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TELEOCIDIN B INHIBITS BINDING OF EPIDERMAL GROWTH FACTOR TO CELLULAR RECEPTORS PROBABLY BY THE SAME MECHANISM AS PHORBOL ESTERS

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SUMMARY: Teleocidin B purified from Streptomyces inhibited the binding of epidermal growth factor(EGF) to rat AH66 hepatoma cells by reducing the receptor affinity. A prolonged treatment of AH66 cells with teleocidin B caused these cells to escape from and to become refractory to teleocidin B-inhibition of EGF binding as seen in TPA-treatment. In addition, those cells refractory to teleocidin B were refractory to a phorbol ester as well, indicating these two compounds with different molecular structure brought similar perturbation of surface structure of AH66 cells.

INTRODUCTION

Teleocidin B, an indole alkaloid, is a toxic substance isolated from the mycellia of Streptomyces(1,2). It is a strong irritant of the skin of human, mice and rabbits(1,2). Fujiki et al. recently reported that dihydroteleocidin B, a derivative obtained by catalytic hydrogenation, stimulated ornithin decarboxylase activity when painted on the skin, and inhibited terminal differentiation of Friend erythroleukemia cells induced by dimethylsulfoxide(3). Since in short term tests dihydroteleocidin B has similar biological effects as 12-O-tetradecanoyl-phorbol-13-acetate(TPA), it is very likely that this is a new type of promoter of carcinogenesis(3). However, the detailed nature of the action of teleocidin B is not known. This paper reports the effects of teleocidin B on rat hepatoma cells with reference to those of TPA, a well known potent tumor promoter.

MATERIALS AND METHODS

Chemicals. Teleocidin B isolated from mycellia of Streptomyces 2A 1563 was obtained from Fujisawa Pharmaceutical Industries, Ltd., Osaka, Japan.

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TPA, 12-O-tetradecanoyl-phorbol-13-acetate; EGF, epidermal growth factor; HEPES, N-2-hydroxyethylpip- erazine-N'-2-ethanesulfonic acid.

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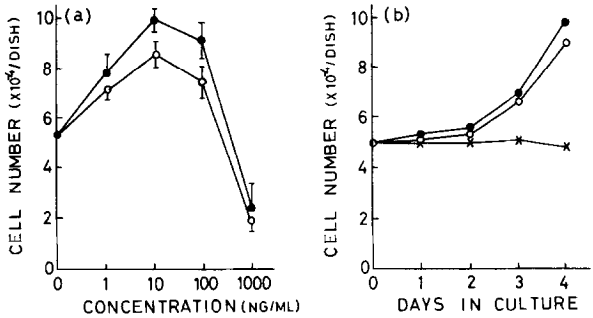

Fig. 1. Effect of teleocidin B on cell proliferation. (a) AH66 cells(5 x 10^4) were cultured for 4 days with different concentrations of teleocidin B(●—●) or TPA(○—○). (b) AH66 cells(5 x 10^4) were cultured for various length of time with none(×—×), 10 ng/ml of teleocidin B(●—●) or 10 ng/ml of TPA(○—○). Cells were counted with hemocytometer.

Teleocidin B was dissolved in 50% ethanol(2 mg/ml) and was stored at -20°C. TPA was purchased from Sigma(St. Louis). The stock solution of TPA was made up in reagent grade dimethylsulfoxide and stored at -20°C. These stock solutions were diluted with the culture medium. The final concentration of these solvents in the culture medium was less than 0.01%.

Cell cultures. Rat AH66 hepatoma cells were cultured in Ham's F12 containing 0.2% fetal calf serum as described previously(4,5).

[^{125}I]EGF binding assay. EGF and [^{125}I]EGF were prepared as reported previously(4,5). Binding assay was performed as reported(5). Briefly, 2 to 5 x 10^5 cells were incubated with [^{125}I]EGF(20,000 cpm) for 90 minutes at 24°C. in 0.5 ml of Ham's F12 + 0.1% bovine serum albumin buffered with 25 mM HEPES (pH 7.4). A vast excess of unlabelled EGF(1 μg/ml) was added to some of the reaction mixtures to asses the amount of non-specific binding.

RESULTS

As shown in Fig. 1a, teleocidin B as low as 1 ng/ml induced an increase in the proliferation of AH66 cells cultured in a low serum medium. The maximum increase in cell number was observed after 4 days of exposure of the cells to 10 ng/ml of teleocidin B(Fig. 1b). The cell number decreased when the cells were cultured with more than 1 μg/ml of teleocidin B. Fig. 1 also shows that similar results were obtained when AH66 cells were cultured with TPA.

Teleocidin B inhibited [^{125}I]EGF binding to the hepatoma cells as demonstrated in Fig. 2. Dose response curve shows that the inhibition was recognized at teleocidin B concentration as low as 0.1 ng/ml, and 100 ng/ml of teleocidin B caused approximately 66% decrease. This inhibitory effect appeared to be similar or even greater than that of TPA(Fig. 1a).

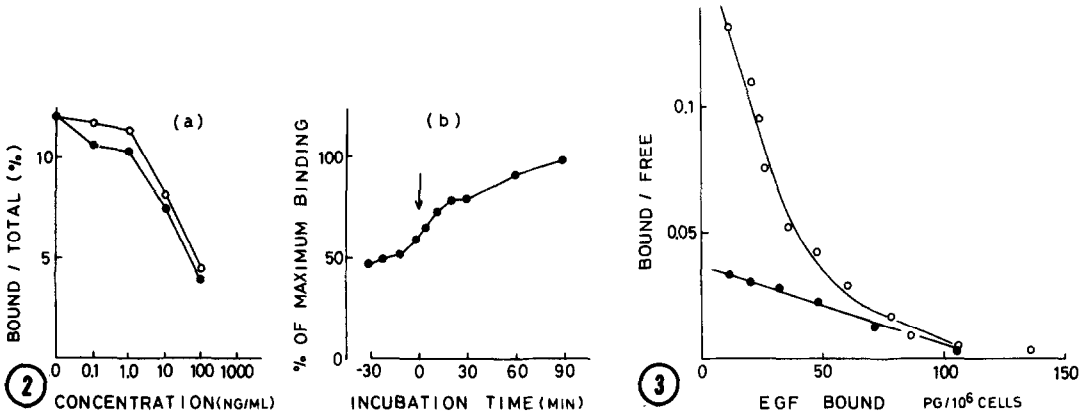

Fig. 2. Inhibition of [^{125}I]EGF binding by teleocidin B. (a) Dose response. AH66 cells(2 x 10^5) were treated for 30 minutes at 37°C with different concentrations of teleocidin B(●—●) or TPA(○—○). Then, [^{125}I]EGF(20,000 cpm) was added and the incubation was continued for further 90 minutes at 24 °C. The value of nonspecific binding(about 100-150 cpm above the instrument back ground counts) was subtracted from all binding assay data. (b) Time course. AH66 cells(2 x 10^5) were incubated with [^{125}I]EGF(20,000 cpm) for 90 minutes (abscissa, 0 to 90) at 37°C. Teleocidin B(10 ng/ml) was added to each tubes before(0 to -30 minutes), after(0 to 90 minutes) or simultaneous with(0 minutes, arrow) the addition of [^{125}I]EGF.

Fig. 3. Scatchard plots of the data about the effects of EGF concentration on the binding to control and teleocidin B-treated AH66 cells. AH66 cells (5 x 10^5) were preincubated for 30 minutes at 37°C with(●—●) or without (○—○) teleocidin B(10 ng/ml). Different amount of [^{125}I]EGF was added and the incubation was continued for further 90 minutes at 24°C.

The effect of the duration of teleocidin B treatment on EGF binding is demonstrated in Fig. 2b. When teleocidin B(10 ng/ml) was added simultaneously with [^{125}I]EGF, there was 40% decrease in the amount of EGF bound to the hepatoma cells. The incubation of cells with teleocidin B more than 30 minutes prior to the addition of [^{125}I]EGF gave 55% decrease. The effects of delayed addition of teleocidin B caused smaller inhibition.

Fig. 3 shows Scatchard plots of the effects of EGF concentrations on the degree of teleocidin B inhibition of EGF binding to the cells. Those cells treated with 10 ng/ml of teleocidin B showed a marked decrease in [^{125}I]EGF binding at the lower concentrations of the added ligand. At higher concentrations of EGF, the effect of teleocidin B on EGF binding was negligible.

On the other hand, those cells cultured for 4 days with 10 ng/ml of teleocidin B bound approximately same amount of [^{125}I]EGF(859 cpm/10^5 cells).

Table 1. Cellular escape from teleocidin B- and TPA-induced inhibition of EGF binding during the prolonged incubation with teleocidin B and TPA.

Growth condition	Assay condition	[^{125}I]EGF bound cpm/10^5 cells	% of control
none	none	808	100
none	TPA	479	59
none	teleocidin B	543	67
TPA	none	859	106
TPA	TPA	830	102
TPA	teleocidin B	767	94
teleocidin B	none	937	115
teleocidin B	TPA	923	114
teleocidin B	teleocidin B	934	115

AH66 cells were seeded at 2 x 10^5 cells per 60 mm dish in 5 ml of Ham's F12 medium containing 0.2% fetal calf serum. After 4 days of culture with or without 10 ng/ml of teleocidin B or TPA, the cells were harvested and centrifuged. The cell count in control, teleocidin B- and TPA-containing culture was 22, 30 and 28 x 10^5 cells per dish respectively. These cells were incubated for 30 minutes at 37°C in 0.5 ml of fresh low serum medium in the presence or absence of 10 ng/ml of teleocidin B or TPA. Then, [^{125}I]EGF (20,000 cpm) was added to each test tube and the incubation was continued for further 90 minutes at 24°C.

Apparently, these cells had escaped from the teleocidin B-inhibiton of EGF binding and were also resistant to fresh teleocidin B added directly to the binding assay(Table 1). Furthermore, these cells were resistant to newly added TPA in addition to teleocidin B. Similarly, the cells cultured with TPA(10 ng/ml) were refractory to newly added teleocidin B(Table 1).

DISCUSSION

We have been investigating the actions of EGF and tumor promoters on α-fetoprotein-producing rat AH66 hepatoma cells as a model for tumor promotion in hepatocarcinogenesis(4,5). Our present study shows that teleocidin B, a possible naturally occurring tumor promoter from Streptomyces, stimulated the proliferation of the hepatoma cells and inhibited the binding of [^{125}I]EGF to the cellular receptors as TPA did. Scatchard analysis showed EGF receptors on AH66 hepatoma cells were heterogenous and teleocidin B inhibited the binding of EGF to the high affinity sites. Similar reduction in the receptor affinity was also recognized when AH66 cells were treated with TPA as reported

previously(5). Such an alteration in the receptor affinity may be due to a change in the microenvironment of ECF receptors as suggested in the case of TPA(6,7).

Lee and Weinstein reported recently that a prolonged treatment of HeLa cells with TPA caused these cells to escape from the TPA-inhibition of EGF binding(escape), and that newly added TPA could not displace [^{125}I]EGF bound to them(refractoriness)(8). In our present experiments, we found that teleocidin B brought about similar changes on the EGF receptors of AH66 hepatoma cells. In addition, we found that the cells showing the refractoriness to teleocidin B after a prolonged treatment with this compound were also refractory to TPA, and so vice vasa.

Our present results show that teleocidin B with a molecular structure different from that of TPA brought about remarkable changes in cell surface structure which are similar to those caused by TPA. Recently, Hoshino et al. reported that dihydroteleocidin B and TPA caused aggregation of human lymphoblastoid cells probably by changing the membrane property(9). Therefore, the target of the action of teleocidin B, a possible naturally occurring tumor promoter, seems to be the plasma membrane and detailed studies about the teleocidin B-modulation of cell surface properties may contribute significantly to the understanding of the mechanism of tumor promotion.

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