Lymphocytes possess an electrogenic H⁺-transporting pathway in their plasma membrane

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The existence of an electrogenic H⁺-transporting pathway similar to that described in the plasma membrane of granulocytes and macrophages is reported in pig peripheral lymphocytes. The function of the H⁺-transport pathway can only be detected when free movement of charge-compensating cations is allowed. H⁺ transport is stimulated by arachidonic acid and various unsaturated fatty acids, and inhibited by bivalent cations, with the

following sequence of efficiency: Zn²⁺ > Cd²⁺ = Co²⁺ = Ni²⁺ > Mn²⁺ > Ba²⁺ = Ca²⁺ = Mg²⁺. The transport pathway is activated by intracellular acidification and by *NN'*-dicyclohexylcarbodiimide, but it is not influenced by phorbol 12-myristate 13-acetate. As pig peripheral lymphocytes are not able to produce O₂^{•-}, it is suggested that the operation of the electrogenic H⁺ conductance does not require the assembly of a functional NADPH oxidase.

INTRODUCTION

A Cd²⁺ and Zn²⁺-inhibitable electrogenic H⁺-conducting pathway has been described in the plasma membrane of human and pig granulocytes [1–7]. The operation of this transport route could be detected fluorimetrically as H⁺ movement in the direction of the prevailing electrochemical gradient. The electrogenic nature of the transport was evidenced by the accompanying changes of the membrane potential and the requirement for charge-compensating ion fluxes [1–5]. In depolarized, but otherwise unstimulated cells the transport route was active only at markedly acidic cytoplasmic pH (pH_i < 6.8) [6]. Stimulation of the cells by protein kinase C (PKC)-activating phorbol esters or arachidonic acid (AA) resulted in a significant rise in the pH_i threshold (0.4 unit) and caused a substantial increase in the rate of H⁺ efflux in the whole pH_i range tested [4–7]. In contrast with the facilitating effect of internal H⁺ ions, external H⁺ ions inhibited the pathway [6].

Detailed electrophysiological studies carried out recently on HL-60 granulocytes [8] and murine macrophages [9] have fully supported the data obtained previously from measurements on cell suspensions. By using the patch-clamp technique in the whole-cell configuration, an entirely H⁺-selective current was detected. The current showed a strict outward rectification: it could be activated only if the H⁺ electrochemical gradient favoured H⁺ extrusion. Both intracellular acidification and extracellular alkalization shifted the voltage threshold of the H⁺ conductance to more negative potentials, whereas Cd²⁺ and Zn²⁺ induced a significant shift to more positive values [8,9].

An electrogenic H⁺ conductance could play an important role in the physiological function of different phagocytic cells. These cells possess the NADPH oxidase enzyme that is rapidly activated following various stimuli. Activation of the enzyme consists of the translocation of three cytosolic proteins (p47_{phox}, p67_{phox} and rac) to the membrane-bound component, the cytochrome b₅₅₈ [10]. The functioning enzyme transfers one electron from intracellular NADPH to extracellular (or intraphagosomal) O₂, thereby producing O₂^{•-} in the external space and H⁺ ions in the internal space. An electrogenic H⁺-conducting pathway could

provide a compensating mechanism for the removal of intracellular H⁺ ions driven by their electrochemical gradient. The electrophysiological properties of the putative H⁺ channel favour its opening upon stimulation of the cell, when PKC and phospholipase A₂ (PLA₂) could be activated and both the membrane potential and pH_i fall, whereas extracellular pH might increase in the vicinity of the cell membrane due to the dismutation of the O₂^{•-} ions produced.

The possibility of a closer relationship between the NADPH oxidase and the H⁺-transporting pathway has been raised on the basis of the following considerations. (1) Both the NADPH oxidase and the H⁺ conductance are activated by phorbol 12-myristate 13-acetate (PMA) and AA, and both are blocked by inhibitors of PLA₂ [6,7,10,11]. (2) Cytochrome components of the mitochondrial respiratory chain were shown to transfer both electrons and H⁺ ions [12]. (3) The electrogenic H⁺-transporting pathway was shown to be defective in neutrophil granulocytes of patients suffering from different forms of chronic granulomatous disease [13]. The granulocytes of these patients are unable to produce O₂^{•-} because of the genetic lack of either the membrane-bound cytochrome or one of the cytosolic factors (p47_{phox} or p67_{phox}) [14]. On the basis of these findings, Nanda and co-workers [13] suggested that the functioning H⁺-transport pathway requires the assembly of an active oxidase.

The aim of the present study was to gain further insight into the relationship of the NADPH oxidase and the putative H⁺ channel by examining the existence of an electrogenic H⁺ transport in a blood-cell type that is unable to produce O₂^{•-}. Our results demonstrate that peripheral lymphocytes possess an electrogenic H⁺-transport pathway which is in many respects similar to that of the phagocytic cells; however, it appears not to be subject to regulation by PKC.

MATERIALS AND METHODS

Chemicals and solutions

2',7'-Bis(carboxyethyl)-5(6)-carboxyfluorescein acetoxymethyl ester (BCECF/AM) and nigericin were obtained from Calbiochem; dimethyl sulphoxide (DMSO), PMA, AA,

Abbreviations used: AA, arachidonic acid; BCECF/AM, 2',7'-bis(carboxyethyl)-5(6)-carboxyfluorescein acetoxymethyl ester; DCCD, *NN'*-dicyclohexylcarbodiimide; DMSO, dimethyl sulphoxide; PKC, protein kinase C; PLA₂, phospholipase A₂; PMA, phorbol 12-myristate 13-acetate; pH_i, intracellular pH.

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peroxidase-labelled anti-rabbit IgG and 4-chloro-1-naphthol were from Sigma; valinomycin was from Serva; Percoll was from Pharmacia.

The PBS medium contained 137 mM NaCl, 8.1 mM Na_2HPO_4 , 2.7 mM KCl, 1.5 mM KH_2PO_4 and 5 mM glucose, pH 7.3. The KCl medium contained 140 mM KCl, 5 mM Tris/HCl and 5 mM glucose, pH 7.4. The NaCl medium consisted of 140 mM NaCl, 5 mM Tris/HCl and 5 mM glucose, pH 7.4.

Cell preparation

Lymphocytes and neutrophil granulocytes were isolated from citrated pig blood basically as described in [15]. Briefly, erythrocytes were eliminated by hypo-osmotic lysis by dilution in 4 vol. of distilled water for 30 s. After restoration of iso-osmotic conditions with concentrated NaCl, cells were pelleted by centrifugation at 290 *g* for 10 min at 20 °C, then washed with PBS, centrifuged again and resuspended in PBS. Samples (10 ml) were layered on the top of 10 ml of 55% (v/v) Percoll in PBS. After centrifugation for 20 min at 1200 *g*, lymphocytes were collected from the top of the Percoll and granulocytes from the bottom layer. Both cell types were washed in PBS, and after pelleting their final concentration was adjusted to 10^9 cells/ml. The purity of the preparations was above 90%.

Measurement of pH_i

Cells (10^9) suspended in 1 ml of PBS were incubated with 4 μM BCECF/AM at 37 °C for 20 min. BCECF-loaded cells were then diluted, washed and resuspended at $(5-7) \times 10^8$ /ml in PBS.

For the measurement of pH_i , cells (1.25×10^7) were suspended in 2.5 ml of KCl or NaCl medium. Fluorescence was monitored with a Perkin-Elmer 3000 spectrofluorimeter, with wavelengths of 495 and 530 nm for excitation and emission respectively. Calibration of the signal in terms of pH_i was carried out as described in [5]. No significant change in the baseline fluorescence was observed up to 4 h from the time of loading the cells with the indicator.

Manipulation of pH_i

Where indicated, the intracellular compartment of BCECF-loaded cells was acidified by using the ammonium pre-pulse technique [16] as detailed in [5]. Cells were finally resuspended in 150 mM choline chloride, 5 mM Tris/HCl and 5 mM glucose, pH 7.4. Acidified cells were kept at 4 °C. At this temperature no change of their membrane permeability properties was detectable up to 4 h.

Measurement of $\text{O}_2^{\cdot-}$ production

Generation of $\text{O}_2^{\cdot-}$ was determined photometrically as the superoxide dismutase-inhibitable reduction of cytochrome *c* [17].

Immunodetection of p47_{phox} and p67_{phox}

Lymphocytes or granulocytes (7.5×10^5 cells) were lysed in hot sample buffer containing 2% SDS and loaded on polyacrylamide gels. The separation gel contained 12% acrylamide. Electrophoretic separation, transfer to nitrocellulose sheets and reaction with the antibodies were carried out as detailed in [18].

RESULTS AND DISCUSSION

Cd^{2+} -sensitive electrogenic H^+ translocation in lymphocytes lacking a functional NADPH oxidase

The capacity for $\text{O}_2^{\cdot-}$ production and the presence of oxidase components were compared in pig peripheral neutrophil granulocytes and lymphocytes. Neutrophil granulocytes, when stimulated by 250 nM PMA or 20 μM AA, produced on average 3.9 ± 1.6 or 2.0 ± 0.7 nmol of $\text{O}_2^{\cdot-}$ /min per 10^6 cells respectively ($n = 13$). No $\text{O}_2^{\cdot-}$ production was detected in the pig lymphocyte preparation stimulated with either PMA or AA (results not shown). In agreement with this observation, the typical cytosolic components of the oxidase (p47_{phox} and p67_{phox}) could not be detected in pig peripheral lymphocytes (Figure 1).

H^+ movements through the plasma membrane of peripheral lymphocytes were measured by monitoring changes in the pH_i under the same conditions as used for the characterization of the H^+ pathway of neutrophil granulocytes [5,7]. Lymphocytes loaded with the fluorescent pH indicator BCECF were acidified to pH_i 6.44 ± 0.16 ($n = 7$) by the ammonium pre-pulse technique [16]. The cells were suspended in a KCl-based medium buffered at pH 7.4, where the membrane potential is decreased by the high external K^+ concentration [19] and the pH gradient of approx. 1 unit serves as the driving force favouring H^+ efflux. Activity of the Na^+/H^+ antiporter was inhibited by the absence of Na^+ from the external medium.

Typical observations are summarized in Figure 2(a). After suspending the cells in the KCl medium, only a slight pH_i increase could be detected, with an average rate of 0.0249 ± 0.0117 $\Delta\text{pH}/\text{min}$ ($n = 5$). Addition of the K^+ ionophore valinomycin stimulated the H^+ extrusion, and the transport rate increased 4–5-fold, reaching an average of 0.106 ± 0.006 $\Delta\text{pH}/\text{min}$ ($n = 8$) (Figure 2a, trace A). When the cells were incubated without valinomycin, no significant change was observed in the transport rate during the same time (Figure 2a, trace B). In the absence of a pH gradient (non-acidified cells or dissipation of the pH gradient by nigericin), valinomycin has no effect on pH_i at all (results not shown). The fact that H^+ efflux is significantly promoted by valinomycin, i.e. by allowing charge-compensating K^+ movement, suggests that the H^+ transport detected is an electrogenic process.

In the presence of 140 mM KCl in the extracellular medium, the concentration gradient of K^+ might be directed inwardly and H^+ extrusion could occur via K^+/H^+ exchange. This possibility was tested by following the reasoning of Nanda and Grinstein

Figure 1 Immunodetection of the cytosolic components of the NADPH oxidase

Granulocytes (GR) and lymphocytes (LY) were solubilized and investigated by SDS/PAGE as described in the Materials and methods section. The positions of p47_{phox} and p67_{phox} are indicated by the arrows '47' and '67' respectively. Results are shown for one representative experiment out of three similar ones.

Figure 2 Effect of valinomycin, Cd²⁺ and PMA on the H⁺ extrusion from lymphocytes

BCECF-loaded acidified cells (3×10^6) were suspended in 2.5 ml of KCl medium. The initial pH_i was 6.43. (a) Comparison of the H⁺ release (A) in the presence and (B) in the absence of valinomycin (Val; 0.8 μM). (C) Inhibition of the valinomycin-induced H⁺ efflux by 2 mM Cd²⁺. (b) Effect of 50 nM PMA on the electrogenic H⁺ transport. Substances were added where indicated by arrows. The Figure shows one representative experiment out of 15 similar ones.

[4]. We obtained a clear H⁺ efflux from acidified cells suspended in the NaCl medium (pH 7.25) when the Na⁺/H⁺ exchanger was blocked by an amiloride analogue and the membrane potential was decreased by gramicidin. In the absence of extracellular K⁺, both the K⁺ and the H⁺ electrochemical gradients point outwards. Under these conditions an exchange of the two univalent cations is highly improbable (results not shown).

The possible contribution of a H⁺-ATPase active in the plasma membrane has also been monitored. Bafilomycin was shown to block the vacuolar-type H⁺ pump at a concentration of 30 nM [20]. In our experiments, up to 400 nM bafilomycin had no effect on the detected H⁺ movement (results not shown).

It is important to note that no electrogenic H⁺ influx could be observed under conditions where the electrochemical gradient favoured H⁺ influx. In NaCl medium, lymphocytes build up a high membrane potential that should drive H⁺ ions inward even in the absence of a concentration gradient. However, no change in the pH_i could be observed when non-acidified cells in NaCl medium and in the presence of an amiloride analogue were treated with valinomycin (results not shown). Depolarization of the cells by gramicidin followed by acidification of the external medium by 0.75 unit also proved to be ineffective (results not shown). These findings are in agreement with the rectifying nature of the H⁺ conductance of macrophages: no inward current could be observed by the patch-clamp technique under conditions where the H⁺ electrochemical gradient favoured H⁺ entry [9].

The Cd²⁺-sensitivity of the detected H⁺ extrusion is shown in Figure 2(a), trace C. Addition of 2 mM Cd²⁺, a concentration that blocks electrogenic H⁺ movements in granulocytes completely, inhibits both basal and valinomycin-promoted H⁺-efflux also in lymphocytes.

Effect of pH_i, PMA and fatty acids on the Cd²⁺-inhibitable H⁺ movement

In granulocytes, the rate of electrogenic H⁺ movement was significantly increased by intracellular acidification, by stimulation of PKC by β-phorbol esters and by AA [4–7]. We tested the effect of all these factors on pig lymphocytes.

First we compared the rate of H⁺ efflux from acidified (pH_i ≈ 6.5) and non-acidified (pH_i ≈ 7.35) cells. The trans-

Figure 3 Effect of AA on the Cd²⁺-inhibitable H⁺ release from lymphocytes

BCECF-loaded acidified cells (3×10^6) were suspended in 2.5 ml of KCl medium. The initial pH_i was 6.4. (a) Stimulatory effect of different concentrations (indicated in μM above the traces) of AA on the valinomycin (Val; 0.8 μM)-induced H⁺ transport. The rate of H⁺ efflux was increased from 0.137 (without AA) to 0.821 ΔpH/min (in the presence of 7.5 μM AA). (b) Inhibitory effect of Cd²⁺ (B) on the AA-stimulated H⁺ extrusion (A). Where indicated by arrows, 0.8 μM valinomycin (Val), 7.2 μM AA and 500 μM CdCl₂ (Cd²⁺) was added. The Figure shows one representative experiment out of nine similar ones.

membrane pH gradient was in both cases 0.85 pH unit, which was achieved for non-acidified cells by sufficient alkalinization of the external medium. The calculated rate of H⁺ efflux was 0.106 ± 0.006 ΔpH/min ($n = 5$) for acidified cells, and 0.054 ± 0.007 ΔpH/min ($n = 4$) if the pH_i was in the physiological range. For granulocytes a similar tendency has been observed, although under comparable conditions the rate showed a 4-fold change [6].

In further experiments we investigated whether activation of PKC can influence the transport rate through our putative channel. The cells were incubated with 50 nM PMA, an efficient activator of PKC, 1 min before addition of valinomycin (Figure 2b) and the rate of H⁺ efflux was compared with that obtained in the presence of valinomycin only. PMA does not seem to influence the H⁺ extrusion from lymphocytes, as the ratio of the transport rates in the presence and absence of PMA was on average 1.01 ± 0.08 ($n = 4$). In accordance with previous observations [21], PMA definitively increased the rate of Na⁺/H⁺ exchange (results not shown), supporting the idea that pig lymphocytes do respond to the phorbol ester.

The results obtained with AA are summarized in Figure 3. Added in the range 1–10 μM, it caused a concentration-dependent elevation of the transport rate (Figure 3a). At a concentration of 5 μM, AA stimulated H⁺ efflux by $443 \pm 47\%$ ($n = 5$). In control experiments, the cells were pelleted after the completion of the transport process and the fluorescence of the cell-free supernatants was compared. No difference could be observed in the presence or absence of AA. This fact indicates that AA did not induce any non-specific leakage of the fluorescent dye from lymphocytes.

The H⁺ movement activated by AA also proved to be Cd²⁺-sensitive. In the presence of 0.5 mM Cd²⁺ (added before valinomycin) the transport rate was decreased by $82.1 \pm 6.1\%$ ($n = 5$) (Figure 3b).

To decide whether the stimulation described is specific for AA, we tested the effect of several saturated and unsaturated fatty acids on the electrogenic H⁺ extrusion. Figure 4 shows some typical results. All the unsaturated fatty acids examined were able to stimulate electrogenic H⁺ transport. When applied in the same concentration, the order of their activatory effect was as follows: elaidic acid > AA > linoleic acid > linolelaidic acid

Figure 4 Effect of different fatty acids on the H^+ efflux from acidified lymphocytes

BCECF-loaded acidified cells (3×10^6) were suspended in 2.5 ml of KCl medium. The initial pH_i was 6.51. Where indicated, fatty acids, $CdCl_2$ and valinomycin were added in the following concentrations: (a)–(d), $8 \mu M$ valinomycin (Val); (b) $5 \mu M$ stearic acid (S); (c) $2 \mu M$ linoleic acid (L) and $60 \mu M$ $CdCl_2$; (d) $4 \mu M$ palmitoleic acid (P) and $400 \mu M$ $CdCl_2$. The Figure shows one representative experiment out of five similar ones.

Figure 5 Effect of different Cd^{2+} concentrations on the valinomycin-induced (●) and on the valinomycin + AA (○)-induced H^+ extrusion from lymphocytes

Acidified cells (3×10^6) were suspended and incubated in 2.5 ml of KCl medium in the absence (●) or presence (○) of AA ($7.2 \mu M$). After incubation for 2 min, $CdCl_2$ was added at different concentrations. At 3 min, cells were treated with $0.8 \mu M$ valinomycin, and the rate of H^+ release was measured: 100% corresponds to $0.073 \Delta pH/min$ in the absence and $0.798 \Delta pH/min$ in the presence of AA. The Figure shows one representative experiment out of four similar ones.

Figure 6 Inhibitory effect of different cations on the H^+ efflux from acidified lymphocytes

Cells (3×10^6) were suspended in 2.5 ml of the KCl medium. Various concentrations of $BaCl_2$ (□), $MnCl_2$ (■), $CoCl_2$ (●), $ZnCl_2$ (▷) and $NiCl_2$ (○) were added after 1 min of incubation. Transport was induced by addition of $0.8 \mu M$ valinomycin at 2.5 min. The Figure shows one representative experiment out of three similar ones.

$\geq$ oleic acid $\geq$ palmitoleic acid $\geq$ linolenic acid $\geq$ palmitelaidic acid. The Cd^{2+} -sensitivity of these processes was similar to that observed previously for AA (see Figure 3). In contrast with this, none of the saturated fatty acids tested (stearic acid and hexanoic acid) was able to influence the transport rate.

Our results suggest that the electrogenic transport activity detected can be enhanced by different unsaturated fatty acids, and this activated transport is also sensitive to the inhibitory bivalent cation, Cd^{2+} . Several of the activating fatty acids are not metabolized either by the cyclo-oxygenase or by the lipoxygenase enzymes; thus derivatives of the AA produced in these pathways seem not to play a role in the regulation of the H^+ conductance. Fatty acids might have a more direct effect on the electrogenic H^+ pathway.

Inhibition of the electrogenic H^+ efflux by different bivalent cations

As shown in the previous Figures, electrogenic H^+ movements were inhibited by Cd^{2+} both under basal conditions (i.e. in the presence of valinomycin only) and when stimulated by different fatty acids. The dependence of the two processes on the concentration of Cd^{2+} proved to be rather similar (Figure 5). Half-maximal inhibition was attained under basal conditions at $40 \mu M$ and in the presence of AA at $80 \mu M$. Both values are near to that reported previously for granulocytes [5].

The specificity of the inhibitory effect of Cd^{2+} was also tested. Figure 6 summarizes the dependence of the rate of basal H^+ transport on the concentrations of Zn^{2+} , Co^{2+} , Ni^{2+} , Mn^{2+} and Ba^{2+} applied. Similar results (not shown) were obtained when H^+ efflux was stimulated by AA. The sequence of decreasing inhibitory effect of the various cations is as follows: $Zn^{2+} > Co^{2+} \geq Cd^{2+} \geq Ni^{2+} > Mn^{2+} > Ba^{2+} \approx Ca^{2+} \approx Mg^{2+}$. This sequence is different from those reported for any previously characterized ion channel in the plasma membrane of lymphocytes [22]. It should be noted that extracellular Ca^{2+} and Mg^{2+} added in the physiological concentration range do not influence the activity of the electrogenic H^+ transport.

The similar sensitivity of basal and fatty-acid-stimulated H^+ -extrusion both to Cd^{2+} and to several other bivalent cations raises the possibility that the electrogenic H^+ efflux occurs in both cases through the same transport protein, which might represent a hitherto undescribed molecular entity of the lymphocyte plasma membrane.

Effect of *NN'*-dicyclohexylcarbodi-imide (DCCD) on the electrogenic H^+ extrusion

Several H^+ transporters, mainly mitochondrial H^+ pumps, are inhibited by DCCD [23], so it was decided to test the effect of this drug on electrogenic H^+ movements in lymphocytes. In contrast with the expectations, DCCD proved to be an efficient stimulator of the H^+ efflux. It caused a concentration-dependent increase in the rate of intracellular alkalinization (Figure 7), levelling off at around 100 – $150 \mu M$ DCCD and achieving a 3.4 ± 0.6 -fold stimulation ($n = 5$) at the concentration of $80 \mu M$. Parallel to the increase in pH_i , extracellular acidification could be detected by a pH electrode, when the cells were suspended in a weakly buffered medium. Thus DCCD stimulated the transmembrane movement of protons. Applied at a concentration of $40 \mu M$, DCCD clearly increased the effect of up to $6 \mu M$ AA (results not shown). At higher concentrations of either compound, non-specific effects were observed.

H^+ release effected by DCCD was also dependent on the presence of valinomycin and could be inhibited by the same

Figure 7 Stimulation of the H⁺ efflux by DCCD

Cells were suspended as described for Figure 6. After 1 min of incubation, DCCD was added at different concentrations. At 2.5 min the cells were treated with 0.8 μM valinomycin. The Figure shows one representative experiment out of three similar ones.

concentrations of Cd²⁺ as documented above for the basal and fatty-acid-induced H⁺ outflow (results not shown). These similarities raise the possibility that DCCD could activate the same H⁺ conductance as did AA and the other fatty acids. The stimulatory effect of DCCD is unexpected, but not unprecedented: in thylakoid membranes DCCD was reported to short-circuit the H⁺ movement within the photosynthetic water oxidase [24] and to activate an endogenous univalent-cation transport system [25]. It is noteworthy that DCCD also activates the NADPH oxidase of neutrophils [25a].

Conclusions

On the basis of our experiments, we propose that pig peripheral lymphocytes possess a H⁺-transporting pathway, the properties of which resemble in the following respects the well-characterized pathway in granulocytes and macrophages.

(1) The transport route is electrogenic, and its functioning becomes apparent only in the presence of suitable charge compensation.

(2) The H⁺ pathway is probably allosterically regulated by pH_i: when the driving force is kept constant, intracellular acidification clearly enhances the transport rate.

(3) It is stimulated by AA and other unsaturated fatty acids [similar results were obtained on granulocytes by K. Suszták, K. Káldi, A. Kapus and E. Ligeti (unpublished work) and on macrophages in [26]].

(4) Under comparable conditions, the rate of intracellular alkalinization in lymphocytes is similar to the values reported for granulocytes [5,6].

(5) The pathway exhibits a unique order of sensitivity towards bivalent cations: it is most effectively inhibited by Zn²⁺, Cd²⁺ and Ni²⁺, whereas it is not influenced at all by Ba²⁺ (a good inhibitor for many K⁺ channels) and by Ca²⁺ and Mg²⁺, the bivalent cations of most physiological relevance [identical results were obtained on granulocytes by K. Suszták, K. Káldi, A. Kapus and E. Ligeti (unpublished work)].

(6) The opening mechanism shows asymmetry: opening induced by changes of the membrane potential and the H⁺-concentration gradient could be achieved only under the conditions when the electrochemical H⁺ gradient favoured H⁺ efflux.

The only significant difference that we observed in the behaviour of the electrogenic H⁺-transport pathway of granulocytes and lymphocytes was their sensitivity towards the PKC activator PMA. Whereas PMA effectively stimulated H⁺ movements in granulocytes, it had no influence on the electrogenic H⁺ transport in lymphocytes. The difference might be due to distinct regulation of PLA₂ in the two cell types. PMA was shown to stimulate the liberation of AA from granulocytes and macrophages [27], but not from lymphocytes [28].

Taking together our results, we propose that lymphocytes possess an electrogenic H⁺-transporting pathway similar to that characterized in granulocytes and macrophages. However, lymphocytes are not able to produce O₂⁻, as they lack the essential cytosolic components. In this view, the molecules responsible for electrogenic H⁺ transport and for electron transport from NADPH to molecular oxygen are probably different entities. It is, however, conceivable that the regulation of the two inter-related processes might include common element(s).

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