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INTERACTION OF MEMBRANE MODIFYING PEPTIDE ANTIBIOTICS FROM TRICHODERMA VIRIDE WITH LEUKOCYTES

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Bovine, rat, and mouse leukocytes were exposed to the pore-forming antibiotics alamethicin, suzukacillin, and trichotoxin isolated from the fungus Trichoderma viride. The compounds caused cell lysis around 5×10^{-5} mol/l; in rat mast cells histamine release was found simultaneously. For bovine and mouse lymphocytes, the compounds inhibited thymidine incorporation into DNA at sublytic concentrations around 2×10^{-6} mol/l. Lymphocyte stimulation by mitogens was inhibited at concentrations below 10^{-6} mol/l. We suggest that pore formation or alterations of membrane conductivity influence the proliferation of B- as well as of T-lymphocytes.

The interaction of a variety of ionophorous substances with the plasma membrane of leukocytes has been shown to influence cell activation; for example, a low molecular weight antibiotic calcium ionophore (A23187) exhibits mitogenicity towards lymphocytes (1). The amphiphilic peptide antibiotics from Trichoderma viride, alamethicin (2), suzukacillin (3), and trichotoxin (4), have also been shown to strongly interact with both natural and synthetic membranes. In millimolar concentrations, they exhibit a lytic action on erythrocytes (5,6); in lower concentrations, they form ion-conducting cooperative pore systems in lipid bilayers (7-10). In this communication, we investigate the interaction of these membrane-modifying antibiotics with leukocytes of different species.

EXPERIMENTAL

Lymphocytes. C3H/HeJ mice were obtained from Jackson Laboratories, Bar Harbor, Maine, USA; bovine lymph nodes from the local

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slaughter house. The organs were removed from the animals within 10 minutes after killing, put into ice cold Minimum Essential Medium Eagle (FLOW), cleaned of adherent connective tissue, cut into small pieces, and macerated gently in a loose fitting tissue grinder (BRAUN MELSUNGEN). The lymphocyte suspension was filtered through a small column of cotton wool, washed twice and suspended in RPMI-1640 (GIBCO).

Agents. alamethicin F-50 (6), trichotoxin A-40 (3), and suzukacillin A (4) were prepared from different strains of the fungus Tichoderma viride as described previously. Mitogens were prepared as described (11).

Cell cultures. Cell cultures were carried out in flat bottom Falcon microtiter plates. Cultures were performed in 0.15 ml aliquots, the cell density adjusted to $3x10^6$ cells/ml in RPMI-1640 medium supplemented with 6.6% heat-inactivated human AB serum (FLOW), fresh glutamine (2 mM/ml), penicillin (100 units/ml), streptomycin (100 µg/ml), and 2-mercaptoethanol ($5x10^{-5}$ mol/l). Cell viability was measured by dye exclusion with trypan blue (0.2% SERVA). Cell proliferation was measured by the incorporation of ^{3}H-thymidine into DNA. Cultures were pulsed for 24 hours before harvesting by the addition to each well of 0.5 µCi ^{3}H-thymidine (AMERSHAM) of the specific activity 40 µCi/mmol. Cultures were harvested with a Mash-II harvester (MICRO-BIOLOGICAL ASSOCIATES). Assays were done at least in duplicate.

Lysis experiments. Rat peritoneal mast cells were obtained from Sprangue-Dawley rats as described by Bloom and Haegermark (12). Histamine was released and determined as described (13, 14). For lysis experiments, cells were incubated in phosphate buffered saline pH 7 with different doses of the antibiotics and subsequently counted microscopically.

RESULTS

Figure 1a presents data on the lytic activity of the antibiotics towards rat peritoneal mast cells. Suzukacillin was the most powerful lytic agent. At a concentration of $2.2x10^{-5}$ mol/l, 50% of the cells were lysed (compare: trichotoxin: $3.8x10^{-5}$ mol/l and alamethicin: $5.4x10^{-5}$ mol/l). For the histamine release of the cells, similar plots were obtained (Figure 1b). Mouse spleen lymphocytes were lysed at concentrations ranging from $3.9x10^{-5}$ mol/l (suzukacillin) to $8x10^{-5}$ mol/l (alamethicin) (Figure 2). Figure 3 shows data on the inhibitory activity of the antibiotics towards lymphocytes cultured for 48 hours and then tested by the incorporation of ^{3}H-thymidine into DNA. 50% inhibition of thymidine incorporation was observed at concentrations around

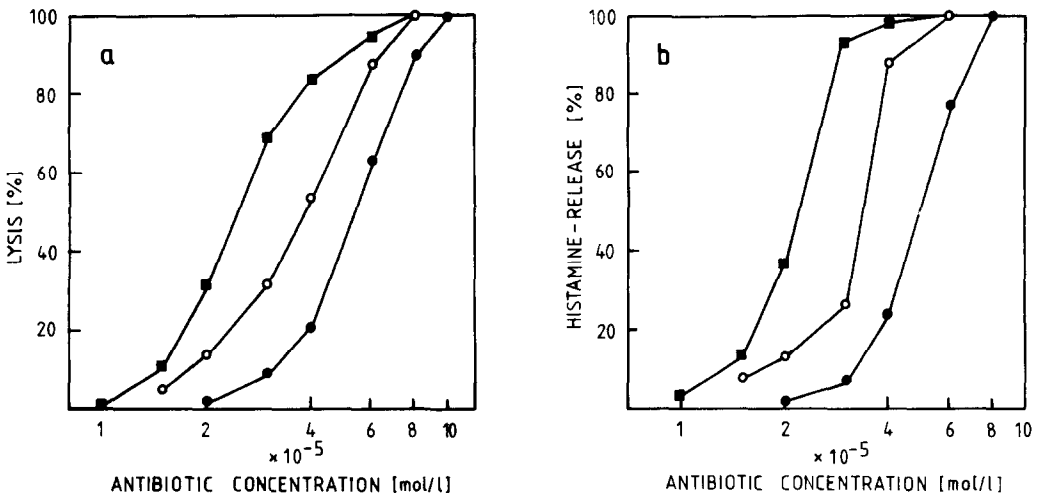

Figure 1. Lysis (a) of and histamin release (b) from rat mast cells by alamethicin (●), suzukacillin (■) and trichotoxin (o) after incubation for 60 minutes at 37°C ($3x10^7$ cells/ml).

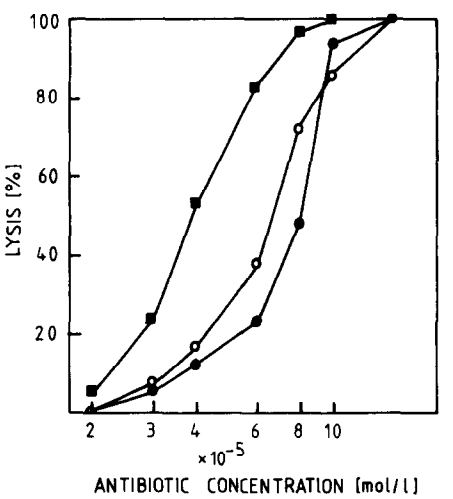

Figure 2. Lysis of mouse spleen lymphocytes by alamethicin (●), suzukacillin (■), and trichotoxin (o) after incubation of $3x10^7$ cells/ml for 60 minutes at 37°C.

$2\text{-}4 \times 10^{-6}$ mol/l (mouse splenocytes; Figure 3a) and $1\text{-}2 \times 10^{-6}$ mol/l (bovine lymphocytes; Figure 3b). At antibiotic concentrations around $8x10^{-6}$ mol/l, thymidine incorporation was almost abolished.

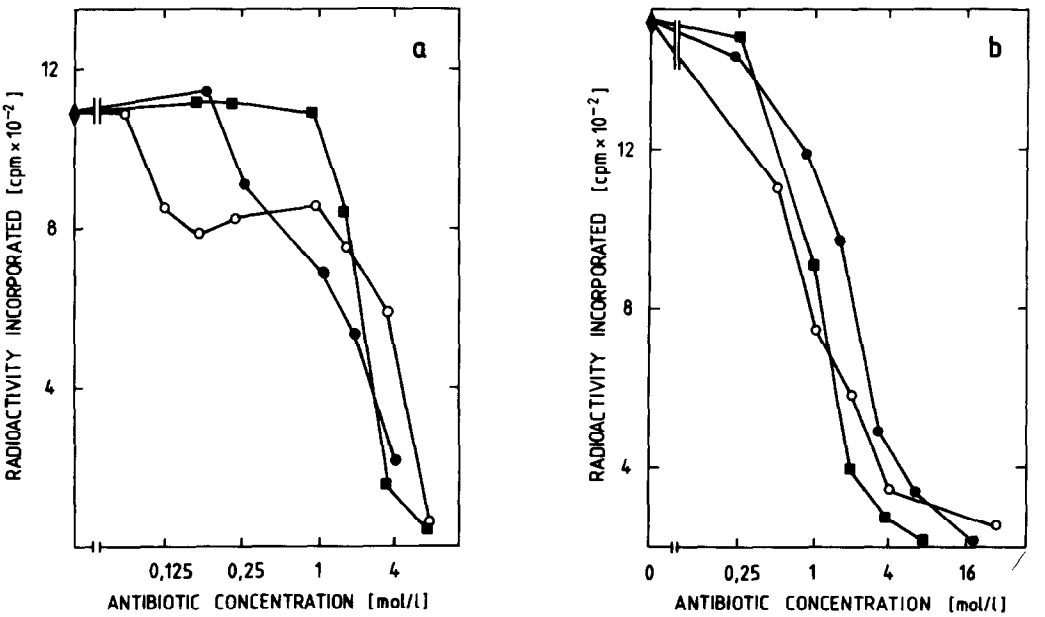

Figure 3. Inhibition of ^{3}H-thymidine incorporation in lymphocytes incubated for 72 hours. a) mouse spleen lymphocytes b) calf lymph node lymphocytes. (●) alamethicin, (■)suzukacillin, (o) trichotoxin.

Figure 4 presents the effects of the peptide antibiotic suzukacillin on lymphocyte stimulation by the T-lymphocyte mitogen Concanavalin A, and the B-lymphocyte mitogen lipoprotein. As seen from the figure, addition of suzukacillin inhibited the stimulation brought about by the T-and B-lymphocyte mitogens starting at concentrations below 10^{-6} mol/l. At a suzukacillin concentration around 3×10^{-7} mol/l, a slight synergistic effect was observed for the mitogenic stimulation by Concanavalin A.

DISCUSSION

The pore-forming membrane-disturbing peptide antibiotics from Trichoderma viride form sequence-analogous polypeptide chains consisting of about 20 amino acids (6). They have been shown to strongly interact with biological membranes and to exhibit a

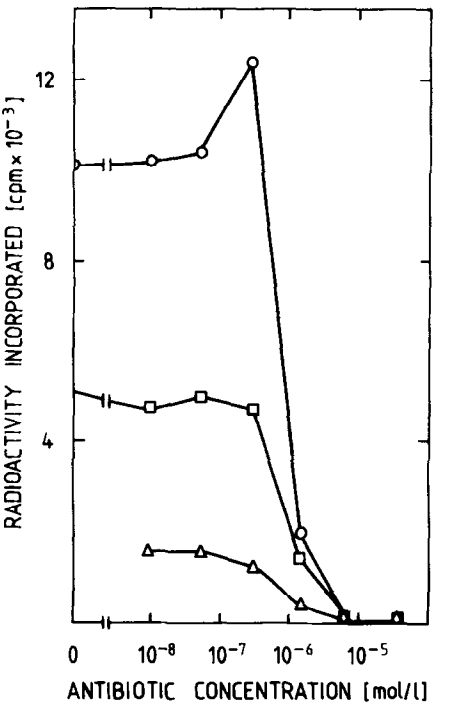

Figure 4. Inhibition of the stimulation by Conanavalin A (●) and lipoprotein (□) of mouse spleen lymphocytes by suzukacillin. Cultures were performed in the presence of 2.5 µg/ml Con A or 75 µg/ml lipoprotein concomitantly with different concentrations of suzukacillin as indicated (72 hours). (Δ) Control cultures without the addition of mitogen.

lytic action on erythrocytes at concentrations around 10^{-5} mol/l (5,6). Here we demonstrate that lytic effects were also exhibited towards leukocytes; rat mast cells, bovine and mouse lymphocytes were lysed by the three antibiotics at concentrations around 5×10^{-5} mol/l. These concentrations are about 5 times higher than the antibiotic concentrations required for erythrocyte lysis, which reflects the higher stability of leukocyte cell membranes.

At sublytic concentrations around $1\text{-}4 \times 10^{-6}$ mol/l, the compounds inhibited thymidine incorporation in bovine and mouse lymphocytes (Figures 3a and 3b). In contrast to other low molecular

weight membrane-disturbing ionophorous substances like the antibiotic Ca^{++}-ionophore A23187 (1), our antibiotics exhibited no mitogenicity by themselves (compare Figures 3a, 3b). Also, no activation was obtained when we incubated the lymphocytes for 2 or 6 hours with the antibiotics and subsequently replaced the supernatants by fresh medium (data not shown). When testing the effect of suzukacillin on mitogenic lymphocyte stimulation, we could demonstrate that the antibiotic was able to inhibit mitogen-induced blastogenesis and proliferation at concentrations starting below 10^{-6} mol/l (compare Figure 4), similar results were found for the structural analoguos molecule alamethicin (data not shown).

It has been demonstrated that, starting at nanomolar concentrations, the peptide antibiotics alamethicin, suzukacillin, and trichotoxin induced the formation of voltage-dependent pores in artificial membranes; several antibiotic molecules are believed to aggregate within the membrane to form ion-conducting channels (7 - 10). Our data suggests that pore formation and/or alterations of membrane conductivity influence lymphocyte proliferation. The fact that the mitogenic stimulation brought about by a B-lymphocyte mitogen is inhibited in a similar way as the activation by a T-lymphocyte mitogen shows that T- as well as B-lymphocyte proliferation is concerned. Consistent with our results, Daniele and Holian (15) recently reported the inhibition of phytohemagglutinin-induced mitogenesis by a potassium ionophore (valinomycin).

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