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STIMULATION OF PROSTAGLANDIN PRODUCTION AND CHOLINE TURNOVER IN HELA CELLS BY LYNGBYATOXIN A AND DIHYDROTELEOCIDIN B

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SUMMARY: Lyngbyatoxin A, isolated from a marine blue-green alga, and dihydroteleocidin B, a hydrogenated derivative of teleocidin, induce ornithine decarboxylase in mouse skin. In addition, dihydroteleocidin B was recently shown to be a potent tumor promoter in mouse skin. The present studies demonstrate that both lyngbyatoxin A and dihydroteleocidin B induce increased prostaglandin release and choline turnover in HeLa cells at concentrations of 6-20 ng/ml, with a time course similar to that of the potent phorbol ester tumor promoter 12-O-tetradecanoyl phorbol-13-acetate. Thus these indole alkaloids, although structurally different from phorbol ester tumor promoters, share several properties with the latter compounds.

INTRODUCTION

12-O-Tetradecanoyl phorbol-13-acetate (TPA), a potent tumor promoter in two-stage carcinogenesis in mouse skin (1-3), induces epidermal ornithine decarboxylase (ODC) (4,5). Lyngbyatoxin A, isolated from a marine green alga, and dihydroteleocidin B, a hydrogenated derivative of teleocidin isolated from Streptomyces, both of which are indole alkaloids and structurally different from TPA, also induce ODC in mouse skin (6,7). In addition, lyngbyatoxin A and dihydroteleocidin B show a number of effects on the phenotype of mammalian cells in culture similar to those of TPA; these include induction of differentiation of human promyelocytic leukemia cells (8), inhibition of differentiation of murine erythroleukemia cells (7), and aggregation of human lymphoblastoid cells (9). The tumor promoting activity of lyngbyatoxin A is

Abbreviations used: TPA, 12-O-tetradecanoylphorbol-13-acetate; ODC, ornithine decarboxylase; DMSO, dimethyl sulfoxide.

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currently being studied in our laboratory. We have already established that dihydroteleocidin B is a potent tumor promoter in two-stage carcinogenesis in mouse skin (6).

Although the mechanisms involved in tumor promotion are not fully understood, it has been suggested that prostaglandin biosynthesis and increased phospholipid turnover are involved in tumor promotion by TPA (10-16). Application of TPA to mouse skin induces increased synthesis of prostaglandins, and inhibition of prostaglandin synthesis by indomethacin results in inhibition of tumor promotion (16). It was recently shown that in rodent cell cultures dihydroteleocidin B induces several membrane changes similar to those produced by TPA, including release of arachidonic acid from membrane phospholipids (17).

In the present studies, we found that lyngbyatoxin A and dihydroteleocodin B induced increased release of prostaglandin and an increased turnover of phospholipid choline in HeLa cells at concentrations similar to those at which TPA produced similar changes. These results provide further evidence that these indole alkaloids exert their effects on cells via the same mechanisms as the phorbol ester tumor promoters.

MATERIALS AND METHODS

Chemicals: Dimethylsulfoxide (DMSO) was obtained from Wako Chemical Industries, Tokyo. Lyngbyatoxin A and teleocidin were isolated and purified from Lyngbya majuscula (18) and Streptomyces mediocidicus (19), respectively. Dihydroteleocidin B was prepared from teleocidin by catalytic reduction (20). TPA was obtained from Consolidated Midland Corporation, Brewster, N. Y. Lyngbyatoxin A, dihydroteleocidin B and TPA were dissolved in DMSO, stored at -20°C and used within 1 month. [1,2-^{14}C]Choline (5mCi/mmol) and Aquasol were obtained from New England Nuclear Corporation, Boston, Mass.

Cell cultures: HeLa cells were maintained as monolayers in Eagle's minimum essential medium containing 10% calf serum under a humidified atmosphere of 5% CO_2 in air.

Release of prostaglandins: HeLa cells were removed from the flasks with 0.025% trypsin in phosphate buffered saline and 1 x 10^5 cells were inoculated into Falcon multiple-well trays of 1.6 cm diameter, containing 0.5 ml of the medium, 48 to 72 hrs before the experiments. The medium was replaced by Eagle's minimum essential medium without serum, and lyngbyatoxin A, dihydroteleocidin B or TPA was added; the concentration of DMSO was always adjusted

to 0.4%. At the times indicated, the medium was removed and centrifuged at 12,000 rpm for 1 min in an Ependorf centrifuge, and the supernatant was used to measure the amount of prostaglandin released. The amount of prostaglandin released was measured serologically, without further extraction, by radioimmunoassay with rabbit anti-prostaglandin E-BSA antiserum (Miles-Yeda Ltd., Rehvot, Israel). The anti-prostaglandin E antiserum was obtained by immunizing rabbits with prostaglandin E_2. It showed only 1.6% cross reaction with prostaglandin $F_{2\alpha}$, but cross reacted significantly with prostaglandins A and B. Therefore, results are expressed as pg of prostaglandin, not of prostaglandin E.

Choline turnover: For study of incorporation of ^{14}C-choline, 5 x 10^5 cells were cultured in 1.5 ml of medium containing 10% calf serum for 24 hrs, and then cultured in 1.5 ml of serum-free medium with one of the test compounds for various periods. ^{14}C-Choline (0.4 μCi/ml) was then added and after the times indicated, the medium was removed, and sodium dodecylsulfate was added to a final concentration of 0.5%. The mixtures were then incubated for 30 min at room temperature. Trichloroacetic acid was added to the lysates at a final concentration of 10% and the precipitates were collected on millipore filters. The radioactivity on the filters was counted in Aquasol. It was reported previously that incorporation of radioactive choline into acid-insoluble materials almost exclusively represents the incorporation into phosphatidylcholine (14,21,22). For study of release of ^{14}C-choline (10,22), the cells were cultured in medium containing 10% calf serum with 0.5 μCi/ml of ^{14}C-choline for 72 hrs. They were then washed three times with phosphate buffered saline and plated in multi-well tissue culture trays at 2 x 10^5 cells per well. After 12 hrs of culture, the cells were incubated in 0.5 ml of serum-free medium in the presence and absence of lyngbyatoxin A, dihydroteleocidin B or TPA. At the indicated times, the medium was collected and centrifuged at 12,000 rpm in an Ependorf centrifuge for 1 min. Aliquots of the supernatant were taken to measure radioactivity in a scintillation counter using Aquasol.

RESULTS

Effects of lyngbyatoxin A and dihydroteleocidin B on release of prostaglandins:

When various concentrations of lyngbyatoxin A, dihydroteleocidin B or TPA were added to HeLa cells in culture, release of prostaglandin into the medium increased rapidly (Fig. 1). With all three compounds the stimulation was apparent within 3 hrs. Dose-response studies on prostaglandin release showed that lyngbyatoxin A, dihydroteleocidin B and TPA all stimulated prostaglandin release in the same concentration range, with optima of about 6 ng/ml, although at 6 ng/ml lyngbyatoxin A and dihydroteleocidin B caused more release than TPA (Fig. 2).

Effect of lyngbyatoxin A and dihydroteleocidin B on choline turnover:

Incorporation of choline into HeLa cells was stimulated by exposing the cells to lyngbyatoxin A, dihydroteleocidin B or TPA for 3 hrs (Fig. 3).

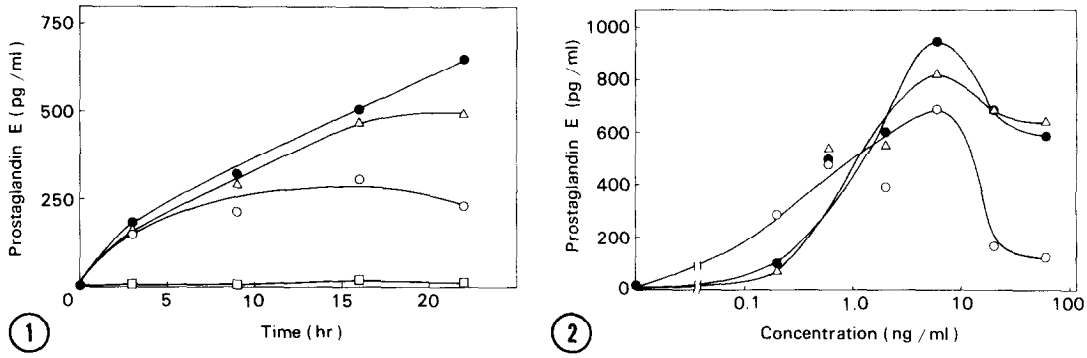

Fig. 1. Effects of lyngbyatoxin A, dihydroteleocidin B and TPA on prostaglandin release. HeLa cells were cultured in multi-well tissue culture trays at a density of 1 x 10^5 cells per 0.5 ml of medium containing 10% calf serum for 48 hrs. Then the medium was changed to serum-free medium containing 6 ng/ml of lyngbyatoxin A (△), dihydroteleocidin B (●) or TPA (○). The cells were also cultured in medium containing 0.4% DMSO (□). At the times indicated, prostaglandin released into the medium was measured.

Fig 2. Effects of lyngbyatoxin A, dihydroteleocidin B and TPA on prostaglandin release. HeLa cells were cultured in multi-well tissue culture trays at a density of 1 x 10^5 cells per 0.5 ml of medium containing 10% calf serum for 72 hrs. Then the medium was changed to serum-free medium containing various concentrations of lyngbyatoxin A, dihydroteleocidin B and TPA. After culture for 18 hrs with lyngbyatoxin A (△), dihydroteleocidin B (●) or TPA (○), prostaglandin released into the medium was measured as described in the text.

Dose-response studies indicated that all three coupounds stimulated choline incorporation in the same concentration range and, as with prostaglandin release, the maximum effect was obtained at about 6 ng/ml (Fig. 4-A).

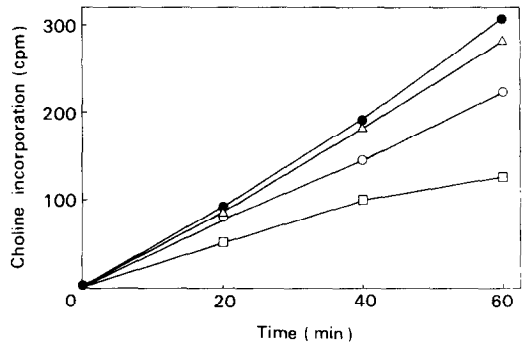

Fig. 3. Time course of choline incorporation into HeLa cells cultured with lyngbyatoxin A, dihydroteleocidin B and TPA. HeLa cells were cultured in serum-free medium for 3 hrs with 6 ng/ml of lyngbyatoxin A (△), dihydroteleocidin B (●), TPA (○) or 0.4% DMSO only (□). ^{14}C-Choline (0.4 μCi/ml) was added to the culture medium and the cells were cultured for the times indicated. Trichloroacetic acid-precipitable counts were measured as described in the text.

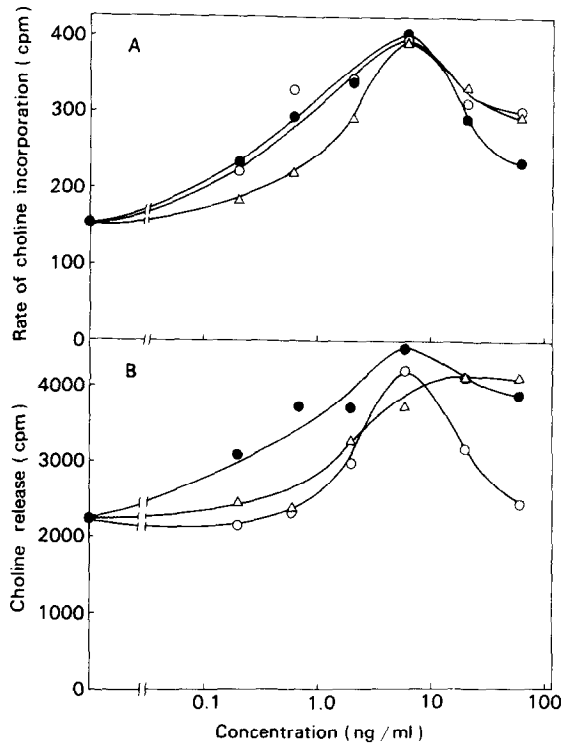

Fig. 4. Effects of the concentrations of lyngbyatoxin A, dihydroteleocidin B and TPA on incorporation of choline (A) and release of radioactivity into the medium from ^{14}C-choline labeled HeLa cells (B). In A, HeLa cells were cultured in serum-free medium containing various concentrations of lyngbyatoxin A (△), dihydroteleocidin B (●) or TPA (○) for 5 hrs. ^{14}C-Choline (0.4 μCi/ml) was added to the culture medium and the cells were cultured for 1 hr. Trichloroacetic acid-precipitable counts were measured as described in the text. In B, ^{14}C-choline labeled HeLa cells were cultured in serum-free medium for 10 hrs with various concentrations of lyngbyatoxin A (△), dihydroteleocidin B (●) or TPA (○). Aliquots of medium were removed to measure radioactivity as described in the text. Results are expressed as counts released per culture.

Recent studies have shown that TPA and related phorbol ester tumor promoters induce rapid release of choline from cellular phospholipids of rodent cell cultures (10,22). We found that when the phospholipid of HeLa cells was prelabelled with ^{14}C-choline, lyngbyatoxin A, dihydroteleocidin B or TPA also stimulated release of radioactivity from the cells (Fig. 4-B). With all three ·mpounds, the increased release was detected within 1 hr after adding the ·nds (data not shown). Dose-response studies indicated that concent- ·bout 6 ng/ml of all three compounds caused maximum release of

radioactivity and that the extents of release by these compounds were similar (Fig. 4-B).

DISCUSSION

Previously we reported that, like TPA, lyngbyatoxin A and dihydroteleocidin B induce ODC in mouse skin, inhibit differentiation of murine erythroleukemia cells, induce adhesion and differentiation of human promyelocytic leukemia cells, and induce aggregation of human lymphoblastoid cells (4-9). We also showed that dihydroteleocidin B is a potent tumor promoter in mouse skin when 7,12-dimethylbenz[a]anthracene is used as initiator (6).

TPA and structurally related phorbol esters with tumor promoting activity in mouse skin two-stage carcinogenesis have numerous effects on the phenotype of cells in culture (for review, see 10). Although the mechanisms involved in tumor promotion by TPA are not fully understood, there is considerable evidence that the primary target of TPA and related compounds is the plasma membrane (10-16). One of the earliest effects exerted by tumor promoting phorbol esters is stimulation of phospholipid metabolism (10-16). Since lyngbyatoxin A and dihydroteleocidin B are indole alkaloids, which are structurally different from the phorbol esters, it was important to examine whether these compounds had the same effects as TPA on phospholipid metabolism.

In the present study, we found that lyngbyatoxin A and dihydroteleocidin B, like TPA, induced rapid increase in release of prostaglandins and in choline turnover in HeLa cells. The dose-responses to lyngbyatoxin A and dihydroteleocidin B of enhancement of prostaglandin release and choline turnover were in the same concentration range as that to TPA, and the maximum responses of prostaglandin release and choline turnover in response to lyngbyatoxin A and dihydroteleocidin B were equal to, or greater than, those observed with TPA. These results are in agreement with previous data showing increased arachidonic acid release in rodent cells exposed to dihydro-

teleocidin B (17), and suggest that, despite marked structural differences, lyngbyatoxin A and dihydroteleocidin B exert their effects on the cellular plasma membrane by the same mechanism as TPA. Consistent with this conclusion is evidence that teleocidin, a parent compound of dihydroteleocidin B, binds to the same, or a similar, set of high affinity membrane receptors as TPA (10,17). The present experiments also support the hypothesis that tumor promoters exert at least some of their effects via changes in cell membrane phospholipid metabolism.

The availability of a new group of compounds that are structurally unrelated to TPA and yet have the same tumor promoting activity as TPA in vivo, and similar biological effects to TPA on cultured cells should be useful not only for understanding the mechanisms underlying tumor promotion, but also for elucidating the molecular mechanisms involved in transmitting cell membrane signals that are involved in modulating phenotypic expression.

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