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Effects of Atropine, Phentolamine and Propranolol on Calcium uptake, Superoxide generation and Phagocytic activity in activated PMN Leukocytes*

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ABSTRACT

Although the release of lysosomal enzymes from activated PMN leukocyte can be regulated by intracellular cyclic nucleotide levels, other responses of PMN leukocyte according to the binding of neurotransmitters to either β-adrenergic or muscarinic receptors are still not clarified. In addition, the function of PMN leukocyte mediated by α-adrenergic receptors is uncertain. Atropine, phentolamine and propranolol inhibited calcium uptake, superoxide generation, NADPH oxidase activity and phagocytic activity in activated PMN leukocyte, whereas carbachol and isoproterenol slightly further stimulated the responses of activated cells. Either carbachol or isoproterenol stimulated superoxide generation was inhibited by their antagonists, atropine and propranolol, respectively. The response of activated PMN leukocyte was inhibited by chlorpromazine, verapamil and dantrolene but slightly stimulated by lithium. On the other hand, chlorpromazine and dibucaine did not affect NADPH oxidase activity. Atropine, phentolamine and propranolol suppressed the calcium dependent phagocytic activity. Thus, the results suggest that atropine, phentolamine and propranolol may inhibit superoxide generation in activated PMN leukocyte by the inhibition of calcium influx and by their direct action on the NADPH oxidase system which is associated with autonomic receptors.

Key Words: Atropine, Phentolamine, Propranolol, Superoxide generation, Calcium influx, Phagocytic activity

INTRODUCTION

A rise in the cytosolic calcium concentrations is considered to be an important factor in the stimulation of PMN leukocyte response. Increased free cytoplasmic calcium ion may involve in the activation of PMN leukocyte functional responses including degranulation and superoxide generation according to surface stimulation by both particulate and soluble agents (Goldstein *et al.*, 1975; Estensen *et al.*, 1976; Newburger *et al.*, 1980).

cAMP appears to exert its intracellular effects by virtue of its activation of specific protein kinases. Changes of cyclic nucleotide level within PMN leukocyte can also influence the release of lysosomal enzymes. It has been observed that agents which elevate the levels within PMN leukocytes of cAMP inhibit the release of enzymes during feeding of zymosan particles coated with immune complexes, whereas agents which elevate the levels within PMN leukocytes of cGMP enhance the release of enzymes (Zurier *et al.*, 1974; Weissmann *et al.*, 1975).

A calmodulin is known as an intracellular calcium receptor that confers sensitivity on various proteins and mediates calcium related reactions. Several reports indicate a possible involvement of calmodulin in phagocytic process. It has been shown that calmodulin is involved in the calcium pump of macrophage phagocytic vesicles (Lew and Stossel, 1980), and the inhibitory effect of phenoth-

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iazines and local anesthetics on the cellular superoxide release and on the particulate NADPH dependent superoxide forming activity is reported (Cohen *et al.*, 1980).

On the other hand, calcium influx may be triggered by the inositide cycle at the plasma membrane. It has been postulated that interaction of catecholamines with α-adrenergic receptors lead to the activation of a phosphatidyl inositol specific phospholipase c in the plasma membrane (Exton, 1983). The resulting breakdown in phosphatidyl inositol is postulated to cause an opening of calcium gates in the plasma membrane (Michell, 1975) and the release of calcium from intracellular storage sites (Blackmore *et al.*, 1982). This suggestion is also demonstrated at muscarinic receptors (Doughney *et al.*, 1987). The chemotactic peptide, fMet-Leu-Phe, cause both lysosomal enzyme secretion and superoxide anion (Lew *et al.*, 1984) from PMN leukocyte through probably the stimulation of phosphatidyl inositol turnover (Takenawa *et al.*, 1985) and the opening of receptor dependent calcium channels (Andersson *et al.*, 1986).

Although the presence of β-adrenergic receptors on PMN leukocytes is well known (Galant and Allred, 1981), functional response according to the binding of catecholamines to their receptors or action sites are still not clarified because epinephrine inhibits release of lysosomal enzymes (Zurier *et al.*, 1974), whereas its antagonist, propranolol inhibits superoxide generation (Taniguchi and Takanaka, 1984). In addition, the responses of PMN leukocytes mediated by α-adrenergic receptors and muscarinic receptors are uncertain. Thus, to elucidate the role of these receptors in the regulation of PMN leukocyte's function, effects of atropine, phentolamine and propranolol on calcium influx, superoxide generation and phagocytic activity in PMN leukocyte were investigated.

MATERIALS AND METHODS

Chemicals

Atropine, carbachol, phentolamine, propranolol, isoproterenol, chlorpromazine, dibucaine, lithium chloride, zymosan (from Saccharomyces cerevisiae), NADPH, ferricytochrome c, nitroblue tetrazolium (NBT) and dextran (M.W. 465,000) were purchased from Sigma Chemical Co.. Murexide was obtained from J.T. Baker Chemical Co.; verapamil from Knoll AG; $CaCl_2$ from Kanto Chemical Co.. Other chemicals were of analytical reagent grade.

Preparation of PMN leukocytes

PMN leukocytes were isolated from heparinized venous blood of healthy donors by dextran (average molecular weight 465,000) sedimentation of erythrocytes and treatment with 0.85% ammonium chloride of previously described (Trush *et al.*, 1978). The purity of PMN leukocyte suspensions averaged 90% as judged by Wright-Giemsa stain.

Preparation of NADPH oxidase containing granule rich fraction of PMN leukocytes

PMN leukocytes activated by opsonized zymosan at 37℃ for 15 min, or control PMN leukocytes were centrifuged at 1,500 g for 3 min and the pellets were resuspended in 0.25 M sucrose to a concentation of 10^8 cells/ml. The cell suspension was the disrupted by sonication for three 15 sec intervals at 25 watts power with a Branson sonifier cell disruptor (Mod. W 185D). Unbroken cells and nuclei were sedimented by centrifugation at 800 g for 5 min. Sucrose was then added to the postnuclear supernatant with constant stirring and the final volume adjusted to the sucrose concentration to 40 % (W/V). The suspension was centrifuged at 48,000 g for 1 h in a Beckman L5-50B ultracentrifuge. The supernatant completely removed and the pellets were resuspended in 0.25 M sucrose. The suspensions were centrifuged at 48,000 g for 1 h and the pellets (granule rich fraction) were suspended in 25% ethylene glycol with a Teflon glass homogenizer (Hohn and Letrer, 1975; Gabig *et al.*, 1982). The protein concentration was determined by the method of Lowry et al. (1951).

Measurement of calcium uptake by PMN leukocytes

Calcium uptake was measured by the spectrophotometric method using an Aminco-Chance dual wavelength split beam spectrophotometer. The reaction mixtures contained 10^6 cells/ml of PMN lekocytes, 50 μM murexide and HBSS buffer or 20 mM HEPES-tris, pH 7.4. After preincubation at 37℃ for 10 min, the reaction was initiated by addition of 1 mg/ml opsonized zymosan concomittent with 1 mM calcium and final volume was a 1.0 ml. The rate and extent of calcium uptake

by PMN leukocytes was measured through the absorbance changes of calcium chelating dye, murexide, at 507-540 nm in a 1.0 ml cuvette (Malmström and Carafoli, 1979).

Assay of superoxide radical generation

The superoxide dependent reduction of ferricytochrome c was measured by the method of Markert et al. (1984). Reaction mixtures in plastic microfuge tubes contained 10^6 PMN leukocytes, 75 μM ferricytochrome c, HBSS buffer (or saline) and 2 mg/ml of opsonized zymosan in a total volume of 500 μl. The reactions were performed in a 37℃ shaking water bath for the stated times. The reactions were then stopped by placing the tubes in melting ice and the cells were rapidly pelleted by centrifuging at 1,500 g for 5 min at 4℃. The supernatants were taken and the amount of reduced cytochrome c was measured at 550 nm in a Golford 260 U.V.-spectrophotometer. The amount of reduced cytochrome c was calculated by using an extinction coefficient of $1.85\times10^4M^{-1}cm^{-1}$ at 550 nm.

The reduction of NBT to purple formazan was also used to measure the generation of superoxide (Baehner, 1975). Reaction mixtures were the same as above description. Superoxide induced reduction of NBT was measured at 560 nm.

Assay of NADPH oxidase activity

The activity of NADPH oxidase was measured as reduction of ferricytochrome c by superoxide radicals produced from oxidation of NADPH by NADPH oxidase. Reaction mixture consisted of 0.2 mg/ml granule rich fraction, 100 μM NADPH, 75 μM ferricytochrome c and 50 mM Tris-HCl, pH 7.4 in a total volume of 500 μl. The reaction mixture was preincubated for 10 min at 37℃ and the reaction was initiated by adding NADPH. Reduction rate of ferricytochromec was measured at 550 nm (Lee *et al.*, 1987).

Assay for phagocytosis

The phagocytic activity of PMN leukocytes was determined by the method of Ishibashi and Yamashita, (1982). A PMN leukocyte suspension (10^7 cells/ml) in HBSS or saline was treated with 2 mg/ml of opsonized zymosan. After incubation for the stated time at 37℃, PMN leukocytes were stained with Wright-Giemsa, and the phagocytosis and attachment were determined microscopically. The phagocytic activity of PMN leukocytes was also assayed with a hemocyanometer.

RESULT

Inhibition of superoxide generation and calcium influx by atropine, phentolamine and propranolol

NADPH oxidase dependent superoxide generation in activated PMN leukocytes was effectively inhibited by autonomic antagonists. Amount of superoxide generated in opsonized zymosan activated PMN leukocytes was 39.57 nmol/10^6 cells/15 min. As shown in Fig. 1, atropine, phentolamine and propranolol at the concentration of 50 μM, inhibited the superoxide generation as 23.6%, 36.1% and 51.5%, respectively.

Since function of PMN leukocytes including the superoxide generation may be affected by

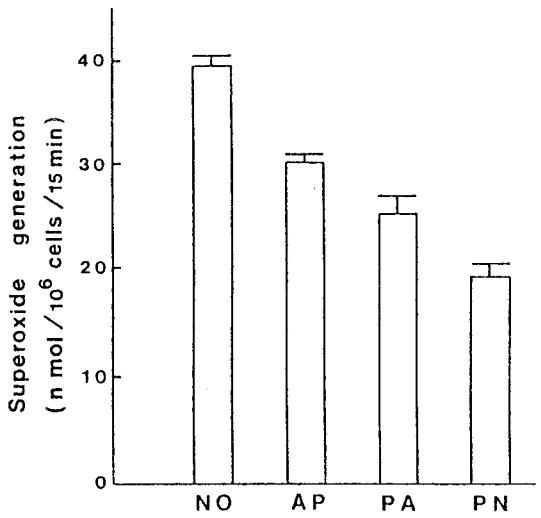

Fig. 1. Inhibition of superoxide generation from activated PMN leukocyte by atropine, phentolamine and propranolol. Reaction mixtures contained 75 μM ferricytochrome c, 10^6 cells, 1 mg opsonized zymosan, HBSS buffer and 50 μM drugs in a total volume of 500 μl. The amount of reduced cytochrome c by superoxide generated in activated PMN leukocytes was spectrophotometrically measured at 550 nm. Superoxide generation by resting PMN leukocyte was 4.28 nmol/10^6 cells/15 min. Data represents mean ± S.E. of 6 experiments. NO, none ; AP, atropine ; PA, phentolamine ; PN, propranolol.

calcium influx and calcium transport at plasma membrane appears to be regulated by adrenergic and cholinergic systems (Reuter, 1983), influence of autonomic antagonists on calcium uptake by activated PMN leukocytes was investigated.

When PMN leukocytes in HBSS were treated with opsonized zymosan, calcium uptake was gradually increased as far as 15 min and at 10 min of incubation time, the amount of calcium uptake was 0.843 μmol/10^6 cells. Fig. 2 shows the inhibitory effect of autonomic antagonists on calcium uptake. Atropine, phentolamine and propranolol of 0.1 mM inhibited the opsonized zymosan induced calcium uptake by 7.7%~31.8%.

To examine the role of autonomic receptors in the superoxide generation in activated PMN leukocyte, the interaction of autonomic agonist with antagonist in activated PMN leukocyte function

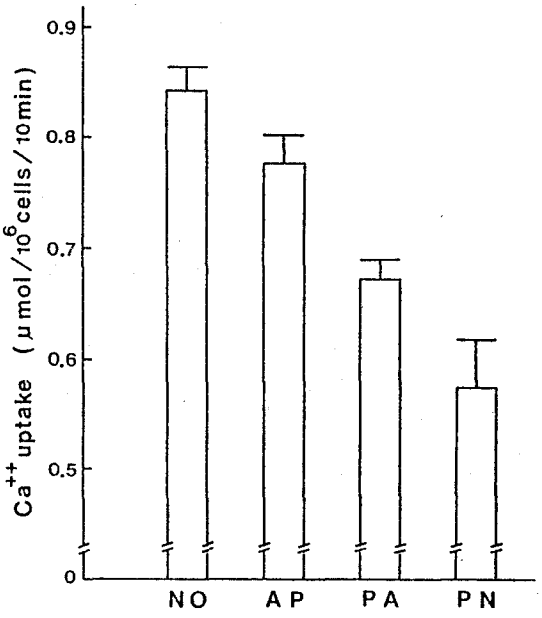

Fig. 2. Inhibition of Ca^{++} uptake by activated PMN leukocyte by atropine, phentolamine and propranolol. Ca^{++} uptake by resting PMN leukocytes was 0.084 μmol/10^6 cells/5 min. PMN leukocytes were preincubated with autonomic antagonists for 10 min at 37°C and Ca^{++} uptake was initiated by addition of 1 mg/ml opsonized zymosan. Ca^{++} uptake by activated PMN leukocyte was measured with dual wavelength spectrophotometer at 507–540 nm. The value represents mean ± S.E. of 5 experiments. NO, none ; AP, atropine ; PA, phentolamine ; PN, propranolol of 0. 1mM.

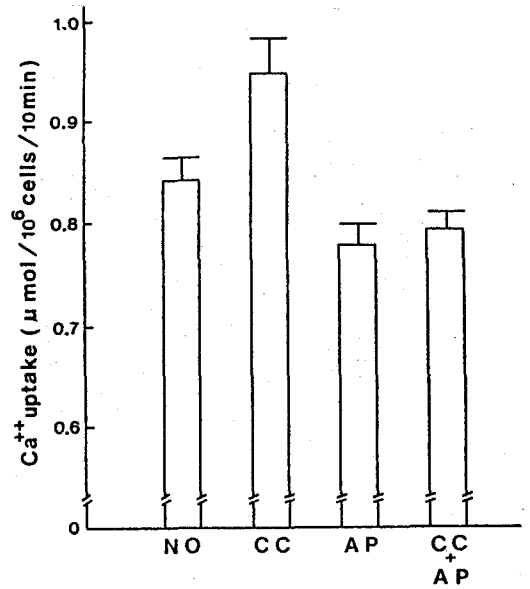

Fig. 3. Inhibition of carbachol induced Ca^{++} uptake by atropine. PMN leukocytes were pretreated with 0.1 mM of drugs for 10 min. Data represents mean ± S.E. of 6 experiments. NO, none ; CC, carbachol ; AP, atropine ; CC + AP, carbachol plus atropine.

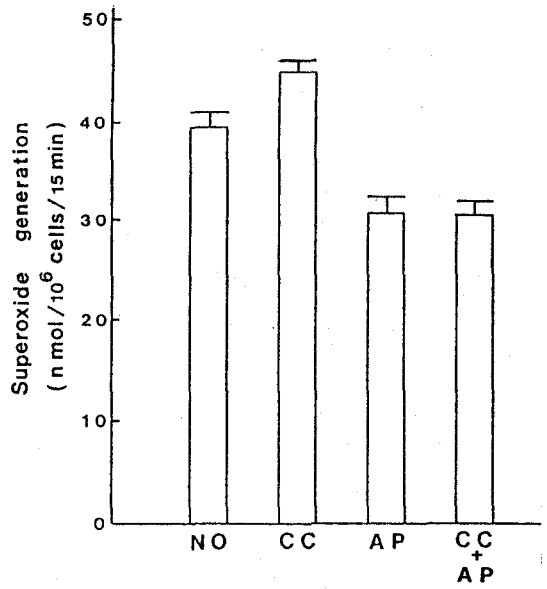

Fig. 4. Inhibition of carbachol superoxide generation by atropine. PMN leukocytes were treated with 50 μM of drugs. Data represents mean ± S.E. of 6 experiments. NO, none ; CC, carbachol ; AP, atropine ; CC + AP, carbachol plus atropine.

was observed. The results represented in Fig. 3 and Fig. 4 indicated that carbachol stimulated calcium influx and superoxide generation was inhibited by atropine. This finding was also investigated in the interaction of isoproterenol with propranolol (Table 1).

Effects of chlorpromazine, verapamil and lithium on calcium uptake and superoxide generation

The inositide cycle at the plasma membrane may trigger calcium influx. α-adrenergic agonists are known to stimulate calcium influx through increased phospholipid turnover. In addition, it is suggested that activation of protein kinase C induce NADPH oxidase activation (Virgilio *et al.*, 1984).

Calcium uptake by and superoxide generation in activated PMN leukocyte was inhibited by chlorpromazine, a protein kinase C and calmodulin inhibitor, verapamil, a calcium channel blocker and dantrolene which inhibit calcium release from intracellular calcium storage sites (Van Winkle, 1976). However, lithium slightly stimulated calcium uptake and superoxide generation.

Table 1. Inhibition of isoproterenol induced superoxide generation by propranolol

Compounds		ΔA of NBT reduction (in 10^6 cells/ 15 min)
None		0.128
Isoproterenol	0.1 mM	0.171
+ Propranolol	0.1 mM	0.051
+ Propranolol	0.01 mM	0.154
Propranolol	0.1 mM	0.043
Propranolol	0.01 mM	0.136

0.5 ml of reaction mixtures contained 0.1 mM NBT, 10^6 leukocytes, 1 mg opsonized zymosan, HBSS buffer and drugs. Reduction of NBT by superoxide generated was spectrophotometrically measured at 560 nm. The value represents the mean absorbancy of 5 experiments.

Table 2. Effects of chlorpromazine, verapamil and lithium on Ca^{++} uptake

Compounds		μmol Ca^{++} uptake/ 10^6 cells/10 min
None		0.861 ± 0.037
Chlorpromazine	0.1 mM	0.670 ± 0.065
Verapamil	1.0 mM	0.668 ± 0.071
Lithium	5.0 mM	1.004 ± 0.023

Drugs are present at incubation period. Ca^{++} uptake by activated PMN leukocyte was measured spectrophotometrically at 507–540 nm. The value represents mean ± S.E. of 5 experiments.

Table 3. Effects of chlorpromazine, verapamil, lithium and dantrolene on superoxide generation

Compounds		Superoxide nmol/ 10^6 cells/15 min
None		39.57 ± 0.62
Chlorpromazine	0.1 mM	9.62 ± 0.28
Verapamil	1.0 mM	5.67 ± 0.49
Lithium	5.0 mM	41.55 ± 0.13
Dantrolene	0.1 mM	31.77 ± 0.41

Drugs are present at incubation period. Superoxide generated from activated PMN leukocyte was measured by the reduction of ferricytochrome c. The value represents mean ± S.E. of 5 experiments.

Table 4. Effects of atropine, phentolamine and propranolol on NADPH oxidase activity

Compounds	Superoxide nmol/ mg protein/10 min
None	55.61 ± 3.34
Atropine	46.15 ± 4.92
Carbachol	67.20 ± 2.32
Phentolamine	37.73 ± 5.41
Propranolol	33.56 ± 4.05
Chlorpromazine	56.29 ± 8.11
Dibucaine	53.49 ± 5.46

Concentration of all drugs is 0.1 mM. Activity of NADPH oxidase from resting PMN leukocytes was 5.42 nmol/mg protein/10 min. Granule rich fraction which obtained from activated PMN leukocytes was preincubated with drugs for 10 min at 37°C and the reaction was initiated by addition of NADPH. Reduction of ferricytochrome c by interaction of NADPH and NADPH oxidase was measured at 550 nm. The value represents mean ± S.E. of 5 experiments.

Table 5. Effects of chlorpromazine, lithium and dantrolene on phagocytic activity

Compounds		Relative percentage for total cells		
		Normal cells	Attached cells	Phagocytosing cells
None		57.6	7.7	34.7
Chlorpromazine	0.1 mM	68.8	3.7	27.5
Lithium	5.0 mM	53.3	9.1	37.6
Dantrolene	1.0 mM	58.4	14.2	27.4

The each value represents an average percentage of 3 experiments.

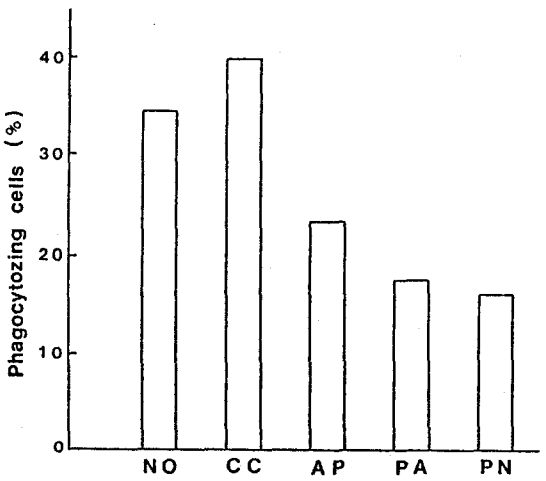

Fig. 5. Effects of atropine, phentolamine and propranolol on phagocytic activity. 10^7 PMN leukocytes/ml were preincubated with drugs for 10 min at 37°C and the reaction was initiated by addition of 2 mg/ml opsonized zymosan. After 15 min of reaction time, phagocytozing cells were microscopically measured. The value represents an average percentage of phagocytozing cells in 5 experiments. NO, none ; CC, carbachol ; AP, atropine ; PA, phentolamine ; PN, propranolol of 0.1 mM.

Effects of atropine, phentolamine and propranolol on NADPH oxidase activity

Since superoxide generation in PMN leukocyte was inhibited by autonomic antagonists, the possibility that these drugs directly act at NADPH oxidase system was investigated. NADPH oxidase activity in PMN leukocyte preincubated with opsonized zymosan was 55.61 nmol/mg protein/5 min and this activity was reduced by atropine, phentolamine and propranolol at the concentration of 0.1 mM as 17.0%-39.7% but increased by carbachol (Table 4). On the other hand, chlorpromazine and dibucaine had no significant effect on NADPH oxidase activity.

Effects of atropine, phentolamine and propranolol on phagocytic activity

Phagocytosis of PMN leukocyte for opsonized zymosan was suppressed by chlorpromazine and dantrolene but enhanced by lithium (Table 5). Fig. 5 shows that atropine, phentolamine and propranolol inhibited phagocytic activity of activated PMN leukocyte, whereas carbachol stimulated it. Thus, the finding obtained is coincided with actions of autonomic antagonists on calcium influx and superoxide generation.

DISCUSSION

The cholinergic agonist carbachol enhances release of lysosomal enzymes from PMN leukocytes and action of carbachol can be blocked by atropine (Zurier *et al.*, 1974). This finding was also seen in superoxide generation, as shown in Fig. 3. when PMN leukocytes are exposed to a phagocytic stimulus or activated immune complements, lysosomal enzyme are released from these cells and is also accompanied by a burst of oxidative metabolism launched by the generation of reactive oxygen radicals, chiefly superoxide anion (Weissmann *et al.*, 1979).

It is observed that endogenous cAMP or agents that increase endogenous cAMP levels (prostaglandin E_1, histamine and isoproterenol) reduce extrusion of lysosomal enzymes, whereas exogenous cGMP and carbachol which increases

endogenous cGMP levels increase enzymes release (Weissmann *et al.,* 1975). Thus, it is clear that the release of lysosomal enzymes is responded to the modulation of exogenous and endogenous cyclic nucleotides. Superoxide generation in activated PMN leukocyte was further stimulated by carbachol but inhibited by atropine, phentolamine and propranolol (Fig. 1 and Fig. 2). In addition, isoproterenol induced superoxide generation was inhibited by propranolol. Accordingly, effect of cholinergic agonist on the superoxide generation is coincided with its action on the release of lysosomal enzymes. However, isoproterenol and propranolol showed a discrepant action on function of PMN leukocyte. Table 1 suggests that inhibitory action of propranolol on superoxide generation may attributed to its action on certain superoxide generating sites which not affected by cyclic nucleotides.

Activation of receptors for several different hormones and neurotransmitters is thought to initiate a cellular response by opening specific calcium channels in the cell membrane (Berridge, 1981; Putney, 1981). It has been suggested that changes in phosphatidyl inositol metabolism may be a key membrane event involved in receptor-mediated changes in cellular permeability to calcium ions (Michell, 1975). α-Adrenergic agonist and cholinergic agonist promote breakdown of phosphatidyl inositol 4, 5-biphosphate leading to a generation of inositol 1, 4, 5-triphosphate. Inositol 1, 4, 5-triphosphate has been reported to induce a release of calcium from intracellular storage sites (Nahorski *et al.,* 1986). In addition, it is suggested that inositol 1, 4, 5-triphosphate mediates transmembrane calcium influx through specific calcium permeable channels in T-lymphocytes (Kuno and Gardner, 1987).

When PMN leukocytes are treated with a variety of soluble or particulate agents, molecular and functional changes take place in the plasma membrane including sodium influx (Showell and Becker, 1976; Korchak and Weissmann, 1980), changes of the membrane potential (Mottola and Romeo, 1982), mobilization of calcium (Bareis *et al.,* 1982) and phospholipid turnover (Gil *et al.,* 1982). It has been demonstrated that phosphatidyl inositol response and arachidonic acid release are close associated with PMN leukocyte activation (Hirata *et al.,* 1979). Thus, in terms of these suggestions effect of autonomic antagonists on calcium uptake by activated PMN leukocyte was investigated. As can be seen in Fig. 2 and Fig. 4, calcium uptake was inhibited by atropine, phentolamine and propranolol but slightly enhanced by carbachol. The results indicate that the permeability of calcium for the plasma membrane of PMN leukocyte may be modulated by autonomic receptors through their possible action on the turnover of inositol phospholipids. However, a direct role of autonomic drugs in calcium conductance at the plasma membrane is uncertain.

On the other hand, two possible intracellular mediators of the respiratory burst, namely calmodulin (Takershige and Minakami, 1981) and protein kinase C (Virgilio *et al.,* 1984), is proposed and they are calcium ion dependent. Both superoxide generation and calcium uptake by activated PMN leuckocyte is inhibited by chlorpromazine, verapamil and dantrolene. Thus, Table 2 and Table 3 suggest that a calcium influx and intracellular redistribution of calcium play an important role in the expression of PMN leukocyte response for the external stimuli.

Lithium blocks the breakdown of myoinositol-l-phosphate and greatly amplify the agonist stimulated phosphorylase c-specific inositol phospholipid hydrolysis in tissue slices (Berridge *et al.,* 1982; Hirasawa and Nishizuka, 1985). Lithium stimulated calcium uptake and superoxide generation, as shown in Table 2 and Table 3. Thus, the results indicate that lithium may act at the process of phospholipid turnover at the plasma membrane. It also propose a possibility that autonomic antagonists may affect on PMN leukocyte function through their action on the phospholipid turnover. Some adrenergic β-blockers may inhibit calcium mobilization or sodium influx in PMN leukocyte by their influence on the action sites other than the adrenergic β-receptors (Taniguchi and Takanaka, 1984). Accordingly, inhibitory effect of propranolol on superoxide generation is probably associated with the inhibition of calcium influx but not the change of cAMP level.

Superoxide generating activity of NADPH oxidase is remarkably inhibited by EGTA (Lee *et al.,* 1987). Therefore, it is suggested that calcium is necessary for the NADPH oxidase activity. Calcium appears to activate the activator of NADPH oxidase system and then stimulate superoxide generation through the oxidation of NADPH by NADPH oxidase actvation. Either activation by carbachol or inactivation by atropine of NADPH oxidase (Table 4) indicates that inhibitory action of autonomic antagonists on superoxide generation is partially ascribed to their direct action on

the NADPH oxidase system and the existence of cholinergic and adrenergic receptors in NADPH oxidase system is assumed.

Suppression of phagocytosis of PMN leukocyte for opsonized zymosan by chlorpromazine and dantrolene (Table 5) suggests that phagocytic activity of activated PMN leukocyte can be affected by both the calcium influx and the intracellular calcium redistribution. Thus, inhibitory effect of autonomic antagonist on phagocytosis is probably associated with their action on calcium transport.

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=국문초록=

Atropine, Phentolamine과 Propranolol이 활성화된 다형핵 백혈구에서의 칼슘 흡수, O_2^- 생성 및 식작용에 미치는 효과

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세포질 내 칼슘 농도의 증가는 다형핵 백혈구의 산화성 대사를 자극하는 주요 인자로 여겨지고 있다. 활성화된 다형핵 백혈구로부터 lysosomal enzyme의 유리는 세포내 cyclic nucleotide농도에 따라 조절될 수 있지만 신경전달물질과 β-아드레날린 또는 무스카린성 수용체의 결합에 따른 그밖의 반응은 아직도 분명하지 않다. 덧붙여, α-아드레날린성 수용체의 증개에 의한 다형핵 백혈구의 기능은 알려져 있지 않다.

Atropine, phentolamine과 propranolol은 활성화된 다형핵 백혈구의 칼슘흡수, superoxide 생성, NADPH oxidase 활성도 그리고 식작용을 억제하였으며, 이에 반하여 carbachol과 isoproterenol은 활성화된 세포의 반응을 약간 더 자극하였다. Carbachol 또는 isoproterenol에 의하여 항진된 superoxide 생성은 각각 그들의 길항제인 atropine과 propranolol에 의하여 억제되었다. 활성화된 다형핵 백혈구의 반응은 chlorpromazine, verapamil과 dantrolene에 의하여 억제되었으나 lithium에 의하여 약간 항진되었다. 한편 chlorpromazine과 dibucaine은 NADPH oxidase 활성도에 영향을 주지 않았다. Atropine, phentolamine과 propranolol은 칼슘에 의존적인 식작용을 억제하였다.

이상의 결과로부터 atropine, phentolamine과 propranolol은 칼슘 유입을 억제하고 자율 신경계의 수용체와 연관이 있는 NADPH oxidase계에 직접 작용함으로써 활성화된 다형핵 백혈구로부터 superoxide 생성을 억제할 것으로 시사되었다.