CORTISOL STIMULATION OF GROWTH HORMONE PRODUCTION BY MONKEY ADENOHYPOPHYSIS IN TISSUE CULTURE

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Many of the factors that affect pituitary hormone secretion in vivo appear to act on the hypothalamus rather than directly on the pituitary. In order to study the effect of cortisol on growth hormone (GH) production in the absence of variable neural and vascular influences, we have examined the growth hormone production by pituitaries in tissue culture. We have found that cortisol stimulates production of immunoreactive growth hormone by monkey adenohypophysis in monolayer culture. This effect occurs at cortisol concentrations as low as 10^{-7}M and appears to require RNA and protein synthesis, but not DNA synthesis.

MATERIALS AND METHODS

Anterior lobes of pituitaries from Macacca mulatta monkeys were finely minced into explants approximately 0.2 mm in diameter. These were cultured in 30 ml plastic flasks in Medium 199 containing 20% fetal bovine serum and 50 units penicillin and 50 µg streptomycin per ml. Studies were performed after the pituitaries had been in culture for one to six months and after confluent monolayers had developed.

The medium was changed every 24 hours during the test periods and frozen for subsequent growth hormone determinations. After four control days, medium containing cortisol was added to the cultures for three to ten days. Cultures treated with combinations of cortisol and cyclo-

heximide, Actinomycin D, or hydroxyurea were pretreated for two hours with media containing the inhibitor alone before the addition of cortisol.

Growth hormone concentrations in the media were measured by radioimmunoassay (1). Human GH (Lot H3475A, Endocrine Study Section, NIH) which cross-reacts with monkey GH (2) was used for iodination and as a standard. The precipitation curve for monkey GH in media was parallel to the curve for the human GH standard in the area used for quantitation. The monkey GH concentrations have been expressed in terms of the human GH standard. Determination of 3H-thymidine incorporation into DNA was measured by methods described previously (3).

RESULTS

After several weeks in culture, daily production of GH by any single

TABLE I

RELATIONSHIP OF CORTISOL CONCENTRATION IN MEDIA TO GROWTH HORMONE PRODUCTION

Cortisol Concentration	Change in GH Content in Media as % of Pretreatment Levels	SD of Ratio
10^{-5}M	272%	20.7%
10^{-6}M	249%	29.4%
10^{-7}M	190%	30.4%
10^{-8}M	71%	15.6%
10^{-9}M	63%	7.5%

Table 1. The GH concentrations in media during cortisol treatment were expressed as a percent of mean pretreatment concentrations. The declines in GH content at 10^{-8}M and 10^{-9}M cortisol are of typical untreated cultures.

culture varied only slightly. GH concentrations in the media of untreated cultures typically showed a slow decline to undetectable levels at four to six months. The addition of cortisol at concentrations as low as 10^{-7}M (Table 1) caused a marked increase in GH content in the media (Fig. 1). This GH increase did not occur after the addition of the biologically inactive analog, 11 α-hydroxycortisol, or after estradiol, testosterone, or progesterone were added to the cultures at similar concentrations (4).

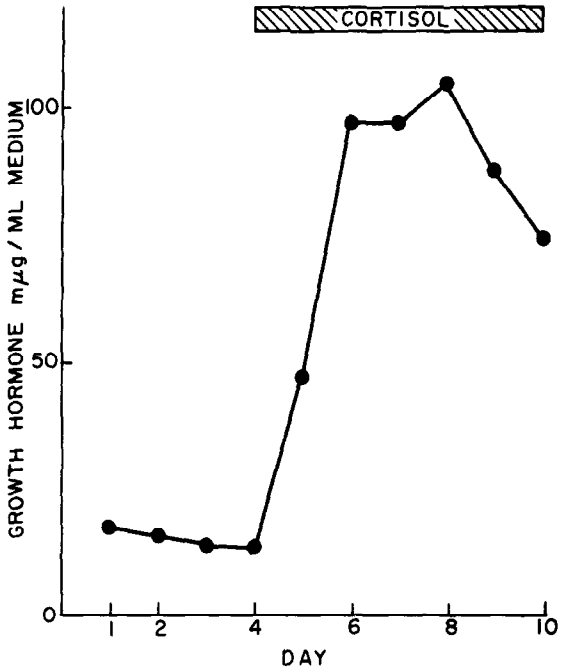

Figure 1. Effect of 5 x 10^{-6}M cortisol in the medium on daily GH production. Complete changes of medium were made daily.

Maximal GH concentrations which varied from 180 to 500% of mean control levels were achieved 96 hours after the addition of cortisol. Peak GH levels were not sustained, but GH concentrations remained higher than baseline levels during the entire period of cortisol treatment.

Cycloheximide at 1.0 μg/ml medium markedly inhibited GH production and blocked the induction of GH by cortisol (Fig. 2). However, when cycloheximide was stopped and cortisol continued, the increase of GH

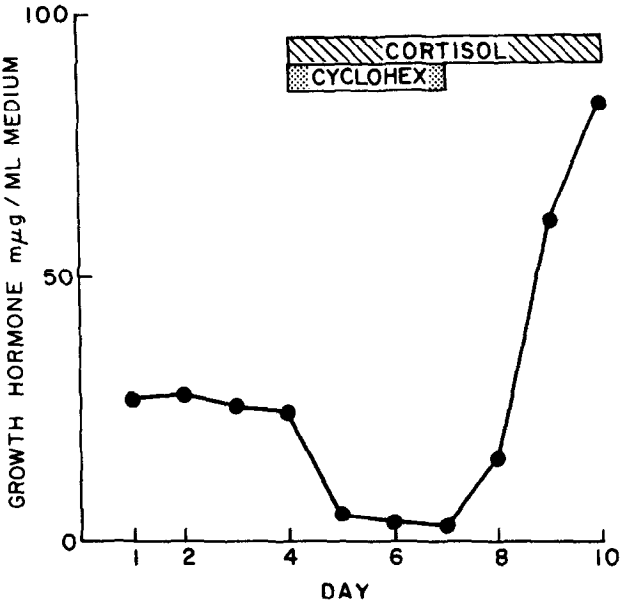

Figure 2. Cycloheximide 1.0 μg/ml was added two hours prior to 5 x 10^{-6}M cortisol. Cycloheximide was stopped after three days and cortisol was continued.

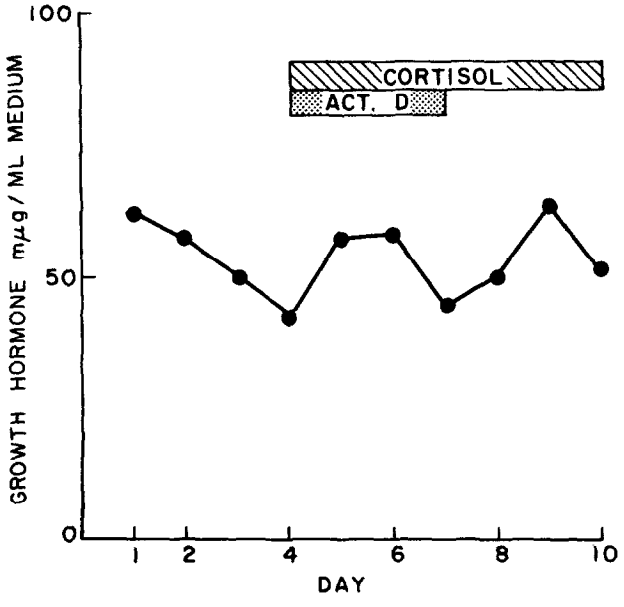

Figure 3. Actinomycin D (Act. D) 0.1 μg/ml was added two hours prior to 5 x 10^{-6}M cortisol. Higher concentrations of Actinomycin D were cytotoxic.

production occurred promptly. The presence of Actinomycin D at 0.1 µg/ml medium blunted the GH response to cortisol (Fig. 3). Hydroxyurea at 10^{-3}M blocked 3H-thymidine incorporation into DNA by more than 55%, but did not block the effect of cortisol on GH production (Fig. 4). At the concentrations used in this study, the inhibitors caused no histologic evidence of toxicity, although concentrations of Actinomycin D higher than 0.1 mg/ml were definitely cytotoxic.

To examine degradation of GH in this system, media from early cultures producing large amounts of hormone was diluted with fresh Medium 199 and added to other actively metabolizing pituitary cultures. Samples were taken at 0, 6, 12, and 24 hours and assayed for GH. After correction for GH produced by the test cultures, disappearance of added GH from the media was found to be less than 10% per 24 hours.

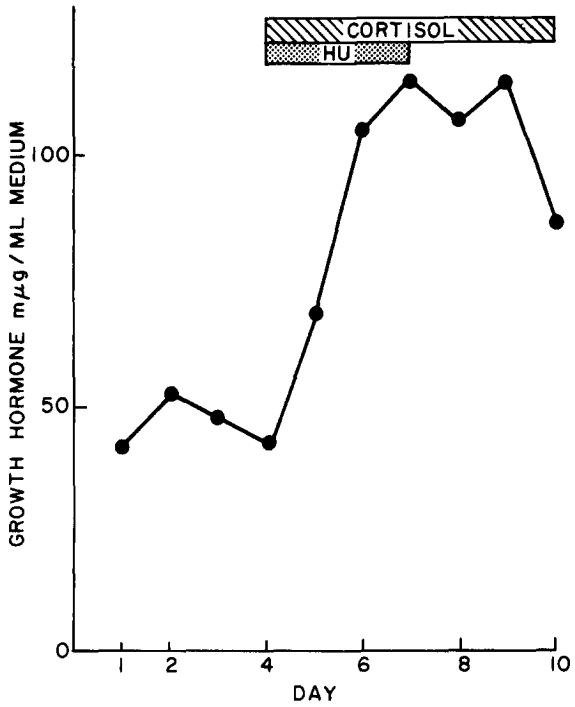

Figure 4. Hydroxyurea (HU) 0.001 M was added two hours prior to 5 x 10^{-6}M cortisol. This concentration of hydroxyurea inhibits DNA synthesis more than 55% in this system.

DISCUSSION

The present studies have demonstrated that the addition of cortisol to monolayer cultures of monkey adenohypophysis causes marked elevations in the GH production by the cultures. The increases of GH levels in the media after cortisol could be the result of either 1) decreased degradation of GH in the media, 2) release of preformed GH from the cells, or 3) synthesis of new protein. The first possibility, that of decreased GH degradation, is inadequate to explain the magnitude of the GH elevation after cortisol because baseline GH degradation is quite low. Although cortisol can apparently alter membrane structure (5,6), the findings that cycloheximide and Actinomycin D blocked the response to cortisol exclude a simple change in membrane permeability resulting in the passive release of preformed GH as the mechanism of action for cortisol. The final hypothesis that cortisol acts through the synthesis of new protein is supported by the blocking effect of cycloheximide on the GH response. Cortisol does cause an increase in GH production after the removal of cycloheximide indicating that the induction can occur with the resumption of protein synthesis. Although Actinomycin D may affect processes other than DNA-directed RNA synthesis (7,8), the inhibition of the GH rise after cortisol by this antibiotic is at least suggestive evidence that RNA synthesis might be a prerequisite for the response. The failure of hydroxyurea to inhibit the GH elevation after cortisol indicates that new DNA synthesis is probably not required for the induction.

It is of interest that glucocorticoids decrease rather than stimulate rat GH secretion *in vivo*, probably by interference with the hypothalamic mechanism through which GH is released from the pituitary (9). However, cortisol has also been shown to inhibit rat GH release by pituitaries *in vitro* during short-term incubations (10). The reasons for the apparent discrepancy between the effect of cortisol during 60-

hour incubations (10) and in the present study are not clear at this time although the systems are quite different. It is possible that cortisol reverses or delays the dedifferentiation of normal cells that occurs with time in culture (11). Glucocorticoids directly induce enzymes (12-14) or are required for expression of differentiated characteristics in other in vitro systems (15).

The results of the present study clearly demonstrate that cortisol can act directly on pituitary cells in culture causing an increase of GH production. These initial studies suggest a subcellular mechanism for the cortisol induction of GH requiring protein and probably RNA synthesis, but not DNA synthesis. This system shows promise as a model with which to study mechanisms of steroid action as well as the interrelationship between glucocorticoids and pituitary hormone production in vitro.

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