Stimulation of human chorionic gonadotropin and progesterone secretion by tumor promoters, phorbol ester and teleocidin B, in cultured choriocarcinoma cells

N. Sasaki, M. Matsuzaki, T. Okabe[1], Y. Imai, Y. Kaneko and F. Matsuzaki

The Third Department of Internal Medicine, Faculty of Medicine, University of Tokyo, Hongo 7-3-1, Tokyo 113 (Japan), June 15, 1982

Summary. Both 12-O-tetradecanoyl-phorbol-13-acetate and teleocidin B stimulated the secretion of human chorionic gonadotropin by cultured choriocarcinoma cells. These tumor promoters also stimulated production of progesterone in the cells. However, the 2 tumor promoters did not exert a marked effect on the cellular binding of epidermal growth factor that also had a stimulatory effect on production of these hormones.

12-O-Tetradecanoyl-phorbol-13-acetate (TPA) is one of the most potent tumor promoting agents, initially isolated from croton oil[2]. This compound has been shown to inhibit the binding of epidermal growth factor (EGF) to cellular receptors[3-6] and share many of the biological properties of EGF in cultured cells[7,8]. Teleocidin B, an indole alkaloid isolated from *Streptomyces*[9,10], is now recognized as a new type of tumor promoter[11]. Teleocidin B has also been reported to inhibit the cellular binding of EGF in some cells[12,13]. Recent studies indicated that EGF stimulated secretion of human chorionic gonadotropin (hCG)[14,15] and progesterone[16] in cultured choriocarcinoma cells. These observations led us to investigate the effects of TPA and teleocidin B on choriocarcinoma cells with reference to those of EGF. In this paper, we report that both TPA and teleocidin B stimulate secretion of hCG and progesterone in choriocarcinoma cells.

Materials and methods. TPA was purchased from Sigma (St. Louis). The stock solution of TPA was made up in reagent grade dimethylsulfoxide and stored at −20 °C.

Teleocidin B isolated from mycelia of *Streptomyces* 2A 1563 was obtained from Fujisawa Pharmaceutical Industries, Ltd, Osaka, Japan. Teleocidin B was dissolved in 50% ethanol (2 mg/ml) and was stored at −20 °C. These stock solutions were diluted with the culture medium. The final concentration of the solvents in the culture medium was less than 0.01%. EGF was purified from submaxillary glands of male mice by the method of Savage and Cohen[17].

Cell cultures: A human choriocarcinoma cell line, T3M-3, has been established by the method described previously[18]. Characterization of the cell line will be published in the near future (manuscript in preparation). All cultures were maintained in RPMI-1640 medium supplemented with 10% fetal bovine serum at 37 °C in a humidified atmosphere of 5% CO_2 in air. Subcultures were performed with 0.25% trypsin and 0.02% EDTA solution as described[18]. To examine the effects of TPA, teleocidin B or EGF, the cells were seeded in 35 × 10 mm plastic dishes (Falcon Plastics, Oxnard, Ca.) in the complete growth medium. 48 h prior to the addition of the samples, the cells were washed with RPMI-1640, and were incubated in serum-free RPMI-1640 medium. The medium from treated cultures was harvested at the time indicated, centrifuged to remove cell debris, and stored at −20 °C until hCG, hCG-beta and progesterone were measured. After collection of the medium, the cell monolayer was washed with 0.9% sodium chloride solution, dissolved in 1 ml of 1 N sodium hydroxide. Protein was measured in these extracts by the method of Lowry et al.[19] using bovine serum albumin as a standard. The concentrations of immunoreactive hCG, hCG-beta and progesterone were determined by the use of the specific radioimmunoassay (RIA) kits (Commissariat a l'Energie Atomique, France) respectively[20,21]. [^{125}I] EGF binding assay: [^{125}I] EGF was prepared as reported previously[22]. Binding assay was performed as reported[23]. Briefly, $1\text{-}2 \times 10^5$ cells were incubated with [^{125}I] EGF (20,000 cpm) for 90 min at 24 °C in 0.5 ml of RPMI-1640 + 0.1% BSA buffered with 10 mM HEPES (pH 7.4). A vast excess of unlabeled EGF (10 μg/ml) was added to some of the reaction mixtures to estimate the amount of nonspecific binding.

Results and discussion. The stimulatory effects of TPA, teleocidin B or EGF on hCG and hCG-beta secretion are shown in figure 1. The maximal stimulatory effects for both hCG and hCG-beta were observed at a concentration of 1 μg/ml or more for TPA, 100 ng/ml for teleocidin B and 10 ng/ml for EGF. The time course of stimulatory effects by TPA (1 μg/ml), teleocidin B (100 ng/ml) or EGF (10 ng/ml) on hCG-beta and progesterone secretion is shown in figure 2. The stimulatory effects of these agents on hCG-beta secretion (fig. 2, a) became more and more prominent by 4 days; there was a maximal 3.5-fold (teleocidin B), 2.7-fold (TPA) or 2.1-fold (EGF) increase above the control. The stimulatory effect of EGF on the secretion of progesterone was significant within 24 h (fig. 2, b). Maximal stimulation by TPA (1.6-fold), teleocidin B (1.3-fold) or EGF (1.5-fold) was attained by 3 days of incubation. Neither TPA, teleocidin B, nor EGF had a stimulatory effect on cellular proliferation, judged by measurements of the protein content of the cultured cells and [^{3}H]-thymidine incorporation into DNA (data not shown).

This study indicated that TPA had a stimulatory effect on the secretion of hCG and progesterone. To our knowledge, this is the first demonstration that TPA stimulated the secretion of hCG and progesterone in human functional

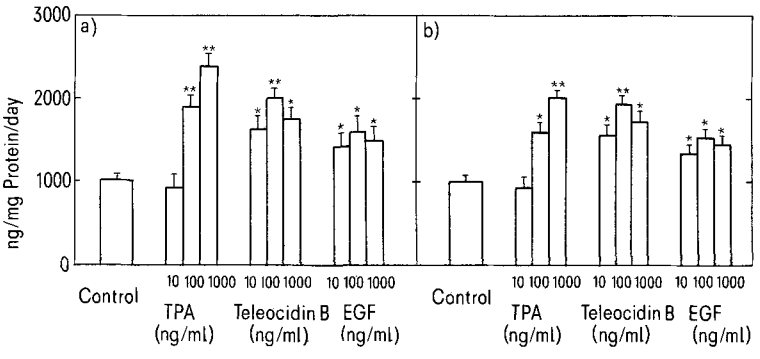

Figure 1. Effects of TPA, teleocidin B or EGF on hCG and hCG-beta secretion. After 2 days of serum-free pre-incubation, the cells were further incubated in a serum-free RPMI-1640 in the absence or the presence of various concentrations of TPA, teleocidin B or EGF. After 24 h, the medium was harvested for hCG (a) or hCG-beta (b) measurements by respective RIA and the cells were also harvested for the protein determinations. Each value represents the mean and SEM of 3 dishes. (* $p < 0.05$, ** $p < 0.02$ compared to control).

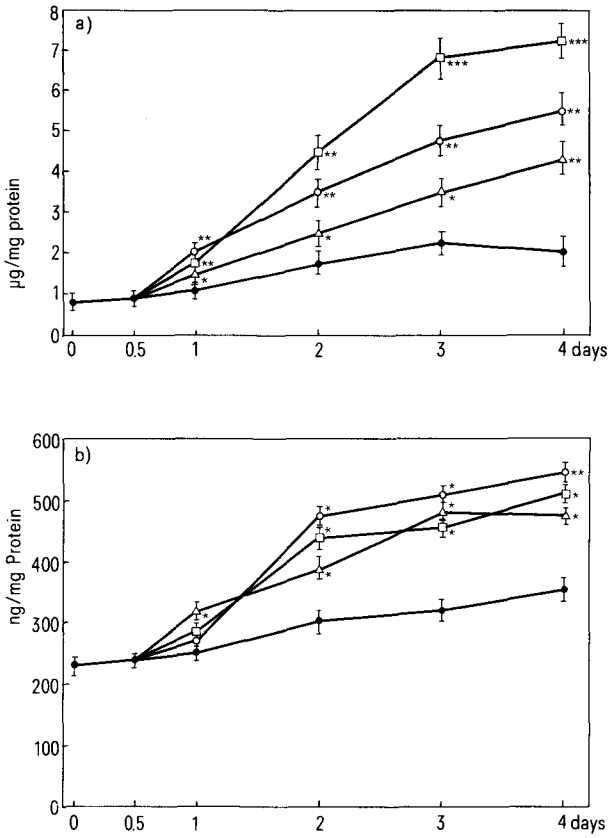

Figure 2. Time course of stimulation of hCG-beta and progesterone secretion by TPA, teleocidin B or EGF. Following 2 days in a serum-free medium, the cells were refed with RPMI-1640 alone (●), RPMI-1640+1 μg/ml TPA (○), RPMI-1640+10 ng/ml teleocidin B (□), or RPMI-1640+ng/ml EGF (△), (day 0). At the indicated time, the medium was harvested for hCG-beta (a) and progesterone (b) measurements and the cells were harvested for protein determinations. Each point represents the mean ± SEM of 3 dishes. (* $p < 0.05$ μm, ** $p < 0.02$, *** $p < 0.01$ compared to control at the time).

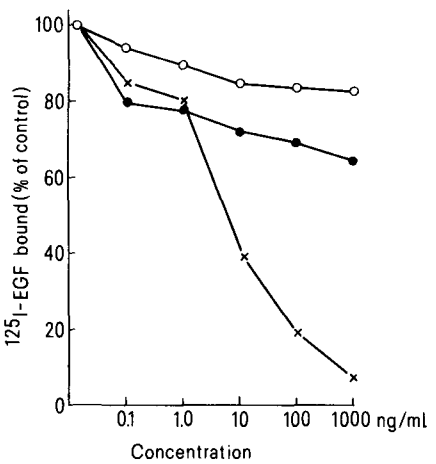

Figure 3. Inhibition of [^{125}I] EGF binding for 30 min at 37 °C with different concentrations of TPA (●), teleocidin B (○), or EGF (×). Then, [^{125}I] EGF (20,000 cpm) was added and the incubation was continued for further 90 min at 24 °C. The value of nonspecific binding was subtracted from all binding assay data.

trophoblastic cells. In addition, a new promoter, teleocidin B was shown to have a stimulatory effect on the secretion of hCG and progesterone for the first time. In this study, the inhibitory effect of TPA or teleocidin B on EGF binding was too small, in comparison with their strong stimulatory effects on the hormone secretion. Recent studies have suggested that phorbol esters possess their own receptor and that the effect of TPA on EGF binding was due to the conformational changes on the plasma membrane rather than direct binding of TPA to the EGF receptor[24-26]. Our observations, together with their reports, strongly suggested that TPA and teleocidin B could act on the choriocarcinoma cells without interacting with the receptor system for EGF.

The biological significance of the effects of the tumor promoters is not fully understood, and awaits further studies for elucidation. The present line of study may provide a clue for better understanding of the mechanism by which tumor promoters affect cell function.

1 To whom reprint requests should be addressed.
2 Hecker, E., in: Method in cancer research, p.439. Academic Press, New York 1971.
3 Lee, L.S., and Weinstein, I.B., Science *202* (1978) 313.
4 Brown, K.D., Dicker, P., and Rozengurt, E., Biochem. biophys. Res. Commun *86* (1979) 1037.
5 Lee, L.S., and Weinstein, I.B., Proc natl Acad Sci. USA *76* (1979) 5168.
6 Shoyab, M., De Larco, J.E., and Todaro, G.J., Nature *279* (1979) 387.
7 Carpenter, G., and Cohen, S., in: Molecular control of proliferation and differentiation, p.13. Academic Press, New York 1978.
8 Lee, L.S., and Weinstein, I.B., Nature *274* (1978) 696.
9 Takashima, M., and Sasaki, H., Bull. agric. chem. Soc. Japan *24* (1960) 647.
10 Takashima, M., and Sasaki, H., Bull. agric. chem. Soc. Japan *24* (1960) 652.
11 Fujiki, H., Mori, M., Nakayasu, M., Terada, M., and Sugimura, T., Biochem. biophys. Res. Commun. *97* (1980) 926.
12 Imai, Y., Kaneko, Y., Matsuzaki, F., Endo, Y., and Oda, T., Biochem. biophys. Res. Commun. *97* (1980) 926.
13 Umezawa, K., Weinstein, I.B., Horowitz, A., Fujiki, H., Matsushima, T., and Sugimura, T., Nature *290* (1981) 411.
14 Benveniste, R., Speeg, Jr, K.V., Carpenter, G., Cohen, S., Linder, L., and Rabinowitz, D., J. clin. Endocr. Metab. *46* (1978) 169.
15 Huot, R.I., Foidart, J.M., Nardone, R.M., and Stromberg, K., J. clin. Endocr. Metab. *53* (1981) 1059.
16 Bahn, R.S., Speeg, Jr, K.V., Ascoli, M., Rabin, D., Endocrinology *107* (1980) 2121.
17 Savage, Jr, C.R., and Cohen, S., J. biol. Chem. *247* (1972) 7609.
18 Okabe, T., Suzuki, A., Ohsawa, N., Kosaka, K., and Terashima, T., Cancer Res. *39* (1979) 41 9.
19 Lowry, O.H., Rosenbrough, N.J., Farr, A.L., and Randall, R.J., J. biol. Chem *193* (1951) 265.
20 Reuter, A.M., Ashoonbrood, J., and Franchimont, P., in: Cancer related antigens, p.237. Excerpta Medica, North-Holland, Amsterdam/New York 1976.
21 Malvano, R., Trojsi, L., Gandoefi, C., Attanasio, A., and Crosignani, P.G., in: Recent progress in reproductive endocrinology, p.229. Academic Press, London 1974.
22 Kaneko, Y., Imai, Y., Matsuzaki, F., and Oda, T., Experientia *35* (1980) 1660.
23 Kaneko, Y., Imai, Y., Matsuzaki, F., and Oda, T., Oncodevl Biol. Med. *1* (1980) 181.
24 Drieger, P.E., and Blumberg, P.M., Proc. natl Acad. Sci USA *77* (1980) 567.
25 Delclos, K.B., Nagle, D.S., and Blumberg, P.M., Cell *19* (1980) 1025.
26 Shoyab, M., and Todaro, G.J., Nature *288* (1980) 451.

0014-4754/83/030330-02$1.50+0.20/0