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The $Cl^-HCO_3^-$ exchanger slows the recovery of acute pH_i changes in rat mast cells

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Abstract

The CO_2/HCO_3^- buffering system is one of the main mechanisms implicated in cytosolic pH (pH_i) regulation. We studied this pH_i-regulatory system in rat mast cells using a fluorescent dye. Mast cells had a more alkaline pH_i in the presence of HCO_3^- than in its absence. The recovery from an acid load was faster in HCO_3^--free conditions than in HCO_3^--containing media. In HCO_3^--buffered conditions the increase of the recovery rate of an acidification in 4,4′-diisothiocyanostilbene-2,2′-disulfonic acid-incubated cells suggested the implication of a Na^+-independent Cl^-/HCO_3^- exchanger. This HCO_3^- transport acidified the cytosol and was also partially responsible for the recovery of intracellular alkalinizations. Moreover, regulation of the recovery rate of an acidification by protein kinase C and calcium signaling pathways depended on the presence or absence of HCO_3^-. The presence of HCO_3^- limits the recovery of acute intracellular acidifications probably through the Na^+-independent Cl^-/HCO_3^- exchanger and modulates the regulation of pH_i by protein kinase C and calcium.

Keywords: Mast cell; Intracellular pH; DIDS; Bicarbonate; Intracellular acidification; Intracellular alkalinization

1. Introduction

Mast cells have a different sensitivity to several stimuli in the presence or absence of HCO_3^- [1]. This fact could be related to some pathologic processes, such as exercise allergy, since this pathology is related to mast cell degranulation [2] and the development of an exercise-induced anaphylactic shock is prevented by pretreatment with sodium bicarbonate [3]. Our recent work demonstrates that intracellular pH (pH_i) is an important intracellular signal for degranulation in mast cells [4]. Thus HCO_3^- ions, which have been demonstrated to modulate mast cell function [5,6], can be related to some pathology through its role on pH_i regulation.

Regarding pH_i regulation, mast cells are endowed with a Na^+/H^+ exchanger, which is the main mechanism responsible for H^+ extrusion [7,8]. The presence of the Na^+-independent Cl^-/HCO_3^- exchanger has been recently demonstrated in peritoneal rat mast cells [5], which acidifies the cytosol in normal conditions and works in a reverse mode when ion gradients are changed [1,5]. However, the Na^+-dependent Cl^-/HCO_3^- exchanger and the Na^+-HCO_3^- cotransporter are absent in serosal rat mast cells [1]. Additionally, in rat mast cells the regulation of pH_i, as recovery of an intracellular acidification, is modulated either by the PKC pathway or by the intracellular Ca^{2+} signal, two important signaling pathways in mast cell function [9,10].

HCO_3^-, which is always present in physiological conditions, plays an important role in the regulation of exocytosis and calcium signaling in rat mast cells [6]. In this work, we study how the presence or absence of HCO_3^- affect the regulation of acute pH_i changes, since cell stimulation is often followed by pH_i changes. We also present further experimental evidences for a HCO_3^--dependent pH_i regulation in serosal rat mast cells, which support previous published results [1,5].

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Abbreviations: BCECF, 2′,7′-bis(carboxyethyl)-5-(6)carboxy fluorescein; DIDS, 4,4′-diisothiocyanostilbene-2,2′-disulfonic acid; $[H^+]_o$, H^+ extracellular concentration; $[H^+]_i$, H^+ intracellular concentration; $[Na^+]_i$, Na^+ intracellular concentration; $[Na^+]_o$, Na^+ extracellular concentration; pH_i, cytosolic pH; pH_o, external pH; PKC, protein kinase C.

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2. Methods

2.1. Chemicals and solutions

BCECF/AM (2,7-bis(carboxyethyl)-5(6) carboxy-fluorescein-acetoxymethyl ester) was purchased from Molecular Probes. Sodium propionate, DIDS and PMA (phorbol 12-myristate 13-acetate) were from Sigma Chemical Co. Thapsigargin was from Alexis and Percoll® was from Pharmacia.

The HCO_3^--buffered medium contains (in mM): Na^+, 142.3; K^+, 5.94; Ca^{2+}, 1; Mg^{2+}, 1.2; Cl^-, 126.1; HCO_3^-, 22.85; $H_2PO_4^-$, 1.2 and SO_4^{2-}, 1.2. The pH was adjusted by bubbling 5% CO_2. The composition of the HCO_3^--free solution was (in mM): Na^+, 146.89; K^+, 5.94; Ca^{2+}, 1; Mg^{2+}, 1.2; Cl^-, 125.94; $H_2PO_4^-$, 7.70; HPO_4^{2-}, 12.49 and SO_4^{2-} 1.2. The pH was adjusted by adding NaOH. Glucose (5.55 mM) was added to the solutions and their pH was adjusted to 7.30. Mast cell purification was performed with a HCO_3^--buffered solution containing BSA 1 mg/mL adjusted to pH 7.20 with HCI.

2.2. Mast cell isolation and purification

Mast cell isolation and purification were performed as previously described [1].

2.3. pH_i measurements

Purified mast cells were incubated in the purification solution with 1 μM BCECF/AM for 20 min at 37° and then washed twice with a BSA-free purification solution. Mast cells were resuspended at 400,000 cells/mL in a quartz cuvette with continuous stirring. Experiments were performed at 37° in a Perkin-Elmer LS-50 B spectrofluorometer with automatic calculation of the ratio values for excitation at 500/440 nm and emission at 530 nm. A calibration curve was obtained using nigericin in a K^+ solution as per Thomas *et al.* [11]. Additionally, pH_i was also measured with ratio imaging microscopy in experiments where the extracellular medium was changed. The imaging equipment consisted of a Nikon DIAPHOT 200 inverted microscope with a 40× immersion objective, two computer controlled filterwheels and an integration camera (Life Science Resources). The light source was a 175 W xenon lamp with optic fiber. Excitation was at 490/440 nm and emission at 535 nm. In this case, mast cells were incubated with 50 nM BCECF/AM for 10 min, since in the previous loading conditions the fluorescence saturated the signal received by the camera of the imaging system. Several experiments performed in both systems gave similar results. Mast cells were attached to the cover glasses by letting them to settle over a 0.001% polylysine bed during 10 min. In these experiments, an average of approximately 40 cells were recorded at the same time. All experiments were carried out at 37°.

2.4. Analysis of the recovery of a cytosolic acidification

A cytosolic acidification was induced by addition of sodium propionate to the extracellular medium as per Livne *et al.* [12] from which cells recover at a characteristic rate. The method of Guggenheim [13], which does not require to know the magnitude of the initial acidification, was employed to determine the velocity constants of the recovery of the acid load. As described before [8], $\log(v'_i - v_i)$ was plotted against time (in min), v_i being pH_i at time i. The slope of the lineal regression is $-k \log_{10} e$, where k is the velocity constant (pH units/min) of recovery.

2.5. Estimation of intracellular buffering power

The intrinsic buffering power of mast cells was calculated as described [14] according to Eq. (1). Five millimolar NH_4Cl was added to BCECF-loaded mast cells suspended in a HCO_3^--free solution. The instantaneous pH_i increment was recorded:

$$\beta_i = \frac{\Delta[NH_4^+]_i}{\Delta pH_i} \quad (1)$$

where β_i is the intrinsic buffering power, ΔpH_i is the recorded pH_i increment, and $\Delta[NH_4^+]_i$ was calculated from $[NH_3]_i$ (Eq. (2)) assuming a pK_a of 9.21 [15].

$$[NH_4^+]_i = [NH_3]_i \times 10^{pK_a - pH_i} \quad (2)$$

Since NH_3 is considered to be in equilibrium across the plasma membrane, $[NH_3]_i$ was considered equal to $[NH_3]_o$, which was calculated according to Eq. (3) [16]:

$$[NH_3]_o = [NH_4^+]_o \times 10^{pK_a - pH_o} \quad (3)$$

The intrinsic buffering power was estimated at different pH_i values. The pH_i of mast cells was manipulated by incubation at several pH_o, values (Fig. 1C, inset).

In HCO_3^--buffered solutions, the total buffering power (β_t) of mast cells is the sum of β_i and β_{CO_2}, which is the buffering power of intracellular CO_2/HCO_3^-. β_{CO_2} was calculated from Eq. (4) [16]:

$$\beta_{CO_2} = 2.3 \times [HCO_3^-]_i \quad (4)$$

where $[HCO_3^-]_i$ is calculated from the actual values of $[HCO_3^-]_o$, pH_i and pH_o, according to Eq. (5) [17]:

$$[HCO_3^-]_i = [HCO_3^-]_o \times 10^{pH_i - pH_o} \quad (5)$$

β_{CO_2} was calculated for each pH_i value recorded in the experiments.

2.6. Calculation of H^+ efflux rate

H^+ efflux rate was calculated to measure the pH_i dependence of acid extrusion. H^+ efflux is equal to the product of intrinsic cellular buffering power (β_i) and the rate of pH_i recovery per minute ($\Delta pH_i/\Delta t$) in a HCO_3^--free medium.

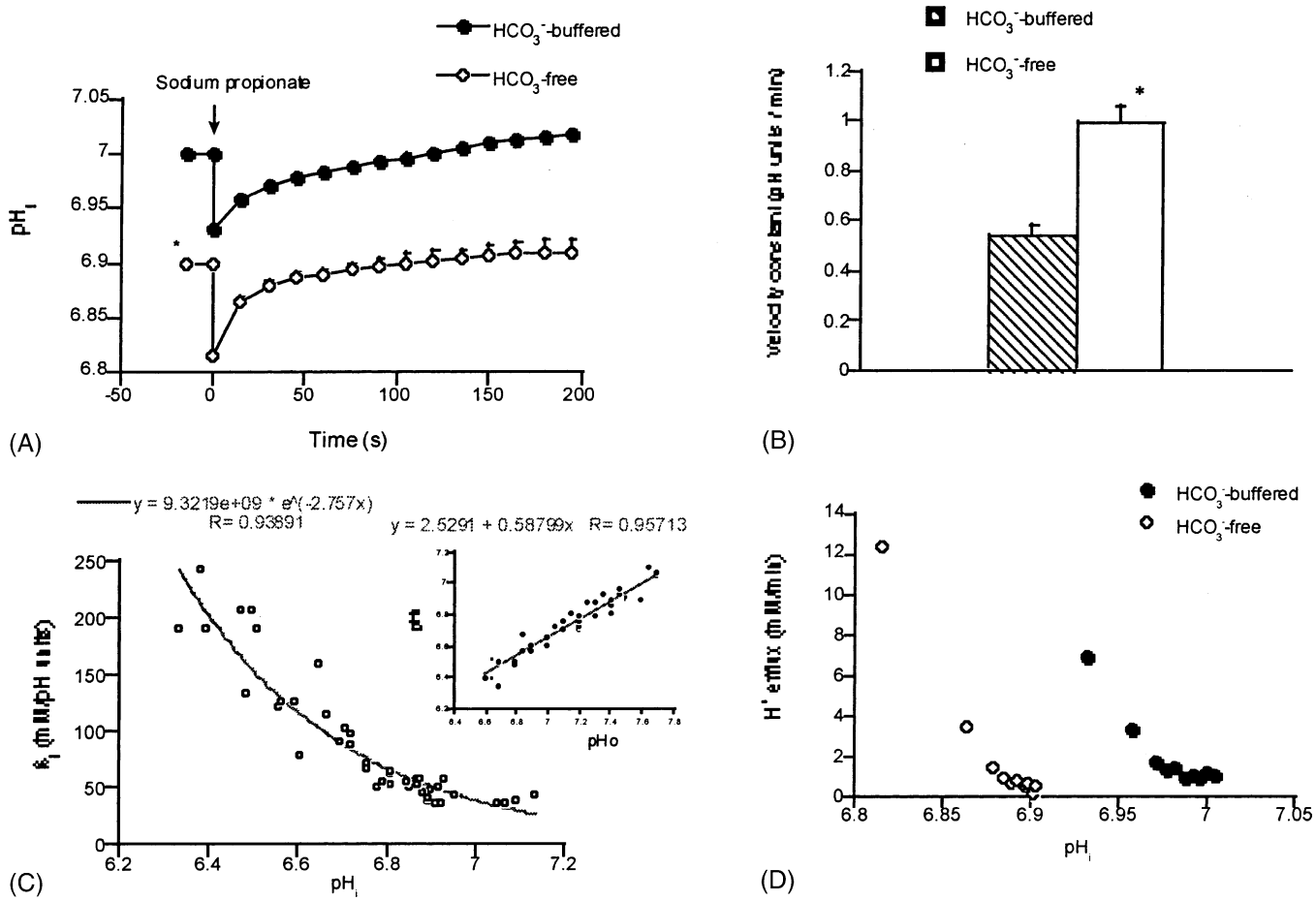

Fig. 1. Intracellular acidification by 20 mM sodium propionate in BCECF-loaded rat mast cells in the presence or absence of HCO_3^- at pH_o 7.30. (A) Mast cells were suspended in a HCO_3^--buffered or a HCO_3^--free medium, and when the pH_i base line was stable, 20 mM sodium propionate was added to the medium (N = 4). (B) Velocity constant of the recovery of the propionate-induced intracellular acidification in a HCO_3^--buffered or a HCO_3^--free medium. (C) Intrinsic buffering power (β_i) of serosal rat mast cells. β_i was calculated for a range of pH_i from 6.38 to 7.13. β_i values were plotted vs. pH_i and adjusted to an exponential equation. pH_i was manipulated by incubating mast cells at different pH_o (inset). (D) H^+ efflux rates were calculated from pH_i data shown in A as described in Section 2. β_i was calculated from equation in C (*, significant differences).

In a HCO_3^--buffered medium H^+ efflux is equal to the product of total buffering power (β_t), calculated as the sum of β_i and β_{CO_2}, and the rate of pH_i recovery per minute ($\Delta pH_i/\Delta t$).

2.7. Statistical analysis

All the experiments were carried out at least three times in duplicate. Results were analyzed using the Student's *t*-test for unpaired data. A probability level of 0.05 or smaller, was used for statistical significance. Results were expressed as the mean ± SEM.

3. Results

In order to study the role of HCO_3^--dependent mechanisms in the recovery of acute pH_i changes, we firstly measured the recovery rate of an acute intracellular acidification in BCECF-loaded mast cells in the presence and absence of external HCO_3^-. BCECF-loaded mast cells were suspended in a HCO_3^--buffered medium or in a HCO_3^--free medium. When mast cells reached a steady-state pH_i, 20 mM sodium propionate was added to the medium. Mast cells have a significantly lower pH_i in a HCO_3^--free medium than in a HCO_3^--buffered medium at pH_o 7.20 (6.91 ± 0.02 and 7.00 ± 0.02, respectively). The addition of sodium propionate to the medium induced a fast intracellular acidification both in the presence and absence of external HCO_3^-. pH_i started to recover reaching initial pH_i basal values in approximately 200 s (Fig. 1A). The propionate-induced intracellular acidification was greater, although not statistically different, in a HCO_3^--free medium than in a HCO_3^--buffered medium (0.083 ± 0.006 and 0.067 ± 0.004, respectively). In order to compare the recovery rate of this propionate-induced intracellular acidification in HCO_3^--free vs. HCO_3^--containing conditions, a velocity constant was calculated for the recovery of the intracellular acidification in each experiment. Calculation was as described in Section 2 and the mean ± SEM is represented in Fig. 1B. The velocity constant was 0.542 ± 0.044 pH units/min in a

HCO_3^--buffered medium and 0.987 ± 0.073 pH units/min in a HCO_3^--free medium (Fig. 1B). H^+ efflux rate was also calculated for data in Fig. 1A to compare the recovery of the intracellular acidification. β_i was calculated for every pH_i value using the equation in Fig. 1C. In order to get this equation, mast cells were suspended in a HCO_3^--free medium at different pH_o ranging from 6.60 to 7.70, after a steady pH_i was reached (pH_i basal values from 6.38 to 7.13), an intracellular alkalinization was induced by addition to the extracellular medium of 5 mM NH_4OH. Initial pH_i and peak pH_i immediately after NH_4OH addition were used to calculate β_i for 44 different pH_i recordings and the β_i values obtained were plotted vs. initial pH_i in Fig. 1C. The H^+ efflux rate was higher in a HCO_3^--free medium than in a HCO_3^--buffered medium (Fig. 1D). The results suggest that HCO_3^- ions have a role in the regulation of the steady-state pH_i and in the recovery of acid loads in rat mast cells. However, the H^+ efflux rate obtained in both media are not completely comparable, because the basal pH_i and the pH_i drop upon additon of sodium propionate, are different in the presence and absence of HCO_3^-. These differences are due to an increase of the intracellular buffering power by intracellular HCO_3^- [18,19].

The Na^+/H^+ exchanger is activated by an intracellular acidification. In order to check if the higher H^+ efflux in a HCO_3^--free medium is really due to the absence of HCO_3^- and not to the lower basal pH_i (significant differences) or the higher pH_i drop (non significant differences), we studied the recovery of an intracellular acidification in mast cells with the same conditions of basal pH_i and pH_i drop both in a HCO_3^--buffered medium and a HCO_3^--free medium. We obtained the same pH_i in mast cells suspended in a HCO_3^--buffered medium at pH_o 7.25 and in a HCO_3^--free medium adjusted to pH_o 7.48. The pH_i drop was induced by 20 mM sodium propionate in a HCO_3^--buffered medium and by 17 mM sodium propionate in a HCO_3^--free medium. The recovery of the acid load was faster in a HCO_3^--free medium than in a HCO_3^--buffered medium (Fig. 2A). H^+ efflux rate was also higher in a HCO_3^--free medium than in a HCO_3^--buffered medium (Fig. 2B). Although Fig. 1D seems to indicate that the acid extrusion mechanism is more sensitive to the intracellular $[H^+]$ in the presence than in the absence of HCO_3^-, it is important to take into account that the recovery of pH_i values is related to the initial resting pH_i in each experiment. This would explain why the H^+ efflux rate in a HCO_3^--buffered medium at pH_i 6.95 is 4-fold higher than in a HCO_3^--free medium at pH_i 6.9 (Fig. 1D), since in the first case the recovery is halfway to pH_i basal values and in the second case the pH_i has completely recovered to basal values. However, when the conditions of initial resting pH_i are equal for both media (Fig. 2B), the determining factor for the difference in H^+ efflux rate seems to be HCO_3^-. However, the difference of pH_o, in Fig. 2 (7.25 and 7.48) could provide a smaller driving force ($[H^+]$ gradient) for the HCO_3^--buffered cells than the HCO_3^--free cells.

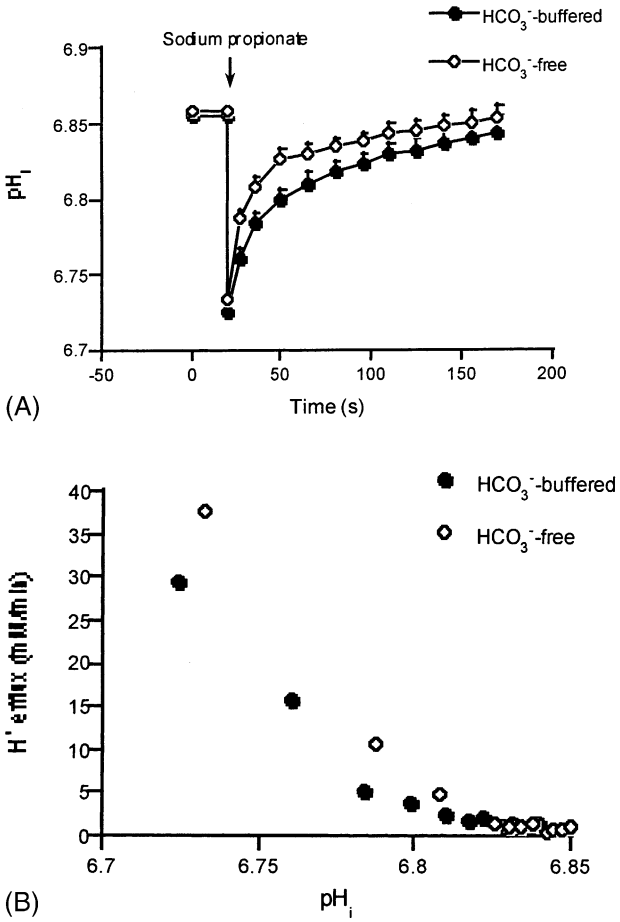

Fig. 2. Intracellular acidification by 20 mM sodium propionate in a HCO_3^--buffered medium and 17 mM in a HCO_3^--free medium in BCECF-loaded rat mast cells at the same pH_i values. (A) Mast cells were suspended in a HCO_3^--buffered or a HCO_3^--free medium, and when mast cells reached a steady pH_i, 20 and 17 mM sodium propionate was added to the medium, respectively (media ± SEM, N = 3). (B) H^+ efflux rates were calculated from pH_i data shown in A as described in Section 2. β_i was calculated from equation in Fig. 1C.

However, the $[H^+]$ gradient ($[H^+]_i/[H^+]_o$) in mast cells suspended in HCO_3^--free medium at pH_o 7.48 and a HCO_3^--buffered medium at pH_o 7.25 is 4.16 and 3.34, respectively. That is a small difference if we consider that the driving force of the Na^+/H^+ exchanger, the main mechanism responsible for the recovery of a cytosolic acidification in mast cells, is the $[Na^+]$ gradient, with a value of around 14 ($[Na^+]_o/[Na^+]_I$). Therefore, we do not believe this small difference in the $[H^+]$ gradient is important in determining a different velocity of recovery, since pH_i is the main regulator of the activity of the Na^+/H^+ exchanger and in this experiment it is identical in both conditions. Overall these results demonstrate that the presence of HCO_3^- reduces the rate of recovery of intracellular acidifications in mast cells.

In the next set of experiments, we used the anion-exchanger inhibitor DIDS to study the role of the Cl^-/HCO_3^- exchanger in the recovery of acute acidifications in rat mast cells. We followed the same experimental

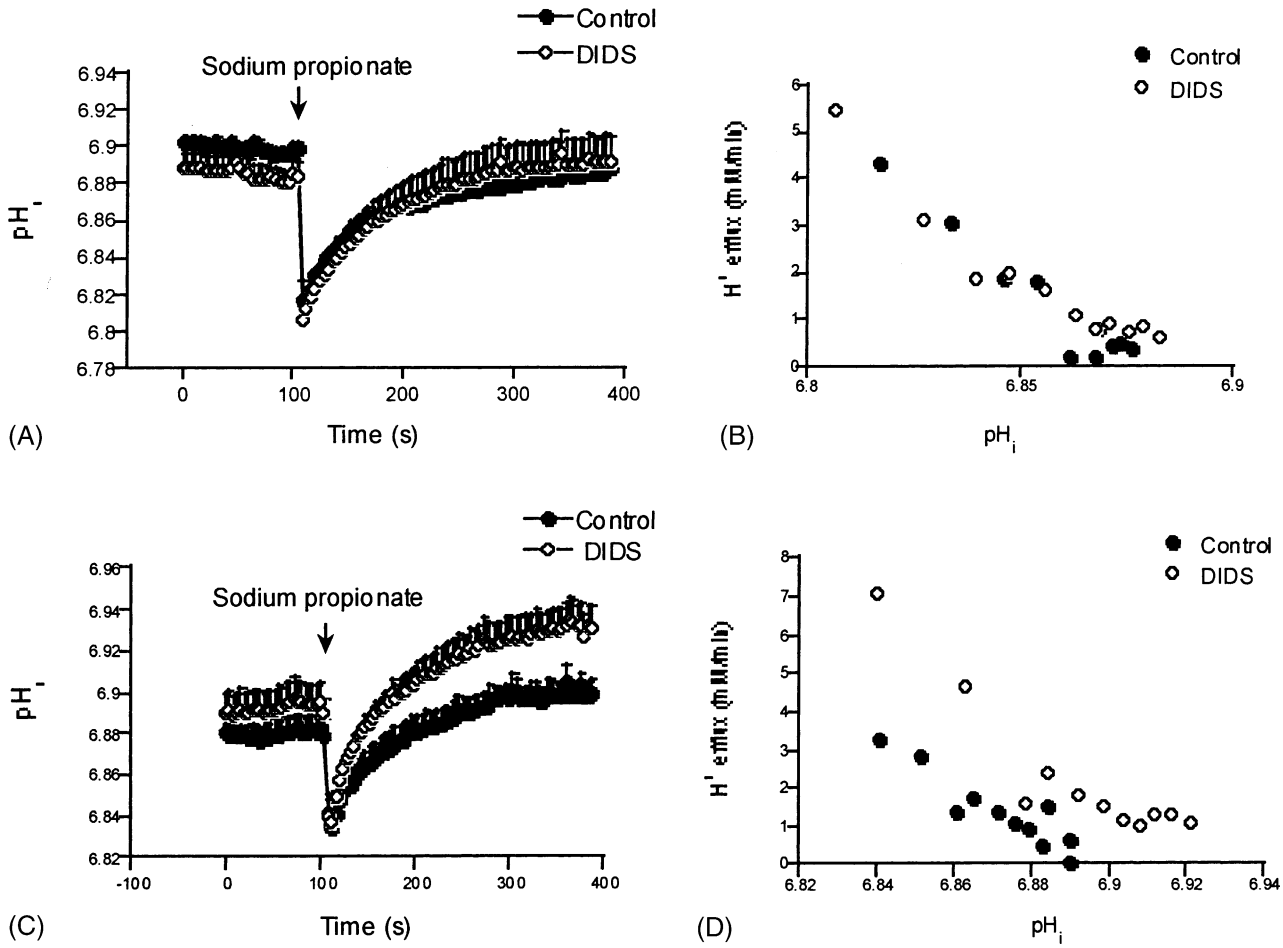

Fig. 3. Effect of DIDS on the recovery of a propionate-induced acidification in the presence and absence of HCO_3^- (A) BCECF-loaded mast cells were preincubated in a HCO_3^--free medium for 5 min with 300 μM DIDS and then 20 mM sodium propionate was added. (B) H^+ efflux rates were calculated from pH_i data shown in A as described in Section 2. β_i was calculated from equation in Fig. 1C. (C) BCECF-loaded mast cells were preincubated in a HCO_3^- buffered medium for 5 min with 300 μM DIDS and then 20 mM sodium propionate was added. (D) H^+ efflux rates were calculated from pH_i data shown in C (media ± SEM, N = 3).

procedure as before, but now a 5-min preincubation with 300 μM DIDS preceded the addition of 20 mM sodium propionate. Control mast cells followed the same time course but in the absence of DIDS. As it was expected, in a HCO_3^--free medium, DIDS had no effect on the steady-state pH_i or on the recovery from an intracellular acidification in mast cells (Fig. 3A), since the Cl^-/HCO_3^- exchangers are already inhibited by the absence of HCO_3^-. Accordingly, in a HCO_3^--free medium H^+ efflux rate was similar in the presence and absence of DIDS (Fig. 3B). On the contrary, in a HCO_3^--buffered medium the recovery of an acid load in DIDS-preincubated mast cells was enhanced vs. control cells (Fig. 3C), although it had no effect on the initial, resting pH_i. Additionally, in a HCO_3^--buffered medium control mast cells recover their pH_i to initial basal values, but in the presence of DIDS the final pH_i is significantly higher than initial basal values (Fig. 3C). Regarding final pH_i, in both HCO_3^--buffered and HCO_3^--free conditions, the difference between them is clear, although there are no significant differences due to the experimental variability. Moreover, the H^+ efflux rate was clearly higher in DIDS-preincubated cells than in control cells in a HCO_3^--buffered medium (Fig. 3D).

The results suggest the presence of a HCO_3^--dependent exchanger or cotransporter that works as an acidifing mechanism. Thus, we studied its role in the recovery of an acute cytosolic alkalinization induced by 5 mM NH_4Cl. In control cells bathed by a HCO_3^--buffered medium, the pH_i started to recover slowly after the alkalinization, but it did not reach the initial pH_i basal values (Fig. 4A). However, 300 μM DIDS-preincubated mast cells were unable to recover the NH_4^+-induced intracellular alkalinization (Fig. 4B). DIDS had no effect on the recovery of a NH_4^+-induced intracellular alkalinization in a HCO_3^--free medium (data not shown). Moreover, the velocity constant of recovery of the NH_4^+-induced alkalinization, which was calculated from experiments carried out in a spectrofluorometer with continuous stirring in order to induce faster pH_i changes, was 0.88 ± 0.28 pH units/min in a HCO_3^--free medium and 1.90 ± 0.11 pH units/min in a HCO_3^--buffered medium (significant differences). The absence of HCO_3^- ions in the medium did not completely

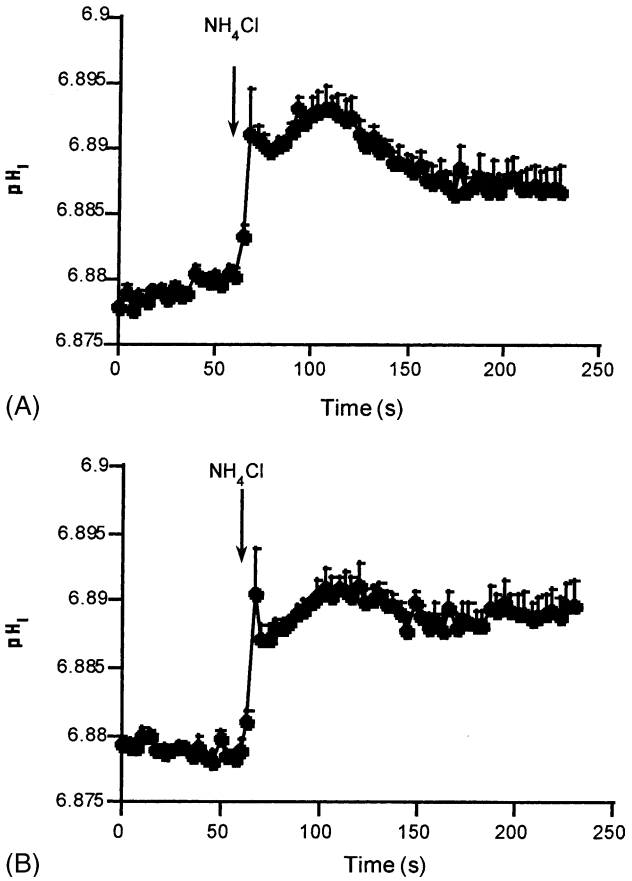

Fig. 4. Effect of DIDS on the recovery of an amonium-induced alkalinization. BCECF-loaded mast cells were bathed with a HCO_3^--buffered solution, after a steady base line was reached, 5 mM NH_4Cl was added to the medium either in (A) the absence of DIDS or (B) after a 5-min incubation with DIDS (media ± SEM, N = 3).

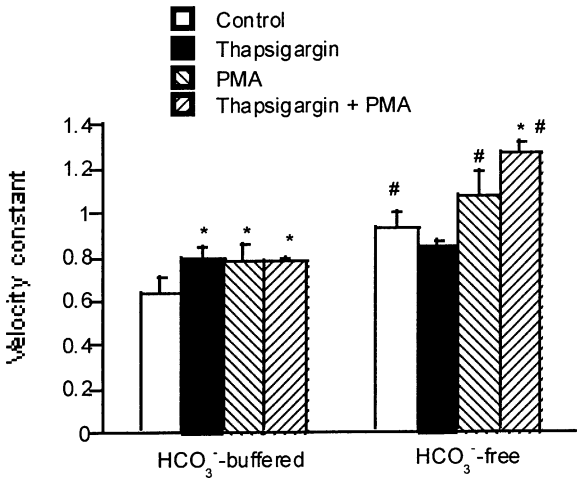

Fig. 5. Effect of thapsigargin and PMA on the velocity constant of the recovery of a propionate-induced intracellular acidification in the presence and absence of HCO_3^-. Mast cells were suspended in a HCO_3^--buffered or a HCO_3^--free medium and preincubated with thapsigargin, PMA or both for 5 min in the presence of 1 mM external Ca^{2+}. Then 20 mM sodium propionate was added to the medium and the velocity constant of recovery was calculated for each experiment. (media ± SEM, N = 4) (*, significant differences vs. control; # significant differences HCO_3^--free vs. a HCO_3^--buffered medium).

inhibit the recovery of an intracellular alkalinization (data not shown), as it did DIDS. This only partial inhibition in a HCO_3^--free medium could be due to HCO_3^- ions generated by cellular metabolism which have been demonstrated to participate in pH_i regulation [20].

In mast cells, the recovery of pH_i changes is regulated by the PKC pathway and the intracellular Ca^{2+} signal. PMA induces an increase of the recovery rate of an intracellular acidification, presumably through a Na^+/H^+ exchanger activation [9]. Regarding the Ca^{2+} signal, the intracellular Ca^{2+}-ATPase inhibitor thapsigargin [21] also increases the recovery rate of an intracellular acidification [9]. Therefore, we compared the effects of both drugs on the recovery of an acidification in the presence and absence of HCO_3^-. A 5-min preincubation with 100 ng/mL PMA, 0.05 μM thapsigargin, or both, preceded the addition of 20 mM sodium propionate. Neither PMA nor thapsigargin modified the basal pH_i of rat mast cells when added to the medium (data not shown). The phorbol ester PMA induced an increase of the velocity of recovery in a HCO_3^-,-buffered medium, but its effect was not significant in a HCO_3^--free medium (Fig. 5). On the other hand, thapsigargin increased the recovery rate in a HCO_3^--buffered solution but slightly decreased it in a HCO_3^--free medium (Fig. 5). Finally, the combination of both drugs increased the velocity constant to the same value as thapsigargin or PMA alone in a HCO_3^--buffered solution. However, in the absence of HCO_3^- thapsigargin and PMA together caused an increase of the recovery rate to higher values than PMA or thapsigargin alone (Fig. 5). Thus, the presence of HCO_3^- modifies the regulation of pH_i by PKC and the Ca^{2+} signal.

4. Discussion

In serosal rat mast cells, basal pH_i values and the recovery of acute pH_i changes are modulated by the presence of HCO_3^- in the external medium. HCO_3^- ions exert an alkalinizing effect on basal pH_i, as it was previously reported for rat mast cells [1] and RBL-2H3 mast cells [22]. Regarding the recovery of an acute intracellular acidification, mast cells suspended in a HCO_3^--buffered medium have a lower velocity of recovery and a lower H^+ efflux rate than mast cells suspended in a HCO_3^--free medium. This effect of HCO_3^- that reduces the speed of pH_i alkalinization after a sudden exogenous acidification, was shown at the same pH_o and also at the same pH_i values. According to our results, the mechanism responsible for this reduction in the rate of alkalinization is HCO_3^--dependent and DIDS-sensitive and works as a cytosol acidifier. The HCO_3^- transporter present in mast cells that matches all these characteristics is the Na^+-independent Cl^-/HCO_3^- exchanger or Cl^-/HCO_3^- exchanger, since the Na^+-HCO_3^- cotransporter and the Na^+-independent Cl^-/HCO_3^- exchanger are not present in mast cells [1].

However, there is no specific inhibitor of the Cl^-/HCO_3^- exchanger so another unknown HCO_3^- transporter or channel could be responsible for this effect of HCO_3^-on pH_i recovery. This HCO_3^- transport works as an acid loader at the same time that the main mechanism responsible for acid extrusion in mast cells, the Na^+/H^+ exchanger [7,10], recovers the pH_i to basal values. Moreover, it is implicated in the maintenance of basal pH_i, since its inhibition by DIDS sets a new steady-state pH_i value after an acute acidification in mast cells suspended in a HCO_3^--buffered medium.

A HCO_3^- transport is also responsible for the recovery of a NH_4^+-induced intracellular alkalinization in mast cells in a HCO_3^--buffered medium, since this recovery is inhibited by DIDS and by the absence of HCO_3^- in the bathing solution. Additionally, as the intracellular alkalinization is not completely recovered, we suggest that there must be another mechanism responsible for the complete recovery of pH_i. The Cl^-/HCO_3^- exchanger has been previously described in rat peritoneal mast cells [1,5], and it was demonstrated to play an important role in basal pH_i determination [5], as the present work also suggests. We present evidence that a HCO_3^- transporter, probably the Cl^-/HCO_3^- exchanger, works as an intracellular acidifier not only during the recovery of an intracellular alkalinization but also during the recovery of an intracellular acidification.

Since the regulation of pH_i is modulated by the intracellular calcium signal and the PKC in rat mast cells [9] we also studied the effect of HCO_3^- on this modulation. We previously knew that the activation of the PKC pathway with PMA and the activation of the Ca^{2+} signal with thapsigargin increase the rate of recovery of an intracellular acidification in the presence of HCO_3^- [9]. The effects of both drugs were not additive. But in a HCO_3^--free medium neither thapsigargin nor PMA have statistically significant effects on the recovery rate of an intracellular acidification. The most interesting difference between the presence and absence of HCO_3^- was found when mast cells were simultaneously stimulated with PMA and thapsigargin. In the absence of HCO_3^- the addition of thapsigargin and PMA together potenciates the recovery rate over PMA or thapsigargin alone, although this potenciation was not observed in the presence of HCO_3^-. This effect of PKC and Ca^{2+} signal is probably due to an increase of the Na^+/H^+ exchanger activity, which has been previously reported in different cellular models [23,24] including mast cells [9], and not to a direct regulation of HCO_3^- transporters. HCO_3^- clearly modifies the effect of PKC and Ca^{2+} on pH_i regulation. Therefore, HCO_3^- is implicated in pH_i regulation not only through HCO_3^- transport but also by modifing the effect of signaling pathways. We could speculate that the change of the intracellular ionic composition may affect the activity of the enzymes or the interactions between molecules involved in these signaling pathways and, thus, their effect on pH_i regulation. Although the mechanism of this triple interaction is unknown, it could have important functional implications, since PKC, the intracellular Ca^{2+} signal and intracellular pH are implicated in the signaling of mast cell exocytosis [4,25,26].

In summary, this work yields more evidence of the presence of a HCO_3^- transport mechanism, probably the Cl^-/HCO_3^- exchanger, that continues acidifying the cytosol even after an acute intracellular acidification. Its function cause the recovery of a sudden intracellular acidification to be slower, so as to soften and reduce cellular distress. Moreover, the presence of HCO_3^- changes the regulation of pH_i by PKC and calcium, which may have important functional consequences.

Acknowledgments

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