i, the larger the subsequent acid-load. Phase 4 (pH_i regulation; see left part of Fig. 2A): the sudden acidosis activates pH_i regulatory proteins in the membrane, for instance Na^+-H^+ exchanger.

In other words, the result of left part of Fig. 2A

indicated that there is a mechanism of acid extrusion in the human monocytes. Removing extracellular Na^+ completely blocked the pH_i recovery from intracellular acidosis following the NH_4Cl pre-pulse, as shown in the middle part of Fig. 2A. The first and second columns of the histogram (Fig. 2B) shows the mean pH_i recovery slope (measured at $pH_i = 6.79 \pm 0.08$) before and after Na^+ removal for 6 experiments that are similar to those whose results are shown in Fig. 2A. This clearly demonstrates that, under nominally CO_2/HCO_3^--free conditions, there is an Na^+-dependent, but CO_2/HCO_3^--independent, acid-extrusion mechanism involved in the pH_i recovery following induced intracellular acidosis in the human monocytes.

To further test if this Na^+-dependent acid extruder is the NHE, we added HOE 694, a specific NHE inhibitor, in the superfusate. As shown in the right part of Fig. 3A, HOE 694 (30 μM) entirely inhibited the pH_i recovery following the induced intracellular acidosis. The pH_i recovery rate (measured at $pH_i = 6.89 \pm 0.06$) of 6 similar experiments, like the result shown in Fig. 3B, were pooled in the first (before HOE 694 addition) and second columns (after HOE 694 addition) of Fig. 3B. Therefore, the present results provide clear pharmacological evidence that NHE1 functionally exists in human monocytes.

The Effect of Indomethacin on Intracellular Resting pH Under HEPES-buffered Tyrode Superfusate

To see whether the indomethacin affects intracellular pH, we added different concentrations of indomethacin (from 1~1000 μM) under HEPES-buffered Tyrode solution. As shown in Fig. 4A, indomethacin showed a concentration-dependent intracellular acidosis, with decreasing 0.1 pH unit to 0.4 pH unit (from 30 μM to 1000 μM, respectively) ($P < 0.01$, n = 6). Note that the significantly intracellular acidosis (~ -0.4 pH unit) induced by 1000 μM indomethacin was completely reversible after washout. The histogram of Fig. 4B shows the mean indomethacin-induced pH_i changes for 6 experiments, similar to that shown in Fig. 4A. The results clearly show that, under HEPES-buffered Tyrode solution, indomethacin (30 μM to 1000 μM) induced a concentration-dependent intracellular acidosis.

The Effect of Indomethacin on Na^+-H^+ Exchanger Activity

Under HEPES-buffered Tyrode condition, NHE1 is the sole acid extruder responsible for pH_i recovery from intracellular acidosis, therefore, we further examine the concentration effect of indomethacin on activity of NHE1 in human monocytes. The activity of NHE1 was measured in the following experiments,

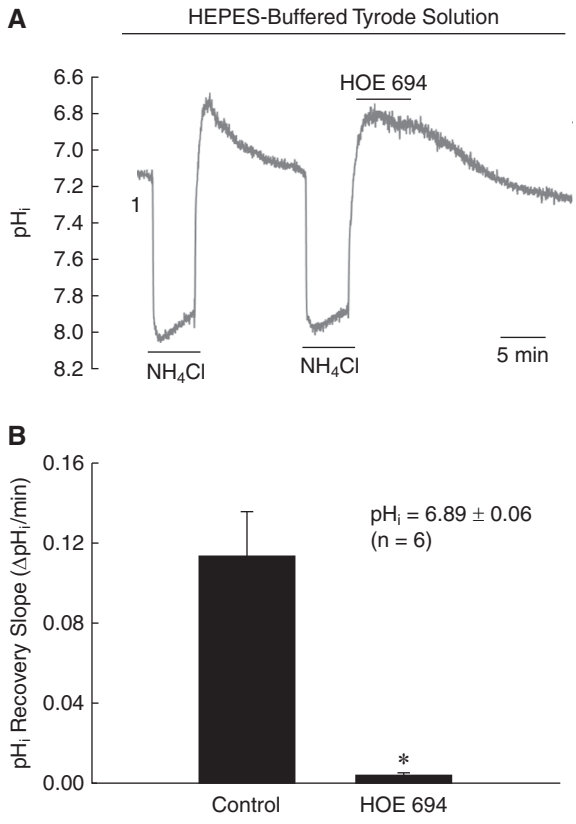

Fig. 3. Effect of 30 μM HOE 694 on pH_i recovery from induced acidosis (evidence of Na^+-H^+ exchanger) in human monocytes superfused with HEPES-buffered Tyrode solution. A: Top bar shows buffer system used in the superfusate. The periods of application of NH_4Cl and 30 μM HOE 694, a NHE exchanger inhibitor, are indicated with bars above or below the trace. The left part of traces A and C show a typical recovery of pH_i-recovery from an intracellular acidosis induced by a 10 min NH_4Cl (20 mM) pre-pulse in HEPES-buffered Tyrode solution ($pH_o = 7.4$, 37°C) in human monocytes. The right parts of traces A represents experiments showing the effect of 30 μM HOE 694 on pH_i recovery in human monocytes. B: Histograms, showing the pH_i recovery slope of acid extrusion after NH_4Cl-induced intracellular acidosis averaged for 6 experiments (measured at $pH_i = 6.89 \pm 0.06$) similar to those shown in A. *: $P < 0.005$ *vs.* control.

which were performed in HEPES-buffered solutions that were nominally free of CO_2/HCO_3^-. As shown in the left part of Fig. 5A, pH_i recovered completely from an intracellular acidosis through the NHE1 in the control. The acute effect of superfusion with indomethacin (3~1000 μM) on the pH_i recovery of the human monocytes is shown in Fig. 5A. Under HEPES superfusate (Fig. 5A), indomethacin treatment caused concentration-dependent changes in the pH_i recovery slope, *i.e.* no change at the lower dose (3 and 10 μM) (data now shown), followed by a significantly de-

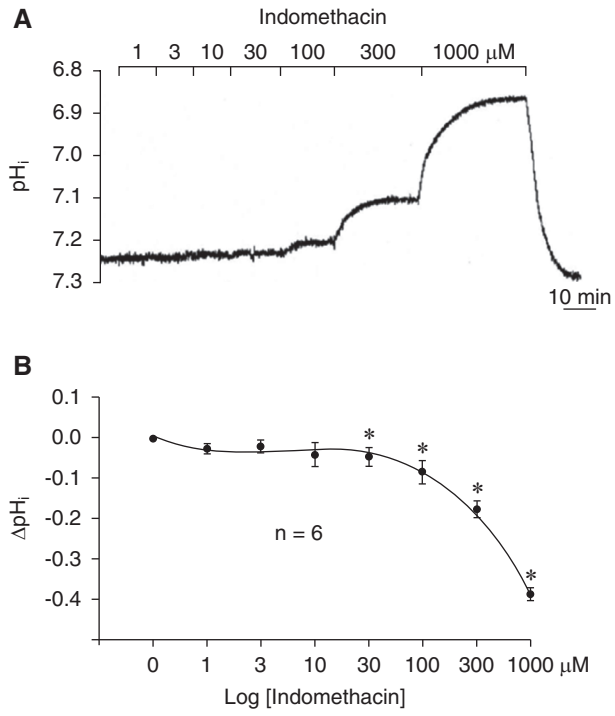

Fig. 4. Effect of indomethacin on resting pH_i in human monocytes superfused with HEPES-buffered Tyrode solution. A: The traces A represents experiment showing the effect of different concentrations of indomethacin (1~1000 μM) on pH_i recovery in HEPES-buffered Tyrode solution (pH_o = 7.4, 37°C) in human monocytes. The top bar shows the buffer system used in the superfusate. The periods of application of indomethacin (1~1000 μM) are shown with bars above the trace in human monocytes. B: Histograms, showing the change in resting pH_i averaged for 6 experiments similar to those shown in A. *: $P < 0.01$ *vs.* control.

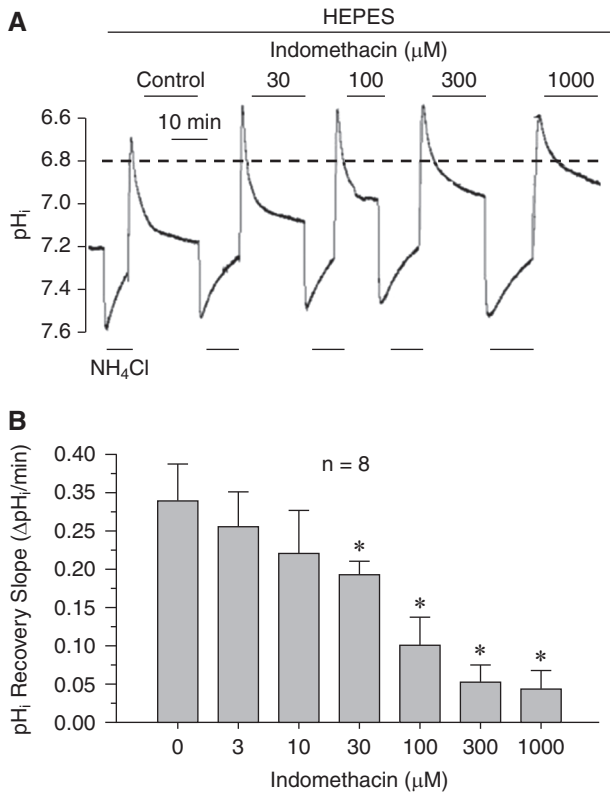

Fig. 5. Effects of indomethacin on NHE1 in human monocytes superfused with HEPES-buffered Tyrode solution. A: The top bar shows the buffer system used in the superfusate. The periods of application of NH_4Cl and indomethacin (30~1000 μM) are shown with bars above or below the trace. Traces A represents experiments showing the effect of different concentrations of indomethacin (30~1000 μM) on resting pH_i in HEPES-buffered Tyrode solution in human monocytes (pH_o = 7.4, 37°C). B: Histograms, showing the pH_i recovery slope of acid extrusion (dpH_i/min) after NH_4Cl-induced intracellular acidosis averaged for 8 experiments similar to those shown in A (measured at $pH_i = 6.79 \pm 0.04$), respectively. *: $P < 0.01$ *vs.* control.

creasing effect on the pH_i recovery slope at higher concentrations of indomethacin (from 30 μM to 1000 μM, respectively), as illustrated in the right parts of Fig. 5A. The histogram of Fig. 5B shows the mean indomethacin-induced pH_i changes on slope for 8 experiments, similar to that shown in Fig. 5B (measured at $pH_i = 6.79 \pm 0.04$; $P < 0.01$, n = 8). The results clearly show that the indomethacin-induced inhibition on NHE1 activity is concentration-dependent between the ranges of 30 and 1000 μM in HEPES-buffered superfusate in human monocytes. Note that the NHE1-independent acidifying that seen in Fig. 4A can be observed from different episodes of resting pH_i of Fig. 5A. In other words, the resting pH_i cannot be recovery back to the level of control after NH_4Cl challenge in the presence of various indomethacin concentrations. It means that the indomethacin induced intracellular acidosis involved another NHE1-independent mechanism.

In conclusion, our present study has demonstrated, pharmacologically and physiologically, that NHE1 functionally exists in human monocytes. Moreover, we found that indomethacin, under HEPES-buffered Tyrode solution, induces a concentration-dependent intracellular acidosis and caused through its summation effects of inhibition effect on NHE1 activity and NHE1-independent mechanism.

Discussion

The Functional Evidence of Acid Extruding Regulator-NHE1

Using the technique of microspectrofluorimetry, we have provided straightforward and convincing pharmacological evidence that NHE1 is functionally

responsible for acid extrusion following induced acidosis in human monocytes. NHE's activity was HCO_3^--independent and Na^+ dependent (the right part of Fig. 2A) (8, 20, 29, 30). This conclusion was confirmed by the finding that the acid extruder could be entirely blocked by HOE 694 (the right part of Fig. 3A), a highly-specific NHE-1 inhibitor (20, 32). Among 9 different members of NHE, *i.e.* NHE 1~9 (2), the NHE1 protein has been identified as an protein which ubiquitously expresses in different tissues, including heart and smooth muscle by molecular biology methods (13, 36). It has been shown that HOE 694 shows a high selectivity for cloned and expressed NHE1 that is two or more orders of magnitude higher than for the other isoforms, such as NHE 2 and 3 (9). Our present results revealed that the functioning NHE in the human monocytes was also sensitive with low concentration of HOE 694 (30 μM) (Fig. 3A). Therefore, our study suggests that NHE isoform is purely NHE1, instead of NHE 2 and NHE 3. One might ask if the present data can exclude a significant presence of other members of NHE (4-9) in human monocytes. The answer would appear to be that it can be excluded, on the grounds that data available so far for NHE 4, 5 indicate that the acid extruder is essentially related insensitive to amiloride and HOE694, and that NHE 6~9 only exists in membrane of intracellular organelles (2). Therefore, using pharmacological maneuvers, our present study has provided direct pharmacological evidence that the native NHE functioning during pH_i-regulation in the human monocytes is the NHE-1 isoform, instead of other members of NHE proteins.

Moreover, the study was performed in the absence of HCO_3^-, and therefore it may not be appropriate to speculate on the effects indomethacin on pH_i in a clinical setting. Whether indomethacin still result in acidosis under physiological conditions, *i.e.* in CO_2/HOC_3^- buffered Tyrode solution, when HCO_3^- is present and NBCs are active waits for further study.

The Implication of Inhibition Effect on NHE1 Activity of Indomethacin in Clinic

The regulation of pH_i is important for normal functions of cells (3, 14, 15, 17, 18, 22, 25). Moreover, irreversible endothelial dysfunction and vascular atherosclerosis have been claimed to be related to pH_i disturbances (3, 43). For example, NHE1 activity has been proven to play a vital role in proliferation, both in carcinogenic and non-carcinogenic cells (11, 24, 39).

NSAIDs reversibly block both isoforms of cyclooxygenase but vary in their degree of selectivity (10, 42). Indomethacin, a highly potent non-selective NSAID, is involved in several physiological and pathological processes, such as several acute and chronic pains (40), rheumatoid arthritis (45), ocular inflammation (19) and various GI disorders. Indomethacin is also implicated to increase the risk of myocardial events in a meta-analysis study (35). Apart from the potent inhibition on cyclooxygenases, the anti-inflammatory and analgesic effects of indomethacin are also achieved through a variety of mechanisms, including regulation of NHE 1 (40). The group of Roginiel *et al*. has further demonstrated that indomethacin specifically enhances proton excretion through regulation of apical NHE-3 and NHE-2 and to a lesser extent on basolateral NHE-1 and NHE-4. They suggested that clinical exposure to NSAIDs may affect colonic tissue at the site of selected NHE isoforms, resulting in modulation of transport and barrier function. Furthermore, an interesting study was reported by Kamachi *et al*. in which NHE inhibitors suppress the LPS-induced production of PGE2 in mouse macrophage cell line (23). In other words, indomethacin not only affects physiological conditions, but also change the pathological situation. Indeed, our present study has found that indomethacin induce monocyte intracellular acidosis significantly, in a concentration-dependent manner (Fig. 4). Moreover, the underlying mechanism of such indomethacin-induced intracellular acidosis is partially due to the directly inhibition effect of NHE1 activity (Fig. 5). Note that the NHE1-independent intracellular acidosis that seen in Fig. 4A can be observed from different episodes of resting pH_i of Fig. 5A, *i.e.* the resting pH_i cannot be recovery back to the level of control after NH_4Cl prepulse in the presence of various indomethacin concentrations. It means that the indomethacin induced intracellular acidosis involved another NHE1-independent mechanism and awaits further study in the future. Moreover, the effect of indomethacin on NHE activity shown in our present result is in contrast to the data shown in rat colonic crypts that reported by Roginiel *et al*. (40). Whether this difference is caused simply by sepsis difference or organ difference wait for further study. Jancic *et al*. have reported that low values of intracellular pH activate human monocytes by enhancing the production of IL-1β (21). Indomethacin is one of the main anti-inflammatory medicines that exerts various inhibition on enzymes and autacoids, such as cyclooxygenases (5) and prostaglandin (12), respectively. Since some indomethacin-induced cardiovascular adverse effects such as prothrombotic stat, are related to indomethacin-induced intracellular acidosis and inhibition on NHE1 activity (38, 47). If a change in NHE1 activity also affects indomethacin-induced impact on cyclooxygenases and prostaglandin, then the development of specific and potent NHE agonists may produce a cure for indomethacin induced-malfunction in a clinic. Therefore, determining whether these indomethacin-induced alternations to cyclooxygenases, peptides and prostaglandin is related to changes

in pH_i and NHE1 activity is worthy of future study.

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