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Research Article

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Inhibitory Effect of Porcine Surfactant on the Respiratory Burst Oxidase in Human Neutrophils

Attenuation of p47^{phox} and p67^{phox} Membrane Translocation as the Mechanism

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Abstract

Surfactant has been shown to inhibit the production of reactive oxygen intermediates by various cells including alveolar macrophages and peripheral blood neutrophils. Superoxide (O_2^-) production by the respiratory burst oxidase in isolated plasma membranes prepared from PMA-treated human neutrophils was significantly attenuated by prior treatment with native porcine surfactant. The effect was concentration dependent with half-maximal inhibition seen at ≈ 0.050 mg surfactant phospholipid/ml. Kinetic analyses of the membrane-bound enzyme prepared from neutrophils stimulated by PMA in the presence or absence of surfactant demonstrated that surfactant treatment led to a decrease in the maximal velocity of O_2^- production when NADPH was used as substrate, but there was no effect on enzyme substrate affinity. Immunoblotting studies demonstrated that surfactant treatment induced a decrease in the association of two oxidase components, p47^{phox} and p67^{phox}, with the isolated plasma membrane. In contrast, surfactant treatment of the cells did not alter the phosphorylation of p47^{phox}. A mixture of phospholipids (phosphatidylcholine and phosphatidylglycerol in a 7:3 ratio) showed similar inhibition of the PMA-induced O_2^- generation. Taken together, these data suggest the mechanism of surfactant-induced inhibition of O_2^- production by human neutrophils involves attenuation of translocation of cytosolic components of the respiratory burst oxidase to the plasma membrane. The phospholipid components of surfactant appear to play a significant role in this mechanism. (*J. Clin. Invest.* 1995. 96:2654–2660.)

Key words: lung surfactant • neutrophils • NAD(P)H oxidoreductase • superoxide • assembly mechanisms

Introduction

Alterations in surfactant composition and function have been well documented in the adult respiratory distress syndrome (ARDS)[1] (1–3). The possibility that these alterations may play an important role in disease pathogenesis has prompted a number of studies of surfactant repletion in animals with experimental lung injury and in patients with ARDS (4–6). One hallmark of ARDS is the intense inflammation detected in the lung as evidenced by the dramatic increase in the number of PMN that are present in histologic sections or recovered in lavage fluid from patients (7). The release of neutrophil-derived proteases and reactive oxygen intermediates (ROI) has also been implicated in disease pathogenesis, and a number of observations have suggested that these inflammatory products may modify structure or function of lung surfactant (8, 9).

The converse possibility, that lung surfactant may regulate the function of phagocytes resident in the lung or migrating into the lung during ARDS, has received less attention. However, studies have shown inhibition of ROI production by rabbit alveolar macrophages or human PMN in the presence of homologous surfactant or a mixture of phospholipids (10–13) and of canine PMN or alveolar macrophages' ROI production by surfactant apoprotein A (SP-A)(14).

The mechanism underlying the inhibitory effect of surfactant on ROI production in phagocytes is unknown. The respiratory burst oxidase, the enzyme responsible for ROI production by phagocytic cells, is a multicomponent enzyme that catalyzes the single electron reduction of O_2 to superoxide (O_2^-) using NADPH as an electron donor (15, 16). The enzyme subunits are distributed in both cytosolic and membrane compartments in the resting neutrophil. Upon stimulation of the cell with any of a variety of soluble or particulate agonists, soluble cytosolic oxidase components, i.e., p47^{phox}, p67^{phox}, and a low molecular weight G-protein are transferred and bound to the membrane, presumably to an integral membrane protein, cytochrome b_{558} (17, 18). This process ultimately leads to the formation of an active enzyme complex which is tightly associated with the membrane. Phosphorylation of multiple serine residues of p47^{phox} occurs before translocation and may be required before the translocation takes place (19, 20). The present study was designed to examine the effect of a native porcine surfactant preparation on assembly of the respiratory burst oxidase in human PMN and to explore the underlying mechanism of inhibition. Porcine surfactant treatment of human neutrophils leads to attenuated translocation of the cytosolic components p47^{phox} and p67^{phox} of the oxidase to the plasma membrane and diminished oxidase activity associated with the plasma membrane.

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1. *Abbreviations used in this paper:* ARDS, adult respiratory distress syndrome; dH_2O, endotoxin-free deionized water; DPPC, 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine; O_2^-, superoxide; POPG, 1-palmitoyl-2-oleyl-*sn*-glycero-3-[phospho-rac-(1-glycerol)]; ROI, reactive oxygen intermediate; SP-A, surfactant apoprotein A.

Methods

Materials. All reagents were obtained from Sigma Chemical Co., St. Louis, MO, unless noted otherwise.

Isolation and stimulation of intact human neutrophils. Blood was harvested from normal donors into acid citrate dextrose–containing syringes, and neutrophils were isolated as previously described (21). After isolation, neutrophils were suspended in Ca^{2+}- and Mg^{2+}- free PBS ($2–5 \times 10^7$/ml) and maintained at 4°C until use. Respiratory burst activity in intact neutrophils in response to PMA was measured as the superoxide dismutase-inhibitable production of superoxide as previously described (22).

Preparation of porcine lung surfactant. Native porcine surfactant was prepared by premortem or immediate postmortem lavage of the lungs of normal 20–28-kg pigs (Gilley Farms, Escondido, CA). The trachea was cannulated and the lungs inflated with sequential aliquots of sterile saline solution (0.15 M NaCl) to a distending pressure of 30 cm H_2O and then drained. The recovered fluid was pooled and centrifuged at 400 *g* for 10 min to remove cells and debris. The supernatant was then centrifuged at 20,000 *g* for 120 min to pellet surfactant aggregates. The crude surfactant pellets were suspended in a 1% NaCl and 23% NaBr solution and further purified over a discontinuous NaCl density gradient as described (23). The porcine surfactant layer was removed from the gradient, pelleted, resuspended at ≈ 5 mg phospholipid/ml, and maintained at −20°C until use. All surfactant concentrations are expressed in terms of phospholipid concentration per milliliter of solution. Porcine SP-A was purified as previously described (24) and consisted of a single band on gel electrophoresis and combined Coomassie and silver staining (25).

Preparation of phospholipid liposomes. Phospholipids were dissolved in chloroform and then dried by evaporation under N_2. Just before use in each experiment, the dried phospholipids were mixed with PBS and dispersed into an even suspension with a probe-type sonicator in ice for 30 s × 2 (10) and then held in a 37°C water bath until use.

Activation of human neutrophils for preparation of membrane fractions. Cells ($4–8 \times 10^7$) were sedimented at 150 *g* for 5 min and then resuspended in 10 ml of PBS containing Ca^{2+}, Mg^{2+}, and 0.2% glucose. The PMNs were equilibrated at 37°C for 15 min and then various amounts of porcine surfactant or phospholipid liposome preparations were added, and the cells incubated for an additional 15 min. PMA was then added to 100 nM and activation of the oxidase allowed to proceed for 15 min before terminating the reaction by the addition of ice-cold PBS and transferring the tube to a 0°C water bath.

Cell disruption and membrane preparation. Neutrophil plasma membranes containing oxidase were prepared as described previously (21, 26). Briefly, cells were sedimented at 150 *g* for 10 min, and the pellet resuspended into 5 ml of 4°C lysis buffer (0.34 M sucrose in H_2O containing 1 mM PMSF). The cell suspension was kept on ice and sonicated twice for 30 s with 60-s cooling periods between bursts (50 W [80% output]) with an ultrasonic cell disrupter with microtip (Heat Systems-Ultrasonics, Inc., Plainview, NY). These conditions resulted in breakage of > 90% of cells. The sonicate was centrifuged at 800 *g* for 5 min to remove intact cells and nuclei before layering the supernatant onto a discontinuous sucrose gradient (30 and 50% sucrose [wt/vol], 10 ml each) and centrifuging at 130,000 *g* for 1 h at 4°C. The turbid plasma membrane fraction located between the two sucrose layers was removed and diluted threefold with 0°C endotoxin-free deionized water (dH_2O). The diluted membrane solution was centrifuged at 27,000 *g* for 30 min. The membrane preparation was resuspended in 80–120 μl of 0.34 M sucrose containing 1 mM PMSF and stored at −70°C until use.

Assay of NADPH-dependent O_2^- production. The O_2^--generating activity of the isolated plasma membrane was assayed as the superoxide dismutase-inhibitable reduction of cytochrome *c* at 550 nm in a 96-well microplate using modifications of published techniques (22, 27). For each assay, 10 μl of plasma membrane suspension was added to duplicate wells containing 20 μl of cytochrome *c* solution (1.0 mM), 140 μl of assay buffer (23 mM potassium phosphate, pH 7.0, 100 mM KCl, 8 mM $MgCl_2$, and 3.3 mM NaCl), and 10 μl of bovine erythrocyte superoxide dismutase (3 mg/ml in dH_2O) or dH_2O. The mixture was incubated at 37°C for 10 min followed by the addition of 20 μl NADPH (2 mM in dH_2O) to initiate the reaction. The reduction of cytochrome *c* was measured in a continuous kinetic mode using a microplate reader (Molecular Devices Corp., Menlo Park, CA). O_2^- production was calculated from the difference between rates of reduction of cytochrome in the presence and absence of superoxide dismutase and expressed as nmol/min per mg membrane protein.

Examination of $p47^{phox}$ phosphorylation by isoelectric focusing and immunoblotting of whole neutrophil lysates. Cells (2×10^7) were prepared and stimulated in the presence or absence of various reagents as described above and then washed and centrifuged twice in ice-cold PBS. The final cell pellet was dissolved in 200–400 μl of lysis buffer (5% CHAPS, 9.5 M urea, 5% 2-mercaptoethanol, NaF 50 mM, 1% pH 3–10 ampholyte, and 1% pH 7–9 ampholyte (Pharmacia LKB Biotechnology Inc., Piscataway, NJ). Isoelectric focusing (600 V maximum, 4–5 h) was performed in a slab gel (55% urea, 3% acrylamide, 2% CHAPS, 2.5% pH 3–10, and 2.5% pH 7–9 ampholytes, 0.033% ammonium persulfate, and 0.2% TEMED) using 0.05 M histidine and 0.01 M glutamic acid as anode and cathode buffers, respectively (28). The separated protein were transferred to membranes and probed with anti-$p47^{phox}$ antibody as described in the following section.

Immunoblotting of $p47^{phox}$ and $p67^{phox}$. The isolated plasma membrane suspensions were solubilized with an equal volume of 2 × SDS sample buffer (0.125 M Tris-HCl, pH 6.8, 4% SDS, 20% glycerol, and 10% 2-mercaptoethanol) and subjected to SDS-PAGE on a 10% polyacrylamide gel (29) followed by electrophoretic transfer onto polyvinylidene membranes (Pharmacia LKB Biotechnology Inc.) and blocking with a 5% milk protein solution (48 mM Tris, pH 7.2, 150 mM NaCl). The transfers were probed with partially purified antibodies (1:2,500 dilution of the Ig fraction of serum) raised in rabbits against synthetic peptides corresponding to the final 12 residues in the carboxyl terminus amino acid sequence of $p47^{phox}$ and $p67^{phox}$. Bound antibody was detected with alkaline phosphatase–conjugated goat anti–rabbit Ig antibodies using a 5-bromo-4-chloro-3-indolyl-1-phosphate and nitroblue tetrazolium kit (Bio-Rad Laboratories Inc., Cambridge, MA).

Results

Effect of porcine surfactant on PMA-induced O_2^- production in neutrophil membrane. When intact neutrophils were stimulated with PMA (100 nM), the presence of porcine surfactant resulted in a concentration-dependent reduction in superoxide production (Fig. 1). Porcine SP-A had no effect on neutrophil superoxide production, even when added at concentrations much greater than might be expected in fully inhibitory preparations of surfactant (Fig. 1). To explore the mechanism of this effect, intact cells were treated with various concentrations of PMA followed by disruption and isolation of the plasma membrane fraction. PMA, at concentrations ranging between 10^{-8} and 10^{-6} M, caused a concentration-dependent increase in the rate of O_2^- production measured in isolated membranes. The concentration for half-maximal activation by PMA was ~ 50 nM, and maximal activation was observed at concentrations $\geq 10^{-7}$ M (Fig. 2). To examine the effect of porcine surfactant, plasma membrane fractions were prepared from cells stimulated with PMA in the presence or absence of porcine surfactant and membrane O_2^- production was examined. The rate of O_2^- production in the plasma membranes prepared from PMA-treated cells was significantly attenuated when cells were stimulated in the presence of porcine surfactant (Fig. 3). The inhibitory effect of porcine surfactant was concentration dependent with a concentration for half-maximal inhibition of ~ 0.05 mg surfactant phospholipid/ml. The inhibitory effect was not due to direct interference of

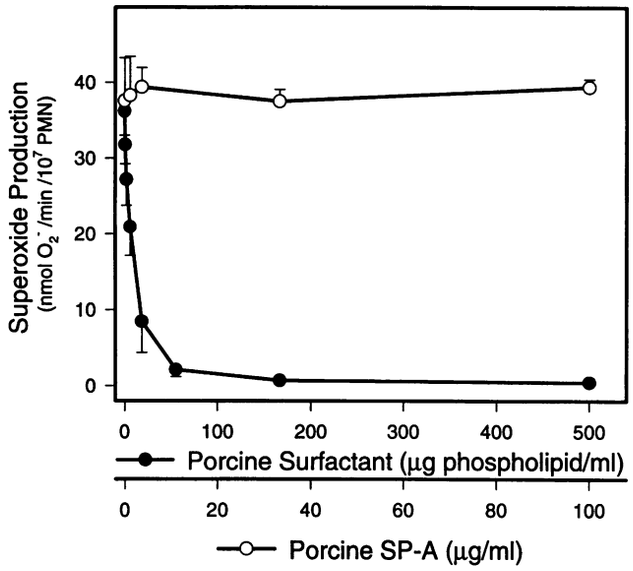

Figure 1. The effect of porcine surfactant on neutrophil O_2^- production elicited by PMA. Normal human neutrophils were incubated at 37°C for 5 min with various concentrations of porcine surfactant before the addition of PMA (100 nM) to initiate the respiratory burst. Maximal rates of O_2^- production after the addition of PMA is expressed as nanomoles per minute per 10^7 cells. Each data point represents the mean value ±SEM of at least four experiments using cells from different donors. The presence of porcine surfactant resulted in a concentration-dependent inhibition of neutrophil O_2^- production as shown. In contrast, the presence of porcine SP-A had no effect on O_2^- production by the cells (*open circles*). SP-A makes up ≈ 3% of the mass of isolated surfactant (30), and the concentrations of SP-A used in these experiments (up to 0.1 mg/ml) greatly exceeds that likely to be present in fully inhibitory amounts of porcine surfactant.

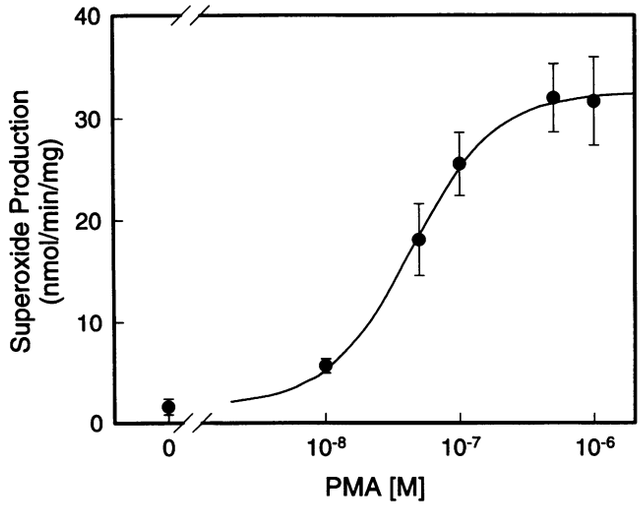

Figure 2. PMA-induced O_2^- production measured in isolated neutrophil plasma membranes. Intact neutrophils (5×10^7) were stimulated with the indicated concentrations of PMA for 15 min before harvesting the plasma membranes and measuring O_2^- production. O_2^- production (*closed circles*) is expressed per milligram of membrane protein and each data point represents the mean value ±SEM of five experiments performed with different membrane preparations.

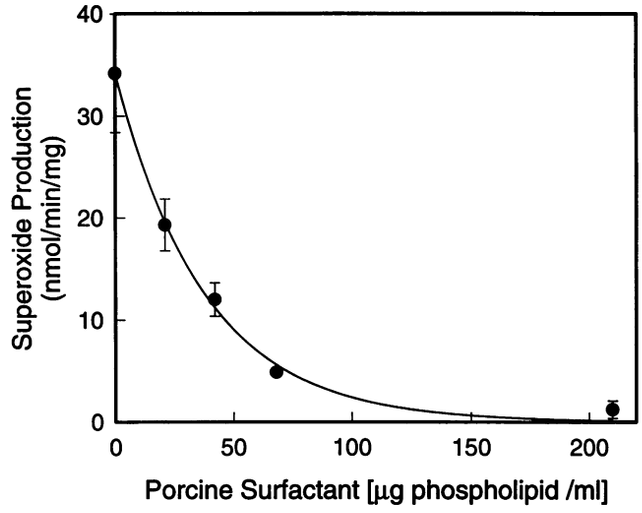

Figure 3. Effect of porcine surfactant on PMA-induced O_2^- production in isolated neutrophil membrane preparations. Cells were pretreated with various concentrations of porcine surfactant at 37°C for 15 min and then stimulated with PMA (5×10^{-7} M) for 15 min. The reactions were terminated and the plasma membrane isolated as described in Methods. A dose-related reduction in rates of O_2^- production was found in membranes isolated from cells stimulated in the presence of surfactant (*closed circles*). Control preparations (*open circles*) were from cells which received neither surfactant exposure nor PMA treatment. Each data point represents the mean value ±SEM of four experiments performed with different membrane preparations.

enzyme activity by residual surfactant possibly carried over during the preparation of the plasma membrane, as the addition of surfactant to the cell suspension at the end of PMA-induced activation (just before sonication) failed to show any inhibition of O_2^- production by the membrane fraction (data not shown).

Enzyme kinetics of the membrane-bound respiratory burst oxidase. Alterations in the activity of the respiratory burst oxidase activity in differentiating mononuclear phagocytes, in cell-free oxidase systems, and in certain forms of chronic granulomatous disease have been associated with altered affinity of the oxidase for its native substrate, NADPH. As part of the exploration of the mechanisms of inhibition of O_2^- production in neutrophils by surfactant, the enzyme kinetics of the membrane-associated oxidase was measured in plasma membrane preparations from PMN stimulated by PMA (5×10^{-7} M) in the presence or absence of a partially inhibiting concentration of surfactant (0.063 mg/ml). As shown in Fig. 4 *A*, increasing concentrations of NADPH caused an increase in O_2^- production, reaching a plateau at an NADPH concentration of ≈ 0.2 mM. Compared to preparations from control cells, the oxidase activity in the membranes of surfactant-treated cells was substantially attenuated at all concentrations of NADPH tested. Lineweaver-Burk analyses (Fig. 4 *B*) of the oxidase activity in membranes prepared from control neutrophils demonstrated a maximal velocity of oxidase-catalyzed O_2^- production using NADPH as the substrate of 52±9 nmol/min per mg with a substrate affinity (K_m) of 0.084±0.007 mM. In membranes prepared in parallel from surfactant-treated cells, the maximal velocity was reduced by 40±2% with no appreciable change in subtrate affinity (K_m = 0.077±0.009 mM). These observations

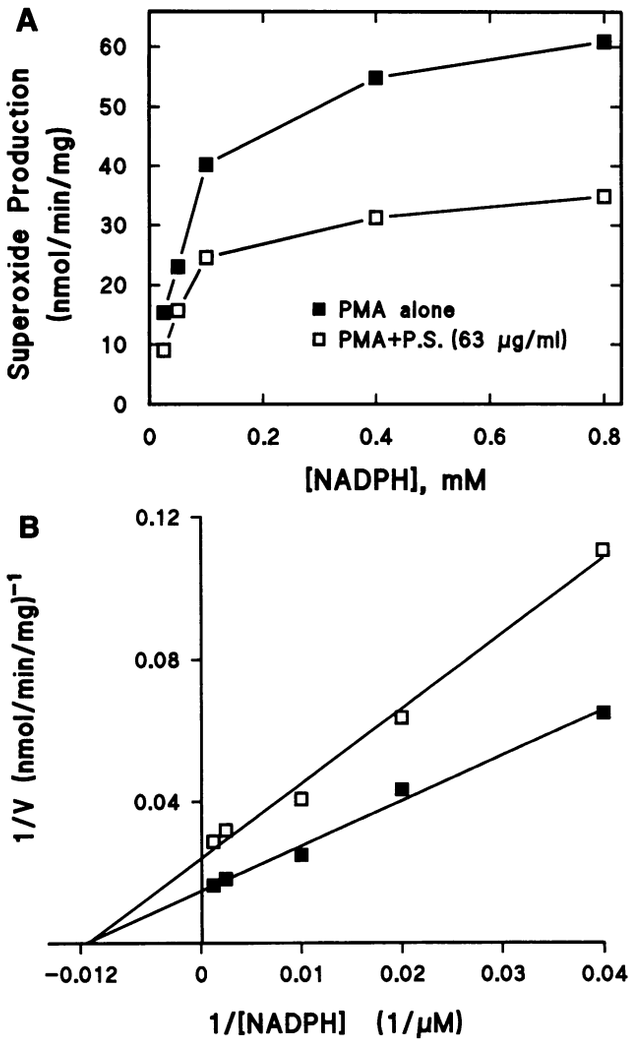

Figure 4. Enzyme kinetics of membrane-associated respiratory burst oxidase. Neutrophils were incubated with 0.06 mg/ml of porcine lung surfactant at 37°C for 15 min followed by 15 min of PMA stimulation before preparation and harvesting of the plasma membranes. The activated plasma membranes isolated from surfactant-treated (*open squares*) or control (*closed squares*) cells were examined for kinetics of the respiratory burst oxidase. Various concentrations of NADPH ranging from 0.025–0.800 mM were added to initiate the reaction. (*A*) Concentration dependency of O_2^- production using NADPH as the substrate. (*B*) Lineweaver-Burk plot of the same data. Although the maximal velocity of O_2^- production was reduced by surfactant treatment, there was no change in enzyme substrate affinity. A representative example of three separate experiments performed with different membrane preparations is shown. *P. S.*, porcine surfactant.

suggest surfactant treatment does not alter oxidase activity through modulation of substrate affinity.

Effect of native porcine surfactant on phosphorylation of p47^{phox} in vivo. Isoelectric focusing of whole neutrophil lysates followed by transfer and immunoblotting for p47^{phox} was used to indirectly examine the phosphorylation of p47^{phox} in vivo. When lysates were prepared from unstimulated cells, p47^{phox} focused to regions of high pH (pI $\approx$ 9.6) as expected for the unmodified protein (Fig. 5, *A–C*, lane *1*) (31, 32). In contrast, when the cells were stimulated with concentrations of PMA > 10 nM (Fig. 5 *A*, lanes *2–6*), minimal p47^{phox} was seen at high pH, and a "ladder" of p47^{phox} isoforms was seen extending to pI $\approx$ 6.3. This technique allowed visualization of multiple (> 9) different p47^{phox} bands corresponding to different numbers of phosphorylated serine residues. The PMA-induced phosphorylation of p47^{phox} was blocked by increasing concentrations of the protein kinase C inhibitor staurosporine (Fig. 5 *B*, lanes *3–6*). No difference in the p47^{phox} banding pattern was seen in lysates prepared from PMA-stimulated cells preincubated with various concentrations of surfactant (0.021–0.47 mg/ml, Fig. 5 *C*).

Attenuation of translocation of cytosolic oxidase components by porcine surfactant. To further explore the mechanism by which porcine surfactant inhibits membrane-associated oxidase activity, translocation to the plasma membrane of the cytosolic oxidase subunits, p47^{phox} and p67^{phox}, was examined in neutrophils stimulated in the absence and presence of surfactant. As shown in Fig. 6, neither p47^{phox} nor p67^{phox} was present in the plasma membrane preparations from unstimulated neutrophils (lane *1*). Stimulation of the cells with PMA was associated with an increase in the amount of p47^{phox} and p67^{phox} bound to the plasma membrane (lane 2). The PMA-induced translocation of p47^{phox} and p67^{phox} was inhibited by various concentrations of porcine surfactant (Lanes *3–6*). The concentration dependency of the surfactant effect on translocation was similar to that seen when membrane-associated O_2^- production was measured (Fig. 3.)

Effect of synthetic phospholipids on PMA-induced superoxide production. To examine whether the phospholipid components contribute to the inhibitory effect of native porcine surfactant, the effect of synthetic phospholipid suspensions on oxidase activity was measured. Various concentrations of a mixture of 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine (DPPC) and 1-palmitoyl-2-oleyl-*sn*-glycero-3-[phospho-rac-(1-glycerol)] (POPG) (DPPC/POPG = 7:3) was added to neutrophils as liposomes before PMA stimulation. As shown in Fig. 7, the phospholipid mixture partially mimicked the effect of native porcine surfactant. Although a concentration-dependent inhibition of PMA-induced O_2^- production was seen in the presence of the phospholipid mixture, the concentration of phospholipid required for half-maximal inhibition was significantly greater than that shown for native porcine surfactant, and the inhibition was incomplete even at relatively high phospholipid concentrations.

Discussion

The present studies were designed to examine the mechanism(s) by which porcine lung surfactant modulates the functions of phagocytes, and specifically the respiratory burst function of human neutrophils. The data presented here demonstrate that prior treatment with porcine surfactant leads to a concentration-dependent decrease of respiratory burst oxidase activity measured in isolated neutrophil plasma membrane. The concentration of surfactant at which these effects are seen is less than the 1–5 mg phospholipid/ml that might be expected in the alveolar lung fluid of the normal lung and much less than might be found when exogenous surfactant is administered to injured lungs (4). The mechanistic studies, through enzyme kinetics analysis and immunoblotting experiments, reveal the inhibitory effect of porcine surfactant is likely due to its attenuating effect

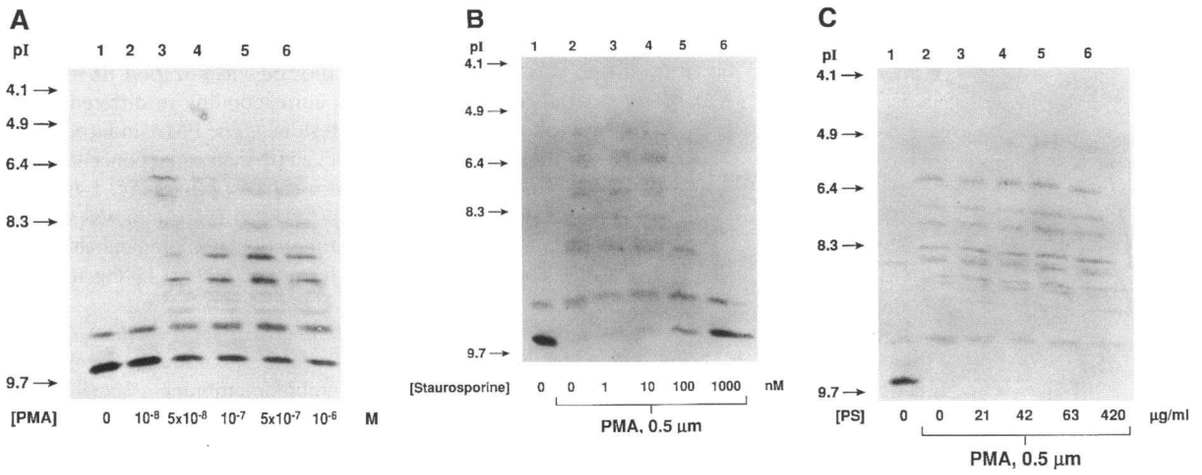

Figure 5. Effect of surfactant on p47^{phox} phosphorylation. The phosphorylation state of p47^{phox} in whole-cell lysates of human neutrophils stimulated with various concentrations of PMA in the presence or absence of surfactant was examined using isoelectric focusing followed by immunoblotting. The expected high pI form of p47^{phox} is found in unstimulated cells (*A–C*, lane *1*). Alterations of the isoelectric point of p47^{phox} as a consequence of phosphorylation were detected using a specific anti-p47^{phox} antibody. The expected fall in the isoelectric point upon stimulation with PMA (at increasing concentrations) is shown in *A* (lanes *2–6*). The addition of various concentrations of the protein kinase C inhibitor staurosporine 5 min before PMA resulted in a decrease in the amount of p47^{phox} found at lower (more phosphorylated) isoelectric points (*B*, lanes *3–6*). In contrast, the presence of surfactant at various concentrations did not alter the apparent quantity of p47^{phox} present at any isoelectric point (*C*, lanes *3–6*). *PS*, porcine surfactant.

on the translocation of the cytosolic components of the oxidase, i.e., p47^{phox} and p67^{phox}, to the plasma membrane.

The mechanism of activation of the respiratory burst oxidase is tightly regulated, complex, and not fully understood (16, 33). The enzyme does not exist in a catalytically active state in unstimulated cells and its separate protein subunits are contained in different subcellular compartments. It is believed that upon activation of cells, the essential cytosolic components of the oxidase, p47^{phox}, p67^{phox}, and a low molecular weight GTP-binding protein, rac2, are transferred to the membrane where they are assembled with a unique integral membrane protein, cytochrome b_{558}, into an active form of the oxidase which is then tightly associated with the plasma or phagosomal membrane (18, 34–38). The triggering mechanism for the membrane translocation of these components is not fully understood. It has been suggested that transfer of p47^{phox} from cytosol to plasma membrane requires phosphorylation of the protein, while transfer of p67^{phox} and rac2 protein requires p47^{phox} (20, 34, 35).

The present finding that porcine surfactant attenuates the transfer of p47^{phox} and p67^{phox} proteins to the plasma membrane provides novel insight into the mechanism by which surfactant modulates the function of phagocytes. PMA, an analogue of diacyl glycerol and activator of protein kinase C, is believed to

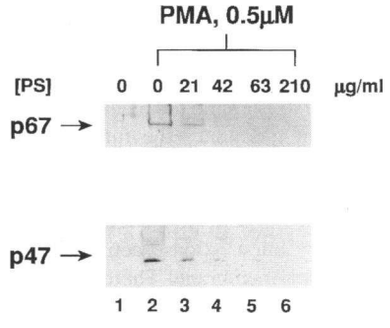

Figure 6. Translocation of p47^{phox} and p67^{phox} to the neutrophil membrane. Normal neutrophils were incubated in the presence (lanes *3–6*) or absence (lanes *1–2*) of various concentrations of porcine lung surfactant before stimulation with PMA (5×10^{-7} M, lanes *2–6*). Equivalent quantities of solubilized membrane proteins were resolved by SDS-PAGE and examined by immunoblotting for the presence of the respiratory burst oxidase components, p47^{phox} and p67^{phox}. A dose-dependent inhibition of the translocation of both oxidase components was seen in the presence of surfactant. A representative example of one of three separate experiments is shown. *PS*, porcine surfactant.

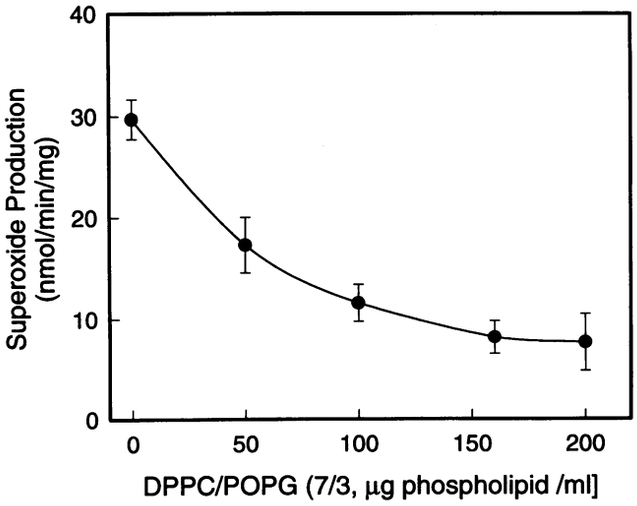

Figure 7. Effect of synthetic phospholipids on PMA-induced O_2^- production. Cells were pretreated with various concentrations of a mixture of DPPC and POPG (DPPC/POPG = 7:3) at 37°C for 15 min followed by stimulation with PMA (5×10^{-7} M) for another 15 min. The membranes were isolated and the oxidase activity measured as described in Methods. Each data point represents the mean value±SEM of four experiments performed with different membrane preparations.

induce translocation of oxidase subunits and oxidase activation through both protein kinase C–dependent and -independent pathways which ultimately lead to phosphorylation of multiple serine residues on $p47^{phox}$ (19, 39, 40). However, the exact relationship between phosphorylation of specific serine residues and subsequent $p47^{phox}$ translocation and oxidase activation has not been established. In the present studies, phosphorylation of $p47^{phox}$, as indicated by changes in its isoelectric point, was unaffected by surfactant treatment of neutrophils. These data suggest surfactant does not interact with protein kinase C or an alternate kinase to modulate oxidase activity via an inhibition of phosphorylation of oxidase components. Given the limits of the resolution of our methods, we cannot completely exclude the remote possibility that surfactant treatment may block the phosphorylation of a small number of critical residues on $p47^{phox}$ or an alternate substrate. These observations also suggest the ability of PMA to induce activation of protein kinase C, a proximal step in the activation of the oxidase, is not altered by porcine surfactant. Therefore, any interaction between surfactant and PMA is insufficient to explain the inhibition of respiratory burst oxidase activity we observed.

The substrate-binding domain of the assembled and catalytically active oxidase is thought to reside on cytochrome b_{558} (41). The oxidase that is assembled in intact cells appears to have different affinity for its native substrate, NADPH, depending on the state of activation of the cell (42). Similarly, certain variant forms of chronic granulomatous disease, an entity characterized by a lack of superoxide production by phagocytic cells, are associated with altered affinity of the oxidase for NADPH (43). This affinity, at least in some studies, may be regulated by cytosolic oxidase components (27). Accordingly, we examined whether the reduced O_2^- production seen in the presence of surfactant was associated with any change in the kinetic function of the enzyme. Although the maximal velocity of the enzyme was reduced in surfactant-treated cells, the substrate affinity was unchanged. Thus, surfactant treatment appears to reduce the total quantity of respiratory burst oxidase assembled on the cell membrane, with the assembled enzyme possessing normal kinetic function and affinity. The mechanism(s) for this reduction may include modification of the conformation of cytochrome b_{558} leading to loss of binding sites for the cytosolic oxidase components, or modulation of the function of the neutrophil cytoskeleton induced by surfactant.

A synthetic phospholipid mixture containing micelles of DPPC and POPG partially duplicates the ability of native porcine surfactant to cause a concentration-dependent attenuation of O_2^- production in neutrophil membranes. This inhibitory effect of the phospholipid on the respiratory burst activity in neutrophils may well explain a previous observation that animals given a DPPC/POPG mixture show attenuated lung phagocytic killing of group B streptococci (10). Some reports have suggested that SP-A enhances respiratory burst activity, phagocytosis, cytokine release, and chemotaxis in macrophages, possibly by acting as an opsonin (44–47), while others have suggested that SP-A may attenuate neutrophil respiratory burst activity (14, 48). In our system, SP-A alone does not appear to modulate respiratory oxidase activity. However, we cannot exclude an additional role for the hydrophobic surfactant apoproteins in the modulation of oxidase activity.

Neutrophils migrate out of the circulation and accumulate in the lung in large numbers during episodes of ARDS (49, 50). These inflammatory cells release various protease and reactive oxygen intermediates which have the potential to damage lung tissue. The observation that lung surfactant can markedly attenuate neutrophil respiratory burst activity through inhibition of oxidase assembly suggests additional rationale for the administration of surfactant to patients with ARDS. The possibility that surfactant might similarly modulate the function of resident lung phagocytes in health provides insights into important roles for surfactant that may complement the simple lowering of alveolar surface tension.

Acknowledgments

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