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CONCANAVALIN-A STIMULATES HUMAN CHORIONIC GONADOTROPIN (hCG) AND hCG-ALPHA SECRETION BY HUMAN CHORIOCARCINOMA CELLS*

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Summary: The plant lectin Concanavalin-A stimulates the secretion of human chorionic gonadotropin and free alpha subunit by cultured human choriocarcinoma cells in a dose dependent and time dependent manner. This stimulation is prevented by alpha-methyl-D-mannopyranoside, a Concanavalin-A specific hapten sugar. This is the first report of a lectin stimulating the secretion of a glycoprotein hormone. Since the stimulation likely occurs subsequent to interactions at the membrane level, Concanavalin-A may represent a probe for studying membrane-related events involved in the control of human chorionic gonadotropin secretion.

INTRODUCTION

The control of secretion of hCG[1] by normal placenta is incompletely understood. Cultured human choriocarcinoma cells retain several characteristics of the normal trophoblast cell (1), and therefore provide a useful model for studies of hCG. Thus choriocarcinoma cells respond to several agents of small molecular weight, such as dbcAMP[1], theophylline and methotrexate with increased hCG secretion (2-6). These agents are thought to act at an intracellular level. We recently reported that EGF[1], a polypeptide with a molecular weight of about 6,000, which specifically binds to membranes of placenta (7), binds also to human choriocarcinoma cells and stimulates hCG secretion with little effect on the secretion of free hCG-alpha subunit (8,9). The presence of a receptor on the plasma membrane for EGF led us to investigate whether Con-A[1], a well-

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[1]Abbreviations: Human chorionic gonadotropin, hCG; dibutyryl cyclic adenosine monophosphate, dbcAMP; epidermal growth factor, EGF; concanavalin-A, Con-A; Dulbecco's modified Eagle medium, DMEM; alpha-methyl-D-mannopyranoside, MAM; radioimmunoassay, RIA.

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characterized plant protein which binds to specific carbohydrate groups on the cell membrane (10), would influence the choriocarcinoma cell function. We report that Con-A stimulates the secretion of hCG and free hCG-alpha by JEG-3 cells.

METHODS

JEG-3 cells were generously provided by Dr. P.O. Kohler (University of Arkansas, Little Rock). Cells were cultured in 60 x 15 mm plastic culture dishes in DMEM[1] enriched with 10% fetal calf serum at 37°C in a 90% air-10% CO_2 admosphere. For each experiment, cells were collected by scraping, pooled, aliquoted to replicate dishes and fed DMEM containing 10% fetal calf serum. At about 30% confluency the cells were transferred to serum-free medium and thereafter medium changes took place each 24 hours. Following 3 days in serum-free medium, cells were exposed for 24 hours to DMEM alone (control) or DMEM containing Con-A (Pharmacia) and/or MAM[1] (Sigma) according to specific experimental manipulations described in the Figure legends. The medium of treated cultures was collected daily, centrifuged to remove cell debris, and stored at -20°C until hCG and hCG-alpha were measured. After removal of culture medium, the cell sheet was collected by scraping, washed with 0.9% sodium chloride, solubilized in 2 ml of 1 N sodium hydroxide and an aliquot was used for protein quantitation by the Bradford method (11) using bovine gamma globulin (Bio-Rad Laboratories) as a standard. The concentrations of immunoreactive hCG and hCG-alpha were determined by homologous RIA[1]; there was no significant cross-reactivity of hCG-alpha in hCG RIA, or of hCG in the hCG-alpha RIA (12). Cell media were diluted fifty-fold in phosphate buffered saline, pH 7.5 containing 1% bovine serum albumin before RIA. In control studies we have shown that undiluted medium containing 20 μg/ml of Con-A was without effect on hCG and hCG-alpha RIAs.

RESULTS

The stimulatory effect of Con-A on hCG and hCG-alpha secretion is shown in Fig. 1. The degree of stimulation was similar for hCG and hCG-alpha. There is a small but reproducible inhibitory effect observed at very low concentrations of Con-A, while levels of 200 μg/ml or greater have a toxic effect upon the cells.

The time course of Con-A stimulatory effect on hCG and hCG-alpha secretion was evaluated at a dose of 10 μg/ml (Fig. 2). The maximal stimulatory effect for both hCG and hCG-alpha takes place 24 hours after removal of Con-A from the medium.

The specificity of the response to Con-A was evaluated by examining the effect of MAM. Results are shown in Fig. 3. This sugar totally inhibited the stimulatory effect of Con-A while having no independent effect on the secretion of hCG or hCG-alpha.

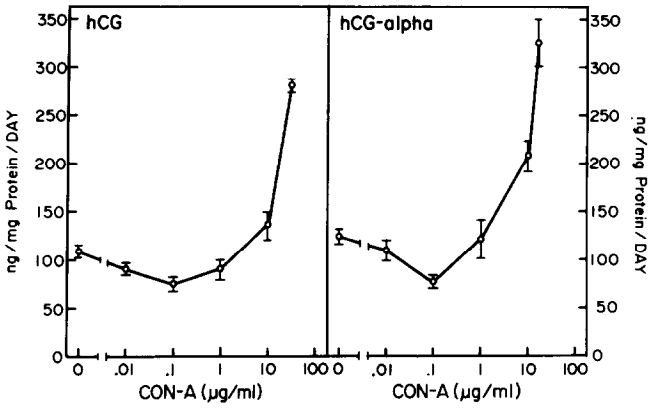

Figure 1. Effect upon hCG and hCG-alpha of varying concentrations of Con-A. After 3 days of serum-free medium, cells received DMEM alone or DMEM containing from 0.01 to 22.5 μg/ml Con-A. After 24 hours, the medium was collected for hCG (left panel) or hCG-alpha (right panel) measurement by specific RIA and the cell sheet collected for protein measurement. Each point represents the mean of three dishes ± S.E.M.

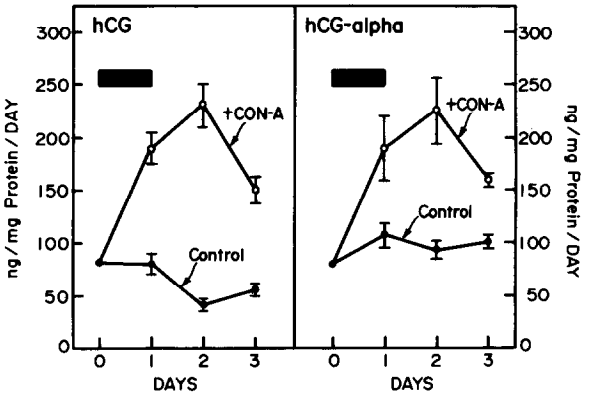

Figure 2. Time course of hCG and hCG-alpha stimulation by Con-A. Following 3 days in serum-free medium, cells were refed with DMEM alone (●) or DMEM + 10 μg/ml Con-A (o) (Day 0). Medium was changed and replaced with DMEM alone at 24 and 48 h (Day 1 and 2, respectively). The black bar represents the length of time Con-A was present on the cells. At the indicated time, medium was collected for hCG (left panel) and hCG-alpha (right panel) measurement and the cell sheet collected for protein measurement. Each point represents the mean of three dishes ± S.E.M.

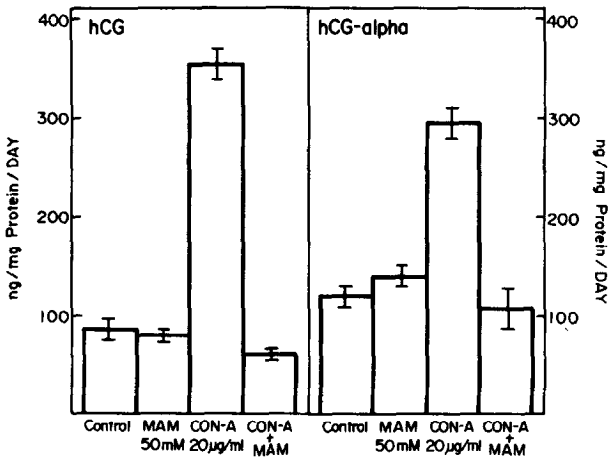

Figure 3. Effect of alpha-methyl-D-mannopyranoside (MAM) on Con-A stimulated secretion. Following 3 days of serum-free DMEM, cells were fed on Day 0 with DMEM alone (Control), DMEM + 50 mM MAM (MAM), DMEM + 20 µg/ml Con-A (Con-A) or DMEM + 50 mM MAM + 20 µg/ml Con-A (Con-A + MAM). On Day 1, medium was removed and replaced with DMEM alone. On Day 2, medium was collected and assayed for hCG (left panel) and hCG-alpha (right panel) and cell sheet collected for protein measurement. Each point represents the mean of three dishes ± S.E.M.

DISCUSSION

Hormonal activity on various target tissues has been reported to be affected by Con-A and other lectins. Czech and Lynn showed that binding of Con-A to isolated adipocytes was associated with insulin-like activity (13). This observation was confirmed by others (14,15) and it was suggested that the insulin-like activity of Con-A on isolated adipocytes was due to its interaction with the insulin receptor on these cells. Costlow and Gallagher demonstrated that Con-A prevents the binding of prolactin to its receptor on hepatocytes (16). Carpenter and Cohen reported that Con-A and other lectins prevent EGF binding to human fibroblasts but do not mimic the mitogenic effect of EGF (17). Recently, Hashimuze et al. (18) reported the influence of Con-A on TSH-induced cAMP formation in mouse thyroid lobes.

Herein we report that the secretion of a glycoprotein hormone by choriocarcinoma cells is influenced by Con-A. In addition to the stimulatory effect on hCG, Con-A also stimulates the secretion of free-hCG alpha subunit. This

stimulation occurs in a dose-dependent and time-dependent manner. The stimulatory effect can be prevented by the addition of MAM, a Con-A specific hapten sugar, and this is consistent with the well-recognized specificity of Con-A to mannose residues on plasma membrane glycoproteins (19). Ewart et al. (20) reported that several lectins induce a rapid release of insulin and glucagon from isolated rat islets of Langerhans. The time course of these effects differs from that associated with the stimulatory effect of Con-A on hCG. This is not surprising when contrasting effects on pancreatic islets where there are significant intracellular hormonal stores with those on the JEG-3 cell which exhibits little hCG storage. Therefore, the mechanisms involved in the overall secretory process may be entirely different with, in this case, the modification at the cell surface influencing delayed biosynthetic processes rather than the more immediate release of stored products of secretion.

The stimulatory effect of Con-A on JEG-3 cells differs with that effect of EGF which stimulates hCG secretion but has little effect on hCG-alpha secretion. In contrast to the long-lived stimulatory effect of Con-A, EGF-initiated hCG secretion declines rapidly with removal of EGF from the medium (8). These observations may reflect a difference in the mechanism of action of these two proteins following their binding to the JEG-3 cells. Interestingly, the stimulatory effect of Con-A ressembles the effect obtained with dbcAMP; dbcAMP stimulates both hCG and hCG-alpha by JEG-3 cells (4) and its maximal stimulation is also observed 24 hours after its removal from the medium (21). The possibility of Con-A raising intracellular cAMP levels which in turn induce hCG secretion is an attractive notion which remains to be demonstrated.

Although the mechanism by which Con-A stimulates gonadotropin secretion remains to be elucidated, Con-A should prove to be a useful probe for studying how transduction of signal from the membrane results in hormone production.

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