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Calcium Ionophore A23187 Inhibits ACTH Secretion from a Human Small Cell Lung Cancer Cell Line,COR-L103

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SUMMARY : The mechanism of ectopic adrenocorticotrophic hormone (ACTH) secretion was examined by studies on the effects of corticotropin-releasing hormone (CRH), dexamethesone, interleukin (IL) -1β and 2, somatostatin, calcium ionophore A23187, 12-O-tetradecanoylphorbol-13-acetate (TPA) and 8-bromo-cAMP on pro-opiomelanocortin (POMC) expression and ACTH secretion from a human small cell lung cancer cell line COR-L103. None of these agents except TPA and A23187 had any effect on ACTH secretion from the cell line in short (0-8 hrs) or long term (1-4 days) cultures. In long term cultures, 1-100 nM TPA stimulated ACTH secretion dose- dependently, whereas 500nM A23187 inhibited ACTH secretion completely. When the cells were incubated with 10nM TPA plus 500 nM A23187, the inhibitory action of A23187 on ACTH secretion was suppressed by TPA. These results suggest that the mechanisms of ACTH secretion by COR-L103 cells and normal pituitary cells are different.

Some small cell lung cancer cells have been reported to produce peptide hormones such as ACTH, vasopressin and calcitonin. ACTH released ectopically stimulates the adrenal cortex resulting in hypercortisolism. The administrations of corticotropin-releasing hormone (CRH) and dexamethasone often have no effect on the plasma ACTH level in patients with the ectopic ACTH syndrome (1).

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Abbreviations: POMC, pro-opiomelanocortin; ACTH, adrenocorticotrophic hormone; CRH, corticotropin-releasing hormone; IL, interleukin; TPA, 12-O-tetradecanoylphorbol-13-acetate.

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Recently, a human small cell lung cancer cell line, COR-L103, was established by Baillie-Johnson et al. (2). This cell line expresses the POMC gene(3), and secretes predominantly ACTH precursor peptides with a small amount of ACTH (3, 4). There is also a report that dexamethasone does not inhibit expression of the POMC gene by these cells(5). On the other hand, glucocorticoid inhibits POMC gene transcription in rat pituitary (6), and ACTH and β-endorphin release from AtT-20 mouse pituitary tumor cells (7). These findings suggest that the mechanism of ACTH secretion by COR-L103 cells differs from that by pituitary cells.

In this study, we examined the effects of various factors on POMC expression and ACTH secretion by the cell line to elucidate their mechanisms of regulation of POMC expression and ACTH secretion.

MATERIALS AND METHODS

Materials

RPMI1640 and glutamine were purchased from Nissui Phamaceutical Co. Tokyo, Japan. HEPES buffer (1M), sodium pyruvate solution(100mM), insulin-transferrin-sodium selenite media supplement, penicillin (10,000U/ml) - streptomycin (10mg/ml) solution, fetal calf serum (FCS), IL-1β, IL-2, 8-bromo-cAMP, TPA and calcium ionophore A23187 were purchased from Sigma Chemical Co. (St. Louis, MO). CRH, dexamethasone, and somatostatin were purchased from the Peptide Institute (Osaka Japan).

Cell Culture

COR-L103 cells were derived from a patient with small cell lung cancer (SCLC) and generously provided by P. Twentyman (Medical Research Council Centre, Cambridge, England). The cells were cultured as previously described (2) in RPMI -1640 supplemented with 2.5% FCS, human transferrin (10mg/ml), bovine insulin (5mg/ml), sodium selenite (3nM), HEPES buffer (10mM), glutamine (4mM), and sodium pyruvate (1mM). Penicillin (100U/ml) and streptomycin (100μg/ml) were also added. Incubation was carried out at 37℃ under 5% CO_2 in air.

Experimental Design

All experiments were performed in quadruplicate. For each experiment, the cells were grown to the stationary phase in a 75 cm^3 flask, and then samples of 1 ml containing 10^5 cells were transferred to 24-well dishes with fresh medium at the same cell density. In the time course study, CRH (1μg/ml), dexamethasone (100nM), somatostatin (1μM), IL-1β (1.0 pg/ml), IL-2 (40 U/ml), 8-bromo-cAMP (2.5mM), TPA (10nM) or A23187 (500nM)(final concentration) was added to the incubation medium, and then incubated for 0-12 hr or 1-4 days. In the dose-response study, TPA (0-100nM) or A23187 (0-5000 nM) was added to the medium and then incubated for 2 days. For

study of the interaction of TPA with A23187, TPA (10 nM) and A23187 (500 nM) were added together, and incubated for 1- 4 days. The medium was stored at -40℃ until measurement of ACTH.

Immunoradiometric Assay (IRMA) of ACTH

ACTH was measured with an ACTH IRMA kit "Mitsubishiyuka" (Mitsubishiyuka, Co. Tokyo, Japan) as reported previously (8).

Statistical Analysis

All data are expressed as means±SEM. statistical significance were assessed by Student's *t* - test, and a vale of $P<0.05$ was regarded as significant.

RESULTS

The number of culture cells of each group did not show any change during the incubation period (0-4 day), and ACTH release from control cells were the same as previouse report (4). CRH, dexamethasone, somatostatin , IL-1β and IL-2 had no effects on ACTH secretion on either short-term (0-8 hr) or long-term (1-4 days) incubation (data not shown). As shown in Fig.1, 8-bromo-cAMP had no effect and TPA and A23187 also had no effects on ACTH secretion on short-term incubation, but on long-term incubation, 500 nM A23187 inhibited ACTH secretion, whereas 10nM TPA induced 2-fold increase in its secretion after 4 days incubation (Fig. 1). TPA stimulated ACTH secretion from the COR-L103 cells dose- dependently at concentrations of

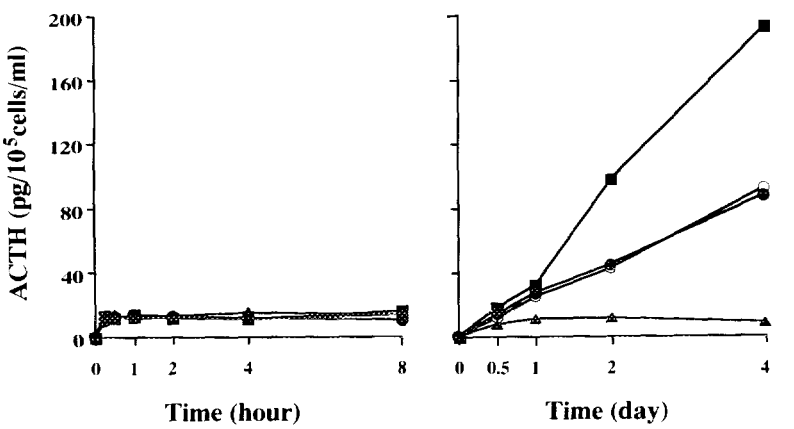

Fig. 1. Effects of 8-bromo-cAMP, TPA and A23187 on ACTH secretion from COR-L103 cells(10^5 cells/ ml) in short-term and long-term incubations. (○-○), control; (●-●), 2.5mM 8-bromo-cAMP; (■-■), 10nM TPA; (▲-▲), 500nM A23187. Values are means for quadruplicate experiments.

1-100nM, whereas, A23187 inhibited ACTH secretion dose- dependently at concentrations of 500 nM and 5 mM (Fig. 2). TPA at 10nM abolished the inhibitory effect of 500 nM A23187 on ACTH secretion (Fig. 3).

DISCUSSION

The mouse pituitary cell line AtT-20 synthesizes ACTH precursor (9), which is finally cleaved to ACTH and β-endorphin (10) during packaging into secretory granules (11), and secretes mature ACTH in response to glucocorticoid (7), CRH, IL-1 and IL-2 (12). On the other hand, ectopic ACTH secretion from human thymic carcinoid tumor cells is not affected by administration of CRH or cortisol (13). Clark et al. also found that glucocorticoid has no effect on POMC gene expression or peptide secretion from COR-L103 cells (5), suggesting that the mechanisms of ACTH secretion by COR-L103 cells and normal pituitary cells or AtT-20 cells are different.

In our experiment, none of the secretagogues examined except TPA and A23187 had any effect on ACTH secretion in short-term or long-term

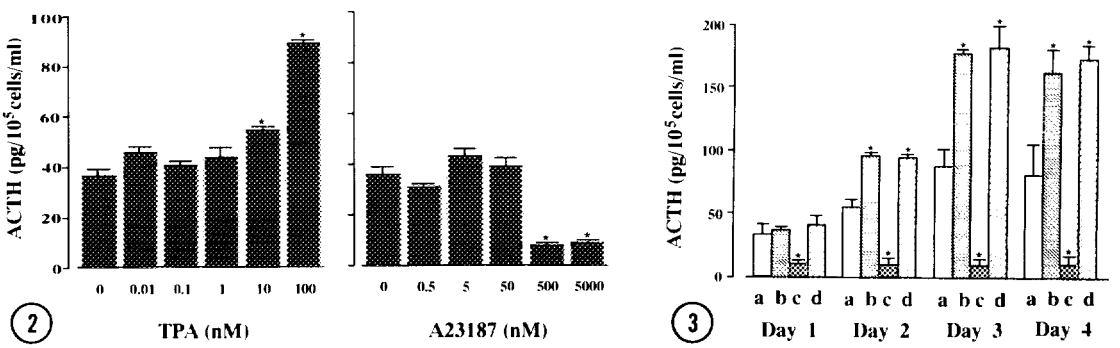

Fig. 2. Effects of various concentrations of TPA (left) and A23187 (right) on ACTH secretion from the COR-L103 cell line (10^5 cells/ ml) after 2-day incubation. Values are means±SEM of quadriplicate experiments. *, P<0.01vs control. Stars show significant differences from the control.

Fig. 3. Effect of TPA on A23187-induced inhibition of ACTH secretion from the COR-L103 cell line (10^5 cells/ ml) after incubation for 1, 2, 3 and 4 days. Incubations: a, control; b, with 10 nM TPA; c, with 500 nM A23187; d, with 10nM TPA + 500 nM A23187. Values are means±SEM for quadruplicate experiments. *, P<0.01 vs control. Stars show significant differences from the control.

incubations. These results suggest that COR-L103 cells release ACTH by unique mechanism.

The calcium ionophore A23187, is known to stimulate the secretions of various hormones from endocrine cells by increasing the intracellular calcium concentration. For example, it stimulates β-endorphin secretion from AtT-20 cells (14). However, Mitchell et al. (15) recently reported that it inhibits endothelin (ET) secretion from normal human umbilical vein endothelial cells in culture. Its inhibitory effect on ET production is highly unusual for at least two reasons. First, no other agents are known to suppress ET production. Second, calcium ionophores usually stimulate hormone secretion. Thus, Mitchell et al. suggested that increase in intracellular calcium may inhibit ET production. On the other hand, TPA, which activates protein kinase C (PKC), stimulates the secretions of most hormones. In our study, A23187 completely inhibited ACTH secretion, and POMC expression (data not shown). This effect may have been due to suppression of POMC production, and/or inhibition of cell proliferation. Since TPA abolished the inhibitory action of A23187 on ACTH secretion in COR-L103 cells, A23187 may inhibit POMC expression and ACTH secretion by an effect at a step prior to PKC. The exact mechanism of the inhibitory action of A23187 remains to be studied.

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