Dansylcadaverine and Rimantadine Inhibition of Phagocytosis, PAF-acether Release, and Phosphatidylcholine Synthesis in Human Polymorphonuclear Leukocytes

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Abstract: *The effect of dansylcadaverine, amantadine, and rimantadine on phagocytosis, release of PAF-acether, phospholipid methylation, and phosphatidylcholine formation by the cholinephosphotransferase pathway was assessed in human peripheral polymorphonuclear leukocytes. All of the drugs induced a dose-dependent and reversible inhibition in the uptake of complement-coated zymosan particles as well as a reduction in the release of PAF-acether. Simultaneously, a marked reduction in the formation of phosphatidylcholine was observed. This was also dose-dependent and reversible, and showed this order of potency: dansylcadaverine > rimantadine > amantadine. Dansylcadaverine reduced phospholipid methylation to a lesser extent than the cholinephosphotransferase pathway. These data show that drugs that block receptor-mediated endocytosis inhibit the response of the human polymorphonuclear cell and suggest that this action may be mediated by their actions on phospholipid metabolism.*

Key Words: Dansylcadaverine; Amantadine; Rimantadine; Phagocytosis; PAF-acether; Phospholipid methylation; Phosphatidylcholine formation; Polymorphonuclear leukocytes.

INTRODUCTION

Human polymorphonuclear leukocytes release platelet-activating factor (PAF-acether) when bound and internalize complement-coated zymosan particles (Lotner et al., 1980; Sanchez Crespo et al., 1980). This process is initiated by the occupancy of receptors on the cell surface and is reminiscent of the process of receptor-mediated endocytosis which occurs in a variety of cellular species, although some differences between these phenomena might be depicted (Pastan and Willingham, 1981).

Recent reports have shown that receptor-mediated endocytosis can be prevented by prior treatment of cells with compounds such as dansylcadaverine, amantadine, and rimantadine (Schlegel et al., 1982). Some of these drugs are inhibitors of the enzyme transglutaminase (Davies

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et al., 1980), but most recent reports suggest that the pharmacological actions of these compounds may be explained on the basis of their actions on phospholipid metabolism (Mato et al., 1983).

In fact, these drugs have been found to block phospholipid methylation and phosphatidylcholine formation through a phosphocholine transferase reaction, and to enhance the turnover of phosphatidylinositol. These changes in phospholipid metabolism are important because during the process of normal activation of the PMN there are important changes in the metabolic rate of the above mentioned process.

In this paper we have studied the effect of dansylcadaverine, amantadine, and rimantadine on particle uptake and release of PAF-acether from human polymorphonuclear leukocytes and we have also analyzed the action of these compounds on some of the changes in phospholipid metabolism which occur during phagocytosis.

MATERIALS AND METHODS

Materials

(^{3}H-methyl)-choline chloride (59.8 Ci/mmol), (^{3}H-methyl)-methionine (15 mCi/mmol), and (^{3}H-5-Hydroxy)-triptamine (16.6 Ci/mmol) were from Amersham International, Lymphoprep was from Nygaard, Norway. Silica gel plates were from Merck, Germany. Dansylcadaverine was from Sigma; amantadine and rimantadine were a gift of Dr. I. Pastan, NIH, Bethesda, MD.

Dansylcadaverine was dissolved in dimethylsulfoxide. Amantadine and rimantadine were dissolved in ethanol. The final concentration of solvents was 0.5% and controls treated the same way were included in each experiment. Under the conditions of the PAF-acether assay employed, interference by the drugs was not observed.

Methods

Human polymorphonuclear leukocytes were isolated from venous blood by density centrifugation on Ficoll-Hypaque cushions (Boyum, 1968) as described (Sanchez Crespo et al., 1980). Assay and characterization of PAF and phagocytosis of complement-coated zymosan particles were performed as described (Alonso et al., 1982). Incorporation of radioactive precursors into phospholipids was carried out as previously described by incubating the cells in the presence of (^{3}H-methyl)-choline (2 μCi/ml) (Garcia Gil et al., 1982) or (^{3}H-methyl)-methionine (10 μCi/ml) (Garcia Gil et al., 1982). Briefly, polymorphonuclear cells at a density of 10^7 cells per milliliter were incubated with the label for 30 min at 37°C and at the end of this period were washed three times. The cell suspension was then incubated in a HEPES-buffered medium in the presence or absence of drugs, before the addition of zymosan particles. After 30 min, samples containing 4×10^6 cells were taken, pipetted into precooled tubes, and centrifuged 5 min at 400 g at 4°C. Phospholipids were extracted from the pellet with 2.9 ml of methanol/water (2/1; v/v) and phase formation was achieved by adding chloroform and water to reach a final composition of chloroform/methanol/water (1/1/0.9; v/v). The chloroform phase was collected, and the upper phase was treated three times with 2 ml of methanol/chloroform (1 : 1, v/v) to achieve complete extraction of phospholipids. The pooled organic fraction was dried under a stream of N_2, and the amount of phosphatidylcholine and methylated phospholipids was quantitated by scintillation spectrometry after separation by thin-layer chromatography on silica gel 60 plates.

Abbreviations. HEPES 4: (2-hydroxyethyl)-1 piperazine ethanesulfonic acid; PAF-acether: platelet-activating factor; PMNs: polymorphonuclear leukocytes.

RESULTS

Effect of Dansylcadaverine, Amantadine, and Rimantadine on Particle Uptake

All of the drugs induced a dose-dependent inhibition of particle uptake at concentrations from 10^{-3} to 10^{-5}M (Figure 1). This inhibition could be reverted by washing, except when 10^{-3} M dansylcadaverine was employed. Under these last conditions, irreversible cell aggregation was

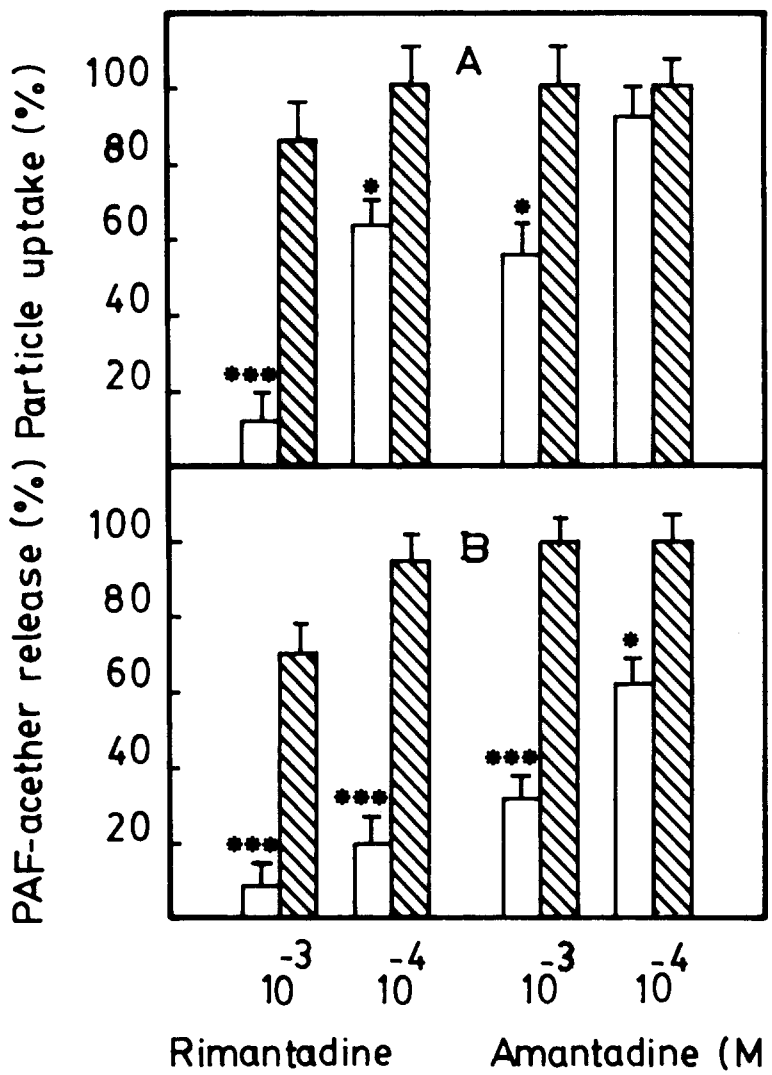

Figure 1 *Effect of amantadine and rimantadine on phagocytosis (A) and PAF-acether release (B). Polymorphonuclears were preincubated with the drugs and then washed with buffer alone (striped columns) or with buffer and drugs (white columns). After resuspension in the same medium employed for washing, they were stimulated as described under Materials and Methods and particle uptake and PAF-acether release were assessed. Results are expressed as percentage of values obtained in controls preincubated in the absence of drugs and treated the same way. Results represent M ± SD of three experiments. (* $p < 0.05$; ** $p < 0.025$; *** $p < 0.005$).*

found and it is likely that adequate washing could not occur. As shown in Figure 1 on molar basis, the order of potency is : dansylcadaverine > rimantadine > amantadine.

In the presence of these drugs, a diminution in the release of PAF-acether was also observed; this was also dose-dependent, reversible, and apparently more sensitive to the blockade than particle uptake. In fact, 10^{-4}M and 10^{-5} dansylcadaverine and 10^{-4}M amantadine induced significant reduction on PAF-acether release without affecting phagocytosis significantly (Figures 1 and 2).

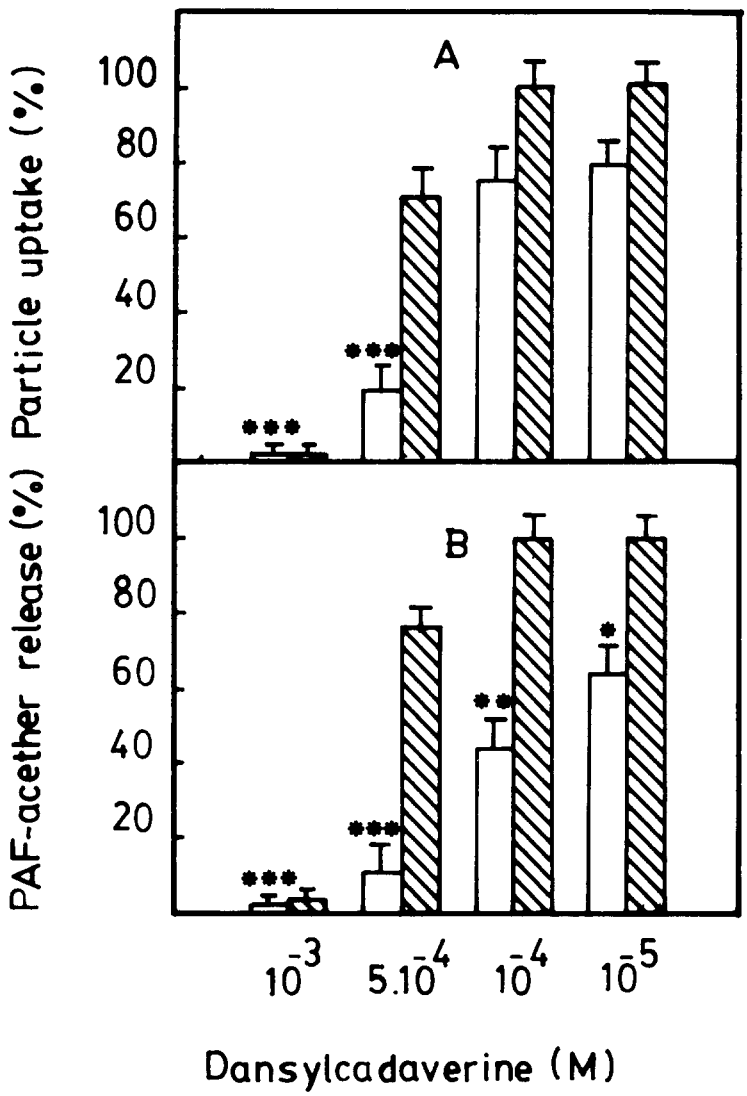

Figure 2 *Effect of dansylcadaverine on phagocytosis (A) and PAF-acether release (B). Experimental conditions in Figure 1. Results represent M ± SD of three experiments. (* $p < 0.05$; ** $p < 0.025$; *** $p < 0.005$).

Effect of Dansylcadaverine, Amantadine, and Rimantadine on Phospholipid Methylation and Phosphatidylcholine Formation by Cholinephosphotransferase Pathway

Preincubationn of cells with dansylcadaverine induced a dose-dependent inhibition of the formation of phosphatidylcholine by the phosphocholinetransferase pathway, measured by the incorporation of (^{3}H-methyl)-choline into phosphatidylcholine (Figure 3). This inhibition reached 78 ± 16% of control values for a concentration of dansylcadaverine of 10^{-3}M. As mentioned above, at lower concentrations, the inhibition could be reverted by washing of the cells (Figure 3). Rimantadine and amantadine also induced a reduction in phosphatidylcholine formation, but to a lesser extent than dansylcadaverine.

As regards the formation of methylated phospholipids by a methyltransferase, only dansylcadaverine was found to be inhibitory in the range of concentrations employed, and the extent of inhibition observed was below that found in the cholinephosphotransferase reaction (Figure 4).

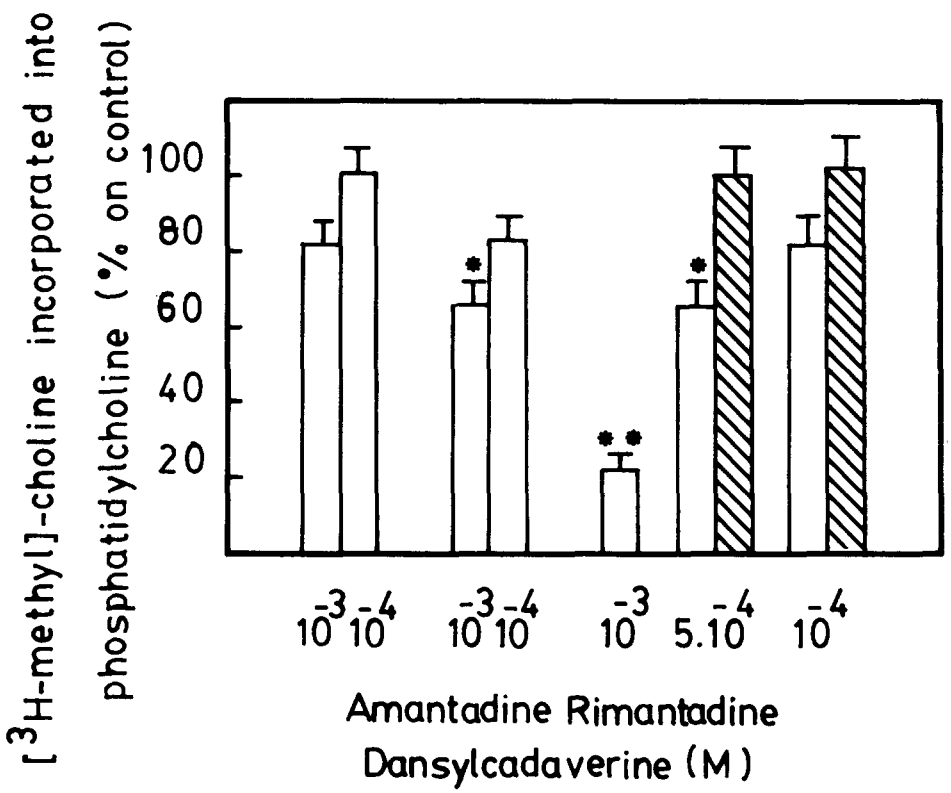

Figure 3 *Effect of dansylcadaverine, amantadine, and rimantadine on phosphatidylcholine formation. Cells were preincubated with 2 μCi/ml (^{3}H-methyl)-choline for 30 min at 37°C. At the end of this period they were washed three times; after washing, the cell suspension was incubated in medium or in medium plus drugs for 15 min at 37°C before the addition of 2 mg complement-coated zymosan particles. In the experiments performed with dansylcadaverine, a new washing was performed with medium (striped columns) or with medium plus dansylcadaverine (white columns) before the addition of zymosan particles, in order to assess the reversibility of the action. Results represent M ± SD of three experiments with amantadine and rimantadine and two experiments with dansylcadaverine (* $p < 0.05$; ** $p < 0.025$).*

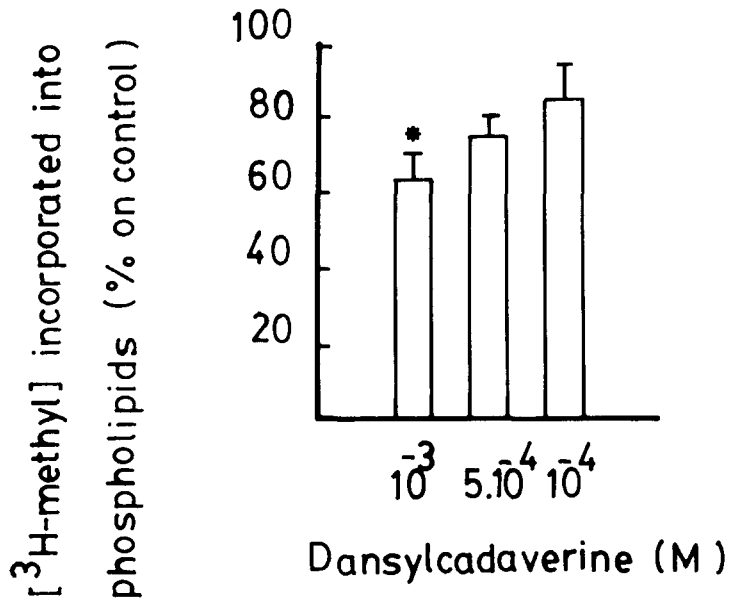

Figure 4 *Effect of dansylcadaverine on phospholipid methylation. Polymorphonuclears at a concentration of 10^7 per ml were incubated with (^{3}H-methyl)-methionine as described in Materials and Methods. Thirty minutes after the addition of 2 mg of complement-coated zymosan particles and samples were taken. The phospholipids were extracted, dried under N_2, and the methylated phospholipids were quantitated. Results are expressed as percent of controls incubated in the absence of dansylcadaverine. Data represent M ± SD of three experiments; 100% corresponds to 15800 d.p.m. (* $p < 0.05$).*

The pattern of phospholipid methylation was similar in the presence or absence of dansylcadaverine. As shown in Figure 5 in a typical chromatogram, 5.10^{-4} dansylcadaverine induced a reduction of 28% in the formation of phosphatidylcholine and of 37% in the formation of the monomethyl and dimethyl derivatives of phosphatidylethanolamine.

DISCUSSION

The data presented in this paper show that compounds having the ability to prevent internalization of protein ligands and viruses (Pastan and Willingham, 1981; Schlegel et al., 1982) in a number of cellular systems also possess the ability to block both phagocytosis of complement-coated zymosan particles and phagocytosis-associated release of the inflammatory mediator PAF-acether.

The pharmacological actions of these drugs have been related to their ability to block the intracellular enzyme transglutaminase (Davies et al., 1980). This enzyme catalyzes the cross-linking of proteins by forming an isopeptide bond between a lysine residue of one protein and a glutamine residue of another, and it has been suggested that intracellular transglutaminase could cross-link the receptor to some intracellular protein. However, the most recent evidence (Mato et al., 1983) might suggest an alternative explanation, since these drugs alter the metabolism of membrane phospholipids. In fact, inhibition of the formation of phosphatidycholine through the methyltransferase pathway has been demonstrated during phagocytosis and PAF-acether release in the human polymorphonuclear leukocyte (Garcia Gil et al., 1981). Further, hydrolysis of

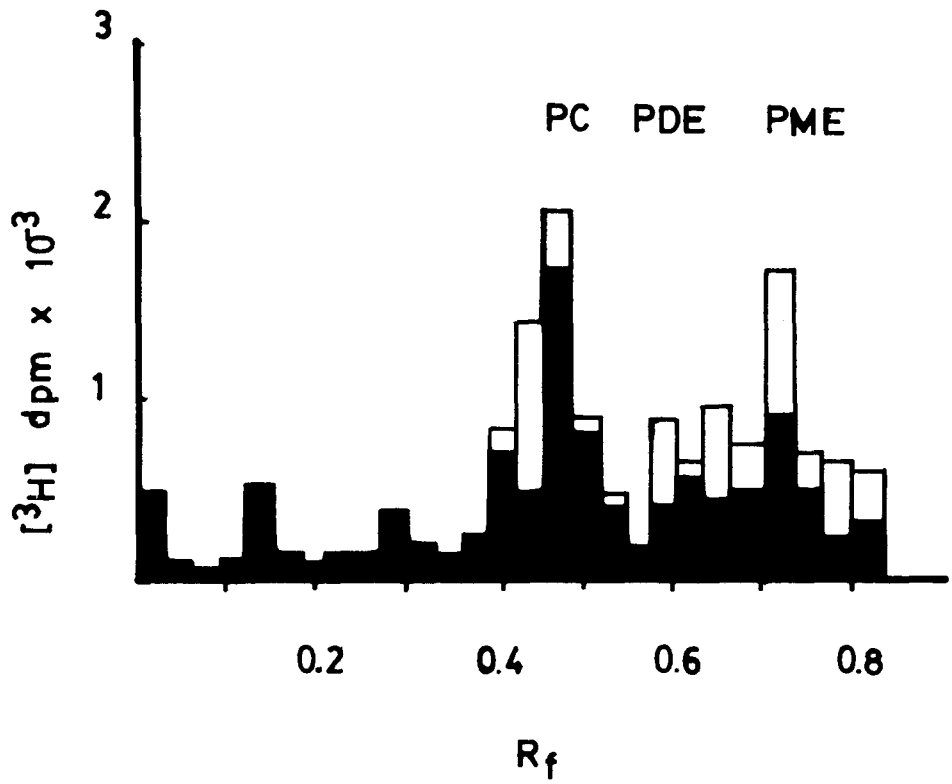

Figure 5 *Separation of methylated phospholipids from polymorphonuclear cells labeled with (^{3}H-methyl)-methionine as described in Materials and Methods. Cells were incubated for 30 min at 37°C in the presence of 5.10^4 M dansylcadaverine (black columns) or with buffer containing 0.5% dimethylsulfoxide (white columns). At the end of this period, samples were taken for phospholipid extraction and thin-layer chromatography on silica gel plates in the mixture of solvents propionic acid/propanol/chloroform/water (2 : 2 : 1 : 1; v/v). PC=phosphatidylcholine; PDE= phosphatidyl N, N dimethyl ethanolamine; PME=phosphatidyl N methyl ethanolamine; RF=retardation factor.*

phosphatidylinositol and increased formation of phosphatidylcholine occur under the same conditions (Garcia Gil et al., 1982). In a recent report (Mato et al., 1983), dansylcadaverine was found to inhibit the formation of phosphatidylcholine as well as chemotaxis and internalization of the attractant.

The present results show that the drugs employed posses the same order of potency as that found to induce changes in phosphatidylcholine synthesis. Further, the same order has been found to block endocytosis in other systems, such as internalization of α_2– macroglobulin, epidermal growth factor, and vesicular stomatitis virus (Schiegel et al., 1982). Both changes in phospholipid metabolism and in biological responses are dose-dependent and reversible, which suggests that they are not the expression of irreversible cell damage and provide the basis for a new pharmacological approach to control secretory and endocytic processes in human PMNs. However, the possibility that these drugs act by a mechanism other than the action on phospholipid metabolism cannot be excluded.

PAF-acether is an inflammatory mediator identified as 1-0-alkyl-2-acetyl-sn-glycero-3-phosphocholine (Demopoulos et al., 1979; Benveniste et al., 1979), which has been found to be released from human PMNs by a number of stimuli that, with few exceptions, act via a receptor ligand interaction (Lotner et al., 1980; Sanchez Crespo et al., 1980). The main biological actions of PAF-acether are: aggregation and degranulation of platelets (Benveniste et al., 1972) and PMNs (Goetzl et al., 1980); hypotension (Blank et al., 1979); and vasopermeability (Sanchez Crespo et al., 1982). All of them occur during inflammation and could be adequately controlled by reducing the release of PAF-acether at inflammatory sites.

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