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Effects of Amitriptyline and Imipramine on Superoxide Generation, Myeloperoxidase Release, Leukotriene B_4 Production and Calcium Mobilization in Human Neutrophils

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ABSTRACT

A number of tricyclic antidepressants appear to have inhibitory action on calmodulin. Although amitriptyline, imipramine and doxepine have been shown to inhibit calcium uptake, oxidative phosphorylation and ATPase activities, effects of amitriptyline, imipramine and doxepine on functional responses of human neutrophils have not been elucidated.

In this study, effects amitriptyline, imipramine and doxepine on superoxide and hydrogen peroxide generation, myeloperoxidase release, leukotriene B4 formation and intracellular calcium level were investigated.

Superoxide and hydrogen peroxide production in heat aggregated IgG-activated neutrophils were inhibited by amitriptyline, imipramine and doxepine. EDTA, EGTA, verapamil and bepredil inhibited heat aggregated IgG-induced superoxide production. Chlorpromazine, trifluoperazine, staurosporine and H-7 also inhibited it. PMA-induced superoxide production was inhibited by amitriptyline, imipramine, doxepine, chlorpromazine and H-7. Amitriptyline, imipramine, chlorpromazine and trifluoperazine inhibited the myeloperoxidase release by heat aggregated IgG. Productions of LTB_4 and 5-HETE in heat aggregated IgG-activated neutrophils were inhibited by amitriptyline, imipramine and doxepine. In neutrophils, elevation of intracellular calcium induced by heat aggregated IgG was inhibited by amitriptyline, imipramine, doxepine, chlorpromazine and EGTA, while verapamil slightly inhibited increase of intracellular calcium and H-7 did not inhibit it.

These results suggest that the inhibitory effect of amitriptyline, imipramine and doxepine on respiratory burst, myeloperoxidase release and LTB4 production in heat aggregated IgG-activated neutrophils appears to be ascribed to the inhibition of calcium mobilization, calmodulin and protein kinase C.

Key Words: Neutrophils, Heat aggregated IgG, Calmo-dulin, Amitriptyline, Imipramine

INTRODUCTION

Neutrophils constitute a first line of defence to microbial infection. Their bactericidal effects are accomplished by a combination of phagocytosis, release of degradative enzyme and oxygen radical forming process known as the respiratory burst (Newberger *et al.*, 1980; Fantone and Ward, 1982). Stimulation of neutrophils with receptor agonists, including (fMLP) leads to the activation of phospholipase C with the production of inositol 1,4,5-trisphosphate and

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1,2-diacylglycerol (Berridge and Irvine, 1984). Inositol 1,4,5-trisphoshate is released into the cytoplasm (Maclntyre and Pollock, 1983) and causes calcium release from the intracellular calcium storage sites (Berridge and Irvine, 1984). Diacylglycerol remains in the plasma membrane and causes the activation of protein kinase C (Nishizuka, 1984). These molecular changes may be involved in the activation of neutrophil responses, like respiratory burst and degraulation (Kramer *et al.*, 1984; Tauber, 1987).

Free calcium in the cytoplasmic space is known to act as an intracellular messenger in neutrophils. The binding of stimuli to receptors causes an increase of intracellular calcium concentration (Conrad and Rink 1986; Randriamampita and Trautmann, 1989). The rise in intracellular calcium is reported to due both to release of calcium from the intracellular stores and to influx from the extracellular medium (Barzaghi *et al.*, 1989; Randriamampita and Trautmann, 1989). The calcium influx is known to be accompained by a simultaneously activated calcium extrusion. Calcium extrusion may be stimulated by either directly the rise of intracellular calcium or indirectly via calmodulin (Niggli *et al.*, 1979; Lew and Stossel, 1980).

Calmodulin is an intracellular calcium modulated protein. The multifunctional (calcium-calmodulin)- dependent protein kinase permits calcium to affect a large number of target enzymes within cell. The role of the (calcium-calmodulin)-dependent protein kinase in transmitting the effects of calcium is similar to that of the cyclic AMP-dependent protein kinases in mediating the effects of cyclic AMP (Barritt, 1992). Several reports indicate a possible involvement of calmodulin in the phagocytic process. It has been shown that calmodulin appears to affect Ca^{2+}-ATPase activity of phagocytic vesicles (Lew and Stossel, 1980), and the inhibitory effects of phenothiazines and local anesthetics on the cellular superoxide release and particulate NADPH-dependent superoxide forming activity are reported (Cohen *et al.*, 1980).

Tricyclic antidepressants, such as imipramine, amitriptyline and doxepine have been shown to inhibit synaptosomal Na^+-K^+ ATPase, Ca^{2+}-Mg^{2+} ATPase and Na^+-Ca^{2+} ATPase (Shin *et al.*, 1986). It was also observed that amitriptyline, imipramine and doxepine inhibit calcium uptake at presynaptic terminals by blockade of calcium channel at probably chlorpromazine sensitive sites (Chung *et al.*, 1988). Phenothiazines and other antipsychotic drugs with a related structure, smooth-muscle relaxants, α-adrenergic antagonists and a number of antidepressants are considered as an inhibitors calcium-calmodulin complex (Barritt, 1992). Although amitriptyline, imipramine, and doxepine have been shown to inhibit calcium uptake, oxidative phosphorylation and ATPase activities, their effects on neutrophil responses have not been elucidated. Thus, effects of amitriptyline, imipramine and doxepine on superoxide and hydrogen peroxide generation, myeloperoxidase release, leukotriene B_4 production and intracellular calcium level in neutrophils were investigated.

MATERIALS AND METHODS

Chemicals

Amitriptyline, imipramine, human IgG, phorbol 12-myristate 13-acetate (PMA), ethyleneglycol-bis (β-aminoethylester), N,N,N',N'-tetraacetic acid (EGTA), verapamil, bepredil, chlorpromazine(CPZ), trifluoperazine staurosporine, 1-(5-isoquinolinysulfonyl)-2-methyl-piper azine dihydrochloride(H-7), ferricytochrome c, scopoletin, o-dianisidine, leukotriene B_4 (LTB_4), hydroxyeicosatetraenoic acid (HETE), quin 2/ AM, dextran (M.W. 465,000), Hank's balanced salt solution(HBSS), Tris were purchased from Sigma Chemical Co.. Doxepine was obtained from Pfizer laboratories; $CaCl_2$ and NaCl from Kanto Chemical Co.; KCl from E Merk. Other chemicals were of analytical reagent grade.

Preparation of human neutrophils

Neutrophils were prepared from fresh whole human blood, anticoagulated with 10% acid-citrate-dextrose, by dextran sedimentation, hypotonic lysis of erythrocytes and ficoll-hypaque density centrifugation (Markert *et al.*, 1984). The neutrophils were suspended in Dulbecco's phos-

phate-buffered saline at a concentration of 1×10^7/ml. Final suspensions of neutrophils were comprised of about 97% neutrophils as judged by Wright-Giemsa stain and viability was more than 98 % as judged by trypan blue dye exclusion.

Assay of superoxide radical generation

The superoxide dependent reduction of ferricytochrome c was measured by the method of Markert *et al.* (1984). The reaction mixtures in plastic tubes contained 10^6 neutrophilis, 0.5 mg heat aggregated IgG, (or 5 ng PMA) 75 μM ferricytochrome c, 20 mM HEPES-tris and HBSS, pH 7.4 in a total volume of 1 ml. The reactions were performed in a 37℃ shaking water bath for 10 or 15 min. The reaction was 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 DU-70 Beckman spectrophometer. The amount of reduced cytochrome c was calculated by using an extinction coeffcient of 2.1×10^4 $M^{-1}cm^{-1}$ at 550 nm.

Assay of hydrogen peroxide

Hydrogen peroxide produced from activated neutrophils was measured by change of scopoletin fluorescence. The reaction mixtures contained 4×10^6 neutrophils, heat aggregated IgG, 2.5 μM scopoletin, 10 μM sodium azide, 5 μg/ml horseradish peroxidase, and HBSS buffer in a total volume of 1 ml. After 5 min of pre-incubation at 37℃ with compounds, the reaction was initiated by the addition of 0.5 mg/ml heat aggregated IgG. The decrease of scopoletin fluorescence by H_2O_2 produced was read at the wavelength of excitation, 343 nm and emission, 460 nm (Root *et al.*, 1975).

Assay of myeloperoxidase release

A 5×10^6/ml neutrophils in HBSS buffer with or without inhibitors were stimulated by adding heat aggregated IgG at 37℃. After 15 min of incubation, 250 μl of 0.2 M phosphate buffer, pH 6.2 and 250 μl of an equal mixture of 3.9 mM O-dianisidine HCl and 15 mM H_2O_2 were added. After 10 min of reincubation, the reaction was stopped by the addition of 250 μl of 1 % sodium azide. The absorbance was read at 450 nm (Spangrude *et al.*, 1985).

Measurement of activity of 5-lipoxygenase

One ml of neutrophils (10^7/ml) in HBSS were incubated with or without amitriptyline, imipramine and doxepine for 10 min at 37℃. The reation was initiated by the addition of 2.5 mg/ml heat aggregated human IgG. After 10 min of incubation, leukotriene synthesis was stopped by the addition of 250 μl of 100 mM citric acid (which lowered the pH to less than 3). Seventy nanograms of 15-HETE was added as internal standard. The leukotrienes and hydroxy acids were extracted into chloroform by the addition of 2.5 ml of 70:30 chloroform: methanol to the acidified cell suspension. The mixture was shaking for 10 min on the shaker and centrifuged at 800 g for 10 min. The bottom chloroform layer was taken and evaporated to dryness with N_2 gas. The extract was reconstituted in 100 μl of methanol and then was centrifuged for the injection into the high performance liquid chromatography.

The HPLC system was composed of Waters U6K injector, 501 pump, automated gradient controller, 484 turnable absorbance detector, and 745B integrator. The column was a C_{18} μ Bondapak. The mobile phase consisted of a 72:28:0.02 mixture of methanol: water: acetic acid with the pH 5.6 with ammonium hydroxide. Ten μl of sample was injected, and the flow rate was 1.5 ml/min. Under these conditons, the LTB_4 and 5-HETE which eluted from column was quantified. LTB_4 eluted was detected at 270 nm, and then the 484 turnable aborbance detector was switched to 234 nm for the detection of 15-HETE. LTB_4 and 5-HETE were quantified by the ratio of component peak height of 15-HETE. These ratios were then compared with ratios obtained from 4 point standard curve (0 ~100 ng for LTB4) (Betts *et al.*, 1990).

Measurement of cytosolic free calcium

Quin 2 loading and fluorescence measurement was performed by the modification of the

method of Tsien *et al.* (1982). Neutrophils (approximately 5×10^7/ml) were loaded with 5 μl of 20 mM quin2/AM at 37℃ for 20 min and 1.0 ml of the reaction mixtures containing 135 mM NaCl, 5 mM KCl, 1 mM $MgCl_2$, 1 mM $CaCl_2$, and 5 mM dextrose. The suspension was then diluted to 10 fold with the above reation mixture and further incubated at 37℃ for 40 min. After loading, the suspension was centrifuged at 1,500 g for 5 min and neutrophils were resuspended in the above reaction mixture as approximately 10^8 cells/100 μl.

Fluorescence measurement was done with a Turner Spectrofluorometer (Model 430). Preloaded neutrophils (5×10^7) were suspended in the same reaction mixture in a final volume of 2.0 ml. After preincubation at 37℃ for 5 min, the reaction was initated by the addition of 0.5 mg/ml heat aggregated IgG. The fluorescence change was read at an excitation wavelengh of 339 nm and emission wavelengh of 492 nm.

RESULTS

Inhibitory effects of amitriptyline and imipramine on superoxide generation in activated neutrophils by heat aggregated IgG

Superoxide generation in human neutrophils was stimulated by heat aggregated IgG, and PMA. As shown in Fig. 1, amitriptyline, imipramine and doxepine inhibited heat aggregated IgG-induced superoxide generation. Amount of superoxide generation in neutrophils activated by 0.5 mg/ml heat agregated IgG was 13.27 ± 0.61 nmole/15 min/10^6 cells (n=6). Amitriptyline, imipramine and doxepine at the con-

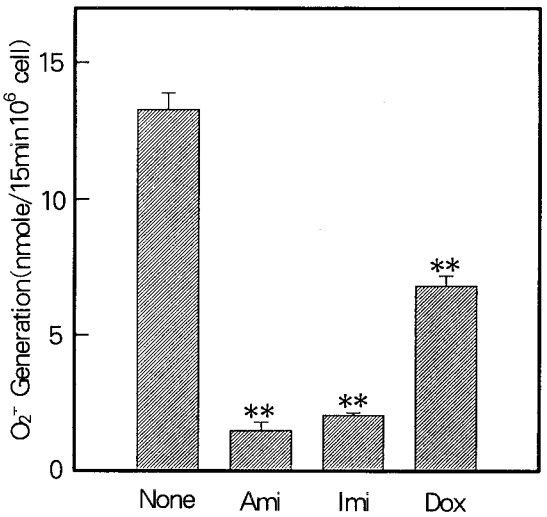

Fig. 1. Inhibition of superoxide generation in heat aggregated IgG-activated neutrophils by amitriptyline, imipramine and doxepine. Neutrophils (10^6 cells/ml) were preincubated with compounds in HBSS buffer for 5 min and then the response was initiated by the addition of 0.5 mg/ml heat aggregated IgG. Data represent mean±S.D., of 5-6 experiments. None, no addition; Ami, amitriptyline; Imi, imipramine; Dox, doxepine of 100 μM with heat aggregated IgG. *p<0.05; **p<0.01 by Student's *t*-test.

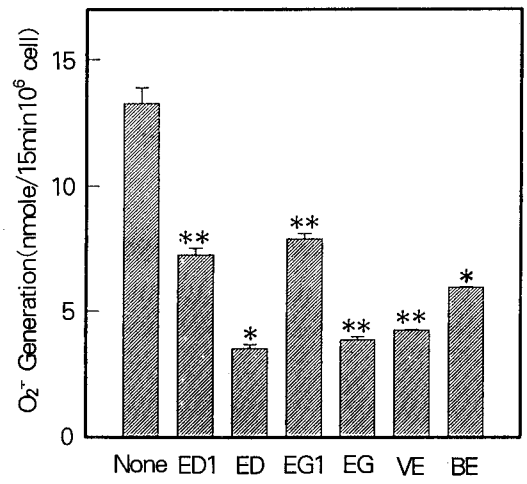

Fig. 2. Inhibitory effects of EDTA, EGTA, verapamil and bepredil on superoxide generation in heat aggregated IgG-activated neutrophils. Neutrophils (10^6 cells/ml) were princubated with compounds for 5 min and then the response was initiated by adding 0.5 mg/ml heat aggregated IgG. Data represent mean±S.D. of 5-6 experiments. None, no addition; ED1, 1 mM EDTA, ED, 10 mM EDTA; EG1, 1 mM EGTA; EG 10 mM EGTA; VE, 1 mM verapamil; BE, 0.1 mM bepredil. *p<0.05; **p<0.01 by Student's *t*-test.

centration of 100 μM inhibited superoxide generation 88.7%, 84.5% and 48.7%, respectively(Fig. 1). Effects of calcium chelators and calcium channel blockers on superoxide generation in activated neutrophils were examined. Fig. 2 shows that EDTA, EGTA, verapamil and bepredil inhibited heat aggregated IgG-induced superoxide generation. Both inhibitors of protein kinase C, 100 μM H-7 and 100 μM staurosporine and calmodulin inhibitors, 50 μM chlorpromazine and 100 μM trifluoperazine inhibited heat aggregated IgG-induced superoxide generation (Fig. 3). A tumor promotor, PMA is known as the activator of protein kinase C (Arai *et al.*, 1993). As can be seen in table 1, superoxide generation stimulated by 5 ng/ml PMA was inhibited by amitriptyline, imipramine, doxepine, H-7 and chlorpromazine of 100 μM. Sodium fluoride is reported to activate G-protein, linked with receptors of the plasma membrane. Sodium fluoride-induced superoxide generation was inhibited by 100 μM imipramine (data not shown).

Table 1. Inhibition of superoxide generation in PMA-activated neutrophils by amitriptyline, imipramine, doxepine, H-7 and chlorpromazine

Compounds		superoxide nmole/ 10 min/10^6 cells
PMA 5 ng/ml		53.43±0.98
+Amitriptyline	100 μM	30.91±0.72
+Imipramine	100 μM	25.87±0.84
+Doxepine	100 μM	40.98±0.94
+H-7	100 μM	21.50±0.53
+Chlorpromazine	100 μM	13.27±0.32

Neutrophils (10^6 cells/ml) were preincubated with compounds in HBSS buffer for 5 min and then the response was initiated by the addition of PMA. Values are mean±S.D. of 4-9 experiments.

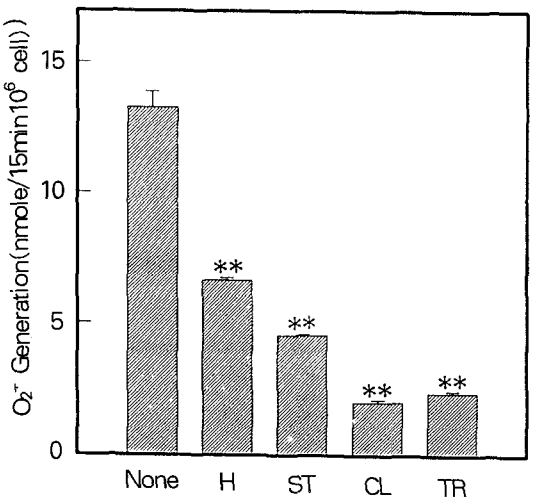

Fig. 3. Inhibitory effects of H-7, staurosporine, chlorpromazine and trifluoperazine on superoxide generation by heat aggregated IgG. Neutrophils (10^6 cells/ml) were preincubated with compounds for 5 min and then the response was initiated by addition of 0.5 mg/ml heat aggregated IgG. Data represent mean±S.D. of 5-6 experiments. None, no addition; H, 0.1 mM H-7; ST, 0.1 mM staurosporine; CL, 50 μM chlorpromazine; TR, 100 μM trifluoperazine, $^*p < 0.05$; $^{**}p<0.01$ by Student's *t*-test.

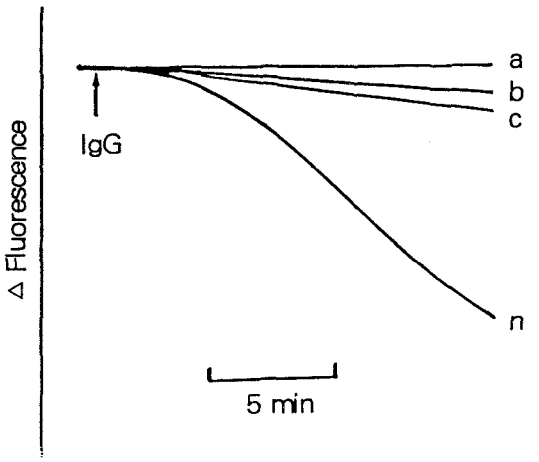

Fig. 4. Inhibitory effects of amitriptyline, imipramine and doxepine on hydrogen peroxide generation by heat aggregated IgG. Neutrophils (2×10^6 cells/ml) were preincubated with compounds for 5 min and then the response was initiated by adding of 0.5 mg/ml heat aggreagted IgG. Fluorescence change of scopoletin was fluorometrically measured at the wavelength pair 343-460 nm. n, no addition; a, amitriptyline; b, imipramine; c, doxepine of 100 μM with heat aggregated IgG.

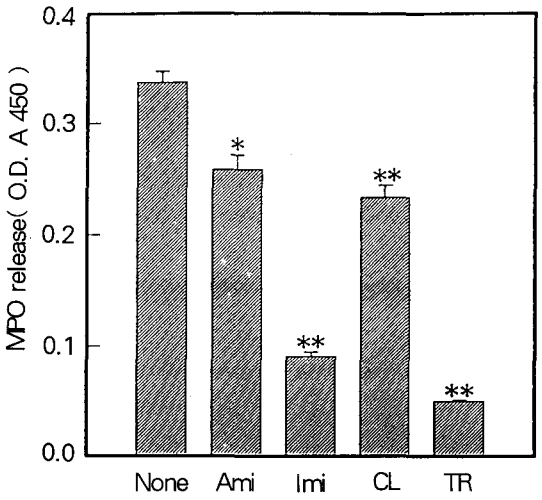

Fig. 5. Inhibitory effects of amitriptyline, imipramine, chlorpromazine and trifluoperazine on myeloperoxidase release by heat aggregated IgG. Neutrophils (5×10^6 cells/ml) were preincubated with compounds in HBSS buffer for 5 min and then the response with initiated by addition of 0.5 mg/ml heat aggregated IgG. Data represent mean±S.D. of 5-6 experiments. None, no addition; Ami, amitriptyline; Imi, imipramine; CL, chlorpromazine; TR, trifluoperazine of 100 μM. *$p<0.05$; **$p<0.01$ by Student's *t*-test.

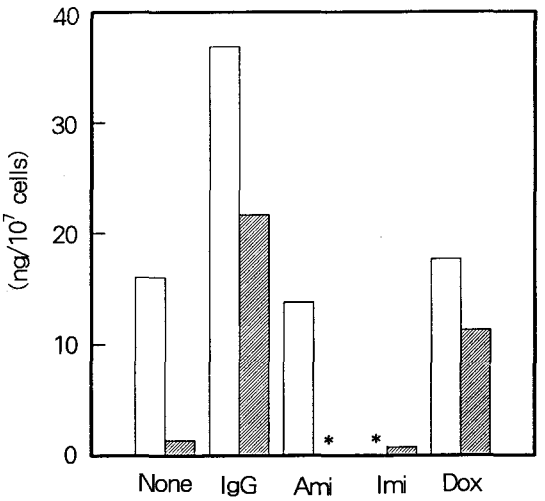

Fig. 6. Effects of amitriptyline, imipramine and doxepine on heat aggregated IgG-induced production of leukotriene and 5-HETE. Neutrophils(10^7) were preincubated with compounds for 10 min and subsequently triggered with 2.5 mg/ml heat aggregated IgG for 10 min. Incubation conditions and RP-HPLC were performed as described in Materials and Methods. The means from 3 determinations were plotted. □, LTB_4; ▨, 5-HETE; *, None detected.

Inhibition of hydrogen peroxide generation in heat aggregated IgG-activated neutrophils by amitriptyline and imipramine

Hydrogen peroxide (H_2O_2) produced, which is attained from dismutation of superoxide anion (Park *et al.*, 1987), was measured by the decrease of scopoletin fluorescence. Heat aggregated IgG induced the oxidation of scopoletin. Hydrogen peroxide generation in activated neutrophils by heat aggregated IgG was inhibited by amitriptyline, imipramine and doxepine of 100 μM (Fig. 4).

Effects of amitriptyline and chlorpromazine on myeloperoxidase release from neutrophils activated by heat aggregated IgG

Secretion of lysosomal enzyme from neutrophils was assayed by measuring myeloperoxidase release. Effects of calmodulin inhibitors on the release of lysosomal enzyme were examined. As can be seen in Fig. 5, heat aggregated IgG induced myeloperoxide release was inhibited by amitriptyline, imipramine, trifluoperazine and chlorpromazine of 100 μM.

Effects of amitriptyline and imipramine on LTB_4 and 5-HETE formation in neutrophils activated by heat aggregated IgG

In human neutrophils, the activation of 5-lipoxygenase pathway in leukotriene synthesis leads mainly to the production of the chemoattractant leukotriene B_4 (LTB_4) and the 5-hydroxyeicosatetraenoic acid (5-HETE) (Betts *et al.*, 1990). LTB_4 and 5-HETE were eluted from the column at approximately 7.88 min of postinjection and 21.95 min, respectively. 15-

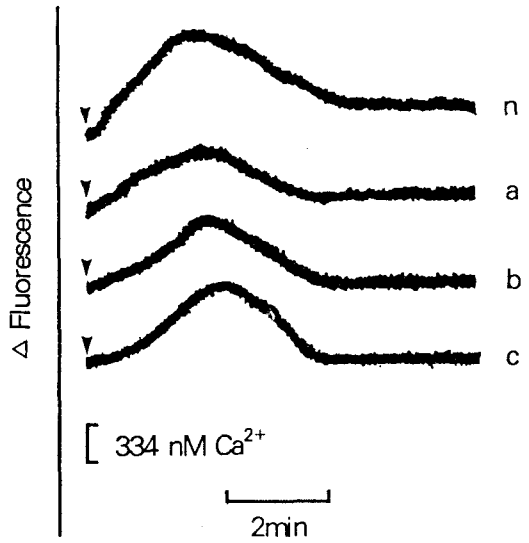

Fig. 7. Inhibitory effects of amitriptyline, imipramine and doxepine on enhanced cytosolic calcium level by heat aggregated IgG in neutrophils. Intracellualr free calcium level in heat aggregated IgG-stimulated neutrophils (5×10^6 cells/ml) was measured as a fluorescence change of quin 2, a specific calcium chelator. The response was initiated by the addition of 0.5 mg /ml heat aggregated IgG in the presence of compounds. Fluorescence of quin 2-calcium complex was read at the wavelength pair 339-492 nm. n, no addition; a, amitriptyline; b, imipramine; c, doxepine of 100 μM

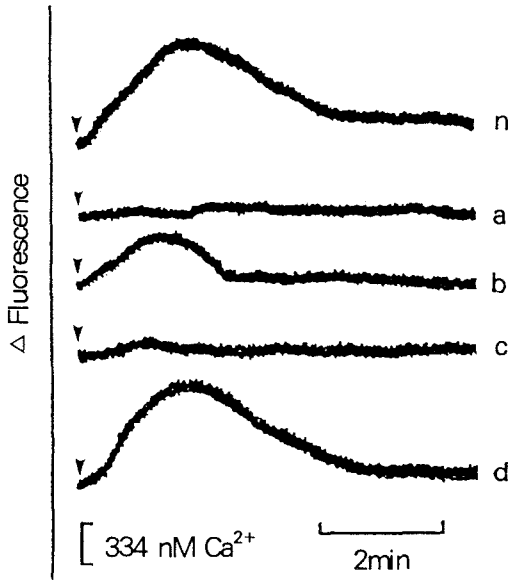

Fig. 8. Effects of EGTA, verapamil, chlorpromazine, H-7 on enhanced cytosolic calcium level by heat aggregated IgG. Neutrophils (5×10^6 cells/ml) were preincubated with compounds and then the response was initiated by adding of 0.5 mg/ml heat aggregated IgG. n, no addition; a, 5 mM EGTA; b, 100 μM verapamil; c, 100 μM chlorpromazine; d, 100 μM H-7

Hydroxyeicosatetraenoic acid (15-HETE) was eluted at 17.28 min. Recovery rate of LTB_4 and 5-HETE was 70% and 68%. Amounts of 5-HETE and LTB_4 in resting neutrophils were 1.39 ng/10^7 cells and 16.07 ng/10^7cells. The production of LTB_4 in neutrophils activated by 2.5 mg/ml heat aggregated IgG was increased to 36.84 ng/10^7 cells and 5-HETE was increased to 21.67 ng/10^7 cells. Fig. 6 shows that amitriptyline, imipramine and doxepine of 100 μM inhibited the production of LTB_4 by 62.5%, 100% and 52 % and 5-HETE by 100%, 96.4% and 47.8%, respectively.

Effects of amitriptyline and imipramine on heat aggregated IgG-induced calcium mobilization

Elevation of intracellular calcium is a early event in the response of phagocytic cells to many agonist, including fMLP (Andersson *et al.*, 1986; Lee *et al.*, 1987; Shimizu *et al.*, 1992). To examine the mechanism of calcium mobilization in heat aggregated IgG-activated neutrophils, effects of amitriptyline, imipramine, doxepine, calcium chelator, calcium channel blocker, calmodulin inhibitor and protein kinase C inhibitor on the elevation of cytosolic calcium level in activated neutophils were investigated.

Intracellular calcium level was measured with quin 2/AM. When neutrophils were exposed to heat aggregated IgG, fluorescence change due to the complex formation of an increased calcium with quin 2 occured, and at 1.5 min of

addition, fluorescence was reached to a peak level (Fig. 7). The increase of intracellular calcium level elicited by 0.5 mg/ml heat aggregated IgG was inhibited by amitriptyline, imipramine and doxepine of 100 μM (Fig. 7). Five mM EGTA, 100 μM chloropromazine and 100 μM verapamil inhibited elevation of intracellular calcium, whereas H-7 did not inhibit it (Fig. 8).

DISCUSSION

Superoxide production in activated neutrophils is due to the activation of NADPH oxidase in the plasma membrane (Horn and Lehrer, 1975). The superoxide-generating enzyme system constitutes a cyanide-insensitive pyridine nucleotide oxidase that utilizes NADPH as the electron donor (Sha'afi *et al.*, 1983; Korchak *et al.*, 1984). In the resting neutrophil, the NADPH oxidase system exists in an inactive state (Tauber, 1987). Exposure of the cell to stimuli, such as phorbol esters and immune complexes results in the activation of the oxidase.

Calcium may be prerequisite for the activation of NADPH oxidase activity, because in calcium free medium the stimulatory effect of heat aggregated IgG on NADPH oxidase, which is obtained from resting neutrophils, was not detected (data not shwon). In addition, heat aggregated IgG-stimulated superoxide generation was inhibited by EDTA, EGTA, verapamil and bepredil. Thus, these findings indicate that intracellular calcium may be involved in the activation of respiratory burst in neutrophils activated by heat aggregated IgG. Since PMA and 1,2-diacylglycerol stimulate superoxide generation, it is suggested that protein kinase C is involved in the activation of superoxide-forming NADPH oxidase (Castagna *et al.*, 1982). Superoxide generation in neutrophils activated by heat aggregated IgG was inhibited by H-7, staurosporine and chlorpromazine. Protein kinase C and calmodulin appear to participate in the activation of neutrophil responses by heat aggregated IgG. Superoxide and hydrogen peroxide generation in neutrophil activated by heat aggregated IgG was inhibited by amitriptyline, imipramine and doxepine. Amitriptyline and imipramine 100 μM showed a similar inhibitory effect with 50 μM chlorpromazine and 100 μM trifluoperazine, effective calmodulin inhibitors on superoxide generation. Activation of NADPH oxidase appears to be regulated by protein kinase C and calcium-calmodulin complex. Sodium fluoride, a G-protein activator, induced superoxide generation and this was inhibited by 100 μM imipramine (data not shown). The result indicates that imipramine may have inhibitory effect on G proteins which are insensitive to pertussis toxin. C5a, fMLP and PAF stimulate phospholipase C (Naccache *et al.*, 1985) which is linked with a pertussis toxin sensitive G protein (Hwang, 1988; Siciliano *et al.*, 1990) and promote the translocation of protein kinase C from the cytosol to the membrane (O'Flaherty *et al.*, 1990; Babior, 1992). Protein kinase C is considered to be involved in the activation process of neutrophils (Sha'afi *et al.*, 1983; Korchak *et al.*, 1984). PMA-stimulated superoxide generation was inhibited by H-7, chlorpromazine, trifluoperazine, amitriptyline, imipramine and doxepine. Amitriptyline, imipramine and doxepine may inhibit the respiratory burst by their inhibitory action on protein kinase C. LTB_4 is a potent proinflammatory mediator which induces chemotaxis, chemokinesis, diapedesis, aggregation and degranulation of neutrophils (Ford-Hutchinson *et al.*, 1980; Goetzl and Pickett, 1980; Pamblad *et al.*, 1981; Rollins *et al.*, 1983), thereby potentially amplifying inflammatory reactions. In neutrophils, arachidonate is converted both to 5-hydroxyperoxyeicosa- tetraenoic acid and to leukotriene A_4 (LTA_4) by the action of 5-lipoxygenase (Rouzer *et al.*, 1986), and then LTB_4 is formed from LTA_4 by LTA_4 5-hydrolase. Calcium ion is known to necessary for the activation of 5-lipoxygenase, and ATP further enhances this stimulation (Rouzer and Samuelsson, 1985; Hogaboom *et al.*, 1986). Calcium and ATP appears to promote the translocation of 5-lipoxygenase to the plasma membrane during the activation of neutrophils. (Dixon *et al.*, 1990) Productions of 5-HETE and LTB_4 in neutrophils stimulated by heat aggregated IgG were markedly inhibited by amitriptyline, imipramine and doxepine. Inhibitory effects of

amitriptyline, imipramine and doxepine on LTB_4 production are probably associated with the inhibition of intracellular calcium mobilization and the inhibition of protein kinase C-dependent phosphorylation of the 5-lipoxygenase.

A rise in the cytosolic calcium level is considered to be an important role in the stimulation of neutrophil responses. Increased intracellular free calcium ion may be involved in the activation of neutrophil responses, including degranulation and superoxide generation due to surface stimulation by both particulate and soluble agents (Smolen *et al.*, 1981). The rise in intracellular calcium is due to both Ca^{2+} from intracellular store and influx from the extracellular medium (Randriamampita and Trautmann, 1989). On the other hand, calcium influx is known to be accompained by a simultaneously activated calcium extrusion which may be accomplished by Ca^{2+}-ATPase and Na^{+}-Ca^{2+} exchanger (Niggli *et al.*, 1979; Randriamampita and Trautmann, 1990). Heat aggregated IgG-induced elevation of intracellular calcium was also inhibited by amitriptyline, imipramine, doxepine, EGTA, verapamil and chlorpromazine but not inhibited by H-7. These results indicate that calcium-calmodulin system may be involved in calcium mobilization in neutrophils activated by heat aggregated IgG, whereas in this response role of protein kinase C is uncertain. Inhibitory effects of amitriptyline, imipramine and doxepine on calcium mobilization appear to be ascribed to their inhibitory actions on calcium-calmodulin system.

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=국문초록=

Amitriptyline과 Imipramine이 호중구에서의 Superoxide 생성, Myeloperoxidase 유리, Leukotriene B_4 생성과 칼슘 동원에 나타내는 영향

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삼환계 항우울제들은 calmodulin 억제 작용을 갖고 있으며, 칼슘 유입, 산화성 인산화 반응및 ATPase 활성도를 억제하는 것으로 제시되고 있지만 사람 호중구에서의 기능 표현에 대한 효과는 밝혀져 있지 않다.

본 연구에서는 amitriptyline, imipramine과 doxepine이 superoxide와 H_2O_2 생성, myeloperoxidase 유리, leukotriene B_4 생성과 세포내 칼슘의 상승에 나타나는 효과를 조사하였다.

변성된 IgG에 의하여 활성화된 호중구에서의 superoxide와 H_2O_2 생성은 amitriptyline, imipramine과 doxepine에 의하여 억제되었고 EDTA, EGTA, verapamil과 bepredil은 superoxide 생성을 억제하였다. Chlorpromazine, trifluoperazine, staurosporine 및 H-7 또한 superoxide 생성을 억제하였다. PMA에 의한 superoxide 생성은 amitriptyline, imipramine, doxepine, chlorpromazine과 H-7에 의하여 억제되었다. Amitriptyline, imipramine, chlorpromazine과 trifluoperazine은 변성된 IgG에 의한 myeloperoxidase 유리를 억제하였다. 변성된 IgG에 의하여 활성화된 호중구에서 LTB_4와 5-HETE 형성은 amitriptyline, imipramine과 doxepine에 의하여 억제되었다. 변성된 IgG에 의한 세포내 칼슘의 증가는 amitriptyline, imipramine, doxepine, chlorpromazine과 EGTA에 의하여 억제되었고, verapamil은 세포내 칼슘의 증가를 약간 억제하였나 H-7은 세포내 칼슘의 증가에 영향이 없었다.

이상의 결과로부터 변성된 IgG에 의하여 활성화된 호중구에서의 respiratory burst, myeloperoxidase 유리와 LTB_4 생성에 대한 amitriptyline, imipramine 과 doxepine 의 억제효과는 칼슘동원, calmodulin과 protein kinase C의 억제에 기인할 것으로 추정된다.