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Inhibition of formyl-methionyl-leucyl-phenylalanine-stimulated respiratory burst in human neutrophils by adrenaline: inhibition of Phospholipase A_2 activity but not p47phox phosphorylation and translocation

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Abstract

The polymorphonuclear neutrophil (PMN)-respiratory burst plays a key role in host defense and inflammatory reactions. Modulation of this key neutrophil function by endogenous agents and the mechanisms involved are poorly understood. This study was designed to analyze the mechanisms involved in the effect of adrenaline on neutrophil superoxide anions production. Using the superoxide dismutase (SOD)-inhibitable cytochrome *c* reduction assay, we report here that the β-adrenergic agonist, adrenaline at physiologic concentrations (5–100 nM) inhibited formyl-methionyl-leucyl-phenylalanine (fMLP)-stimulated but not phorbol-myristate-acetate (PMA)-stimulated PMN superoxide anion production. The inhibitory effect of adrenaline runs in parallel with an increase in intracellular levels of cAMP which was reversed by the protein kinase A (PKA) inhibitor H-89, suggesting a role for PKA in mediating the inhibitory effect of adrenaline on fMLP-induced superoxide production. Adrenaline at physiological concentrations did not inhibit the fMLP-stimulated membrane translocation of the NADPH oxidase components p47phox and p67phox, nor the fMLP-stimulated phosphorylation of p47phox. However, adrenaline strongly depressed the activity of the cytosolic isoform of Phospholipase A_2 ($cPLA_2$). We suggest that adrenaline inhibits fMLP induced superoxide production upstream of the NADPH oxidase via a mechanism involving PKA and $cPLA_2$.

Keywords: Neutrophil; Respiratory burst; NADPH oxidase; Adrenaline; PKA; PLA_2

1. Introduction

Neutrophils are phagocytic cells of the innate immune system that are important in host defence and inflammatory responses. They use a combination of reactive oxygen species, anti-microbial proteins, and peptides to kill and destroy ingested microorganisms. They are stimulated by a variety of agents and depending on their stimulus their activation may result in chemotaxis, phagocytosis, degranulation, and the activation of superoxide anions production in a metabolic event known as the respiratory burst [1]. The respiratory burst can be stimulated by a variety of agonists such as the chemoattractant peptide fMLP, the calcium ionophore A23187 and the tumor promoting agent phorbol-myristate-acetate (PMA) [2,3]. The biochemical events leading to these processes involve the phosphorylation of several proteins [4,5].

The respiratory burst is a coordinated series of metabolic events whereby the activated phagocyte reduces molecular oxygen to a variety of toxic oxygen-derived products. The initial product of the respiratory burst is superoxide anion (O^{2-}) and subsequent reactions lead to the formation of other toxic agents including H_2O_2, HOCl, and possibly the hydroxyl radical ($OH^{\bullet}$) [2,6]. The enzyme catalyzing the one electron reduction of oxygen is the NADPH oxidase [2,6], a multicomponent enzyme which is dormant in

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Abbreviations: PMN, polymorphonuclear neutrophil; fMLP, formyl-methionyl-leucyl-phenylalanine; PMA, phorbol-myristate-acetate; phox, phagocyte oxidase; $cPLA_2$, Phospholipase A_2; PKA, protein kinase A.

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resting cells, in which its components are divided between the cytosol and the membrane [7]. Following activation of the neutrophil, the cytosolic components translocate to the membrane where they join with the membrane bound components to form a fully functional oxidase. The membrane bound components are comprised of cytochrome b558, a heterodimer consisting of a 22 kDa protein and a 91 kDa protein (p22phox and gp91phox, respectively), and rap 1A, a low molecular weight GTP-binding protein. The cytosolic components of the NADPH oxidase are comprised of the phagocyte oxidase proteins p47phox, p67phox, p40phox, and the GTP-binding protein p21rac [2,4,6].

Activation of the NADPH oxidase is associated with the phosphorylation of p47phox, p67phox and with translocation of the cytosolic components including p21rac to the membrane [2,7]. Agonist-mediated activation of the respiratory burst in human PMN involves several signal transduction pathways in the cell including enzymes such as PLD, PLC, PLA_2, and PI3 kinase [8]. These enzymes produce second messengers which in turn activate downstream effectors such as PKC and PKB. PMNs have been shown to release arachidonic acid specifically from their phospholipids on cell stimulation. An 85 kDa $cPLA_2$ has been identified in the cytosol of PMNs and $sPLA_2$ has been shown to be present in the secretory granules of PMNs [9,10]. Inhibition of either PLA_2 or PLD activity attenuates the respiratory burst suggesting that their products PA and AA are required for signal transduction resulting in activation of the NADPH oxidase.

Neutrophil NADPH oxidase activation can be modulated by a multitude of endogenous biological agents such as proinflammatory cytokines, lipids and hormones [11,12]. Adrenaline and other β-adrenergic agonists such as isoproterenol have been shown to supress the production of reactive oxygen species by neutrophils [13–16] However, inhibitory effects were demonstrated only at high pharmacological concentrations and in the presence of theophylline, thus the physiological significance of these observations was unclear and the mechanisms are poorly understood.

The aim of the study reported here was to investigate the mechanisms of adrenaline induced inhibition of the respiratory burst in human PMN at physiological concentrations. We analyzed the effect of adrenaline on various intermediates in the transduction pathways regulating the PMN respiratory burst including the phosphorylation and translocation of the NADPH oxidase components and PLA_2 activity.

2. Materials and methods

2.1. Materials

Cytochrome *c*, cytochalasin B, catalase, SOD, fMLP, PMA, adrenaline ((+) bitartrate salt), H-89, Triton X-100, sodium pyrophosphate, glycerophosphate, leupeptin, pepstatin, aprotonin, diisopropylfluorophosphate, DNAse 1, PMSF, BSA, and streptolysin-O were purchased from Sigma. Lymphoprep was purchased from Nycomed, Dextran T500 from Pharmacia. HBSS, HEPES, and glucose from Gibco, ^{32}Pi and ^{3}H-arachidonic acid from Amersham. The anti-p47phox antibody was kindly supplied by Dr. B. Babior (Scripps Research Institute).

2.2. Isolation of human neutrophils

Venous blood was collected from healthy adult volunteers and neutrophils were isolated by Dextran sedimentation and density gradient centrifugation as described previously [5,7]. Erythrocytes were removed by hypotonic lysis. Following isolation, the cells were resuspended in the appropriate medium such as Hank's Balanced Salt Solution (HBSS). A cell count was performed and cell viability was determined using the Trypan Blue Exclusion method.

2.3. Superoxide anion production assay

Following isolation, the cells were resuspended in HBSS at a concentration of 2 million per milliliter. Superoxide production was determined by measuring cytochrome *c* reduction [7]. Cell suspension (1 mL) was placed in a cuvette with 1 mL HBSS preheated to 37°, 80 μM cytochrome *c*, in the presence or absence of SOD (150 U/mL) and in the presence or absence of 2.5 μg/mL cytochalasin B, the cuvette was placed in the thermostatted chamber of the spectrophotometer (Beckman DU640) and allowed to stabilize at 37°. After a baseline was established, the cells were stimulated with either 10^{-7} M fMLP or 100 ng/mL PMA. Changes in absorbance readings at 550 nm were measured over a 10-min period. Results were calculated as nanomoles of superoxide produced per 2 million cells over 10 min for total superoxide production and nmol superoxide per 2 million cells per minute as initial rate over the first 60 s of the burst. To determine the effect of adrenaline on the respiratory burst the cells were first preincubated at 37° with 5, 10, 100, or 500 nM adrenaline for 10 min in the thermostatted spectrophotometer chamber. Superoxide production, was determined as described above. To determine the effect of H-89, neutrophils were preincubated for 10 min at 37° with 5 μM/H-89 followed by incubation for a further 10 min with adrenaline. The cells were then stimulated with fMLP and superoxide production assessed by following the reduction of cytochrome *c* at 550 nm.

2.4. cAMP measurement

cAMP was measured following the technique described by Brown *et al.* [17].

PMN were resuspended in ice-cold incubation buffer containing IBMX (1 mM) and digitonin (0.5 μM) in the presence of varying concentrations of adrenaline and left on ice for 15 min. ATP (1 mM) was added and the samples

placed at 37° for 10 min. The reaction was stopped by boiling, samples were centrifuged and supernatants assayed for cAMP by competitive protein binding assay.

2.5. p47phox phosphorylation and immunoprecipitation

Freshly isolated cells were resuspended in loading buffer (20 mM HEPES pH 7.4, 150 mM NaCl, 5 mM KCl, 10 mM glucose, and 0.025% BSA) in the presence of 1 mCi ^{32}Pi for 1 hr at 30° and the cells were then washed with loading buffer [5]. Cells (60 million) were resuspended in loading buffer + Ca^{2+} + Mg^{2+} and preincubated at 37° in the presence or absence of adrenaline (100 or 500 nM) for 10 min. fMLP (10^{-7} M) was added to some of the samples and following a further 2.5 min at 37°. The reaction was stopped with a large excess of ice-cold loading buffer. Samples were centrifuged at 4°, 400 *g* for 10 min and the pellets resuspended in Lysis Buffer (20 mM Tris pH 7.5, 150 mM NaCl, 5 mM EDTA, 5 mM EGTA, 0.5% (w/v) Triton X-100, 2.5 mM sodium pyrophosphate, 1 mM glycerophosphate, 5.4 mM $NaVO_3$, 50 μM leupeptin, 25 μM pepstatin, 25 μM aprotonin, 1 mM diisopropylfluorophosphate (DFP), 1 mg/mL DNAse 1) and sonicated. The sonicate was ultracentrifuged at 100,000 *g* for 30 min. Immunoprecipitation of p47phox was performed as described previously [5,7]. Protein G sepharose beads were washed in PBS and equilibrated in Immunoprecipitation buffer containing 1% (w/v) BSA then incubated with the lysate in the presence of 5 μL anti-p47phox antibody, incubated overnight at 4° with end-over-end rotation. The samples were washed four times with immunoprecipitation buffer.

2.6. p47phox and p67phox translocation

PMN were resuspended in HBSS and incubated at 37° for 10 min in the presence or absence of adrenaline (100 nM), then stimulated with PMA or fMLP. The cells were lysed and fractionated as previously described [7], the membrane fraction subjected to SDS–PAGE, and proteins transferred to a nitrocellulose membrane which was then immunoblotted with anti-p47phox antibody.

2.7. Western blotting and autoradiography

p47phox was eluted by adding Laemmli sample buffer to the samples followed by vortexing and boiling. Samples were then centrifuged to remove the beads. Samples were electrophoresed on a 10% SDS–PAGE. Proteins were electrotransferred to nitrocellulose membrane which was exposed to X-ray film overnight [5,7].

2.8. Phospholipase A_2 activity

Phospholipase A_2 activity in PMNs was measured as release of arachidonic acid from the membrane, based on a method reported by Nielson *et al*. [18]. Freshly isolated PMNs were resuspended in loading buffer pH 7.4 (20 mM HEPES, 150 mM NaCl, 10 mM glucose, 5 mM KCl, 12 mM Tris, and 0.1% fat-free BSA). Cells were labeled with 1 μCi ^{3}H-arachidonic acid at 30° for 1 hr. Samples were centrifuged at 400 *g* for 10 min and cells washed twice and resuspended in loading buffer supplemented with 1.2 mmol/L $CaCl_2$, 0.49 mmol/L $MgCl_2$, and 0.49 mmol/L $MgSO_4$. Cell suspension (2 million cells) was added to p47phox permeabilization buffer pH 6.8 (20 mM Pipes, 140 mM KCl, 5.5 mM glucose, 1 μM $CaCl_2$, 3 mM EGTA, and 0.1% fat-free BSA) including 10.8 U/mL streptolysin-O and 2.5 μg/mL cytochalasin B. These cells were then preincubated in the presence of adrenaline for 10 min. The cells were then stimulated at 37° for 5 min with 10^{-7} M fMLP and released arachidonic acid was trapped by albumin in the permeabilization buffer. The reaction was stopped after 5 min by the addition of ice-cold 0.9% saline. Samples were centrifuged for 5 min at 1750 *g*. 0.5 mL of supernatant was added to 5 mL Ecoscint A scintillation fluid and radioactivity was measured in dpm using a LKB Wallac 1217 Rackbeta liquid scintillation counter. By determining the total arachidonic acid release (in the absence of 4-BPB) and AA available from sources other than the activities of PLA_2 (in the presence of 4-BPB), the release of AA resulting from PLA_2 activity only was determined.

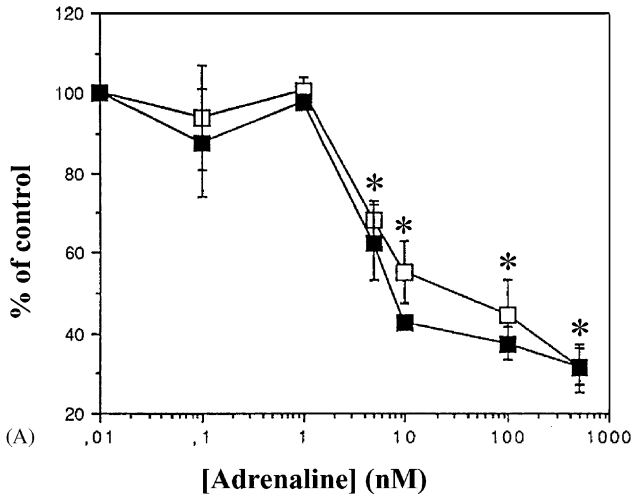
2.9. Statistical analysis

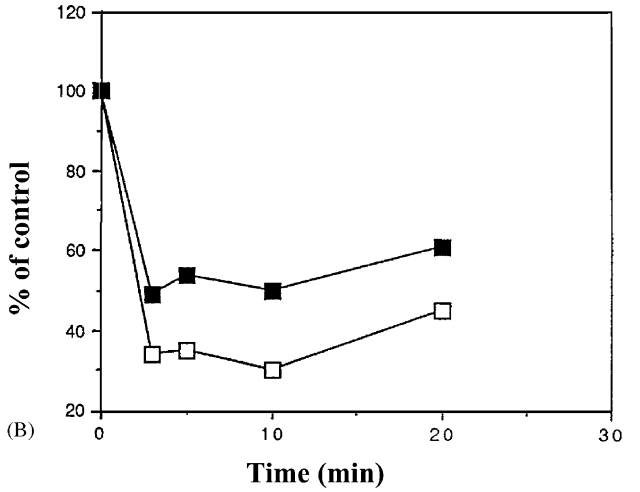
Values are given as mean ± SEM of at least three duplicate experiments. Statistically significant differences between conditions were compared using the Mann–Whitney test. A *P* value of less than 0.05 was considered significant.

3. Results

3.1. Adrenaline inhibits fMLP- but not PMA-induced superoxide anion production of human neutrophils

To assess the effect of adrenaline on human neutrophil superoxide anion production, neutrophils were incubated for 10 min with different concentrations of adrenaline, stimulated and superoxide release was quantified by cytochrome *c* reduction assay. Control neutrophils stimulated by fMLP (10^{-7} M) elicited rapid superoxide production. The initial rate of superoxide production was 4.77 ± 2.5 nmol/min/2 million cells with a total production of 31.2 ± 4 nmol/2 million cells over 10 min. As shown in Fig. 1A, adrenaline inhibits fMLP-stimulated superoxide production in a concentration-dependent manner starting as low as 1–1000 nM. The inhibitory effect was rapid occurring in less than 2.5 min (Fig. 1B). Both initial rate and total production were equally inhibited. However,





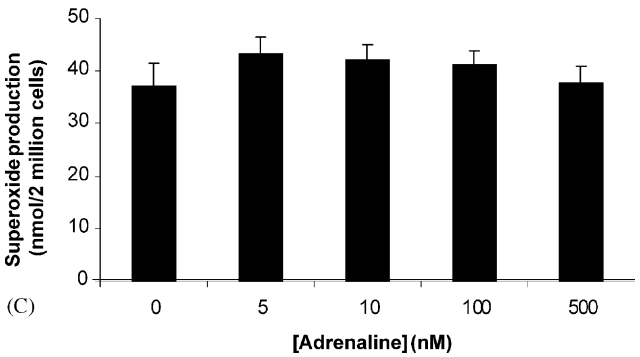


Fig. 1. Effect of adrenaline on fMLP- and PMA-induced superoxide anion production by human neutrophils. (A) Concentration-dependent effect: neutrophils were preincubated with varying concentrations of adrenaline at 37° for 10 min. The cells were then stimulated with fMLP (10^{-7} M) and superoxide anion production measured by following the reduction of cytochrome *c*. Initial rate (open square) and total production (closed square). (B) Time course of the effect of adrenaline: neutrophils were preincubated with 100 nM of adrenaline at 37° for varying times. The cells were then stimulated with fMLP (10^{-7} M) and superoxide anion production measured by following the reduction of cytochrome *c*. (C) Effect of adrenaline on PMA-induced superoxide anion production: human neutrophils were preincubated with varying concentrations of adrenaline at 37° for 10 min. The cells were then stimulated with PMA (100 ng/mL) and superoxide anion production measured. The results shown are from five independent experiments ($^{*}P < 0.05$ for N = 5).

when neutrophils were stimulated by the protein kinase C activator PMA, adrenaline failed to inhibit superoxide production (Fig. 1C). PMA caused a total production of 37.108 ± 4.3 nmol superoxide per 2 million cells over 10 min. These results show that adrenaline depresses the chemoattractant-receptor-dependent activation of NADPH oxidase and suggest that it does not affect PMA-activated PKC isoforms activities.

3.2. Adrenaline induces an increase in intracellular level of cAMP and the protein kinase A (PKA) inhibitor H-89 reverses its inhibitory effect

β-Adrenergic agonists are known to be powerful adenylate cyclase activators inducing production of cAMP which may inhibit the respiratory burst through activation of PKA. To check this hypothesis, cAMP was measured in human neutrophils treated with adrenaline. Figure 2 shows that adrenaline induced a concentration-dependent increase of cAMP in human neutrophils. This process was observed in the same range of adrenaline concentrations which inhibits fMLP-stimulated superoxide production. To check that PKA activity is implicated in this inhibitory process, neutrophils were preincubated with the PKA inhibitor H-89. The results show (Fig. 3) that H-89 caused blockade of the adrenaline inhibitory effect on the fMLP-stimulated respiratory burst. H-89 (5 μM) alone had a moderate potentiating effect on fMLP-induced superoxide production (in the presence of H-89, the response was $145.6 \pm 6.5\%$ as compared to fMLP (100%) alone, mean ± SEM, N = 3). Taken together these results suggest a role for PKA in mediating the inhibitory effect of adrenaline on fMLP-induced superoxide production.

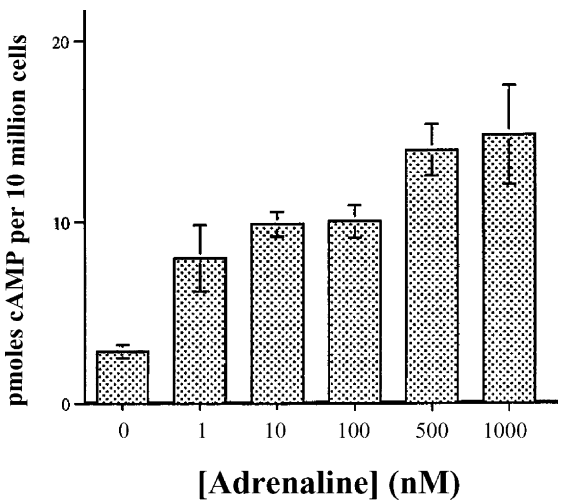


Fig. 2. Effect of adrenaline on cAMP production in human neutrophils. Human neutrophils were incubated with varying concentrations of adrenaline at 37° for 10 min. The reaction was stopped by boiling and supernatants assayed for cAMP by competitive binding assay. The results shown are from eight independent experiments.

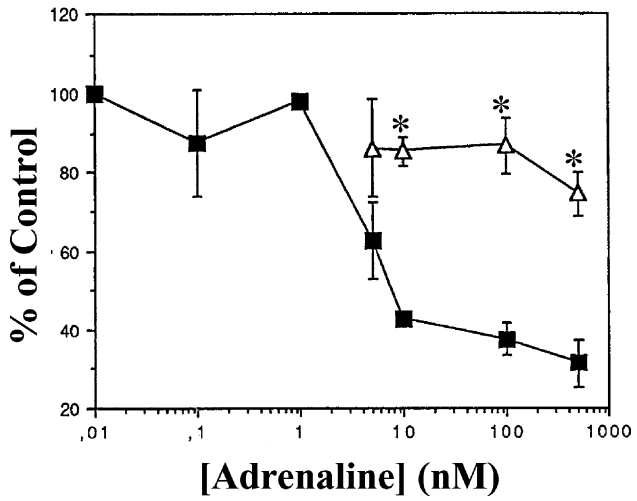


Fig. 3. Effect of the protein kinase A inhibitor H-89 on adrenaline inhibition of superoxide anions production. Human neutrophils were preincubated with 5 μM H-89 followed by incubation for a further 10 min with varying concentrations of adrenaline. The cells were then stimulated with fMLP(10^{-7} M) and superoxide anions production measured by following the reduction of cytochrome *c* at 550 nm in the absence (closed square) or presence (open triangle) of H-89. The results shown are from three independent experiments ($^{*}P < 0.05$ for N = 3).

3.3. Effect of adrenaline on the phosphorylation of p47phox and translocation of p47phox and p67phox

NADPH oxidase activation is dependent on the phosphorylation and translocation of its cytosolic components. To determine whether adrenaline targets the early activation step, we first analyzed the phosphorylation of p47phox in adrenaline-treated neutrophils. Figure 4 shows that in resting cells there was a very low level of phosphorylation of p47phox, adrenaline at 100 and 500 nM alone did not induce further phosphorylation of p47phox. fMLP (10^{-7} M) caused potent phosphorylation of p47phox, adrenaline at 100 and 500 nM did not cause any appreciable change in the phosphorylation of p47phox stimulated with fMLP.

Membrane translocation of p47phox and its binding to cytochrome b558 is a second important step for NADPH oxidase activation. Figure 5 shows that translocation of p47phox and p67phox are not affected by adrenaline, suggesting that it did not affect this assembly step in fMLP- or PMA-stimulated neutrophils.

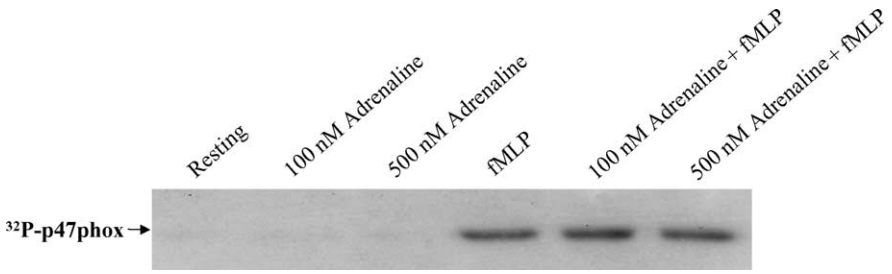


Fig. 4. Effect of adrenaline on p47phox phosphorylation in human neutrophils. ^{32}Pi-labeled neutrophils were incubated with adrenaline for 10 min followed by stimulation with fMLP. p47phox was then immunoprecipitated with a specific antibody, subjected to SDS–PAGE, blotted on nitrocellulose, and detected by autoradiography. Data are representative of three different experiments.

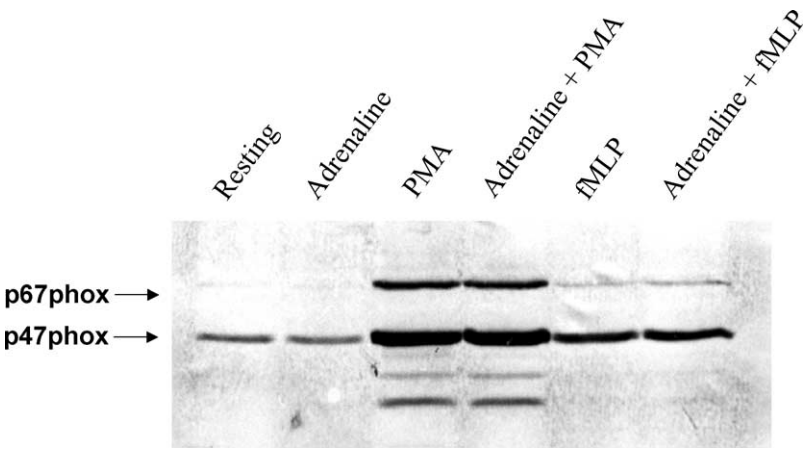


Fig. 5. Effect of adrenaline on p47phox translocation in human neutrophils. Neutrophils were incubated with adrenaline for 10 min followed by fMLP stimulation. Cells were lysed and cytosol and membranes were separated as described in Section 2. p47phox was subjected to SDS–PAGE, blotted on nitrocellulose, and detected with a specific antibody and by autoradiography. Data are representative of three different experiments.

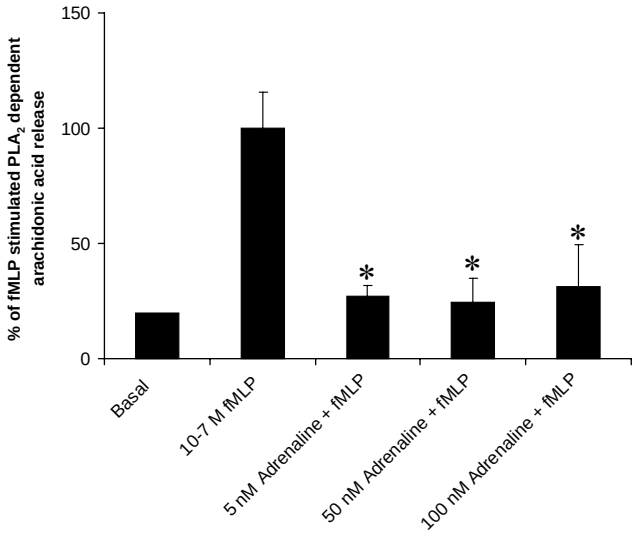


Fig. 6. Effect of adrenaline on PLA_2 activity in human neutrophils. ^{3}H-Arachidonic acid-labeled neutrophils were incubated with adrenaline for 10 min followed by fMLP stimulation. Released arachidonic acid was trapped by albumin in the permeabilization buffer. 0.5 mL of supernatant was added to 5 mL Ecoscint A scintillation fluid and radioactivity was measured in dpm using a LKB Wallac 1217 Rackbeta liquid scintillation counter. This result is representative of four independent experiments ($^*P < 0.05$ for N = 4).

3.4. Adrenaline inhibits Phospholipase A_2 activity in human neutrophils and exogenous arachidonate restores superoxide production in adrenaline-treated neutrophils

It is well established that two main parallel routes govern NADPH oxidase activation: p47phox phosphorylation and PLA_2 activation. Since adrenaline did not affect p47phox phosphorylation and translocation, we analyzed its effect on PLA_2 activity. As shown in Fig. 6, fMLP (10^{-7} M) caused an 80% increase in PLA_2 activity over basal levels. Adrenaline strongly decreased the magnitude of the fMLP-stimulated PLA_2 activity at doses as low as 5 nM. This result strongly suggests that adrenaline inhibits neutrophil superoxide anions production by inhibiting PLA_2 activity. Indeed we tested the effect of exogenous arachidonic acid on adrenaline depressed neutrophils. The results show that adrenaline (10 nM) inhibited fMLP-induced superoxide production by $80.5 \pm 6.5\%$, in the presence of 10 μM arachidonate, adrenaline has only an inhibitory effect of $25.5 \pm 4.5\%$ (mean ± SEM, N = 3).

4. Discussion

In this study, we show that the β-adrenergic agonist, adrenaline, at physiological and low pharmacological concentrations (5–500 nM) inhibited the fMLP-stimulated respiratory burst in human neutrophils. Adrenaline did not, however, inhibit the PMA-stimulated superoxide production. fMLP and PMA activate the respiratory burst via different signal transduction pathways. As PMA is known to be a direct activator of PKC [19,20] it is likely that events upstream or different from those involving PKC are attenuated by adrenaline.

Adrenaline induced a concentration-dependent increase of the PKA activator cAMP in neutrophils. This process occurs in the same range of concentration as the inhibitory effect of adrenaline on the fMLP-stimulated superoxide production. Furthermore, when neutrophils were pre-treated with the PKA inhibitor H-89, adrenaline inhibition of the burst was reversed, advocating a role for PKA in down regulation of the fMLP-stimulated respiratory burst. Simchowitz *et al.* [21] previously reported that fMLP and the complement fragment C5a induced a transient increase in intracellular levels of cAMP. Chemoattractant elicited increase in intracellular cAMP concentrations may provide a termination signal since treatment of leukocytes with cAMP-elevating drugs inhibits cellular responses to chemotactic factors and an anti-inflammatory role for a pathway involving cAMP has been suggested [22].

NADPH oxidase activation is dependent on the phosphorylation and translocation of its cytosolic component p47phox. Membrane translocation of p47phox and its binding to cytochrome b558 is the final step for NADPH oxidase activation. The results presented here clearly show that adrenaline did not affect these key steps of oxidase activation. It is believed that throughout activation, the NADPH oxidase is continuously deactivated and reactivated accompanied by phosphorylation and dephosphorylation of p47phox [2]. p47phox has phosphorylation consensus sequences for PKC, MAP kinase and also potential phosphorylation sites for PKA [5,23,24]. High doses of dbcAMP caused p47phox phosphorylation in PMN cytoplasts [25,26] but did not induce superoxide production. PKA-dependent phosphorylation may thus be inhibitory preventing reassembly of the oxidase. Physiological concentrations of adrenaline did not induce or inhibit phosphorylation of p47phox suggesting that other pathways may be affected by low concentrations of cAMP.

It is well established that two main parallel routes govern NADPH oxidase activation: p47phox phosphorylation and PLA_2 activation [27]. Our results show that adrenaline at the concentrations tested did not affect p47phox phosphorylation and translocation but inhibited fMLP-induced PLA_2 activity and arachidonic acid generation. This result strongly suggest that adrenaline inhibits neutrophil superoxide production by inhibiting PLA_2 activity. other targets like PLC and PLD were not affected by adrenaline at physiological concentrations (data not shown). Cytosolic PLA_2 is present in PMNs [28] and the main product of its activity, arachidonic acid, is released in PMN cytosol in response to stimulation. Inhibition of the human neutrophil respiratory burst is achieved by the specific PLA_2 inhibitor AACOCF3 [29], and this study (data not shown) implying a role for this isoform of PLA_2 in the respiratory burst. Our results show that PMA-induced NADPH oxidase activation was not affected by adrenaline contrary to fMLP-induced

activation. They also suggest that PMA stimulation is not affected by cAMP-elevating agents in agreement with the literature. However the requirement for PLA_2 and arachidonate for PMA activation is not ruled out. As neutrophils express at least three different PLA_2 isoforms ($cPLA_2$, $iPLA_2$, and $sPLA_2$) [30,31], fMLP and PMA could activate different isoforms, the one activated by fMLP is cAMP sensitive. In a recent paper by Zhao *et al.* [32], authors show that arachidonate is required for translocation of NADPH oxidase components to the membrane, but not phosphorylation in human monocytes. Opsonized zymosan was used to stimulate the cells, the requirement for arachidonate for translocation of NADPH oxidase subunits could depend on the stimuli used. Opsonized zymosan is a particle which triggers pathways different from fMLP or PMA.

Arachidonic acid has been shown to activate the respiratory burst in the cell free system [33–35] and in intact cells [36,37]. Arachidonic acid may interact with the oxidase components to induce a conformational change and evoke a respiratory burst. Shiose and Sumimoto [38] suggested that arachidonic acid synergises with phosphorylation of p47phox to facilitate the interaction with p22phox, thereby activating the NADPH oxidase. Indeed inhibition of either p47phox phosphorylation or arachidonic acid production can block NADPH oxidase activation.

In conclusion, agonist-mediated activation of the respiratory burst in human PMN is an important aspect of the pro-inflammatory response. The pharmacological manipulation of this neutrophil function is strategic in view of several disorders involving neutrophils. This work suggests that adrenaline caused inhibition of the fMLP-mediated respiratory burst via production of cAMP and activation of PKA which subsequently inhibited $cPLA_2$-dependent arachidonic acid production. The pharmacological potential of cAMP-elevating agents as inhibitors of neutrophil pro-inflammatory functions and arachidonic acid release in general is very important.

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