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Modification of Cellular Membrane Functions by Pendolmycin

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Pendolmycin is a new indole alkaloid isolated from *Nocardiopsis* as an inhibitor of phosphatidylinositol turnover. Structurally, pendolmycin is similar to teleocidin B and lyngbyatoxin A. Pendolmycin, teleocidin B and 12-O-tetradecanoylphorbol-13-acetate all inhibited phosphatidylinositol turnover in A431 cells. Pendolmycin inhibited binding of epidermal growth factor, activated arachidonic acid release and hexose transport, and inhibited binding of phorbol-12,13-dibutylate in C3H10T1/2 cells. Thus, pendolmycin was as potent as teleocidin B and phorbol ester tumor promoters in modification of cell membrane functions, while having the simplest structure among them.

Key words: Pendolmycin — Teleocidin — Tumor promotion — Phosphatidylinositol turnover — Epidermal growth factor

In the course of our screening of phosphatidylinositol turnover inhibitors, we have isolated a potent inhibitor from a strain of *Nocardiopsis*.[1] A structural study revealed it to be a new indolactam-V derivative (Fig. 1), and we named it pendolmycin, since it contained an isopentene structure having antibacterial activity. The structure of pendolmycin is similar to those of teleocidin B[2] and lyngbyatoxin A.[3]

Teleocidin B and lyngbyatoxin A are potent tumor promoters in mouse skin.[4] They both consist of the indolactam-V portion and a diisopentene unit structure. In cell culture, teleocidin B induces arachidonic acid release and 2-deoxyglucose transport, and inhibits cellular binding of epidermal growth factor (EGF) and phorbol-12,13-dibutylate (PDBu).[5] Thus, we have studied these biological effects of pendolmycin in cell culture.

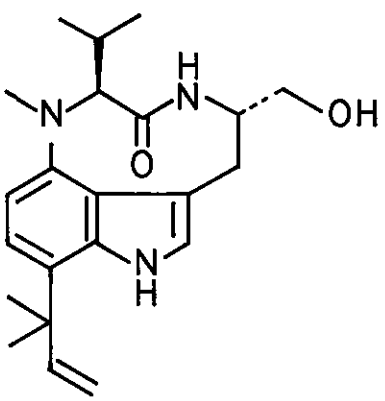

Fig. 1. Structure of pendolmycin.

For phosphatidylinositol turnover assay A431 cells (3×10^5) grown in 35-mm plastic dishes for 16 h beforehand were preincubated in 1 ml Hepes-buffered saline containing [^{3}H]inositol (14 Ci/mmol, 1μCi/ml) at 37°C for 30 min. Then, a test chemical and EGF (400 ng/ml) were added, and the incubation was continued at 37°C for 60 min. Then, 0.5 ml of 10% trichloroacetic acid containing 0.01 *M* sodium pyrophosphate was added, and the acid-insoluble fraction was scraped off from the dish in 1.0 ml of H_2O. The lipid was extracted from the acid-insoluble fraction by the addition of $CHCl_3$ and CH_3OH (1:1) and [^{3}H]inositol-labeled lipids were counted by liquid scintillation. For EGF binding assay C3H10T1/2 or A431 cells (5×10^5) were plated in 35-mm plastic dishes 18 h before use and washed twice with phosphate-buffered saline (PBS), then one ml of serum-free DMEM containing 0.1% bovine serum albumin, referred to as binding medium, and 0.05 μCi of [^{125}I]EGF (100 μCi/μg) were added. A test chemical was added just prior to the addition of [^{125}I]EGF. The plates were incubated for 30 min at 37°C in a 5% CO_2 atmosphere, then the medium was removed. The cells were washed 3 times with cold DMEM and solubilized with 0.5 ml of a solution containing 1% Triton X-100, 5 mg/ml trypsin, and 2 mg/ml EDTA. After a 30-min incubation, the cell lysate was collected and 0.25 ml of 1% SDS was added. The A431 cells were scraped off with a rubber policeman. The radioactivity of the total lysate was determined by liquid scintillation counting. Arachidonic acid release, 2-deoxyglucose uptake and PDBu binding assay were carried out with C3H10T1/2 cells as described before.[5] All values are means of duplicate samples.

Pendolmycin inhibited EGF-induced phosphatidylinositol turnover at about 1 ng/ml as shown in Fig. 2. 12-O-Tetradecanoylphorbol-13-acetate (TPA) showed similar inhibitory activity, and teleocidin B showed the

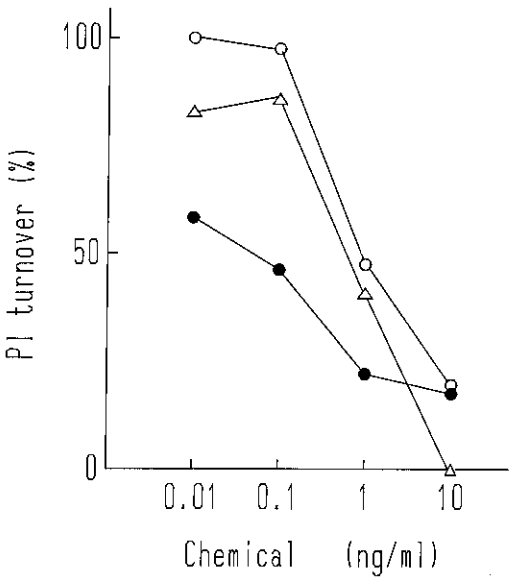

Fig. 2. Inhibition of phosphatidylinositol turnover by pendolmycin and skin tumor promoters. The A431 cells were incubated for 60 min at 37°C with pendolmycin (○), teleocidin B (●) or TPA (△).

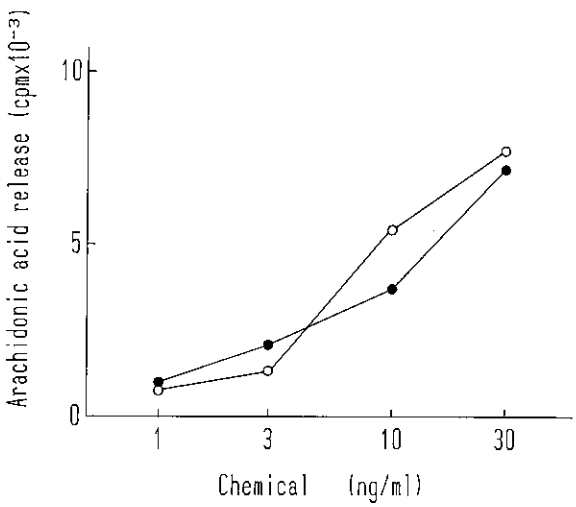

Fig. 4. Induction of arachidonic acid release by pendolmycin. The C3H10T1/2 cells prelabeled with [^{3}H]arachidonic acid were incubated with pendolmycin (○) or teleocidin B (●) for 30 min at 37°C.

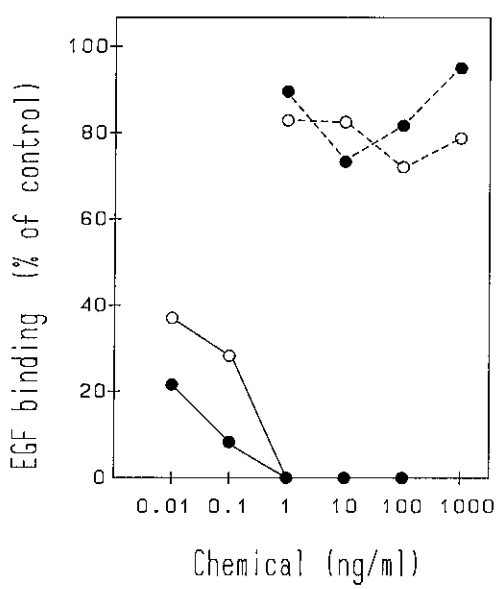

Fig. 3. Effect of pendolmycin on EGF binding in C3H10T1/2 and A431 cells. The C3H10T1/2 (solid line) or A431 (dotted line) cells were incubated with [^{125}I]EGF for 30 min at 37°C with pendolmycin (○) or teleocidin B (●).

strongest activity with IC_{50} of about 0.1 ng/ml. The phorbol ester receptor is known to be down-regulated by addition of TPA.[6] When the cells were preincubated with 100 ng/ml of TPA for 18 h, EGF-induced phosphatidylinositol turnover was not inhibited by TPA or pendolmycin (data not shown).

Pendolmycin also inhibited binding of EGF to the receptor at about 1 ng/ml in C3H10T1/2 cells, like teleocidin B, as shown in Fig. 3. However, pendolmycin did not inhibit binding of EGF in A431 cells up to 1 μg/ml.

C3H10T1/2 cells were preincubated with radioactive arachidonic acid overnight to label membrane phospholipids. When pendolmycin and teleocidin B were added to the prelabeled cells they induced arachidonic acid release from the membrane phospholipids at 3–30 ng/ml, as shown in Fig. 4. Pendolmycin and teleocidin B also enhanced cellular uptake of deoxyglucose about 50% at 10–30 ng/ml as shown in Fig. 5. The binding of PDBu was blocked by both pendolmycin and teleocidin B at about 10 ng/ml as shown in Fig. 6.

Inhibition of phosphatidylinositol turnover by pendolmycin should be due to the binding of the ligand to the phorbol ester receptor, since no inhibition was observed in the receptor-down-regulated cells. As reported before,[7] mouse skin tumor promoters do not inhibit EGF

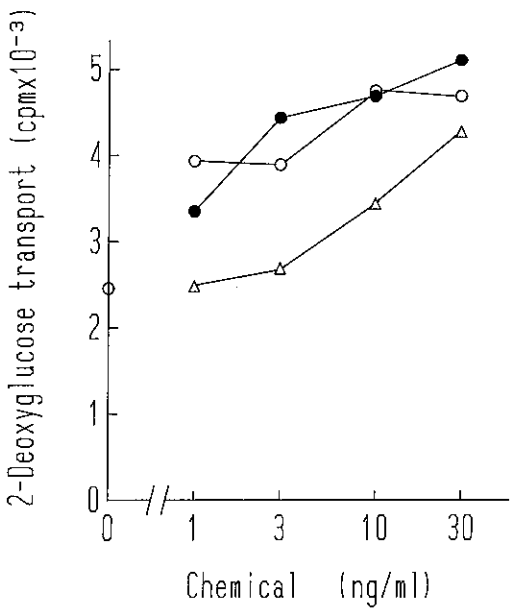

Fig. 5. Enhancement of 2-deoxyglucose transport by pendolmycin. The C3H10T1/2 cells were incubated with [^{3}H]2-deoxyglucose and pendolmycin (○), teleocidin B (●) or TPA (△) for 10 min at 37°C.

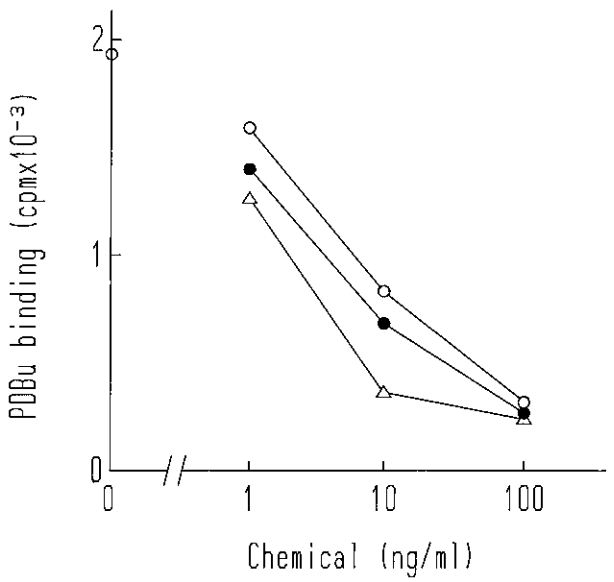

Fig. 6. Inhibition of PDBu binding by pendolmycin. The C3H10T1/2 cells were incubated with [^{3}H]PDBu and pendolmycin (○), teleocidin B (●) or TPA (△) for 30 min at 37°C.

binding in A431 cells. Pendolmycin also does not inhibit EGF binding, and its inhibition of EGF-induced phosphatidylinositol turnover should not be due to the decrease of EGF binding. Although tumor promoters block tyrosine-specific phosphorylation of the EGF receptor,[7] this mechanism may not explain inhibition of phosphatidylinositol turnover, since erbstatin, a tyrosine kinase inhibitor, does not inhibit EGF-induced phosphatidylinositol turnover in A431 cells.[8] More direct inhibition of phosphatidylinositol turnover pathways by activation of protein kinase C may be more likely. It was also reported that TPA inhibited phosphatidylinositol turnover in HL60 cells.[9]

Indolactam-V having no hydrophobic portion shows only a weak *in vitro* and *in vivo* tumor-promoting activity.[10] However, pendolmycin showed almost the same *in vitro* tumor-promoting activity as teleocidin B in cell culture. Teleocidin B has 4 stereoisomers because of the presence of two asymmetric carbons in the hydrophobic diisopentene moiety, and lyngbyatoxin A has 2 stereoisomers.[11] Pendolmycin has no asymmetric carbon in the hydrophobic moiety. The *in vivo* tumor promoting activity of pendolmycin is being studied.

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