Cytosolic pH regulation in mouse macrophages

Characteristics of HCO_3^- -dependent mechanisms

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Mechanisms regulating cytosolic pH (pH_i) in adherent resident mouse macrophages have been characterized by use of the pH-sensitive fluorescent probe 2',7'-bis(carboxyethyl)-5(6)-carboxyfluorescein (BCECF). Na^+/H^+ exchange was activated after an acid load of the macrophage cytosol. However, when Na^+/H^+ exchange was the only pH_i -regulatory mechanism operative, recovery did not proceed beyond a pH_i of approx. 6.6. The mechanisms found to be operative at physiological pH_i levels were alkalinizing Na^+ -dependent and acidifying Na^+ -independent $\text{Cl}^-/\text{HCO}_3^-$ exchangers and a H^+ -ATPase further characterized in the accompanying paper [Tapper & Sundler (1992) *Biochem. J.* 281, 245–250]. Acid extrusion via $\text{Na}^+/\text{Cl}^-/\text{HCO}_3^-$ exchange was demonstrated by the dependence on external Na^+ and HCO_3^- and on internal Cl^- and by the sensitivity to 4-acetamido-4'-isothiocyanatostilbene-2,2'-disulphonic acid (SITS) and 4,4'-di-isothiocyanatostilbene-2,2'-disulphonic acid (DIDS). By monitoring pH_i changes upon Cl^- removal and re-addition, the pH-dependence and sensitivity to SITS were found to differ for the alkalinizing and the acidifying $\text{Cl}^-/\text{HCO}_3^-$ exchangers.

INTRODUCTION

Rapid advance has been made regarding our understanding of the mechanisms by which mammalian cells maintain and regulate their cytosolic pH (pH_i) [1–3]. This is in part due to new techniques for pH_i measurement, in particular a fluorescent pH indicator [4,5], but is also a result of the growing awareness of their importance as regulatory mechanisms for metabolic and other cellular processes [3,6–8]. The best characterized proton-translocating mechanism described to date is Na^+/H^+ exchange [2,9], which is present in nearly all cell types investigated. Lately, the importance of mechanisms other than Na^+/H^+ exchange for the maintenance of pH_i has been realized [10–13]. Among these are several alkalinizing mechanisms that utilize HCO_3^- but differ in regard to their dependence on other ionic species, i.e. Na^+ and/or Cl^- . In addition to Na^+/H^+ exchange, most cell types possess one of these and also acidifying $\text{Cl}^-/\text{HCO}_3^-$ exchange.

We have recently found a regulatory role for pH_i in lysosomal enzyme secretion from macrophages [14]. When macrophages reside in an acidic environment, such as certain inflammatory foci [15] and solid tumours, special demands may be placed upon their pH_i regulation. Only Na^+/H^+ exchange and a mechanism identified as a H^+ -translocating ATPase, sensitive to inhibitors of vacuolar-type H^+ -ATPase, have been described in macrophages [13,16,17], but the presence of HCO_3^- -dependent mechanisms in this cell type has not previously been reported. The accompanying paper [18] focuses on H^+ -ATPase-mediated pH_i regulation, whereas the present paper deals with the contribution of the various mechanisms to pH_i regulation in macrophages, with special emphasis on the presence and characteristics of HCO_3^- -dependent mechanisms.

MATERIALS AND METHODS

Materials

BCECF and its tetra-acetoxymethyl ester (BCECF-AM) were purchased from Molecular Probes, Eugene, OR, U.S.A. Hepes and NEM were obtained from Merck. Amiloride, DIDS, SITS,

nigericin, choline and gluconate salts, *N*-methyl-D-glucamine and D-gluconic acid lactone were from Sigma. All materials for cell culture were purchased from Flow Laboratories.

Experimental agents were dissolved in either dimethyl sulphoxide (BCECF and amiloride) or water and were added to the experimental media in a volume not exceeding 0.5 %. When used in Cl^- -free solutions, amiloride was dissolved in water and passed through an ion-exchange column to yield a HCO_3^- salt, which was added to the experimental media in a volume never exceeding 5 %. When cells were acid-loaded by preincubation with amine, osmotic compensation by decreasing the concentration of the major salt in the medium was performed.

Experimental media

Na^+ -based solution with nominal HCO_3^- (Na-medium) contained: NaCl, 127 mM; KH_2PO_4 , 1.2 mM; KCl, 5.4 mM; MgSO_4 , 0.8 mM; CaCl_2 , 1.8 mM; glucose, 5.6 mM; Hepes, 10 mM. Adjustment of pH to the indicated value was performed at 37 °C.

In Na^+ -free solution (Ch-medium) NaCl was replaced by choline chloride.

In Cl^- -free solution (Glu-medium) all chloride salts were replaced by their gluconate counterparts.

In Na^+ - and Cl^- -free solution (NMGGlu-medium) NaCl was replaced by *N*-methyl-D-glucamine and D-gluconic acid lactone and was otherwise identical with Glu-medium.

Solution based on presumed intracellular ionic composition (K-medium) contained: KCl, 127 mM; NaH_2PO_4 , 1.2 mM; NaCl, 5.4 mM; otherwise identical with Na-medium.

Bicarbonate-containing solutions (Na/ HCO_3^- -medium, Ch/ HCO_3^- -medium, Glu/ HCO_3^- -medium and NMGGlu/ HCO_3^- -medium) were identical with their bicarbonate-free counterparts except for replacement of 18.5 mM of the major salt by NaHCO_3 or choline HCO_3 and omission of Hepes. Adjustment and maintenance of pH in bicarbonate-containing solutions was performed by equilibration with appropriate $p\text{CO}_2$ at 37 °C. The pH attained was verified by pH-electrode and also by comparing the fluorescence ratio of BCECF in medium devoid of bicarbonate with that obtained in the CO_2 -equilibrated solution. Also,

Abbreviations used: BCECF-AM, 2',7'-bis(carboxyethyl)-5(6)-carboxyfluorescein tetra-acetoxymethyl ester; DIDS, 4,4'-di-isothiocyanatostilbene-2,2'-disulphonic acid; NEM, *N*-ethylmaleimide; pH_e , extracellular pH; pH_i , cytosolic pH; SITS, 4-acetamido-4'-isothiocyanatostilbene-2,2'-disulphonic acid.

during experiments, media were maintained in equilibrium with a gas phase of appropriate $p\text{CO}_2$.

Degassed media were prepared by degassing nominally bicarbonate-free solutions under vacuum for 2 h, after which they were kept under argon. Before and during experiments, the media were bubbled with O_2/N_2 (21:79).

Macrophage culture and measurement of pH_i with BCECF

Harvesting of peritoneal cells (3 ml of cell suspension/mouse), plating (2 ml/35 mm culture dish), isolation of resident macrophages by adherence and conditions for cell culture were as described previously [14].

Loading with BCECF and experimental conditions for measurement and calibration of pH_i were as described previously [14]. Recordings of pH_i were highly reproducible, and traces presented are representative of at least three separate experiments. Traces presented together emanate from the same experiment.

To determine intrinsic buffer capacity, i.e. the buffering power of all buffers other than $\text{CO}_2/\text{HCO}_3^-$, experiments were performed essentially as described previously [19,20]. After cells had been preincubated in Ch-medium for 30 min, pH_i was monitored in Ch-medium supplemented with $75\text{ }\mu\text{M}$ -NEM. After addition of NH_4Cl ($\text{p}K_a = 9.25$), changes in pH_i upon subsequent stepwise decrease in its extracellular concentration were recorded.

RESULTS AND DISCUSSION

Acid-extruding pH -regulatory mechanisms: sensitivity to inhibitors

Macrophages loaded with the pH -sensitive fluorescent probe BCECF [4] were monitored by the fluorescence-ratio technique, and continuous perfusion of medium was employed to minimize the contribution from probe leaking out from the cells. To study pH_i -regulatory mechanisms active at low pH_i , cells were preincubated with NH_4^+ to yield a cytosolic acid load [21]. Fig. 1 shows that recovery after such an acid load was sensitive to inhibitors of Na^+/H^+ exchange (amiloride), H^+ -ATPase (NEM)

Fig. 1. Inhibition of recovery from acid load by amiloride, DIDS or NEM

All traces were recorded at $\text{pH}_e 7.2$ in Na/HCO_3^- -medium. Cells were acid-loaded by preincubation for 15 min with 30 mM (a) or 20 mM (b) amine. In (a) the traces show (top to bottom): recovery with no additions, in the presence of $400\text{ }\mu\text{M}$ -amiloride and in the presence of $400\text{ }\mu\text{M}$ -amiloride plus $600\text{ }\mu\text{M}$ -DIDS. In (b) the traces show (top to bottom): recovery with no additions, in the presence of $75\text{ }\mu\text{M}$ -NEM and in the presence of $75\text{ }\mu\text{M}$ -NEM plus $600\text{ }\mu\text{M}$ -DIDS. For traces showing effect of DIDS, cells were preincubated with DIDS for 10 min.

Fig. 2. Na^+ - and HCO_3^- -dependence of recovery from acid load in the presence of amiloride

All traces were recorded at $\text{pH}_e 7.2$. In (a) and (b) cells were acid-loaded by preincubation for 15 min with 10 mM, in (c) with 30 mM and in (d) with 25 mM amine. All traces were recorded in the presence of $600\text{ }\mu\text{M}$ -amiloride. The traces show (top to bottom): (a) recovery in Na/HCO_3^- -medium, in Ch/HCO_3^- -medium and in Ch/HCO_3^- -medium with $75\text{ }\mu\text{M}$ -NEM; (b) recovery in Na/HCO_3^- -medium with $75\text{ }\mu\text{M}$ -NEM and in Ch/HCO_3^- -medium with $75\text{ }\mu\text{M}$ -NEM; (c) recovery in Na/HCO_3^- -medium, in Na/HCO_3^- -medium with $100\text{ }\mu\text{M}$ -NEM and in degassed Na -medium; (d) recovery in degassed Na -medium, in degassed Ch -medium, in degassed Na -medium with $50\text{ }\mu\text{M}$ -NEM and in degassed Ch -medium with $50\text{ }\mu\text{M}$ -NEM.

and also of HCO_3^- -dependent mechanisms (DIDS). It may also be noted that a lower pH_i was attained after recovery in the presence of either NEM or a combination of NEM and DIDS (Fig. 1b), whereas a similar pH_i was eventually attained in the presence as compared with the absence of amiloride (Fig. 1a). The concentrations of inhibitors used in this study were not toxic, as indicated by the retention of both lactate dehydrogenase (EC 1.1.1.27) and loaded BCECF, as well as by the preservation of H^+ gradients (results not shown).

Bicarbonate-dependent pH_i recovery; identification of $\text{Na}^+/\text{Cl}^-/\text{HCO}_3^-$ exchange

In order to characterize the DIDS-inhibitable part of the recovery from an acid load, we had to discriminate between several possible candidates such as Na^+ -dependent or -independent $\text{Cl}^-/\text{HCO}_3^-$ exchange and $\text{Na}^+/\text{HCO}_3^-$ co-transport (Cl^- -independent). In a medium devoid of Na^+ (Fig. 2a, middle trace), H^+ -ATPase rather than alkalinizing Na^+ -independent $\text{Cl}^-/\text{HCO}_3^-$ exchange was responsible for recovery, as indicated by the strongly inhibitory effect of NEM (Fig. 2a, lowest trace) and also by the similar recovery in the presence as compared with

Fig. 3. $\text{Na}^+/\text{Cl}^-/\text{HCO}_3^-$ exchange: dependence on intracellular Cl^- and inhibition by SITS

All traces were recorded at pH_e 7.2. In (a) and (b) amiloride ($600 \mu\text{M}$) was added as HCO_3^- salt. In (a) the top trace shows recovery in $\text{Glu}/\text{HCO}_3^-$ medium (acid load with 20 mM amine, 15 min), middle trace shows recovery in $\text{Glu}/\text{HCO}_3^-$ medium [preincubated Cl^- -free for 75 min and acid-loaded with 40 mM $(\text{NH}_4)_2\text{SO}_4$ for 20 min], and in the bottom trace cells were treated as in the middle trace, but recovery was in a medium supplemented with amiloride. After depletion of intracellular Cl^- , higher amine concentrations had to be applied to achieve comparable acid loads. In (b) cells were acid-loaded (15 min) with 10 mM (two top traces) or 20 mM amine (two lower traces). The cells in the two lower traces were preincubated for 120 min in medium devoid of Cl^- . Traces show (top to bottom): recovery in Na/HCO_3^- medium with amiloride, in Na/HCO_3^- medium with amiloride and $75 \mu\text{M}$ NEM, in $\text{Glu}/\text{HCO}_3^-$ medium with amiloride and NEM and in Na/HCO_3^- medium with amiloride and NEM. In (c) cells were acid-loaded by preincubation with 10 or 20 mM amine for 15 min . All traces were recorded in Na/HCO_3^- medium in the presence of $600 \mu\text{M}$ amiloride and $75 \mu\text{M}$ NEM. In the second trace from top and in the bottom trace, cells were preincubated for 10 min in the presence of 1 mM SITS, and SITS was also included in the recovery media. The arrow denotes cross-over of traces.

the absence of HCO_3^- and the lack of inhibition by either DIDS or SITS (results not shown). In the presence of amiloride ($600 \mu\text{M}$), pH_i recovery still showed dependence on Na^+ (Figs. 2a and 2b), and this was more pronounced if NEM was also present (Fig. 2b). Recovery of pH_i in a medium containing Na^+ and amiloride was in addition enhanced by HCO_3^- (Fig. 2c, upper traces). In the absence of HCO_3^- , no Na^+ -dependence could be seen either in the presence or in the absence of NEM (Fig. 2d), showing that the Na^+ - and HCO_3^- -dependencies were coupled to each other, and furthermore that amiloride at $600 \mu\text{M}$ indeed inhibited Na^+/H^+ exchange effectively. Thus macrophages can alkalinize their cytosol by means of a Na^+ - and HCO_3^- -dependent mechanism that is insensitive to amiloride and NEM (Fig. 2b).

In order to discriminate between two such mechanisms reported for other cell types, i.e. $\text{Na}^+/\text{Cl}^-/\text{HCO}_3^-$ exchange and $\text{Na}^+/\text{HCO}_3^-$ co-transport [1,3,10,12], we addressed the question whether a dependence on intracellular Cl^- could be demonstrated. Fig. 3 shows that recovery was not impaired in a medium devoid of Cl^- unless the cells were preincubated in such medium. That Na^+/H^+ exchange was operative after such treatment is evidenced by the sensitivity to amiloride shown in Fig. 3(a), middle and lowest traces. The inhibition of recovery by preincubation in Cl^- -free medium indicates a requirement for intracellular Cl^- as reported for $\text{Na}^+/\text{Cl}^-/\text{HCO}_3^-$ exchange [1,22]. Further inhibition of pH_i recovery could be achieved by imposing an inward-directed Cl^- gradient after preincubation in Cl^- -free medium (Fig. 3b, lowest trace). This indicates either that a 2 h preincubation alone causes insufficient depletion of intracellular Cl^- (and only partial inhibition of recovery), or that an inward-directed Cl^- gradient can reverse $\text{Na}^+/\text{Cl}^-/\text{HCO}_3^-$ exchange to make it acidifying.

To characterize $\text{Na}^+/\text{Cl}^-/\text{HCO}_3^-$ exchange further, inhibition with SITS was attempted, and as shown in Fig. 3(c) this inhibitor was very effective. Similar results were obtained with DIDS (not shown). It may also be noted that $\text{Na}^+/\text{Cl}^-/\text{HCO}_3^-$ exchange provoked a more rapid recovery of pH_i when the pH_i was

decreased to 6.1 rather than 6.6 . Other results indicated that HCO_3^- -dependent mechanisms had the capacity to operate also when the HCO_3^- concentration was rather low, such as in a nominally HCO_3^- -free medium in equilibrium with atmospheric CO_2 , whereas it was severely impaired in a medium actively depleted of CO_2 by degassing.

pH_i -dependence of Na^+/H^+ exchange, H^+ -ATPase and $\text{Na}^+/\text{Cl}^-/\text{HCO}_3^-$ exchange

Under conditions where only Na^+/H^+ exchange was operative, recovery did not proceed beyond approx. pH 6.6 (Fig. 4a, lower traces). Also, amiloride had no effect on the recovery from a smaller acid load ($\text{pH}_i > 6.6$), whereas NEM in the absence of HCO_3^- inhibited such recovery to a similar extent whether Na^+ was present or not (results not shown). These findings indicated that the set-point for Na^+/H^+ exchange was well below the steady-state pH_i of resting cells (approx. 7.0). When H^+ -ATPase was also operating, recovery proceeded to a higher level (Fig. 4a, upper trace), and a higher level was also attained when the only operative mechanism would be $\text{Na}^+/\text{Cl}^-/\text{HCO}_3^-$ exchange (Fig. 4b, upper trace). This leads us to propose that H^+ -ATPase and $\text{Na}^+/\text{Cl}^-/\text{HCO}_3^-$ exchange, rather than Na^+/H^+ exchange, operates to maintain pH_i at steady-state. This may not be true under all conditions, since Na^+/H^+ exchange has been shown to undergo activation and attain a higher set-point in response to, e.g., activators of protein kinase C [2,9]. This appears to be the case also in macrophages (results not shown).

Cytosol acidification after inhibition of alkalinizing mechanisms

An alternative way to study acid-extruding pH-regulatory mechanisms, complementary to the use of an amine-induced acid load, would be to monitor the effect of inhibitors on spontaneous cytosol acidification. As shown in Fig. 5, amiloride had no effect on such acidification, as opposed to NEM. This is in accordance with the notion that Na^+/H^+ exchange is active only at more acidic pH . Reversibility of $\text{Na}^+/\text{Cl}^-/\text{HCO}_3^-$ exchange was

Fig. 4. H^+ -ATPase and $Na^+/Cl^-/HCO_3^-$ exchange are active at higher pH_i than Na^+/H^+ exchange

All traces were recorded at pH_e 7.2. Cells were acid-loaded by preincubation for 15 min with 10 mM amine, except for the bottom trace in (a), where cells were acid-loaded with 30 mM amine. Traces show (top to bottom): (a) recovery in degassed Na-medium (upper trace) and in degassed Na-medium with 75 μ M-NEM (middle and lower traces); (b) recovery in Na/ HCO_3^- -medium supplemented with 600 μ M-amiloride and 75 μ M-NEM and in degassed Na-medium with 75 μ M-NEM.

Fig. 5. Effect of inhibitors of H^+ -extruding mechanisms on cytosolic acid-loading rate

All traces were recorded at pH_e 7.2. Traces show (top to bottom) pH_i of cells transferred to: (a) Na/ HCO_3^- -medium, Na/ HCO_3^- -medium with 600 μ M-amiloride, Na/ HCO_3^- -medium with 75 μ M-NEM, Na/ HCO_3^- -medium with amiloride and NEM, Ch/ HCO_3^- -medium with amiloride, Ch/ HCO_3^- -medium with amiloride and NEM. (b) Na-medium, Na-medium with amiloride and NEM, Ch-medium, Ch-medium with amiloride and NEM, Ch-medium with NEM. The media used in (b) were degassed before the experiment.

indicated by the rapid initial acidification occurring in Na^+ -free medium in the presence, but not in the absence, of HCO_3^- . The pronounced acidification in Na^+ -free medium without amiloride (Fig. 5b, bottom trace) indicated reversibility also of Na^+/H^+ exchange. A higher cytosolic buffer capacity in HCO_3^- -containing media is suggested by the different slope of the curves obtained in the presence and absence of HCO_3^- in Na^+ -free media containing amiloride and NEM (cf. Figs. 5a and 5b). The intrinsic buffer capacity was found to be approx. 30 mM/pH unit at pH_i 7.0 and increased slightly with decreasing pH_i , whereas the calculated total buffer capacity (including HCO_3^-/CO_2) was

Fig. 6. pH_i -dependence of pH_i changes induced by Cl^- removal and re-addition in the absence of Na^+

All traces were recorded at pH_e 7.2. Experiments were started in either NMGGlu/ HCO_3^- -medium (top traces) or Ch/ HCO_3^- -medium, and after 10 min were performed in Ch/ HCO_3^- -medium. All media contained 400 μ M-amiloride. In (b)–(d) cells were acid-loaded (15 min) with 2.5 mM (b), 5 mM (c) or 10 mM (d) amine before experimentation.

somewhat larger and increased steeply at more alkaline pH_i (results not shown).

pH-dependence of $Na^+/Cl^-/HCO_3^-$ exchange and Na^+ -independent Cl^-/HCO_3^- exchange

Whether Na^+ -dependent and -independent Cl^-/HCO_3^- exchangers can regulate pH_i or not is determined by the gradients of the transported ionic species over the plasma membrane and possibly also by pH-sensitive intrinsic properties of the exchangers. Removal and re-admittance of extracellular Cl^- is often used in order to demonstrate the presence and characteristics of these Cl^- -dependent pH-regulatory mechanisms. However, the assumption must be made that the mechanism responsible for Cl^- -flux-induced pH_i changes is able to partake in pH_i regulation also at physiological Cl^- concentrations under otherwise similar conditions. It has been shown above that macrophages possess an alkalinizing mechanism with the characteristics of $Na^+/Cl^-/HCO_3^-$ exchange. Under conditions where this exchanger would be operative, removal of extracellular Cl^- would lead to Cl^- efflux coupled to Na^+ -dependent influx of HCO_3^- and an increase in pH_i . When Cl^- is re-admitted, this process would be reversed. If Na^+ -independent Cl^-/HCO_3^- exchange was present, similar but Na^+ -independent changes in pH_i would be expected.

At elevated extracellular pH (pH_e), Cl^- -flux-provoked pH_i changes were Na^+ -independent (results not shown), whereas at

Fig. 7. pH_i -dependence of pH_i changes induced by Cl^- removal and re-addition in the presence of Na^+

All traces were recorded at pH_e 7.2. Experiments were started in either Glu/ HCO_3^- -medium (top traces) or Na/ HCO_3^- -medium, and after 10 min were performed in Na/ HCO_3^- -medium. All media contained $400 \mu\text{M}$ -amiloride. In (b) cells were acid loaded for 15 min with 5 mM amine before experimentation. Arrows denote cross-over of traces.

physiological pH_e both Na^+ -dependent and -independent exchangers participated, since larger pH_i changes were induced in the presence than in the absence of Na^+ (cf. Figs. 6a and 7a, upper traces). This implies that pH_i in the physiological range could be determined by the activity of both exchangers. At pH_e 6.8, Cl^- -flux-induced pH_i changes were much decreased (results not shown), indicating that also Na^+ -dependent $\text{Cl}^-/\text{HCO}_3^-$ exchange was inhibited at low pH_e .

To determine the pH_i -dependence of $\text{Cl}^-/\text{HCO}_3^-$ exchange at physiological pH_e , pH_i was adjusted to different values before the experiment (Fig. 6). As pH_i was lowered, the pH_i changes induced by Cl^- flux were much decreased. The pH_i -dependence for the Na^+ -independent $\text{Cl}^-/\text{HCO}_3^-$ exchanger would appear to be similar to that reported for other cell types [23]. The rapid initial acidification in the lower traces in Figs. 6(a)–6(c) is probably due to reversal of $\text{Na}^+/\text{Cl}^-/\text{HCO}_3^-$ exchange, since this phase was not present in parallel experiments where the cells had been preincubated in Na^+ -free medium for 30 min (results not shown).

In the presence of Na^+ , the pH_i changes provoked by Cl^- removal were not inhibited to a similar extent by lowering of pH_i (Fig. 7). When both Na^+ and Cl^- were present in the medium, as in the lower traces of Fig. 7, $\text{Na}^+/\text{Cl}^-/\text{HCO}_3^-$ exchange could operate. Also, when Cl^- is re-administered in the upper traces, operational $\text{Na}^+/\text{Cl}^-/\text{HCO}_3^-$ exchange could act to limit the Cl^- -flux-provoked fall in pH_i . Even so, the pH_i changes are inhibited less by cytosol acidification in the presence (Fig. 7b) than in the absence (Fig. 6c) of Na^+ . The activation of $\text{Na}^+/\text{Cl}^-/\text{HCO}_3^-$ exchange by cytosol acidification has also been indicated elsewhere in this paper (Fig. 3c).

Bicarbonate-dependence of $\text{Cl}^-/\text{HCO}_3^-$ exchangers and differential inhibition by SITS

To characterize the $\text{Cl}^-/\text{HCO}_3^-$ exchangers further, conditions were sought where dissociation of the Na^+ -dependent and Na^+ -independent mechanisms could be achieved. In Fig. 8, the top traces were recorded in the absence of sodium and at an elevated pH_e , conditions that should favour the Na^+ -independent exchanger. In the lower traces both mechanisms would be operative, but with predominance for $\text{Na}^+/\text{Cl}^-/\text{HCO}_3^-$ exchange.

Fig. 8. HCO_3^- -dependence and inhibition by SITS or DIDS of Na^+ -dependent and -independent $\text{Cl}^-/\text{HCO}_3^-$ exchangers

In (a), (c) and (d), experiments were started in either Glu/ HCO_3^- -medium (lower trace) or NMGGlu/ HCO_3^- -medium. After 10 min this medium was replaced with either Na/ HCO_3^- -medium (lower traces) or Ch/ HCO_3^- -medium. pH of the HCO_3^- -containing media was adjusted and maintained with CO_2 at either 7.1 (Glu/ HCO_3^- -medium and Na/ HCO_3^- -medium) or 7.7 (NMGGlu/ HCO_3^- -medium and Ch/ HCO_3^- -medium). All media contained $400 \mu\text{M}$ -amiloride (HCO_3^- salt). In (b) traces were recorded in media containing nominal bicarbonate and experiments were performed under atmospheric $p\text{CO}_2$. Otherwise, conditions were as in (a). In (c) all media were supplemented with DIDS; top trace contained $100 \mu\text{M}$ and middle and lowest traces contained $600 \mu\text{M}$. In (d) all media were supplemented with $600 \mu\text{M}$ -SITS.

In nominally HCO_3^- -free medium both mechanisms were slowed considerably, but not completely inhibited (Fig. 8b). The larger pH changes evoked here may reflect decreased cytosolic buffer capacity in nominally HCO_3^- -free medium. Fig. 8(c) shows that both transporters were inhibited by DIDS, and Fig. 8(d) (lower trace) shows that Na^+ -dependent exchange was also inhibited by SITS. However, the Na^+ -independent mechanism was inhibited by SITS to a much lesser extent. After an acid load by amine preincubation, $\text{Na}^+/\text{Cl}^-/\text{HCO}_3^-$ exchange was inhibited to a similar extent by SITS and DIDS also at pH_e 7.7 (results not shown). Differential inhibition by SITS could provide a means to discriminate between the exchangers, in addition to the difference in Na^+ -dependence.

Concluding remarks

The importance of HCO_3^- -dependent mechanisms for maintenance of steady-state pH_i in many cell types has only recently been realized. In the present paper, both Na^+ -dependent and -independent $\text{Cl}^-/\text{HCO}_3^-$ exchangers were found to operate at physiological pH_i levels in macrophages, whereas the Na^+/H^+

exchanger appeared to be quiescent and operative only after a larger acid load of the cytosol. In addition, macrophages extruded protons by a H^+ -ATPase, further characterized in the accompanying paper [18]. Na^+ -dependent Cl^-/HCO_3^- exchange was activated by cytosol acidification, whereas a low pH_e was inhibitory. Since low pH_e is inhibitory also to Na^+/H^+ exchange [2,9], H^+ extrusion by H^+ -ATPase may be of crucial importance in acidic environments encountered by macrophages under certain pathophysiological conditions.

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