Effects of Various Drugs on Superoxide Generation, Arachidonic Acid Release and Phospholipase A2 in Polymorphonuclear Leukocytes

Katsuhiko TANIGUCHI, Misuzu URAKAMI and Koichiro TAKANAKA

Department of Toxicology, Niigata College of Pharmacy, 5829 Kamishineicho, Niigata 950-21, Japan

Accepted December 7, 1987

Abstract—The effects of variety of drugs on metabolic burst and phospholipase A2 in polymorphonuclear leukocytes (PMNs) were investigated. The stimulation of PMNs by n-formyl-methionyl-leucyl-phenylalanine (FMLP) causes arachidonic acid (AA) to be released in the cells concomitantly with the generation of superoxide anion. These variables were effectively diminished with some clinically employed drugs including chlorpromazine, trifluoperazine, azelastine, clemastine and mepacrine at the lower concentration of 20 μM. In contrast, indomethacin and procaine were ineffective even at the higher concentration of 100 μM. Subcellular fractionation of PMNs revealed that phospholipase A2 activity was located both in the plasma membrane-rich fraction as well as the granule-microsome-rich fraction, and the potency of inhibition of membrane-bound phospholipase A2 by the above mentioned drugs was: indomethacin (IC50=3 μM)<chlorpromazine<azelastine and clemastine (IC50>100 μM). The low potency of antipsychotropic drugs and antihistaminic drugs in inhibiting the fractionated phospholipase A2 contrast with the high efficiency with which they inhibit the superoxide generation and the AA release from stimulated PMNs. The AA releases from the PMNs stimulated by FMLP or calcium ionophore (A23187) were almost equally diminished by various drugs at the lower concentration. From these observations, it appeared likely that these drugs might inhibit the metabolic stimulations of PMNs at the sites of the Ca2⁺-dependent activation processes of the enzymes responsible for the AA release and the superoxide generation.

Polymorphonuclear leukocytes (PMNs) are known to play a central role in host defensive mechanisms including bactericidal functions as well as inflammatory processes (1–3). The activation of polymorphonuclear leukocytes (PMNs) by particulate or soluble stimuli induce a series of biochemical events such as generation of superoxide anions, release of lysosomal enzymes and initiation of the arachidonic acid (AA) cascade (4–6). Phospholipases mediate release of AA from membrane phospholipids, and its subsequent metabolism via cyclooxygenase or lipoxygenase pathways have been suggested as one of the mechanisms for the response of PMNs to inflammatory stimuli (7). The possible enzymes responsible for the release of AA from stimulated PMNs are phospholipase A2 and/or phospholipase C with subsequent involvement of glyceridelipase (8, 9). AA is released from a variety of cells concomitantly with the transmembrane control mediated by increased Ca2⁺ concentrations in the cytosol (10, 11).

Most of the metabolic stimuli of PMNs including chemotactic peptide (FMLP), calcium ionophore (A23187), and phagocytic particles commonly activate both the generation of superoxide anions as well as the release of AA. The only known exception is phorbol myristate acetate (PMA) which is a potent protein kinase C activator, but does not stimulate AA release (12, 13). Very recent studies have presented a stimulus-specific in-
duction of the AA metabolism in terms of the activations of the cellular phospholipases (14). Furthermore, it has been demonstrated that the reaction products of phospholipase A₂ could be responsible for the initiation of the respiratory burst of PMNs (12, 15, 16). Although it has been widely accepted that the site of action of the nonsteroidal antiinflammatory agents such as indomethacin and aspirin is the cyclooxygenase in the AA cascade, there are some reports suggesting that some of these drugs inhibit the phospholipase A₂ (17) and superoxide generation (18, 19). We have previously demonstrated that some antinflammatory drugs and antihistamines inhibit superoxide generation as well as changes of membrane potential of PMNs (20).

In this study, we have examined the effects of a variety of drugs on the AA release and the generation of superoxide from the stimulated PMNs. Furthermore, effects of the drugs on the membrane-bound phospholipase A₂ have been examined using the fractionated PMNs. The results are discussed in relation to the inhibitory effects of the same drugs on superoxide generation as well as AA release from PMNs.

Materials and Methods
Preparation of PMNs: A rabbit was injected intraperitoneally with 250 ml of a 0.2% (wt./Vol.) solution of glycogen in sterile saline. After 14–16 hr, peritoneal exudates containing PMNs were collected and filtered through three layers of cheese cloth. The cell suspension was centrifuged (400 x g, 5 min), and the pellet was resuspended in Hanks’ solution (Nissui Pharmaceutical Co., Tokyo, Japan). Two volumes of ice-cold distilled water was added, and the suspension was left to stand for 1 min to lyse contaminating erythrocytes. After restoring the osmolarity with 1.8% (wt./Vol.) NaCl solution, the suspension was centrifuged (400 x g, 2 min), and the pellet was resuspended in Hanks’ solution (3 x 10 ml). After collection, the cells were suspended in Gey’s balanced salt solution containing 0.1% (wt./Vol.) bovine serum albumin and 0.01 M HEPES buffer at pH 7.4 (modified Gey’s solution), at a concentration of 1 x 10⁷ cells/ml. This procedure yielded leukocytes (5 x 10⁶ –10⁹) containing over 90% of PMNs.

Chemicals: [³H]-Arachidonic acid (80 Ci/mmol) was obtained from New England Nuclear. Azelastine and clemastine were obtained from the Eisai Co. (Tokyo, Japan). FMLP, A23187, indomethacin, procaine, chlorpromazine, trifluoperazine and mepacrine were purchased from Sigma Chemical Co. p-Bromophenacyl bromide was purchased from Wako Pure Chemical Industries (Osaka, Japan). Indomethacin and A23187 were dissolved in dimethyl sulfoxide and diluted with medium prior to each experiment. p-Bromophenacyl bromide was dissolved in ethanol.

Preparation of [³H]-AA labelled cells: [³H]-AA (final concentration, 0.25 μCi/ml) was transferred into a plastic test tube; the ethanol was evaporated under a stream of nitrogen; and immediately, a known volume of cell suspension (10⁷ cells in 1 ml) was added. The cell suspension was incubated at 37°C for 10 min. At the end of the incubation period, five volumes of ice-cold modified Gey’s solution was added, and the suspension was centrifuged (400 x g, 2 min). The pellet was washed with modified Gey’s solution (2 x 10 ml) and resuspended in the appropriate solution to give a final concentration of 1 x 10⁷ cells/ml [110,000±14,000 dpm/10⁷ cells/ml (n=16)].

Assay of arachidonic acid release: The labelled cell suspension was preincubated with various drugs at 37°C for 5 min. The reaction was started by addition of 500 nM A23187 or 100 nM FMLP and then carried out for a further 5 min, before termination by the addition of 3.5 ml of methanol/chloroform (2:1; Vol./Vol.). Extraction and separation of radioactivity were carried out according to the method described by Bligh and Dyer (21). The chloroform extract was evaporated under a stream of nitrogen, and the residue was spotted on a TLC plate (silicagel, 20 x 20 cm) that was then developed in petroleum ether/diethyl ether/acetic acid (80:20:1, Vol./Vol.). Spots detected by iodine vapor were scraped into counting vials, and the radioactivity was measured in a liquid scintillation counter after the addition of 10 ml of Scintisol (Wako Pure Chemical Industries) to each vial. The values of FMLP and A23187 stimulated release of AA
were 17,900±4,800 dpm (n=4) and 42,900±
3,700 dpm (n=4), respectively. In the absence
of stimuli, the control value was 6,200±1,500
dpm (n=4).

Assay of superoxide generation: Superoxide
anion production was measured as described previously (20) using 100 nM
FMLP, 20 μM ferricytochrome c and 10^6 cells
in the presence of various drugs.

Fractionation of PMNs: To lyse the PMNs,
the pellet was resuspended in 0.34 M sucrose
solution and agitated 15–20 times with a
Pasteur pipet. After centrifugation (400×g,
5 min), the supernatant fraction contained the
cell lysate. This procedure was repeated 3–4
times until the supernatant became clear. The
pooled supernatant fraction was layered on
top of a 30%, 40% and 50% (wt./wt.) discon-
tinuous sucrose gradient containing 1 mM
EGTA and 10 mM HEPES (pH 7.2) and
centrifuged at 105,000 g for 2 hr in a swing
type rotor (Hitachi RPS-28). The resulting
bands on the top of 30%, 40% and 50% of
sucrose were collected as band I, band II and
band III, respectively.

Assay of phospholipase A2: Phospholipase
A2 activity was estimated according to the
method of Victor et al. (22). The phospholi-
pids of Escherichia coli strain S17r were
labeled with [1-14C]oleic acid (specific ac-
tivity 54 Ci/mol, New England Nuclear Co.).
After labelling, the organisms were autoclaved
for 15 min at 120°C in order to inactivate
bacterial phospholipase and used as the
substrate for the enzyme assay. Assay mix-
tures of 0.5 ml containing 80 mM Tris-HCl
buffer (pH 7.5), 10 mM CaCl2, 30–40 μg
protein and 10,000–15,000 dpm of the above
mentioned substrate were employed. Incuba-
tions were carried out at 37°C for 15 min and
terminated by addition of 3 ml of methanol/
chloroform (2:1, vol./vol.). Extraction, sepa-
ration and assay of radioactivity were carried
out as described by Bligh and Dyer (21).
Briefly, the chloroform extracts were evaporat-
ed under a stream of nitrogen. The residues
were spotted on TLC plates (silica gel 20 cm×
20 cm) which were developed in petroleum
ether/diethyl ether/acetic acid (80:20:1, Vol./
Vol.). Spots detected by iodine vapor were
scraped into scintillation vials, and their radio-
activity measured after the addition of 10 ml
scintillation fluid (Scintizol). Hydrolysis of
phospholipids was expressed as % of the
fraction of free fatty acid in the total radio-
activity. The enzyme activity was expressed as
% of hydrolysis/mg protein. Protein was deter-
mimed by the Lowry method (23).

Results

Figure 1 shows the release of [3H]AA from
prelabelled PMNs. In the absence of any
stimulation, there was a slow time-dependent
release of AA (~3000 dpm at 0 min, ~4000
dpm at 5 min and ~6000 dpm at 10 min).
PMNs preincubated for 5 min and then chal-
enged with FMLP or A23187 for a further 5
min showed a 7-fold and 20-fold increase in
[3H]AA release, respectively, over the unchal-
enged control. The release of AA from both
resting and stimulated PMNs was almost
totally dependent on the presence of calcium
in the medium. Addition of the antiallergic
drug clemastine completely abolished [3H]-
AA release from FMLP stimulated PMNs. In
fact, the release of the radiolabel at the end of
10 min in the presence of both clemastine and
FMLP was less than the release of [3H]AA

Fig. 1. FMLP and A23187 induced release of
arachidonic acid from PMNs. Cells were preincubated
for 5 min, and FMLP (–○–) or A23187 (–△–)
was challenged at the point indicated by the arrow.
The release of arachidonic acid in the absence of any
stimuli (–●–) was employed as a control. In the
presence of 100 μM clemastine, the cells were
stimulated with FMLP (–■–). Releases of [3H]AA
were estimated at 0 time, 5 min and 10 min after the
additions of stimuli as described in Methods. Results
represent the mean±S.D. of four different experi-
ments.
from unchallenged cells (Fig. 1). In such cases, the data is expressed as a negative value (see Table 1).

The effects of phospholipase A2 inhibitors and a variety of drugs on the release of [3H]-AA from FMLP or A23187 stimulated PMNs are summarized in Table 1. Preincubation of PMNs with the well-known phospholipase inhibitors p-bromophencayl bromide and mepacrine substantially inhibited [3H]-AA release when added at the low concentration of 20 μM. At a higher concentrations of 100 μM, they almost completely abolished the release of [3H]-AA from FMLP or A23187 stimulated PMNs.

The antipsychotropic drugs chlorpromazine and trifluoperazine and the antiallergic drugs azelastine and clemastine when added at a lower concentration of 20 μM substantially inhibited [3H]-AA release (50-75%) from stimulated PMNs. At a higher concentration of 100 μM, they completely abolished [3H]-AA release. The IC50s of chlorpromazine and azelastine in inhibiting [3H]-AA release from FMLP stimulated PMNs were found to be 10 μM and 25 μM, respectively (Fig. 2). The antiinflammatory drug indomethacin and the local anesthetic procaine were ineffective as inhibitors of [3H]-AA release when used up to a concentration of 100 μM, the highest concentration tested.

To investigate the sites of these inhibitory effects of drugs in the cells, PMNs were lysed by 0.34 M sucrose treatment and the subcellular fractionation was carried out with the 30, 40 and 50% of discontinuous sucrose
gradient. The three bands (bands I, II and III from the top) appeared separately and each of the bands were subjected to electron microscopic examination as well as assays for enzyme activities (data in detail will be submitted in separate paper). The electron microscopic observations revealed that most of the structures in band II were small fragments from membranes, and subcellular granule structures such as mitochondria and electron dense granules were observed in band III. Band I contained small fragments of membrane and the recovery was so small that it was not subjected for the assays of phospholipase A2 in this study.

Phospholipase A2 activity was found mainly in the band III fraction in terms of absolute activities. The specific activity in band III was 3-fold greater than in the original homogenate and approximately 75% of the phospholipase A2 activity in the layered sample was recovered in band III. The distribution pattern of phospholipase A2 activity was different from that of glucose-6-phosphatase and Na\(^+-\)K\(^+\) ATPase, and it appeared to be present both in band III as well as in band II. Detectable amounts of phospholipase A2 activity were not seen in the resultant supernatant fraction following ultracentrifugation.

The optimal pH for the phospholipase A2 activity in both bands II and III was found to be pH 7.5. The time course of the assay of phospholipase A2 was linear up to at least 15 min of incubation under the assay conditions described in the Methods Section.

The effect of various drugs and chemicals on phospholipase A2 activity in the plasma membrane and granule-microsome fractions (bands II and III) was quite similar (Table 2). Low concentrations (<20 \(\mu M\)) of the anti-inflammatory drug indomethacin were very effective in substantially inhibiting phospholipase A2 activity in both fractions and its IC50 was 3 \(\mu M\) in the band II fraction. The antipsychotropic drug chlorpromazine and trifluoperazine also significantly inhibited the activity in both fractions at a concentration of 100 \(\mu M\). The antiallergic drugs clemastine and azelastine exerted only weak inhibitory effects at concentrations of 100 \(\mu M\). Mepacrine at concentrations of 100 \(\mu M\) only slightly inhibited phospholipase A2 activity in bands II and III. As far as examined in this study, indomethacin was the only effective inhibitor of the phospholipase A2 of PMNs with an IC50 of less than 20 \(\mu M\) (Table 2).

The effect of various drugs on superoxide anion production in FMLP-stimulated PMNs is shown in Table 3. Chlorpromazine and trifluoperazine were the effective drugs inhibiting superoxide anion production at the low concentration of 20 \(\mu M\). Azelastine and clemastine were slightly effective at 20 \(\mu M\),

| Chemicals          | % of control activity |
|--------------------|-----------------------|
|                    | Band II  | Band III |
|                    | 20 \(\mu M\) | 100 \(\mu M\) | 20 \(\mu M\) | 100 \(\mu M\) |
| Indomethacin       | 28±6 (5)  | 25       | 20±16 (5) | 4 |
| Chlorpromazine     | 76±13 (6) | 29±8 (6) | 78±5 (4) | 35±18 (4) |
| Trifluoperazine    | 68        | 23       | 100       | 21 |
| Clemastine         | 102       | 74       | 74        | 68 |
| Azelastine         | 107±17 (4) | 82±24 (4) | 80        | 74 |
| p-Bromophenacyl bromide | 72±10 (4) | 79       | 64±7 (4) | 72 |
| Mepacrine          | 112±4 (4) | 98       | 85±35 (4) | 72 |

Assay mixtures of 0.5 ml containing 80 mM Tris-HCl buffer, 10 mM CaCl\(_2\), 30–40 \(\mu g\) protein from the band II or III and 10,000–15,000 dpm of [1-\(^{14}\)C] oleic acid were incubated in the presence of various concentrations of drugs or chemicals for 15 min at 37°C. Hydrolysis of phospholipid was estimated as the % of the free fatty acid fraction (6,000±500 dpm) in the total radioactivity. The effects of chemicals were calculated from the control activity (2180±215 dpm) which was 20.6±2.2% of the total radioactivity. The data accompanied by the number of assays in parenthesis represents the mean±S.D.
but completely abolished superoxide anion production at 100 µM. Indomethacin was somewhat less effective, causing approximately 50% inhibition at 20 µM and a 82% inhibition at 100 µM. Procaine caused only a slight inhibition of superoxide anion production at the concentration of 100 µM.

Typical examples of the inhibitory effects of the drugs are summarized in Fig. 3 in terms of the effects on the AA release, phospholipase A2 activity and superoxide generation.

**Discussion**

The activation of PMNs by soluble or particulate stimuli leads to a series of biochemical changes including superoxide generation and AA metabolism. From the experimental observations in this study, the AA release by activated PMNs has been shown to be inhibited by the following agents: antipsychotropic drugs (chlorpromazine and trifluoperazine), antiallergic drugs (clemastine and azelastine), p-bromophenacyl bromide and mepacrine (19, 24-26). The most effective inhibitor of the AA release is the antipsychotropic drug trifluoperazine. On the contrary, indomethacin and procaine are hardly effective on the first of the AA cascade, i.e., the release of the AA release, although these chemicals are known to inhibit prostaglandin formation.

To gain further insight on the inhibitory effects of these drugs on the AA release, attempts were made to investigate the effects of the above mentioned drugs on the subcellularly fractionated phospholipase A2 as well as the superoxide generations from PMNs. In fact, the properties of phospholipase A2 in membrane fractions from PMNs are quite different from that present in snake venom. First, in agreement with previous studies by others, the phospholipase A2 activity in PMNs is not significantly inhibited by the well-known inhibitors of snake venom phospholipase A2 such as p-bromophenacyl bromide and mepacrine (27, 28). Secondly, indomethacin, which is a potent inhibitor of phospholipase A2 in PMNs (Table 2), is much less effective on the snake venom enzyme (17). Our results also indicated that over 5-fold higher concentration of p-bromophenacyl bromide is required to inhibit the phospholipase A2 activity to a similar degree as the AA release from the stimulated cells. In view of this, the inhibition of the AA release and superoxide generation by p-bromophenacyl bromide could be due to some other reasons or it may act only when the phospholipase A2 is activated (28).

The order of potency of a variety of drugs in terms of the inhibition of phospholipase A2 activity and the AA release does not show parallel relationships. As far as we have examined in this study, drugs that are potent phospholipase A2 inhibitors are relatively weak inhibitors of the AA release and vice versa.

---

**Table 3. Effect of various drugs on FMLP-induced superoxide anion production by PMNs**

| Chemicals                     | % of control |
|-------------------------------|-------------|
|                               | 20 µM       | 100 µM     |
| Trifluoperazine               | 0±0         | 0±0        |
| Chlorpromazine                | 11±14       | 0±0        |
| Azelastine                    | 71±20       | 0±0        |
| Clemastine                    | 68±33       | 0±0        |
| Procaine                      | 73±8        | 65±7       |
| Indomethacin                  | 49±13       | 18±14      |
| p-Bromophenacyl bromide      | 0±0         | 0±0        |
| Mapacrine                     | 26±5        | 0±0        |

Incubations were carried out as described in the Methods Section. Absolute amount of superoxide anion production by FMLP was 1.4±0.2 nmoles/min/10^6 cells. Control (blank) rate of cytochrome c reduction was estimated by the addition of SOD, and it was always less than 1% of the rates in the absence of cytochrome c. Results represent the mean±S.D. of four different experiments.
Fig. 3. Comparisons among the inhibitory effects of drugs on the AA release, phospholipase A₂ and superoxide generation. Typical examples of the results demonstrated in Tables 1, 2 and 3 are summarized in this figure. Results represent the remaining activities of the AA release (---○---), phospholipase A₂ in band II (---●---) and superoxide generation (---®---).

As seen Tables 1 and 3 and Fig. 3, although the degrees of effectiveness were not identical, antipsychotropic drugs and antiallergic drugs commonly depress the AA release in relatively good correlation with superoxide generation. In other words, it appears likely that action sites of these drugs on the AA release as well as the superoxide generation are very close. On the contrary, indomethacin, a well-known inhibitor of cyclooxygenase (7, 29, 30) and phospholipase A₂ (17), effectively diminishes superoxide generation and phospholipase A₂ activity, but no inhibitory effects on the AA release are seen even at 5-fold greater concentration of the chemical inhibiting the AA release. These results are in agreement with previous studies by other researchers (17, 31) demonstrating that the AA release is not inhibited by indomethacin, although it is a potent phospholipase A₂ inhibitor of PMNs. These suggest that the AA release from PMNs is not phospholipase A₂-mediated, but we must be cautious in making such an interpretation because the relationship between results obtained with fractionated membrane bound enzymes to the behavior and role of the enzymes in whole cells remain to be defined. At present, questions still remain about whether phospholipase A₂ is involved in the AA release from membranous phospholipid.

Several clinically important drugs have been shown to inhibit the release of AA from platelets and PMNs, although the mechanism for this inhibition is not clear (32–35). Although the role of several different phospholipases in initiating the AA cascade has been suggested and investigated for many years, it is not as yet clear whether phospholipase A₂ or diglyceride lipase is primarily responsible for the AA release (36, 37). Currently, it is suggested that the influx of calcium ions and/or the presence of diglyceride formed by the action of phospholipase C leads to the phosphorylation of lipocortin which in turn activates phospholipase A₂ leading to the release of AA (38, 39).

The release of AA from PMNs is known to require the influx of extracellular Ca²⁺, and the role of phosphate has been suggested in the stimulation mechanism of FMLP (10). It is likely, therefore, that the inhibitory effects of the drugs and chemicals on AA release from PMNs is related to the intracellular function of increased calcium ion concentrations, since both FMLP and A23187 stimulations were depressed by removal of Ca²⁺ from the reaction medium. Drugs or chemicals inhibiting any site of the activation process of AA release would lead to lower the AA release from the stimulated cells. These sites would include: (i) the process of Ca²⁺ influx, (ii) Ca²⁺-dependent activation (40, 41), and (iii) the
catalytic action of phospholipase A₂ or C. As demonstrated in this study, some antipsychotropic drugs and antihistamines are potent inhibitors of the AA release and superoxide generation from PMNs, but weak inhibitors of phospholipase A₂. This suggests the possibility that these agents might inhibit the AA cascade at the sites of the activation process of phospholipases (so called transmembrane control systems) and/or the protein kinase c mediated activation process. Since the AA release from the PMNs stimulated by A23187 is effectively diminished by the antipsychotropic drugs as well as antiallergic agents, it is likely that these drugs might interrupt the AA release as well as superoxide generation at the sites of the Ca²⁺-dependent activation process which is followed by activations of phospholipases and superoxide generating enzymes (42, 43).

Although further investigations are necessary to elucidate the mechanisms in detail, these inhibitory effects may contribute to the actions or the side actions of these drugs in clinical applications.

References

1 Babior, B.M., Kipnes, R.S. and Curnutte, J.T.: Biological defense mechanisms. The production by leukocytes of superoxide, a potential bactericidal agent. J. Clin. Invest. 52, 741–744 (1973)

2 Weiss, J., Victor, M., Stenchal, O. and Elsbach, P.: Killing of gram-negative bacteria by polymorphonuclear leukocytes. J. Clin. Invest. 69, 969–970 (1982)

3 Oyanagui, Y.: Participation of superoxide anions at the prostaglandin phase of carrageenan foot-oeedema. Biochem. Pharmacol. 25, 1465–1472 (1976)

4 Bainton, D.F.: Sequential degranulation of the two types of polymorphonuclear leukocyte granules during phagocytosis of microorganisms. J. Cell Biol. 58, 249–264 (1973)

5 Estensen, R.D., White, J.G. and Holmes, B.: Specific degranulation of human polymorphonuclear leukocytes. Nature 248, 347–348 (1974)

6 Hirata, F., Corcoran, B.A., Venkatasubramanian, K., Sciffmann, E. and Axelrod, J.: Chemotacticants stimulate degranulation of methylated phospholipid and release of arachidonic acid in rabbit leukocytes. Proc. Natl. Acad. Sci. U.S.A. 76, 2640–2643 (1979)

7 Siegel, M.I., McConnel, R.T., Porter, N.A., Selph, J.L., Truax, J.F., Vineger, R. and Cuattrecasas, P.: Aspirin-like drugs inhibit arachidonic acid metabolism via lipoxygenase and cyclo-oxygenase in rat neutrophils from carrageenan pleural exudates. Biochem. Biophys. Res. Commun. 92, 688–695 (1980)

8 Bills, T.K., Smith, J.B. and Silver, M.J.: Metabolism of [¹⁴C]arachidonic acid by human platelets. Biochim. Biophys. Acta 424, 303–314 (1976)

9 Simmons, S.R.: Production of diglyceride from phosphatidylinositol in activated human platelets. J. Clin. Invest. 63, 580–587 (1979)

10 Bareis, D.L., Hirata, F., Sciffmann, E. and Axelrod, J.: Phospholipid metabolism, calcium flux, and the receptor-mediated induction of chemotaxis in rabbit neutrophils. J. Cell Biol. 93, 680–697 (1982)

11 Rubin, R.P.: Calcium-phospholipid interactions in secretory cells: a new perspective on stimulus-secretion coupling. Fed. Proc. 41, 2181–2187 (1982)

12 Curnutte, J.T., Badwey, J.A., Robinson, J.M., Karnovsky, M.J. and Karnovsky, M.L.: Studies on the mechanism of superoxide release from human neutrophils stimulated with arachidonate. J. Biol. Chem. 259, 11851–11857 (1984)

13 Walsh, C.E., Waite, B.M., Thomas, M.J. and DeChatelet, L.R.: Release and metabolism of arachidonic acid in human neutrophils. J. Biol. Chem. 256, 7228–7234 (1981)

14 Godfrey, R.W., Manzi, R.M., Clark, M.A. and Hoffstein, S.T.: Stimulus-specific induction of phospholipid and arachidonic acid metabolism in human neutrophils. J. Cell Biol. 104, 925–932 (1987)

15 Yoshimoto, S., Yoshimoto, T. and Tsubura, E.: Arachidonic acid-induced chemiluminescence of human polymorphonuclear leukocytes. Biochem. Biophys. Res. Commun. 107, 777–784 (1982)

16 Marionneau-Parini, I. and Tauber, A.I.: Activation of NADPH-oxidase by arachidonic acid involves phospholipase A₂ in intact human neutrophils but not in the cell-free systems. Biochem. Biophys. Res. Commun. 138, 1099–1105 (1986)

17 Kaplan, L., Weiss, J. and Elsbach, P.: Low concentration of indomethacin inhibit phospholipase A₂ of rabbit polymorphonuclear leukocytes. Proc. Natl. Acad. Sci. U.S.A. 75, 2955–2958 (1978)

18 Hoffman, M. and Autor, A.P.: Effect of cyclooxygenase inhibitors and protease inhibitors on
phorbol-induced stimulation of oxygen consumption and superoxide production by rat pulmonary macrophages. Biochem. Pharmacol. 31, 775–780 (1982)

19 Smolen, J.E. and Weissmann, G.: Effects of indomethacin, 5,8,11,14-eicosatetraynoic acid and β-bromophenacyl bromide on lysosomal enzyme release and superoxide anion generation by human polymorphonuclear leukocytes. Biochem. Pharmacol. 29, 533–538 (1980)

20 Taniguchi, K. and Takanaka, K.: Inhibitory effects of various drugs on phorbolmyristate acetate and n-formylmethionyl leucyl phenylalanine induce O₂⁻ production in polymorphonuclear leukocytes. Biochem. Pharmacol. 33, 775–780 (1982)

19 Smolen, J.E. and Weissmann, G.: Effects of indomethacin, 5,8,11,14-eicosatetraynoic acid and β-bromophenacyl bromide on lysosomal enzyme release and superoxide anion generation by human polymorphonuclear leukocytes. Biochem. Pharmacol. 29, 533–538 (1980)

20 Taniguchi, K. and Takanaka, K.: Inhibitory effects of various drugs on phorbolmyristate acetate and n-formylmethionyl leucyl phenylalanine induce O₂⁻ production in polymorphonuclear leukocytes. Biochem. Pharmacol. 33, 533–538 (1984)

22 Victor, M., Weiss, J., Klempner, M.S. and Elsbach, P.: Phospholipase A₂ activity in the plasma membrane of human polymorphonuclear leukocytes. FEBS Lett. 136, 298–300 (1981)

23 Lowry, O.H., Rosebrough, N.J., Farr, A.L. and Randall, R.J.: Protein measurement with the Folin phenol reagent. J. Biol. Chem. 193, 265–275 (1951)

24 Ochs, D.L. and Reed, P.W.: Inhibition of the neutrophil oxidative burst and degranulation by phenotiazine. Biochem. Biophys. Res. Commun. 102, 958–962 (1981)

25 Korchak, H.M., Rutherford, L.E. and Weissmann, G.: Stimulus response coupling in the human neutrophil. (I) Kinetic analysis of changes in calcium permeability. J. Biol. Chem. 259, 4070–4075 (1984)

26 Tauber, A.I. and Simons, E.R.: Dissociation of human neutrophil membrane depolarization, respiratory burst stimulation and phospholipid metabolism by quinacrine. FEBS Lett. 156, 161–164 (1983)

27 Roberts, M.F., Deems, R.A., Mincey, T.C. and Dennis, E.A.: Chemical modification of the histidine residue in phospholipase A₂. J. Biol. Chem. 252, 2405 (1977)

28 Lanni, C. and Becker, E.L.: Inhibition of neutrophil phospholipase A₂ by β-bromophenacyl bromide, nordihydroguaiaretic acid, 5,8,11,14-eicosatetraynoic acid and quercetin. Int. Arch. Allergy Appl. Immunol. 76, 214–217 (1985)

29 Ferreira, S.H., Moncada, S. and Vane, J.R.: Indomethacin and aspirin abolish prostaglandin release from the spleen. Nature New Biol. 231, 237–239 (1971)

30 Smith, J.B. and Willis, A.L.: Aspirin selectively inhibits prostaglandin production in human platelets. Nature New Biol. 231, 235–237 (1971)
Cell Biol. 99, 1212–1220 (1984)

42 Wong, P.Y.K. and Cheung, W.Y.: Calmodulin stimulates human platelet phospholipase A₂. Biochem. Biophys. Res. Commun. 90, 473–480 (1979)

43 Okamura, N., Ohashi, S., Nagahisa, N. and Ishibashi, S.: Changes in protein phosphorylation in guinea pig polymorphonuclear leukocytes by treatment with membrane-pertubing agents which stimulate superoxide anion production. Arch. Biochem. Biophys. 228, 270–277 (1984)