Abnormal activation of H+ conductance in NADPH oxidase-defective neutrophils
(proton channel/chronic granulomatous disease/proton pump/Na/H exchange/superoxide/ granulocytes)

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ABSTRACT To combat bacterial infection, phagocytes generate superoxide (O2•−) and other microbicidal oxygen radicals. NADPH oxidase, the enzyme responsible for O2•− synthesis, is deficient in chronic granulomatous disease (CGD) patients. Although O2•− generation is accompanied by a large burst of metabolic acid production, intracellular pH (pHi) remains near neutrality due to the concomitant stimulation of H+ extrusion. Three major pathways contribute to pHi regulation in activated phagocytes: Na+/H+ exchange, vascular-type H+ pumps, and a H+ conductance. The present study analyzed the relationship between activation of the NADPH oxidase and stimulation of the H+ extrusion mechanisms in human blood neutrophils. Phorbol ester-induced activation of Na+/H+ exchange and H+ pumping occurred normally in cells from CGD patients. Unlike normal individuals, however, CGD patients were unable to activate the H+ conductive pathway. Thus, activation of the H+ conductance appears to be contingent on the assembly of a functional NADPH oxidase. These findings imply a dual role of the NADPH oxidase in O2•− synthesis and in the regulation of pHi. The oxidase (or some components thereof) may itself undertake H+ translocation or, alternatively, may signal the activation of a separate H+ conducting entity.

When confronted by invading microorganisms, phagocytic cells catalyze the one-electron reduction of molecular oxygen. The resultant superoxide anions (O2•−) and various reduced oxygen metabolites derived therefrom have potent microbicidal effects. A multisubunit complex known as the NADPH oxidase is the molecular entity responsible for O2•− generation (1–6). The importance of this enzyme is underscored by individuals afflicted with chronic granulomatous disease (CGD). The phagocytes of these patients fail to assemble a functional oxidase and are therefore unable to mount a respiratory burst. Because microbial killing is impaired, CGD patients suffer from severe and recurrent bacterial infections (1).

The NADPH oxidase is composed of at least five, and possibly more, subunits. Three of these are cytosolic and have molecular masses of 47 kDa (p47-phox), 67 kDa (p67-phox), and 22 kDa (rac-1, rac-2, or krev-1) (7–9). In addition, there is a membrane-associated cytochrome b59s moiety, composed of 91 kDa (gp91-phox) and 22 kDa (p22-phox) subunits. The existence of a membrane-bound flavoprotein component has been suggested (reviewed in ref. 3) but not yet demonstrated conclusively. Upon stimulation of the phagocyte, the cytosolic components migrate to the membrane, where they associate with the cytochrome, thereby rendering the oxidase complex functional. Oxidation of NADPH to NADP+ ensues, which is accompanied by release of H+ into the cytosol. These H+, together with the acid equivalents produced during regeneration of NADPH by the hexose monophosphate shunt, impose a severe strain on the maintenance of the intracellular pH (pHi). To alleviate this acid stress, H+ are extruded from the cells by at least three independent mechanisms: a Na+/H+ antiport (10, 11), a vacuum- (or V-type) H+-ATPase (12), and a H+ conductance (13). The antiport performs the electroneutral exchange of intracellular H+ for extracellular Na+ and is exquisitely susceptible to inhibition by amiloride and its analogs (10, 11, 14). Vacular H+-ATPases are electrogenic pumps that can be selectively blocked by bafilomycins, a group of macrolide antibiotics (12, 15). Flux through the conductive pathway is passive, driven by the electrochemical H+ gradient. It is insensitive to amiloride or bafilomycin but is instead blocked by Zn2+, thus resembling H+ channels described earlier in invertebrate neurons (16, 17). The activity of these three transport pathways is enhanced by stimulation of the cells. This effect is particularly apparent in the case of the V-type pump and the H+ conductance, which are virtually undetectable in unstimulated cells. Because activation of the NADPH oxidase occurs concomitantly, an association between these events seemed possible. The purpose of the experiments described below was to establish whether assembly of a competent oxidase was required for activation of the H+ transporting systems.

MATERIALS AND METHODS

Materials. Bafilomycin A1 was the kind gift of K. Altendorf (University of Osnabruck, Osnabruck, Germany). N-Methyl-N-(2-methyl-2-propenamino)amiloride (MMPA) was a gift from Merck, Sharp & Dohme. Diphenylene iodonium (DPI) was synthesized in our laboratory as described (18).

Solutions. Na+ medium contained 140 mM NaCl, 3 mM KCl, 1 mM MgCl2, 1 mM CaCl2, 10 mM glucose, and 20 mM Heps, titrated to pH 7.5 with concentrated NaOH. K+ medium was made by isoosmotic replacement of NaCl with KCl and was titrated to the same pH with KOH. The osmolarity of all solutions was adjusted to 295 ± 5 milliosmolar with the major salt.

Methods. Neutrophils were isolated from freshly drawn blood of normal or CGD donors, as described (19). pHi was measured fluorimetrically using the pH-sensitive probe 2',7'-bis(2-carboxyethyl)-5(6)-carboxyfluorescein. Neutrophils were loaded with the probe by preincubation with 2 µg/ml of the precursor 2',7'-bis(2-carboxyethyl)-5(6)-carboxyfluorescein acetoxyethyl ester for 10 min at 37°C. After washing,

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Abbreviations: CGD, chronic granulomatous disease; pHi, intracellular (cytosolic) pH; NBT, nitroblue tetrazolium; DPI, diphenylene iodonium; TPA, 4β-phorbol 12-tetradecanoate 13-acetate; MMPA, N-methyl-N-(2-methyl-2-propenamino)amiloride; FCCP, carbonyl cyanide p-trifluoromethoxyphenylhydrazone.

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RESULTS AND DISCUSSION

Neutrophils from normal and CGD donors were compared to assess the possible role of a functional NADPH oxidase in the activation of H\(^+\) transport. Although all of the CGD patients studied were unable to synthesize reduced oxygen metabolites, as demonstrated by the nitroblue tetrazolium (NBT) test (Table 1), two distinct subgroups could be identified. Patients 1 through 6 contained normal levels of immunoreactive p47-phox (Table 1) and p67-phox (not shown) but had severely deficient expression of gp91-phox (leading to a secondary loss of p22-phox as well) and consequently undetectable levels of cytochrome b\(_{558}\) (Table 1). In accordance with the current classification system (1), these patients belong to the X91\(^{-}\) type. As described earlier (1, 2), neutrophils from carriers of the mutant gp91-phox gene are heterogeneous. A subpopulation of the cells expresses normal levels of gp91-phox, has a functional oxidase, and can therefore reduce NBT, whereas the remainder is defective. As a result, the overall levels of these parameters are only a fraction of those in normal individuals (see carriers 1A and 6A in Table 1). A second group of CGD patients (7 through 9) contained no detectable levels of p47-phox, whereas cytochrome b\(_{558}\) levels were normal (Table 1). The defect in these patients had an autosomal mode of inheritance and was hence classified as A47\(^{-}\) (1).

Fig. 1A illustrates the burst of metabolic acid generation observed in normal neutrophils stimulated with 4\β-\)phorbol 12-tetradecanoate 13-acetate (TPA), a potent activator of protein kinase C. Under conditions where effective pH\(_{i}\) regulation is precluded by omission of Na\(^+\) and addition of bafilomycin (thereby impairing Na\(^+\)/H\(^+\) exchange and H\(^+\) pumping through the V-type ATPase), respectively; see Table 2), TPA induced a marked cytosolic acidification, at an average rate of 0.077 ± 0.010 pH unit/min (Table 3). The acidification was even more pronounced when the third H\(^+\) regulatory pathway, the H\(^+\) conductance, was blocked by addition of Zn\(^{2+}\) (not shown). That these acid equivalents are generated by the NADPH oxidase and/or the associated acceleration of the hexose phosphate shunt was confirmed by stimulation of normal cells in the presence of DPI, a flavoprotein antagonist that is a highly selective inhibitor of the oxidase (21). In the presence of DPI, addition of TPA had little effect on pH\(_{i}\) (upper trace of Fig. 1A). The role of the oxidase in the acidification was further demonstrated in CGD neutrophils. As shown earlier (22), stimulation of these cells was not accompanied by cytosolic acidification, whether in the absence (Fig. 1E) or presence of DPI (not shown). It is noteworthy that the pH\(_{i}\) of normal and CGD cells prior to stimulation was indistinguishable (Table 3) as was the intrinsic buffering power\(^\text{5}\) of the two groups of cells. Thus, the highly significant (P < 0.001) difference in the rate of acidification cannot be attributed to differences in these parameters and is more likely due to the failure of CGD cells to mount a respiratory burst. Consistent with this notion, the acidification rate of X91\(^{-}\) carriers was intermediate between that of control and CGD individuals (Table 3).

The activation of the three H\(^+\) transporting systems upon stimulation by phorbol ester was studied next. In these experiments DPI was included to prevent acid generation by the oxidase, thereby facilitating detection of the H\(^+\) extrusion systems and optimizing the comparison of normal and CGD cells. Though not strictly required, DPI was also present in experiments using CGD neutrophils, to maintain the same conditions used for normal cells. Activation of the Na\(^+\)/H\(^+\) antiport was analyzed first (Table 2). In normal cells suspended in Na\(^+\)-rich medium, addition of TPA induced a rapid cytosolic alkalinization (Fig. 1B, upper trace) with an average initial rate of 0.16 ± 0.02 pH per min (Table 3). The rate and magnitude of the pH\(_{i}\) change are considerably greater than reported earlier for cells not treated with DPI (22). This implies that, in the absence of the flavoprotein antagonist, metabolic acid generated by the oxidase offsets the net H\(^+\) extrusion by the antiport. As shown in Fig. 1B (lower trace), the phorbol ester-induced alkalinosis was completely obliterated.

The cytoplasmic H\(^+\) buffering power was measured by titration with NH\(_{4}^+\) (23) and was consistently between 30 and 38 mmol of H\(^+\) per liter of cells per pH unit in normal and CGD neutrophils suspended in nominally bicarbonate-free solutions.

Table 1. Summary of CGD patients and carriers analyzed in this study

<table>
<thead>
<tr>
<th>Subject</th>
<th>Sex</th>
<th>% positive cells</th>
<th>Cytochrome b(_{558}), pmol per 10(^6) cells</th>
<th>p-47 immunoblot(^\dagger)</th>
<th>CGD type(^\dagger)</th>
<th>Mutant oxidase gene</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patient</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1. S.K.</td>
<td>♂</td>
<td>0</td>
<td>0</td>
<td>N</td>
<td>X91(^{-})</td>
<td>gp91-phox</td>
</tr>
<tr>
<td>2. C.C.</td>
<td>♂</td>
<td>0</td>
<td>0</td>
<td>N</td>
<td>X91(^{-})</td>
<td>gp91-phox</td>
</tr>
<tr>
<td>3. M.W.</td>
<td>♂</td>
<td>0</td>
<td>0</td>
<td>N</td>
<td>X91(^{-})</td>
<td>gp91-phox</td>
</tr>
<tr>
<td>4. J.H.</td>
<td>♂</td>
<td>0</td>
<td>0</td>
<td>N</td>
<td>X91(^{-})</td>
<td>gp91-phox</td>
</tr>
<tr>
<td>5. J.W.</td>
<td>♂</td>
<td>0</td>
<td>0</td>
<td>N</td>
<td>X91(^{-})</td>
<td>gp91-phox</td>
</tr>
<tr>
<td>6. J.B.H.</td>
<td>♂</td>
<td>0</td>
<td>0</td>
<td>N</td>
<td>X91(^{-})</td>
<td>gp91-phox</td>
</tr>
<tr>
<td>7. G.P.</td>
<td>♂</td>
<td>51.5</td>
<td>0</td>
<td>A47</td>
<td>gp47-phox</td>
<td></td>
</tr>
<tr>
<td>8. R.G.</td>
<td>♂</td>
<td>60.1</td>
<td>0</td>
<td>A47</td>
<td>gp47-phox</td>
<td></td>
</tr>
<tr>
<td>9. J.C.</td>
<td>♀</td>
<td>66.7</td>
<td>0</td>
<td>A47</td>
<td>gp47-phox</td>
<td></td>
</tr>
<tr>
<td>Carrier</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1A. W.H.</td>
<td>♀</td>
<td>48</td>
<td>ND</td>
<td>ND</td>
<td>X91(^{carrier})</td>
<td>gp91-phox</td>
</tr>
<tr>
<td>6A. S.H.</td>
<td>♀</td>
<td>26</td>
<td>38</td>
<td>ND</td>
<td>X91(^{carrier})</td>
<td>gp91-phox</td>
</tr>
</tbody>
</table>

Nine patients with CGD (six with X chromosome-linked absence of cytochrome b and three with autosomal recessive inheritance of p47-phox deficiency) were studied. Their respiratory burst oxidase properties are summarized here. See text for further details. ND, not determined.

\(^\text{a}\)Normal % NBT-positive cells is 99–100%. In all CGD cases, no O\(_2\) was detectable by the cytochrome c assay.

\(^\text{b}\)Normal range, 73.0 ± 4.8 pmol per 10\(^7\) cells (mean ± SE, n = 42).

\(^\text{c}\)N = normal.

\(^\text{d}\)CGD type: in this nomenclature (1), the first letter represents the mode of inheritance [X-linked (X) or autosomal (A)] and the number indicates the phox component that is genetically affected. The superscript symbols indicate whether the level of protein component affected is undetectable (*), diminished (~), or normal (+) as measured by immunoblot analysis.

\(^\text{e}\)Carriers 1A and 6A are the mothers of patients 1 and 6, respectively.
antipor activity. This lower rate may be related to the somewhat alkaline resting pH of this patient (Table 3), as the activity of the exchanger is exquisitely sensitive to the cytosolic pH (10, 11). Nonetheless, significant activation of the Na+/H+ antiport was observed in both types of CGD.

In assaying the activity of the V-type H+-ATPase, it was necessary to minimize H+ efflux through the other H+ extrusion mechanisms as well as H+ generation by the NADPH oxidase. For this purpose, Na+/H+ antiport activity was precluded by removal of external Na+ while the H+ conductance was eliminated by treating the cells with Zn2+ (Table 2). Valinomycin was added to provide a path for counterion (K+) permeation, thereby neutralizing the electronegative nature of the H+ fluxes. Under these conditions, a bafilomycin-sensitive extrusion of H+ can be attributed to the action of a vacuolar-type pump (12). As shown in Fig. 1 C and G, stimulation of normal and CGD neutrophils with the phorbol ester resulted in a delayed alkalinization that was completely inhibited by bafilomycin A1. Moreover, addition of the inhibitor prior to stimulation precluded the response (lower traces, Fig. 1 D and H), implying activation of a V-type H+-ATPase by the phorbol ester.9 No significant difference in the activity of the pumps was noted between normal neutrophils and those from A47° or X91° CGD cells (Table 3). Together with the results described above, these indicate that assembly of a functional NADPH oxidase is not essential for the activation of the Na+/H+ antiport or the V-type H+-ATPase. Inasmuch as CGD cells and normal cells treated with DPI fail to acidify the cytosol (see above), it can also be concluded that the activation of these transporters occurs directly in response to TPA, presumably through stimulation of protein kinase C, and is not an indirect consequence of the intracellular pH changes generated by the NADPH oxidase.

Strikingly different results, however, were obtained with the H+ conductance. The activity of this pathway was determined in cells treated with DPI and suspended in a K+-rich medium (Table 2). The latter served to block Na+/H+ antiport activity and, in combination with valinomycin, also depolarized the cells and provided a pathway for counterion permeation to neutralize the rheogenic nature of the H+ fluxes. Bafilomycin was also added, to inhibit the V-type H+ pump. Under the conditions selected for estimation of the activity of the conductive pathway, pH was stabilized at 6.9 prior to stimulation. Because the membrane potential is near 0 mV and the pH of the external medium was 7.5, the net H+ electrochemical gradient is directed outward. Thus, an increase in conductance to H+ equivalents is predicted to result in an outward flux (see ref. 13 for detailed rationale). Under these conditions, treatment of normal neutrophils with TPA promoted a large and rapid alkalinization (Fig. 1 D, upper trace). The involvement of the conductive pathway in this pH change is supported further by the effects of Zn2+, which largely eliminated the net H+ extrusion (Fig. 1 D, lower trace). Addition of the exogenous protonophore FCCP readily bypassed the blocking effects of Zn2+, verifying the outward direction of the electrochemical H+ gradient and confirming the integrity of the cells and the sensitivity of the pH measurements.

In contrast to normal neutrophils, cells from CGD patients displayed only a minute change in H+ conductance upon

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9 The slight initial acidification noted in normal cells likely reflects residual metabolic acid generation, due to incomplete inhibition of the oxidase by the dose of DPI used. Under these conditions, DPI inhibited ~95% of the oxidase activity, as determined from the rate of TPA-induced O2 consumption, measured polarographically. This component was not detectable in Fig. 1 A and B likely because of concomitant acid extrusion through the conductive pathway, which was not blocked in these instances.

**Fig. 1.** pH determinations in TPA-activated neutrophils. Human neutrophils from normal (A–D) or X91° CGD donors (E–H) were isolated from whole blood and pH was measured fluorimetrically using 2',7'-bis(2-carboxyethyl)-5(6)-carboxyfluorescein and calibrated as described in the text. Assays for metabolic acidification (A and E), Na+/H+ antipor (B and F), V-type H+-ATPase (C and G), and H+ conductance (D and H) are shown, using the conditions outlined in Table 2. Neutrophils (2 × 106) were sedimented and suspended in either K+ medium (A, C–E, G, and H) or Na+ medium (B and F). Where indicated below the traces, 100 nM bafilomycin A1 (BAF), 3 μM DPI, 1 μM valinomycin (VAL), or 50 μM ZnCl2 (Zn2+) was present in the cell suspension from the outset, 1–5 min prior to activation with the phorbol ester. In A, DPI (3 μM) was added to the upper trace only. The amiloride analogue MMPA (1 μM) was present prior to stimulation in the lower trace of B and F, whereas Zn2+ (50 μM) was present from the outset in the lower traces of D and H. Additions of TPA (100 nM), bafilomycin (BAF; 100 nM), or carbonylcyanide p-trifluoromethoxyphenyldrazine (FCCP; 1 μM) are indicated by arrows. Traces in A–D are representative of six experiments using normal neutrophils from different donors; traces in E–H are from X91° patients and are representative of all of the CGD patients (X91° and A47°) studied (see Table 3). Temperature was 37°C. The scale time applies to all traces.
stimulation with phorbol ester (Fig. 1H). In X91° and A47°
CGD cells the rate of pH change, a measure of the conduc-
tance, was ≤15% of the control rate (Table 3). As in Zn2+-
treated control cells, addition of FCCP to CGD neutrophils
resulted in a sizable alkalinization, confirming the presence of
an outwardly directed H+ electrochemical gradient. Patients
heterozygous for the mutant gp91-phox gene were also
tested. As indicated in Table 3, the two X91°carrier individuals
showed a H+ conductance that was intermediate between
that of normal subjects and homozygous X91° patients.

Together, the above results suggest a strong correlation
between the presence of a functional NADPH oxidase and
stimulation of the H+ conductance. Our findings imply that
p47-phox and gp91-phox are necessary to activate the H+
conductance. We interpret this requirement to be an indica-
tion that either (i) the functional oxidase complex itself
(possibly the cytochrome b) provides the conductive pathway
for H+ translocation or (ii) association of the cytosolic
components with the cytochrome initiates a series of events
leading, on one hand, to O2 generation and, on the other,
to activation of a distinct entity responsible for the conduc-
tive H+ efflux. Oxidase assembly would be impaired in CGD
cells, but not in control neutrophils treated with DPI, since
this inhibitor prevents electron flow but not association of
the oxidase subunits. In fact, assembly of a functional oxidase
appears to be required for DPI to exert its inhibitory effect
(24). On the other hand, a catalytically active oxidase com-
plex does not appear to be required for H+ translocation,
inasmuch as increased conductance is detected in DPI-
treated cells.

Regardless of the specific mechanism involved, simulta-
neous activation of the conductance and the oxidase would
serve to coordinate the acid-generating and acid-extruding
processes during neutrophil activation. Such orchestration
would preclude opening of the conductance under conditions
where the electrochemical H+ gradient is directed inward—
i.e., at the resting membrane potential (=−60 mV), which
would be detrimental to pH homeostasis. In addition, be-

Table 2. Properties and assay conditions for metabolic acidification and H+ transport systems of activated neutrophils

<table>
<thead>
<tr>
<th>Metabolic process or transporter</th>
<th>Properties</th>
<th>Measurement conditions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Na+/H+ antiport</td>
<td>Na+-dependent</td>
<td>Inhibit Na+/H+ exchange (high K+/Na+-free medium)</td>
</tr>
<tr>
<td></td>
<td>Amiloride-sensitive*</td>
<td>Inhibit Na+ medium</td>
</tr>
<tr>
<td>V-type H+-ATPase</td>
<td>Electrogenic</td>
<td>Inhibit Na+/H+ exchange (high K+/Na+-free medium); Clamp E_m at 0 mV (K+ medium plus valinomycin)</td>
</tr>
<tr>
<td></td>
<td>Bafilomycin-sensitive*</td>
<td>Inhibit oxidase (DPI)</td>
</tr>
<tr>
<td>H+ conductance</td>
<td>ΔpH+-driven</td>
<td>Inhibit H+ conductance (Zn2+)</td>
</tr>
<tr>
<td></td>
<td>Zn2+-sensitive*</td>
<td>Clamp E_m at 0 mV (K+ medium plus valinomycin)</td>
</tr>
</tbody>
</table>

Under the conditions shown, the property indicated by an asterisk (*) was the criterion used to detect the specified process or transporter in our experiments. To inhibit the Na+/H+ antiport, the more specific amiloride analogue MMPS was used. The inhibitors or ionic manipulations used to achieve the specified conditions are indicated in parentheses. The pH of all media was 7.50 at 37°C.

Table 3. Determinations of metabolic acidification and H+ transporting systems of activated neutrophils

<table>
<thead>
<tr>
<th>Subject</th>
<th>Defect</th>
<th>Resting pH</th>
<th>Metabolic acidification, pH unit/min</th>
<th>Na+/H+ antiport, pH unit/min</th>
<th>H+-ATPase, pH unit/min</th>
<th>H+ conductance, pH unit/min</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patient</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 S.K.</td>
<td>X91°</td>
<td>7.26 ± 0.01 (6)</td>
<td>0.077 ± 0.010 (5)</td>
<td>0.160 ± 0.021 (3)</td>
<td>0.045 ± 0.006 (4)</td>
<td>0.107 ± 0.007 (6)</td>
</tr>
<tr>
<td>2 C.C.</td>
<td>X91°</td>
<td>7.34</td>
<td>ND</td>
<td>0.185</td>
<td>0.059</td>
<td>0.021</td>
</tr>
<tr>
<td>3 M.W.</td>
<td>X91°</td>
<td>7.18</td>
<td>ND</td>
<td>0.079</td>
<td>ND</td>
<td>0.013</td>
</tr>
<tr>
<td>4 J.H.</td>
<td>X91°</td>
<td>7.23</td>
<td>0.010</td>
<td>ND</td>
<td>0.064</td>
<td>0.009</td>
</tr>
<tr>
<td>5 J.W.</td>
<td>X91°</td>
<td>7.28</td>
<td>0.007</td>
<td>ND</td>
<td>0.049</td>
<td>0.011</td>
</tr>
<tr>
<td>6 J.B.H.</td>
<td>X91°</td>
<td>7.26</td>
<td>ND</td>
<td>ND</td>
<td>0.077</td>
<td>0.016</td>
</tr>
<tr>
<td>Mean ± SE</td>
<td>X91°</td>
<td>7.26 ± 0.02 (6)</td>
<td>0.005 ± 0.003 (3)*</td>
<td>0.121 ± 0.032 (3)</td>
<td>0.062 ± 0.006 (4)</td>
<td>0.016 ± 0.003 (6)*</td>
</tr>
<tr>
<td>7 G.P.</td>
<td>A47°</td>
<td>7.23</td>
<td>0</td>
<td>ND</td>
<td>0.054</td>
<td>0.012</td>
</tr>
<tr>
<td>8 R.G.</td>
<td>A47°</td>
<td>7.22</td>
<td>ND</td>
<td>ND</td>
<td>0.044</td>
<td>0.012</td>
</tr>
<tr>
<td>9 J.C.</td>
<td>A47°</td>
<td>7.36</td>
<td>ND</td>
<td>0.088</td>
<td>0.049</td>
<td>0.015</td>
</tr>
<tr>
<td>Mean ± SE</td>
<td>A47°</td>
<td>7.27 ± 0.05 (3)</td>
<td>0.049 ± 0.003 (3)</td>
<td>0.013 ± 0.001 (3)*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Carrier</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1A W.H.</td>
<td>X91°carrier</td>
<td>7.26</td>
<td>0.038</td>
<td></td>
<td>0.068</td>
<td></td>
</tr>
<tr>
<td>6A S.H.</td>
<td>X91°carrier</td>
<td>7.21</td>
<td>0.022</td>
<td></td>
<td>0.030</td>
<td></td>
</tr>
</tbody>
</table>

pH was measured fluorimetrically using 2',7'-bis(2-carboxyethyl)-5(6)-carboxyfluorescein (see text). The metabolic acidification was determined as the phorbol ester-induced generation of acid when the regulatory H+ extrusion processes Na+/H+ exchange and V-type H+-ATPase were inhibited. The activity of the Na+/H+ antiport was estimated as the phorbol ester-induced, MMPA-sensitive net rate of pHi increase under the conditions described in Table 2. Similarly, the activities of the V-type H+-ATPase and the H+ conductance were estimated as the phorbol ester-induced net rate of pHi alkalinizations that were sensitive to bafilomycin and Zn2+, respectively, using the conditions described in Table 2. Since the buffering power in the range of pHi studied was approximately equal in all subjects tested, the rates of change of pHi are directly proportional to the transmembrane fluxes of H+. Numbers are either individual values or means ± SE of the number of determinations indicated in parentheses. ND, not determined.

*P < 0.001 vs. control.

Carriers 1A and 6A are the mothers of patients 1 and 6, respectively.
cause neutrophil activation is associated with membrane potential changes (see ref. 25 for review), the conductance would be operative exclusively in depolarized cells, where the electrochemical H⁺ gradient is directed outward. Furthermore, depolarization would also facilitate H⁺ pumping through the electrogenic V-type ATPase, by reducing the opposing electrical potential. The resulting H⁺ efflux would contribute not only to net acid extrusion but also to neutralization of the membrane potential changes generated during the respiratory burst, favoring continued synthesis of O₂⁻.

In summary, the present study has revealed a tight correlation between the assembly of a functional NADPH oxidase and the activation of a H⁺ conductive pathway in human neutrophils. This relationship is indicative of a dual role of the NADPH oxidase in superoxide generation and in the regulation of intracellular pH. It remains to be defined whether the oxidase itself (or some component thereof) undertakes H⁺ translocation or signals the activation of a separate H⁺ conducting entity.

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