Biochem. J. (1992) 281, 245–250 (Printed in Great Britain)

Cytosolic pH regulation in mouse macrophages

Proton extrusion by plasma-membrane-localized H^+-ATPase

Hans TAPPER and Roger SUNDLER
Department of Medical and Physiological Chemistry, Lund University, P.O. Box 94, S-221 00 Lund, Sweden

Recent evidence indicates that H^+ extrusion in macrophages is in part accomplished by a H^+-ATPase of vacuolar type. The presence and plasma-membrane localization of such a mechanism in adherent resident macrophages was verified by inhibition of H^+ extrusion, monitored by changes in both cytosolic pH (pH_i) and extracellular pH, with low concentrations of the H^+-ATPase inhibitors *N*-ethylmaleimide and 7-chloro-4-nitrobenz-2-oxa-1,3-diazole. The H^+-ATPase was operative at physiological pH_i levels, thus contributing to maintenance of steady-state pH_i. It was further shown to be sensitive to the plasma-membrane potential, with hyperpolarization being strongly inhibitory. In addition, H^+ extrusion mediated by the H^+-ATPase and the generation and release of lactic acid caused acidification of the pericellular space and could enable secreted lysosomal hydrolases to act extracellularly.

INTRODUCTION

Mechanisms that extrude H^+ equivalents from cell cytosol are vital, since many enzymic reactions have narrow pH optima in the physiological range [1,2]. These mechanisms must counteract the tendency of cells to accumulate protons due in part to the plasma-membrane potential, which sets the electrochemical gradient for protons such that pH_i would be well below that normally maintained [2,3]. Also, metabolic processes can acidify the cytosol if not counterbalanced by H^+ extrusion. Special demands may be put on the pH_i-regulatory capacity in cells such as macrophages that reside in an acidic environment under certain pathophysiological conditions. Macrophages possess, in addition to Na^+/H^+ exchange and $Na^+/Cl^-/HCO_3^-$ exchange [4], an alkalinizing mechanism which is Na^+- and HCO_3^--independent and tentatively identified as a H^+-extruding ATPase of vacuolar type [5,6]. Such a H^+-ATPase is most likely present also in osteoclasts, where it is responsible for the acidification of their resorptive lacunae [7].

In the present paper, H^+ extrusion by plasma-membrane-localized H^+-ATPase in macrophages has been characterized with respect to pH_i-dependence, to sensitivity to low concentrations of H^+-ATPase inhibitors and to manipulations of plasma-membrane potential. Since the plasma-membrane H^+-ATPase of macrophages could also be important for an acidification of the pericellular space, a possible prerequisite for degradative processes employing secreted lysosomal enzymes [8], its ability to cause extracellular acidification along with extruded lactic acid has also been assessed.

MATERIALS AND METHODS

Materials and methods not described below were identical with those in the preceding paper [4]. Compositions of experimental media, their abbreviated designations and the presentation of experimental results were the same as in the previous paper [4].

Materials

Fluorescein isothiocyanate–dextran (FD; average M_r 42000; 9 mmol of fluorescein isothiocyanate/mol of glucose residue), 4-methylumbelliferyl *N*-acetyl-β-D-glucosaminide, carbonyl cyanide *p*-trifluoromethoxyphenylhydrazone (FCCP) and reagents for determination of lactate and lactate dehydrogenase (LDH, EC 1.1.1.27) were purchased from Sigma. Valinomycin was obtained from Molecular Probes, Eugene, OR, U.S.A. NBD-Cl was from Aldrich. FCCP and valinomycin were dissolved in ethanol, and NBD-Cl was dissolved in dimethyl sulphoxide, and added to the experimental media in a volume not exceeding 0.5%.

Measurement of LDH

Experiments were performed with cells on culture dishes, the culture media were collected and the cells were lysed with 1 ml of 0.1% (v/v) Triton X-100 and scraped off with a Teflon policeman. The samples were centrifuged at 5000 rev./min for 5 min, and the supernatants collected and put on ice. LDH activity in media relative to cell lysates was assayed by a modification of a method described previously [9], and taken as an indication of cellular integrity [10]. Assay was performed on the day of the experiment, and the release of enzyme was corrected for the release observed in controls (< 5%). Unless otherwise stated, conditions used in pH experiments with cells on coverglasses were, in parallel experiments on culture dishes, found to cause negligible release of LDH.

Measurement of *N*-acetyl-β-D-glucosaminidase (EC 3.2.1.30)

Experiments were performed as described above for the LDH assay, and *N*-acetyl-β-D-glucosaminidase in media and cell lysates was determined as described previously [8].

Measurement of lactate

For these experiments, cells were seeded on culture dishes with a 3-fold increased cell density. Cell protein in each well after overnight culture was approx. 150 μg as measured by the Bradford [11] method. Experiments were performed in 1 ml of experimental medium for 30 min, after which medium and cell lysate were obtained as described above for the LDH assay. Lactate in media was determined by a modification of a method described previously [12] and related to protein content in the same well. The amount of lactate in the cell lysate was negligible.

Abbreviations used: BCECF-AM, 2′,7′-bis(carboxyethyl)-5(6)-carboxyfluorescein tetra-acetoxymethyl ester; FCCP, carbonyl cyanide *p*-trifluoromethoxyphenylhydrazone; FD, fluorescein isothiocyanate–dextran; LDH, lactate dehydrogenase; NBD-Cl, 7-chloro-4-nitrobenz-2-oxa-1,3-diazole; NEM, *N*-ethylmaleimide; pH_e, extracellular pH; pH_i, cytosolic pH.

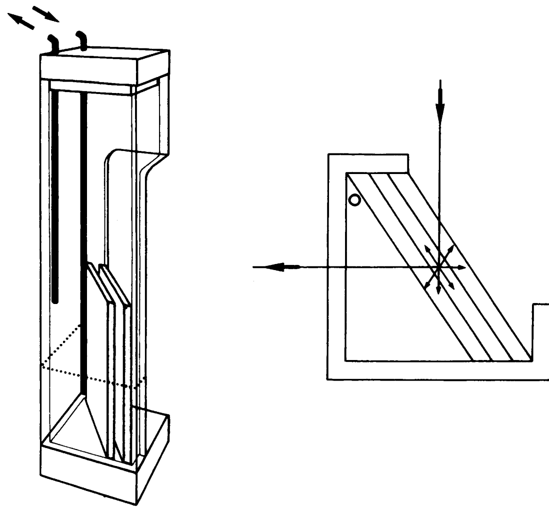

Fig. 1. Fluorimeter cuvette for measurement of H^+ extrusion from cells in monolayer

A coverglass with cells was placed in a chamber at approx. 30° angle relative to the light beam with the cells facing the exciting light and the opposing side of the coverglass being in close contact with one wall of the chamber. Water at 37 °C was continuously perfused beside the chamber on the side of emitted light. The dotted line represents the plane of cross-section.

Measurement of lysosomal pH with FD

Loading with FD and experimental conditions for measurements and calibration of lysosomal pH were as described previously [8].

Measurement of H^+ extrusion

Cells seeded with 3-fold increased cell density on coverglasses were assayed for H^+ extrusion in a specially built fluorimeter cuvette (Fig. 1). The experimental media used were similar to Na- or Ch-medium, except for decreasing Hepes to 100 μM and supplementation with 400 nM-BCECF (free acid). Experiments were performed in 125 μl of medium, degassed and equilibrated with O_2/N_2 (21:79) before registration of H^+ extrusion. Uptake of probe by the cells was negligible, as judged by fluorescence microscopy and also by the similar fluorescence intensity recorded from the BCECF-containing media at excitation wavelength 456 nm before and after the experiments. It was also assessed that the fluorescence ratio of BCECF had a similar pH-dependence under these conditions as when used to monitor pH_i.

RESULTS

pH_i-dependence of H^+-ATPase and inhibition by NEM and NBD-Cl

The presence of a Na^+-independent alkalinizing pH-regulatory mechanism in macrophages was indicated by a substantial pH_i recovery in Na^+-free medium (Fig. 2*a*, upper trace). Under these conditions, after acid-loading to different pH_i levels in the range 6.1–6.6, no difference in recovery rate was seen (results not shown). However, if a larger acid load was applied (Fig. 2*a*, lower trace) recovery was severely inhibited. The mechanism responsible for pH_i recovery under Na^+-free conditions is probably a H^+-ATPase, since in the presence of NEM (75 μM), an inhibitor of H^+-ATPase of the vacuolar type [13], no pH_i recovery was seen (Fig. 2*a*, middle trace).

In Na^+-free medium, pH_i recovery was enhanced in the presence of amiloride compared with in its absence (cf. Figs. 2*b* and 2*a*), indicating that reversed Na^+/H^+ exchange driven by the outward-directed Na^+ gradient could acidify the cytosol and that this H^+ influx was inhibited by amiloride. However, the H^+-ATPase was not inhibited by amiloride, since H^+ extrusion showed similar sensitivity to NEM under these conditions (cf. Figs. 2*b* and 2*a*). Furthermore, the apparent inactivation of the H^+-ATPase when pH_i approached approx. 6.0 was evident also in the presence of amiloride (Fig. 2*b*, lowest trace).

As shown in Fig. 3(*a*), recovery of pH_i in a Na^+- and HCO_3^--free medium was inhibited by NEM already at concentrations below 75 μM. This was true also for NBD-Cl (Fig. 3*b*), another inhibitor of H^+-ATPase of the vacuolar type [13]. In earlier studies of macrophage H^+-ATPase [6,14], NEM was used at a concentration of 1 mM. This high concentration was not necessary in our system. Instead, it was desirable to use low concentrations

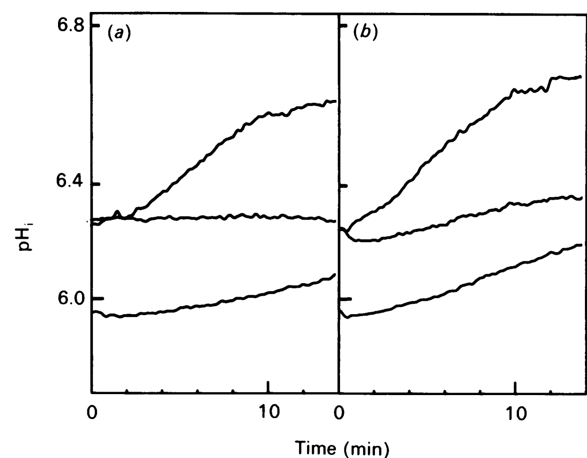

Fig. 2. pH_i-dependence of H^+-ATPase and inhibition by NEM or NBD-Cl

Cells were acid-loaded for 15 min with 10 mM (upper two traces) or 25 mM (lower trace) amine. Traces show recovery of pH_i in Ch/HCO_3-medium (pH 7.2). The middle traces were recorded in the presence of 75 μM-NEM. In (*b*) all traces were recorded in the presence of 600 μM-amiloride.

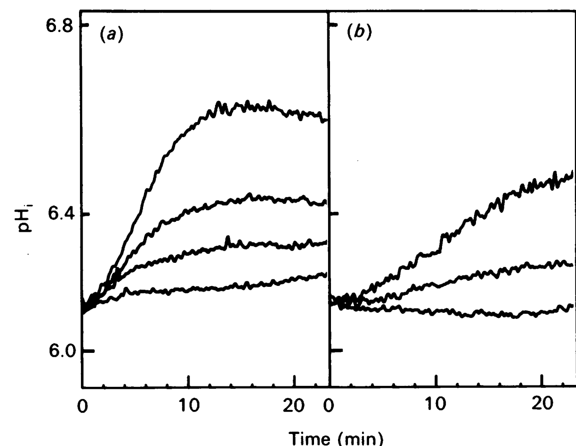

Fig. 3. Dose-dependent inhibition of H^+-ATPase by NEM or NBD-Cl

Cells were acid-loaded for 15 min with 15 mM amine. Traces show recovery in Ch-medium (pH 7.2) in the presence of 600 μM-amiloride. In (*a*) was included in the second trace from top 25 μM-NEM, in the third trace 75 μM-NEM and in the bottom trace 150 μM-NEM. In (*b*) was included in the top trace 5 μM-NBD-Cl, in the middle trace 25 μM-NBD-Cl and in the bottom trace 75 μM-NBD-Cl.

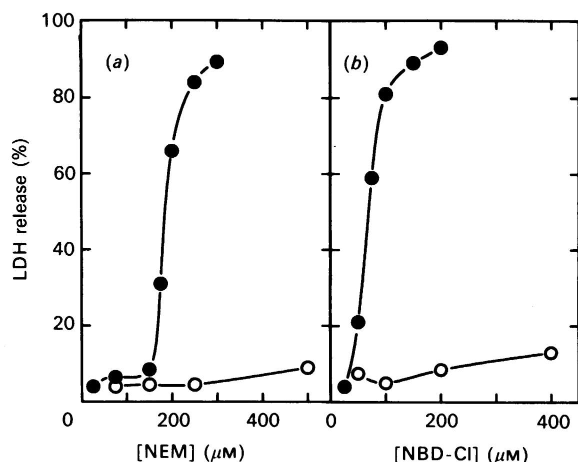

Fig. 4. Toxicity of NEM or NBD-Cl is decreased after acid-loading of the cytosol

Cells were incubated in Ch/HCO_3-medium (pH 7.2) in the presence of indicated concentrations of either NEM (*a*) or NBD-Cl (*b*) for 30 min, and the release of LDH was assessed (●); ○, release from cells that had been acid-loaded with 10 mM-amine for 15 min before exposure to NEM or NBD-Cl.

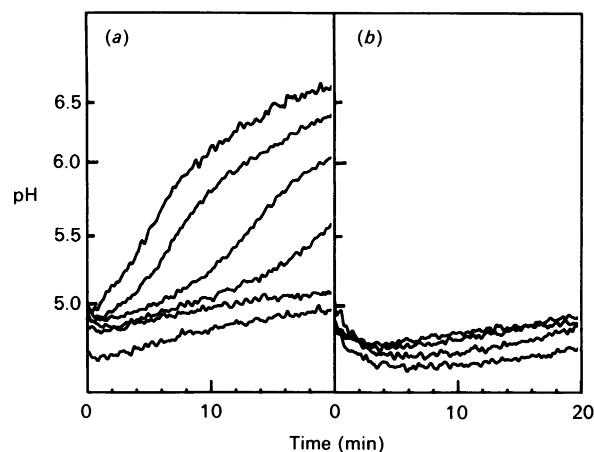

Fig. 5. Effect of NEM and NBD-Cl on lysosomal pH

Cells were loaded with FD as described in the Experimental section. Traces were recorded (*a*) in Na/HCO_3-medium (pH 7.2) containing (top five traces, downward): 1 mM-, 500 μM-, 250 μM-, 150 μM- or 75 μM-NEM. Bottom trace is recorded in Na/HCO_3-medium containing 600 μM-amiloride and 75 μM-NEM after preincubation with 15 mM amine for 15 min. In (*b*) traces were recorded in Ch-medium (pH 7.2) supplemented with 600 μM-amiloride and (top trace to bottom): 0, 75 μM-, 25 μM- or 75 μM-NBD-Cl. Before recording of the top and the two bottom traces, cells were preincubated with 10 mM amine for 15 min.

of NEM and NBD-Cl, since both inhibitors caused extensive release of LDH from the cells at concentrations above 150 μM and 25 μM respectively (Fig. 4). On the other hand, acidification of the cytosol by an NH_4Cl pre-pulse partially protected the cells from the deleterious effects of NEM and NBD-Cl (Fig. 4), although acid-loaded cells also released LDH at higher concentrations of NEM or NBD-Cl after a time delay (results not shown). Since the reactivity of these agents is pH-dependent [15,16], protection of intracellular proteins from covalent modification was possibly obtained under conditions of acidic pH_i. Under no conditions where NEM or NBD-Cl otherwise was used in this or the preceding paper [4] was there any release of LDH above that in control cultures, even when the time of exposure was tripled. Also, similar retention of BCECF and preservation of H^+ gradients by the cells (cf. Figs. 3 and 5) confirmed the lack of cytotoxicity.

Inhibition of cytosolic H^+ extrusion does not raise lysosomal pH

Vacuolar-type H^+-ATPase is primarily localized to intracellular membranes of the endocytic pathway, but might also be present in the plasma membrane [5,6,17]. In order to determine whether the NEM- or NBD-Cl-inhibitable H^+ transport described above was carried out by H^+-ATPase present in vacuolar membranes or the plasma membrane, we monitored proton flux into the lysosomal compartment (Fig. 5) and into the extracellular environment (Fig. 6). As shown in Fig. 5(*a*), only higher concentrations of NEM caused an increase in lysosomal pH. Since both LDH and the lysosomal enzyme *N*-acetyl-β-D-glucosaminidase were released under these conditions, and roughly in parallel (results not shown), this pH increase most likely reflects cell damage. The bottom trace (Fig. 5*a*) was recorded after an acid load of the cytosol and in the presence of NEM (75 μM), and did not differ from traces recorded in the absence of NEM (not shown). Neither did traces differ when recorded without prior acid-loading of the cytosol, in the absence or presence of a non-toxic concentration of NEM (75 μM, Fig. 5*a*). Thus lysosomal pH was unaffected by changes in the rate of H^+ extrusion from the cytosol, induced by acid loading or treatment with NEM (75 μM). Neither was lysosomal pH affected by NBD-Cl, at concentrations sufficient to inhibit cytosolic H^+ extrusion (Fig. 5*b*); only higher (toxic) concentrations of NBD-Cl led to a rise in lysosomal pH similar to that seen with toxic concentrations of NEM (results not shown). Possibly, a lower effective concentration of inhibitor is attained at the lysosomal membrane as compared with the plasma membrane, owing to protection by intracellular thiol-containing agents, e.g. glutathione.

H^+ extrusion by plasma-membrane-localized H^+-ATPase

Direct evidence for the existence of a H^+-ATPase localized in the plasma membrane could be obtained if H^+ extrusion into the extracellular medium could be attributed to its operation. By using a special fluorimeter cuvette (Fig. 1) and weakly buffered media containing BCECF (free acid), it was possible to monitor H^+ extrusion from cells in monolayer into the extracellular medium. Results from such experiments are presented in Fig. 6. In a medium containing glucose (Figs. 6*a* and 6*b*) the H^+-extrusion rate was found to be larger than in a glucose-free medium (Figs. 6*c* and 6*d*). This implied that H^+ equivalents were generated by glucose-requiring metabolic pathways. Glycolysis generating transportable acid equivalents in the form of lactic acid would be such a pathway. That resident macrophages produced and released lactate (approx. 1 nmol/30 min per μg of cell protein) and that this did not occur in a medium devoid of glucose were confirmed experimentally (results not shown).

In a Na^+- and glucose-containing medium, no effect of NEM was seen on H^+ extrusion (Fig. 6*a*) either with (two lower traces) or without prior acid-loading of the cytosol. This is most likely due to a masking effect of lactic acid release and possibly also to extrusion of H^+ equivalents by Na^+-dependent mechanisms operative in addition to the H^+-ATPase. The rapid initial acidification seen in the two lower traces of Fig. 6(*a*) was probably due to activation of Na^+/H^+ exchange by prior acid loading of the cytosol. Amiloride was not used in these experiments, since it acted as a buffer ($pK_a = 8.7$). As shown in Fig. 6(*b*) (upper trace), in a Na^+-free medium containing glucose an inhibitory effect of NEM was evident after enhancement of H^+ extrusion by prior acid-loading of the cytosol. Again, the lack

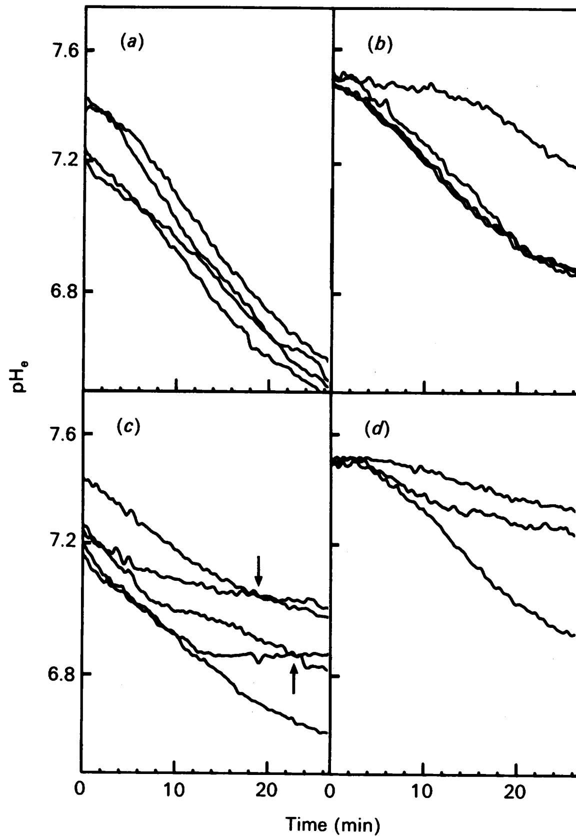

Fig. 6. Extracellular acidification by H^+ extrusion via Na^+/H^+ exchange and H^+-ATPase

H^+ extrusion was measured as described in the Experimental section in (*a*) weakly buffered Na-medium, (*b*) weakly buffered Ch-medium, (*c*) weakly buffered Na-medium without glucose and (*d*) weakly buffered Ch-medium without glucose. From top trace to bottom, inclusion of 150 μM-NEM and/or prior acid loading (NH_4Cl for 15 min) was as follows: (*a*) no treatment, NEM, acid load (20 mM), acid load (20 mM)+NEM; (*b*) acid load (20 mM)+NEM, acid load (20 mM), NEM, no treatment; (*c*) no treatment, acid load (20 mM)+NEM, acid load (20 mM), acid load (40 mM)+NEM, acid load (40 mM); (*d*) acid load (20 mM)+NEM, no treatment, acid load (20 mM). Arrows denote cross-over of traces.

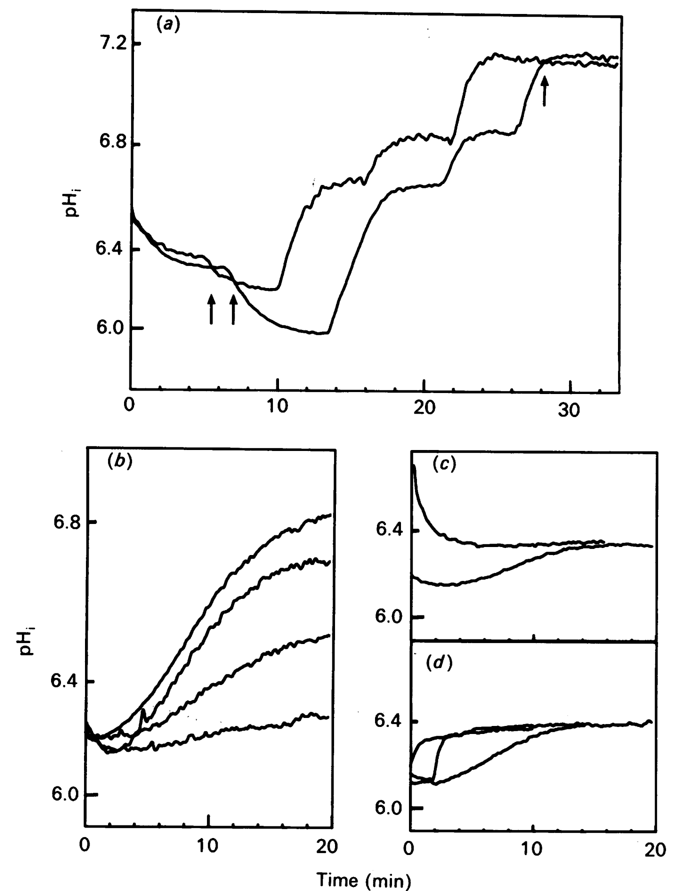

Fig. 7. Inhibition of H^+-ATPase by hyperpolarization

In (*a*) and (*b*), media with indicated K^+ concentrations were obtained by replacing choline chloride in Ch-medium with KCl. Medium with low KCl was obtained by replacing KCl in Ch-medium with choline chloride. All traces in (*a*)–(*c*) were recorded in the presence of 400 μM-amiloride. In (*a*), traces show pH_i in media containing 10 μM-FCCP in the absence (top trace) or presence of 10 μM-valinomycin. Recording was started in medium containing 11.6 mM-K^+, which was replaced by successive perfusion with media containing 1.2 mM-, 37.0 mM-, 62.4 mM- and 138.6 mM-K^+. Arrows denote cross-over of traces. Traces in (*b*) show recovery of pH_i after amine-induced acid load (15 mM, 15 min) in media containing (top trace to bottom) 37.0 mM-, 11.6 mM-, 1.2 mM- and 1.2 mM-K^+. The bottom trace was recorded in the presence of 10 μM-valinomycin. In (*c*), traces show pH_i in the presence of 10 μM-FCCP in Ch-medium. The bottom trace was recorded after preincubation with 15 mM amine for 15 min. pH_i in the presence of 10 μM-FCCP in various media after preincubation with 15 mM amine for 15 min is shown in (*d*). In the bottom and middle traces, recording was started in Ch-medium, which after 2 min was replaced by Na-medium (by perfusion). Bottom trace was recorded in the presence of 400 μM-amiloride. Top trace was recorded in Na/HCO_3-medium.

of inhibition of H^+ extrusion by NEM when cells were not acid-loaded (Fig. 6*b*) would be due to masking by lactic acid release. If so, the unmasking of NEM inhibition after an acid load would indicate that the formation of lactate was inhibited by cytosol acidification [1,2]. Lactate production in a Na^+-free medium was decreased at extracellular pH (pH_e) below neutrality, probably owing to a decrease in pH_i, since acid-loading of the cytosol by preincubation with amine also decreased lactate production (results not shown). Under conditions of prolonged cytosol acidification, as would occur after an acid load in a Na^+-free medium containing NEM, lactate production was severely inhibited (> 50%). It may also be noted that, after acid-loading in Na-medium, NEM (75 μM) only slightly impaired glucose metabolism to lactate.

An alternative way of inhibiting lactate production would be to remove glucose from the medium. As shown in Fig. 6(*c*), an inhibitory effect of NEM was seen in Na^+-containing glucose-free medium after a prior acid-loading of the cytosol (second and fourth traces from top). It may also be noted that the initial rapid acidification ascribed to Na^+/H^+ exchange was larger when a larger acid load was applied. The inhibitory effect of NEM on H^+ extrusion was, however, most clearly demonstrated in a Na^+- and glucose-free medium (Fig. 6*d*), where the basal H^+ extrusion was much increased by an acid load (lower trace) and largely inhibited by NEM (upper trace).

In parallel experiments, where pH_i changes were monitored, no difference in pH_i recovery mediated by either Na^+/H^+ exchange or H^+-ATPase was seen in glucose-free as compared with glucose-containing medium (results not shown).

H^+-ATPase-mediated H^+ extrusion is sensitive to plasma-membrane potential

In mammalian cells, pH_i is maintained at a level considerably higher than that expected from the passive distribution of protons

according to the electrochemical gradient [2,3]. After addition of a protonophore, such as FCCP, a H^+ influx down this gradient would be expected, with pH_i approaching the pH set by the gradient [18]. Accordingly, addition of $10\,\mu$M-FCCP to macrophages resulted in a rapid and large acidification of the cytosol (Fig. 7*a*). The change in pH_i elicited by FCCP would be expected to be sensitive to changes in the plasma-membrane potential, provided that pH_e is maintained at a fixed level [19]. Macrophages have been shown to possess a number of K^+-conductive pathways [20,21] and, like other cells, to depolarize when extracellular K^+ is elevated. When extracellular K^+ was decreased in the presence of FCCP, a decrease in pH_i was recorded, whereas increases in extracellular K^+ led to graded increases in pH_i (Fig. 7*a*). In the presence of the K^+-ionophore valinomycin, pH_i fell even further than in its absence in medium low in K^+ (Fig. 7*a*, lower trace), indicating that the exit of K^+ from the cells, leading to hyperpolarization, was enhanced by valinomycin.

H^+ transport via vacuolar-type H^+-ATPase has been shown to be influenced by the membrane potential [13,22,23]. Thus sensitivity to changes in the plasma-membrane potential of the H^+-ATPase mediating pH_i recovery in macrophages would further support its localization to the plasma membrane. In medium low in K^+, pH_i recovery was clearly depressed, and it was virtually abolished when valinomycin was also included (Fig. 7*b*). On the other hand, in medium high in K^+, recovery was only somewhat accelerated, but proceeded to a higher level (Fig. 7*b*). This indicated that the H^+-ATPase is more strongly inhibited by a hyperpolarization than it is accelerated by a depolarization. In medium with 11.6 mM-K^+ valinomycin caused only slight inhibition, in agreement with the data in Fig. 7(*a*), whereas no inhibitory effect of valinomycin was seen in medium with 37.0 mM-K^+ (results not shown).

The lag period exhibited by H^+ extrusion via H^+-ATPase (Fig. 7*b*) and the inhibition of pH_i recovery seen after a larger acid load (cf. Fig. 2) could be explained if acid load of the cytosol caused plasma-membrane hyperpolarization. In agreement with this possibility, the lag phase was decreased when cells were exposed to medium high in K^+ for 5 min before recording of pH_i was started (results not shown). A transient hyperpolarization was also indicated by pH_i in the presence of FCCP after an acid load (Fig. 7*c*). However, when Na^+/H^+ exchange was operative, the hyperpolarization indicated by FCCP was rapidly nullified (Fig. 7*d*). Finally, the very similar pH_i levels recorded in the presence of FCCP in Na-medium, Na/HCO_3-medium and Ch-medium containing amiloride (Figs. 7*c* and 7*d*) indicate that the membrane potential is similar when cells are maintained in these media.

DISCUSSION

Macrophages possess an alkalinizing pH_i-regulatory mechanism, dependent on neither Na^+ nor HCO_3^-, that has been characterized as a H^+-ATPase of vacuolar type primarily on the basis of its inhibitory profile [5]. In the accompanying study [4], the H^+ pump was found to be operative at physiological pH_i, since pH_i was lowered in the presence of the H^+-ATPase inhibitor NEM. Thus the H^+-ATPase appears to be operating continuously and to contribute to the maintenance of steady-state pH_i together with $Na^+/Cl^-/HCO_3^-$ exchange [4]. This conclusion differs from that of Swallow *et al.* [24], who found no effect of H^+-ATPase inhibition on steady-state pH_i. However, these authors employed thioglycolate-elicited macrophages in suspension and a different inhibitor of the H^+-ATPase.

In order to ascertain that the pH_i-regulatory H^+ transport occurred over the plasma membrane, H^+ extrusion into the medium and the internal pH of lysosomes were monitored. No evidence was found, related to changes in pH_i, for changes in lysosomal H^+ transport in response to relevant concentrations of the H^+-ATPase inhibitors NEM and NBD-Cl, neither with nor without prior acid-loading of the cytosol. However, extracellular acidification was enhanced after a cytosolic acid load, and was inhibited by NEM under conditions where lactic acid production by the cells was decreased. Glycolysis is quite active in macrophages also under aerobic conditions [25] and, as shown in the present paper, contributes significantly to an acidification of the pericellular space. However, inhibition of glycolysis by glucose starvation did not affect H^+-ATPase-mediated pH_i regulation, indicating that its operation was not critical in furnishing ATP under these conditions. Activated macrophages have been found able to seal off an acidic extracellular compartment through adherence [26], much like osteoclasts that create an acidic resorptive lacuna on the surface of bone, into which they secrete lysosomal enzymes [7,27]. The H^+-ATPase as well as the generation of lactic acid may be important in the acidification of such a pericellular space.

Changes in the plasma-membrane potential were found to affect pH_i regulation mediated by the H^+-ATPase, with a hyperpolarization being strongly inhibitory. By the use of the protonophore FCCP to monitor the membrane potential indirectly, a transient hyperpolarization was recorded after acid load of the cytosol in the presence of amiloride or in a medium devoid of Na^+. Operative Na^+/H^+ exchange rapidly nullified this hyperpolarization, possibly owing to influx of Na^+. Thus acid loading by preincubation with amine results in a hyperpolarization which might be responsible for the lag period seen for H^+-ATPase-mediated pH_i recovery, and also for the inhibition of recovery seen after a larger acid load. A hyperpolarization upon acidification of macrophage cytosol has previously been recorded by using potential-sensitive probes and was ascribed to the operation of the H^+-ATPase [5]. However, electrogenic H^+ transport should not affect the membrane potential in the presence of a high concentration of protonophore. Accordingly, in the presence of $10\,\mu$M-FCCP, similar pH_i recordings to those in Figs. 7(*c*) and 7(*d*) were obtained if H^+-ATPase-mediated H^+ extrusion was inhibited by $150\,\mu$M-NEM or $75\,\mu$M-NBD-Cl (results not shown), suggesting an alternative cause of the observed hyperpolarization. The transient nature of the hyperpolarization after a moderate acid load raises the possibility that it is established during the pretreatment with amine, e.g. by modulation of some pH-sensitive K^+ channel [28].

In urinary epithelial cells, H^+-ATPase is mobilized to the plasma membrane, by exocytotic insertion of vesicle membrane containing H^+-ATPase, secondary to an increase in cytosolic Ca^{2+} [17]. In macrophages, H^+-ATPase appears to be constitutively present in the plasma membrane, since it is operative in resident cells at steady-state pH_i. Also, pH_i recovery mediated by H^+-ATPase was similar after acid loading obtained by either exposure to acidic pH_e or preincubation with amine (results not shown). It has been shown that treatment with amine induces massive exocytosis of lysosomal contents from macrophages and that acidic pH_i is strongly inhibitory [8]. Thus recruitment of H^+-ATPase from the lysosomal membrane in response to an acid load of the cytosol appears unlikely. The same conclusion was reached by Swallow *et al.* [5] on the basis of other experimental evidence.

In certain pathophysiological environments, such as infectious abscesses and solid tumours, special demands may be posed upon the pH_i regulation in macrophages, owing to relative anaerobiosis, acidosis [29] and, possibly, decreased bicarbonate concentration. Na^+/H^+ exchange and $Na^+/Cl^-/HCO_3^-$ exchange would both be ineffective under these conditions, and the

macrophage would then depend on an accelerated H^+ extrusion via the H^+-ATPase, provided that ATP can be furnished by anaerobic glycolysis. Although lactate production by macrophages is sensitive to acidosis, it is only partially inhibited, as shown in the present paper. Further studies on pH_i regulation in macrophages under conditions of acidic pH_e and the possible role of an elevated K^+ concentration [29,30] are needed.

Financial support by the Swedish Medical Research Council (grant nr. 5410 and 7830), the Crafoord Foundation, the A. Påhlsson Foundation, the A. Österlund Foundation and the Medical Faculty, Lund University, and the technical assistance by Marianne Peterson and Maria-Luisa Prieto-Linde, are gratefully acknowledged.

REFERENCES

1. Busa, W. B. (1986) Annu. Rev. Physiol. **48**, 389–402
2. Madshus, I. H. (1988) Biochem. J. **250**, 1–8
3. Roos, A. & Boron, W. F. (1981) Physiol. Rev. **61**, 296–434
4. Tapper, H. & Sundler, R. (1992) Biochem. J. **281**, 239–244
5. Swallow, C. J., Grinstein, S. & Rotstein, O. D. (1990) J. Biol. Chem. **265**, 7645–7654
6. Bidani, A., Brown, S. E. S., Heming, T. A., Gurich, R. & DuBose, T. D., Jr. (1989) Am. J. Physiol. **257**, C65–C76
7. Baron, R., Neff, L., Louvard, D. & Courtoy, P. J. (1985) J. Cell Biol. **101**, 2210–2222
8. Tapper, H. & Sundler, R. (1990) Biochem. J. **272**, 407–414
9. Reeves, W. J. & Fimognari, G. M. (1963) J. Biol. Chem. **238**, 3853–3858
10. Cook, J. A. & Mitchell, J. B. (1989) Anal. Biochem. **179**, 1–7
11. Bradford, M. M. (1976) Anal. Biochem. **72**, 248–254
12. Lundholm, L., Mohme-Lundholm, E. & Vamos, N. (1963) Acta Physiol. Scand. **58**, 243–249
13. Mellman, I., Fuchs, R. & Helenius, A. (1986) Annu. Rev. Biochem. **55**, 663–700
14. Swallow, C. J., Grinstein, S. & Rotstein, O. D. (1988) J. Biol. Chem. **263**, 19558–19563
15. Riordan, J. F. & Vallee, B. L. (1967) Methods Enzymol. **11**, 541–548
16. Birkett, D. J., Price, N. C., Radda, G. K. & Salmon, A. G. (1970) FEBS Lett. **6**, 346–348
17. van Adelsberg, J. & Al-Awqati, Q. (1986) J. Cell Biol. **102**, 1638–1645
18. McLaughlin, S. G. A. & Dilger, J. P. (1980) Physiol. Rev. **60**, 825–863
19. Macey, R. I., Adorante, J. S. & Orme, F. W. (1978) Biochim. Biophys. Acta **512**, 284–295
20. Gallin, E. K. & Sheehy, P. A. (1988) in Inflammation: Basic Principles and Clinical Correlates (Gallin, J. I., Goldstein, I. M. & Snyderman, R., eds.), pp. 651–664, Raven Press, New York
21. Gallin, E. K. (1989) Am. J. Physiol. **257**, C77–C85
22. Al-Awqati, Q. (1986) Annu. Rev. Cell Biol. **2**, 179–199
23. Moriyama, Y. & Nelson, N. (1987) J. Biol. Chem. **262**, 9175–9180
24. Swallow, C. J., Grinstein, S., Sudsbury, R. A. & Rotstein, O. D. (1990) Surgery **108**, 363–369
25. Newsholme, P., Gordon, S. & Newsholme, E. A. (1987) Biochem. J. **242**, 631–636
26. Silver, I. A., Murrills, R. J. & Etherington, D. J. (1988) Exp. Cell Res. **175**, 266–276
27. Blair, H. C., Kahn, A. J., Crouch, E. C., Jeffrey, J. J. & Teitelbaum, S. L. (1986) J. Cell Biol. **102**, 1164–1172
28. Lang, F., Oberleithner, H., Kolb, H. A., Paulmichl, M., Völkl, H. & Wang, W. (1988) in pH Homeostasis: Mechanisms and Control (Häussinger, D., ed.), pp. 27–42, Academic Press, London
29. Bryant, R. E., Rashad, A. L., Mazza, J. A. & Hammond, D. (1980) J. Infect. Dis. **142**, 594–601
30. Zimmerli, W. & Gallin, J. I. (1988) Inflammation **12**, 37–43

Received 28 January 1991/20 June 1991; accepted 1 August 1991