PRODUCTION OF BOTH PROLACTIN AND GROWTH HORMONE BY CLONAL STRAINS OF RAT PITUITARY TUMOR CELLS

Differential Effects of Hydrocortisone and Tissue Extracts

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ABSTRACT

Several established clonal strains of rat pituitary cells which produce growth hormone in culture have been shown to secrete a second protein hormone, prolactin. Prolactin was measured immunologically in culture medium and within cells by complement fixation. Rates of prolactin production varied from 6.6 to 12 μg/mg cell protein per 24 hr in four different cell strains. In these cultures ratios of production of prolactin to growth hormone varied from 1.0 to 4.1. A fifth clonal strain produced growth hormone but no detectable prolactin. Intracellular prolactin was equivalent to the amount secreted into medium in a period of about 1–2 hr. Both cycloheximide and puromycin suppressed prolactin production by at least 94%. Hydrocortisone (3×10^{-6} M), which stimulated the production of growth hormone 4- to 8-fold in most of the cell strains, reduced the rate of prolactin production to less than 25% of that in control cultures. Conversely, addition of simple acid extracts of several tissues, including hypothalamus, to the medium of all strains increased the rate of production of prolactin six to nine times and decreased growth hormone production by about 50%. We conclude that multifunctional rat pituitary cells in culture show unusual promise for further studies of the control of expression of organ-specific activities in mammalian cells.

INTRODUCTION

A number of clonal strains of rat pituitary tumor cells have been established in culture (1, 2). Each of these strains of epithelial cells carries out organ-specific function in vitro; namely, they synthesize growth hormone and secrete the protein into the culture medium. Three of these strains have been serially propagated for over 4 yr without exhibiting any loss of ability to produce growth hormone. In fact, the rate of growth hormone production for the most extensively studied strain, GH_3, has actually increased by a factor of three to five times since the line was established (3).

Recent experiments have suggested that these

cell strains may prove to be useful model systems in which to study control mechanisms in the biosynthesis and secretion of proteins, such as hormones, made specifically for export from the cell of origin. For example, Bancroft, Levine, and Tashjian (3) and Kohler et al. (4) have examined the characteristics of the stimulation by hydrocortisone of growth hormone production in the GH_3 and GH_1 strains, respectively.

When the cultured pituitary cells are injected into female rats of the Wistar/Furth strain, they almost invariably give rise to tumors (5). Concomitant with the appearance of the tumor, both intact and hypophysectomized rats begin to grow rapidly (5). All organs are greatly enlarged, as is the length of the long bones. The tumor-bearing animals may have body weights which are two to four times greater than those of control rats. In the course of examining the organs of tumor-bearing animals it was noted that there was extensive development of the mammary glands. This was first attributed to excessive production of growth hormone by the tumor. However, because the tumor (MtT/W5) from which the cultured cells were derived was reported to have marked mammary gland-stimulating properties (6, 7), the possibility existed that the culture-derived tumors were also producing, in addition to growth hormone, a substance which stimulated the mammary gland specifically, namely prolactin. We therefore undertook to determine whether these pituitary cells in culture produced prolactin, a protein hormone which is synthesized by the pituitary gland, and in the rat is chemically distinct from growth hormone (8–10).

In this report we describe the results of experiments which show that most of the existing clonal strains of growth hormone-producing cells also synthesize and secrete prolactin at rates comparable to those of growth hormone. In addition, we have found that while hydrocortisone stimulates the production of growth hormone in all but one of these cell strains, this steroid hormone suppresses the production of prolactin. Conversely, the addition of simple extracts of several different tissues to the culture medium has been found to stimulate the production of prolactin and suppress the production of growth hormone. We believe that the ability to stimulate the production of one exportable protein while suppressing the production of another makes these cell strains promising model systems for studying the mechanisms which underlie the control of expression of differentiated function in mammalian cells.

MATERIALS AND METHODS

Materials

Hydrocortisone sodium succinate was obtained from the Upjohn Co., Kalamazoo, Mich. Stock solutions of HC[1] were made in 0.15 M NaCl at a concentration of 9×10^{-5} M. They were stored at 4°C for no more than 1 wk. Cycloheximide and puromycin dihydrochloride were purchased from Nutritional Biochemicals Corp., Cleveland, Ohio. Highly purified rat prolactin (Fraction D) was kindly donated by Dr. Albert F. Parlow (UCLA). This preparation of prolactin has specific biological activity of 15–20 international units/mg as determined by the intradermal pigeon crop sac assay method (10). It was assayed immunologically for contamination with growth hormone by complement fixation (see below) and was found to contain about 0.2% growth hormone. This small degree of contamination does not introduce a significant error in estimates for prolactin when this material is used as standard in our immunoassay method. Likewise, since rat growth hormone is an extremely poor immunogen in rabbits (11), this preparation of prolactin, when used for immunization, did not give rise to detectable antibodies to growth hormone (see below).

Methods of Culture

The origin and methods of culture of three of the rat pituitary tumor cell strains (GH_1, GH_1C_1, and GH_3) have been described in detail previously (1, 2, 5). The GH_1 cells which were used in the present experiments had been frozen for 34 months. They were thawed and placed in culture 5 months before these studies (2). Two new strains, not previously described, have also been used in this report. These strains are designated GH_3C_1 and GH_3C_6. They are subclones of GH_3 which were established in June 1969 by plating single GH_3 cells in micro-Petri dishes (6 × 10 mm, Linbro Chemical Co., New Haven, Conn.) containing 0.1 ml of medium (F. C. Bancroft and A. H. Tashjian, Jr. In preparation).

Experiments were performed in plastic tissue-culture dishes (50 × 15 mm, Falcon Plastics, Division of B-D Laboratories, Inc., Los Angeles, Calif.) containing 3 ml of Ham's F 10 medium (12) supplemented with 15% horse serum and 2.5% fetal calf serum. The dishes were incubated at 37°C in a humidified atmosphere of 5% CO_2 and 95% air.

Experiments designed to measure cell function were performed in the following way. Equal samples

[1] Abbreviations used: HC, hydrocortisone sodium succinate; C′, complement; GH, growth hormone.

of a homogeneous suspension of cells were inoculated into a number of replicate dishes. Cells were grown for a minimum of 3 or 4 days before starting an experiment. Thereafter, medium, either with or without specific additions (such as HC or tissue extracts, see below), was added and changed every 1, 2, or 3 days. Dishes from which medium was saved for hormone assays were washed three times with 0.15 M NaCl and stored at −20°C until cell protein was determined by the method of Lowry et al. (13). Duplicate dishes rarely varied by more than 3% in total cell protein.

Assay Method for Prolactin

Rat prolactin was measured immunologically in culture medium or in cells disrupted with sonic oscillation by the method of complement (C′) fixation (14). This method is analogous to techniques previously described in detail which are used in our laboratories for the measurement of a variety of growth hormones (11, 15–17) and rat serum albumin (18). The antiserum used in the present experiments was obtained in a single bleeding from a rabbit immunized with a total of 1.0 mg of rat prolactin. The antigen was emulsified in complete Freund's adjuvant (Difco Laboratories, Inc., Detroit, Mich.) and injected in the toe pads and intramuscularly. The antiserum (Ra 410C-2) was used in C′-fixation experiments at a final dilution of 1:400. No evidence for antibodies to contaminating substances was observed (see Results) when this antiserum was examined by double diffusion (19) or by C′ fixation with large amounts of crude antigen (20). The reproducibility of the immunoassay method for prolactin as performed in these experiments is ± 20%.

Culture medium for immunoassay was stored at −20°C. No loss of prolactin was detected during storage for periods of up to 5 months; however, most assays were performed within 1–2 wk after collecting the medium. Samples were prepared for assay by dilution (1:3 to 1:25) in the C′-fixation buffer followed by heating at 60°C for 20 min to eliminate any anticomplementary activity in the medium. This procedure did not qualitatively or quantitatively affect prolactin in the medium. The prolactin standard was treated in the same manner.

Assay for Growth Hormone

Rat GH was assayed immunologically by C′ fixation using monkey antiserum as described previously (1, 11). This assay method is specific for GH and does not detect rat prolactin. The reproducibility of the growth hormone assay is ±20%.

Preparation of Tissue Extracts

Extracts of four bovine (calf) tissues were prepared. The tissues used were hypothalamus, cerebral cortex, liver, and kidney. They were obtained at a local abattoir within seconds after death of the animal. They were transported in ice to the laboratory where they were processed within 1–2 hr. All tissues were handled in a similar manner.

A partially defatted powder was made by homogenizing the tissue with acetone (200 ml/gm fresh weight) at 4°C. After stirring with acetone for 18 hr, the insoluble material was removed by filtration. It was washed on the filter three times with fresh cold acetone and once with ether and then dried over phosphorus pentoxide. The dry powder was extracted by stirring with 0.1 N HCl (10 ml/gm) for 30 min at 25°C. The insoluble material was removed by centrifugation at 10,000 *g* for 30 min. The supernatant solution was the material added to culture medium.

The acidic extracts were added to medium in one of two ways. In one method, the pH of the extract was adjusted to 7.5 by the gradual addition of NaOH. The precipitate which formed on neutralization was removed by centrifugation, and the clear neutral solution was added to medium (1 part extract to 20–160 parts medium). In the second method, the acidic extract was added gradually to culture medium with maintenance of the pH above 7.2 by the simultaneous addition of NaOH. The final volume ratio of extract to medium was again 1 to 20–160. As described previously, insoluble material was removed by centrifugation. Medium containing extracts was sterilized by filtration. Results obtained with extracts added to medium in the two different ways were qualitatively and quantitatively the same.

RESULTS

Demonstration of the Production of Prolactin by Rat Pituitary Cells in Culture

The C′-fixation curves obtained with antisera raised against prolactin and GH and their homologous antigens are shown in Fig. 1. Fig. 1 also shows that GH did not fix C′ with anti-prolactin, and that prolactin did not fix C′ with anti-GH. Results of previous studies have shown the usefulness of the C′-fixation assay method for measuring GH production by rat (1, 3) and human (17, 21) pituitary cells in culture. This method, using antiserum against rat prolactin, was thus employed to determine whether the rat pituitary cells produced prolactin.

The data in Fig. 2 show that material was pres-

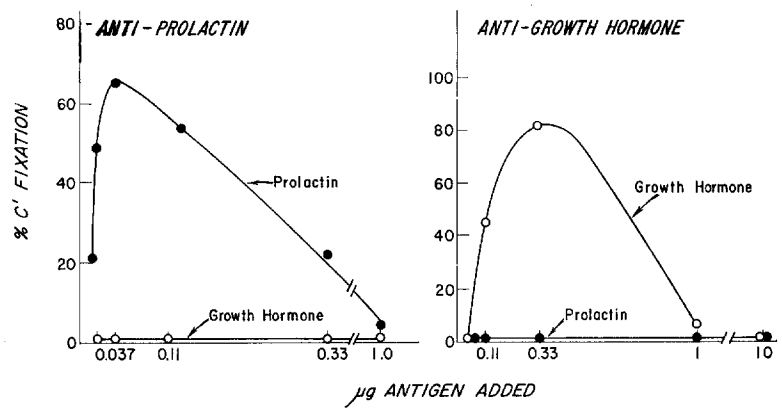

FIGURE 1 C′-fixation curves obtained with rabbit antiserum against rat prolactin (diluted 1:400), and monkey antiserum against rat GH (diluted 1:1000).

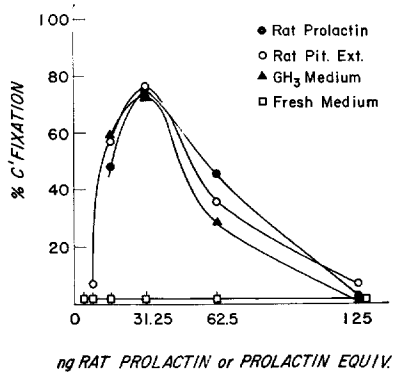

FIGURE 2 C′-fixation curves obtained with antiserum against rat prolactin (diluted 1:400) and rat prolactin (●), crude rat pituitary extract (○), and medium from the GH_3 strain of pituitary cells (▲). Fresh, uninoculated medium (□) did not fix C′ with anti-prolactin. The rat pituitary extract was prepared by homogenizing 10 fresh pituitary glands in 3 ml of neutral 0.15 M NaCl. The insoluble material was removed by centrifugation (10,000 *g*, 20 min) and the clear supernatant solution was used without further fractionation. Starting with dilutions of this extract as low as 1:4, only one peak of C′ fixation was observed.

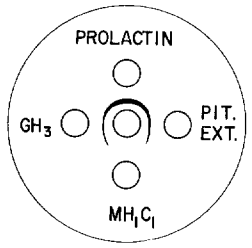

FIGURE 3 Tracings of bands of precipitation seen in agar by double diffusion for 48 hr at 25°C (19). Undiluted rabbit anti-prolactin was placed in the center well. Purified rat prolactin (20 μg), 0.15 ml undiluted crude pituitary extract, 0.15 ml medium from rat hepatoma cells (MH_1C_1), and 0.15 ml medium from rat pituitary cells (GH_3) were placed in the top, right, bottom, and left wells, respectively. No precipitation was seen when rat GH (50 μg) was allowed to diffuse against anti-prolactin.

ent in medium of GH_3 cells and in crude rat pituitary extract, but not in fresh medium, which fixed C′ with anti-prolactin. Furthermore, the maximum C′ fixed was the same for rat prolactin, GH_3 medium, and pituitary extract. These results indicate strongly that the same antigen is present in all three samples. The identity of the immune systems was also shown by double diffusion (Fig. 3). Only one band of precipitation was seen with undiluted antiserum and prolactin, medium from GH_3 cells, and crude pituitary extract. These three bands of precipitation merged without spur formation. No precipitation was observed with medium from a nonpituitary strain (MH_1C_1) of rat cells.

The specificity of the production of prolactin by cells in culture was examined by assaying immunologically medium from a variety of cell types. The results, summarized in Table I, reveal that, of the cell types tested, only rat pituitary cells produce prolactin.

Stability of Prolactin

The stability of rat prolactin under the conditions of incubation used in the present studies was

TABLE I

Specificity of the Production of Rat Prolactin by Animal Cells in Culture

Cell type	Rat prolactin
	μg/ml medium per 24 hr
Rat pituitary (GH_3)	0.3–2.2*
Rat fibroblast (R_5)	nd‡
Rat hepatoma (MH_1C_1)	nd
Mouse adrenal (Y_{11})	nd
Human fibroblast	nd
Human medullary thyroid carcinoma	nd
Human pituitary	nd
Fresh medium alone	nd

* The lower value given for GH_3 cells was obtained at low population density (0.2 mg cell protein/dish); the higher value was obtained in a more mature culture (1.0 mg cell protein/dish). All media from cell lines other than GH_3 were collected from dishes which had more than 1.0 mg cell protein/dish.

‡ nd, none detected. The assay method would have detected 0.01 μg prolactin/ml medium per 24 hr.

determined. Medium containing added prolactin was placed in dishes which contained either no cells or GH_3 cells. After a 3 day incubation period, the medium was collected and assayed for prolactin. The results (Table II) show that 92% of the prolactin originally added was recovered from the cell-free dishes and 86% from those containing GH_3 cells. Since medium was usually changed at least every 3 days in the experiments reported here, nearly all of the prolactin secreted into the medium during an incubation period was still present when the medium was collected and assayed.

Intracellular vs Extracellular Levels of Prolactin

Intracellular prolactin caused the same maximum amount of C′ to be fixed with anti-prolactin as did either prolactin secreted into the medium or the prolactin standard. Intracellular levels and the rate of production[2] of prolactin are shown in Table III. It can be seen that intracellular levels of prolactin are equal to the amount produced in about 1–2 hr, assuming that the rate of production is constant during the collection period.

[2] By "rate of production" of prolactin or GH we mean the rate of appearance of either protein in the growth medium of the cells in culture (see [3] and Discussion).

TABLE II

Stability of Prolactin

Conditions	Prolactin	Recovery
	μg/ml	%
Stock solution	49	—
GH_3 cells alone	9.4 ± 0.3	—
Stock solution incubated without cells	45 ± 4.0	92
Stock solution incubated with GH_3 cells	51 ± 7.0	86

A stock solution was made by adding rat prolactin to F 10 medium. 3-ml samples of the stock solution were added to two cell-free dishes and to two duplicate dishes containing GH_3 cells. The remaining solution was frozen. Two duplicate dishes of GH_3 cells which received only F 10 medium served as controls to determine the amount of prolactin produced by the cells. After a 72 hr incubation, the medium was removed from each dish and frozen. The collected media and the stock solution were then assayed for prolactin. The ranges of the results in duplicate dishes are shown.

TABLE III

Intracellular vs Extracellular Levels of Prolactin

Experiment No.	Prolactin production	Intracellular prolactin
	μg/mg cell protein per 24 hr	*μg/mg cell protein*
I	23	2.2
II	25	0.90

These results represent mean values of assays of duplicate cultures. Medium was changed and fresh medium was added for a 24 hr period. The cells were then scraped from the dishes, suspended in 3 ml of isotonic saline and treated for 5 min at 1–2°C in a Raytheon Model DF 101 sonic oscillator. After removal of samples for determination of protein, the cell sonicates and the 24 hr medium were assayed for prolactin.

Requirement for Protein Synthesis for Production of Prolactin

The requirement for protein synthesis for the appearance of both prolactin and growth hormone

in medium was verified by an experiment with two inhibitors of protein synthesis. Fig. 4 shows that, following a 30 min preincubation with inhibitor, either cycloheximide or puromycin inhibited the rates of appearance of prolactin and GH in medium by at least 94%. In this experiment, incorporation of labeled amino acids into trichloroacetic acid-precipitable material was inhibited by 93% and 98% by cycloheximide and puromycin, respectively (3).

Rate of Production of Prolactin by Different Clonal Strains of Rat Pituitary Cells

The results given in Table IV show that four of the five clonal strains examined produce at least as much prolactin as GH. One strain, GH_12C_1, which is morphologically more spindle-shaped than the others (1, 2), appears to produce little or no prolactin.

Control of Prolactin Production

Much of our interest in these cells lies in their potential use as model systems in which to study

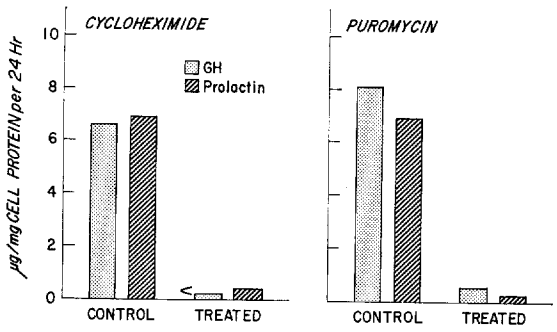

FIGURE 4 Effects of inhibitors of protein synthesis on prolactin and GH production by GH_3 cells. At zero time, fresh medium which had been equilibrated at 37°C in a 5% CO_2 atmosphere was added to each of duplicate dishes. At 12 hr the medium was collected for prolactin and GH assay, and equilibrated medium containing either no additions, cycloheximide (10 μg/ml, 3.6×10^{-5} M), or puromycin (200 μg/ml, 3.7×10^{-4} M) was added. 30 min later this medium was removed and discarded, and fresh medium with the same additions was added. 12 hr later this medium was collected and saved for prolactin and GH assay, and the dishes were washed and frozen for cell protein determination. The control bars show the results without inhibitor. The treated bars show the results after exposure to the inhibitor.

TABLE IV

Production of Prolactin and Growth Hormone by Five Different Clonal Strains of Rat Pituitary Cells

Strain	Prolactin	Growth hormone	Ratio
	μg/mg cell protein per 24 hr		*P/GH**
GH_3	12	12	1.0
GH_1	10	3.8	2.6
GH_12C_1	<0.05	1.7	<0.03
GH_3C_1	15	6.7	2.2
GH_3C_6	6.6	1.6	4.1

* The ratio of the rate of production of prolactin (P) to growth hormone (GH). All cells were in the early stationary growth state (3) at the time these measurements were made.

the control of differentiated function in animal cells. The following experiments were performed in order to define the response of the cells to two different sorts of stimuli. GH_3 cells were used in all of these experiments (unless otherwise stated); however, GH_1 cells exhibited similar responses.

Medium containing hydrocortisone (HC) was added to GH_3 cells. The characteristic stimulation (3) of the rate of GH production was observed (Fig. 5). At about 110 hr, GH production was about eight times that in control cultures. In contrast to the effect on GH, HC in medium suppressed the production of prolactin. By 110 hr the rate of prolactin production in HC-treated cultures was only about 25% that in controls.

Table V shows that, in addition to its characteristic effect on the GH_3 strain, HC suppressed prolactin production in two other strains (GH_3C_1 and GH_3C_6) of pituitary cells. It is interesting to note that GH production by cells of the GH_3C_6 strain was not stimulated by HC. Further studies of this variant line are in progress.

The effects of various concentrations of HC on hormone production were examined. Medium containing HC at concentrations from 5×10^{-10} M to 5×10^{-4} M was added to GH_3 cells. The relationship between dose and response is shown in Fig. 6. Within the range tested, doses of HC which stimulated GH production suppressed the production of prolactin. In addition, it is noteworthy that the greater the stimulation of GH, the greater was the suppression of prolactin.

A series of experiments was undertaken in order to assess the usefulness of the functional rat pituitary cell system for studies of the action of hypo-

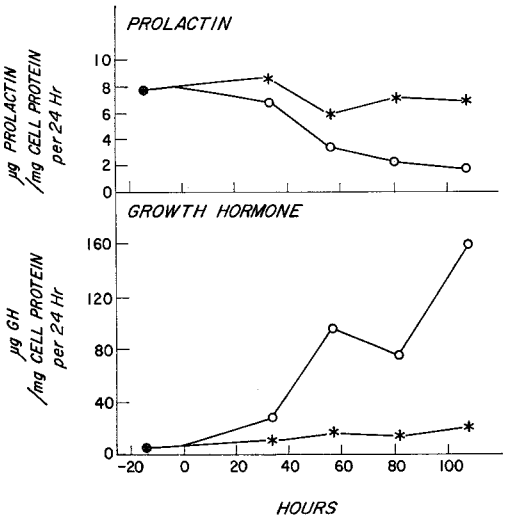

FIGURE 5 Effects of HC on prolactin and GH production. Duplicate dishes were used for each point. At zero time, fresh medium either containing HC (3×10^{-6} M) or lacking HC was added to each dish. Medium was collected at intervals from HC-treated (○) and control (*) dishes and frozen for hormone assays. These dishes were washed and frozen for determination of cell protein.

TABLE V

Effects of Hydrocortisone on Three Different Clonal Strains of Rat Pituitary Cells

Strain	Prolactin		Growth hormone	
	−HC	+HC	−HC	+HC
	μg/mg cell protein per 24 hr			
GH_3	12	4.4	12	72
GH_3C_1	15	2.4	6.7	47
GH_3C_6	6.6	0.7	1.6	1.9

Cells were inoculated in replicate dishes, and medium was changed at least every 3 days. 8 days later, medium containing either no HC (−HC) or HC (+HC, 3×10^{-5} M) was added. Media were changed approximately every 1–2 days thereafter. The results reported are for the interval 117–139 hr after adding HC.

thalamic factors which affect the release and possibly the synthesis of pituitary hormones (22). The results of a representative experiment are shown in Fig. 7. Little or no effect of the hypothalamic extract on cell growth was observed; however, extracts did have a marked effect on the morphology of the cells. Within 4 hr after adding medium containing extract the cells appeared to be more

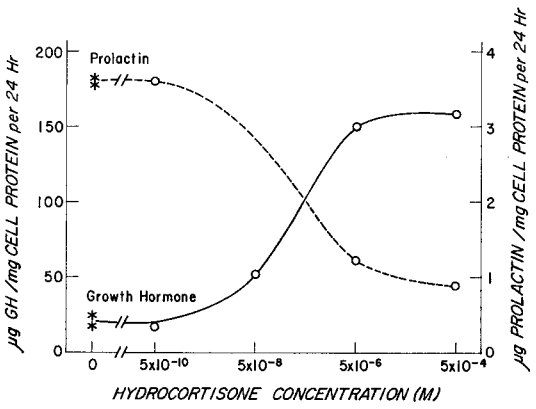

FIGURE 6 Effects of various concentrations of HC on prolactin and GH production. Conditions were similar to those described in Fig. 5, except that medium containing HC at final concentrations from 5×10^{-10} M to 5×10^{-4} M was added to experimental dishes (○), and medium lacking HC was added to duplicate control dishes (*). Results are given for the interval 96–121 hr.

highly stretched (or adherent to the plastic surface), and no rounded-up cells could be seen. This effect persisted as long as the cells were grown in extract-containing medium.[3] The time-course of the effect of hypothalamic extract on prolactin production by GH_3 cells was similar to the time-course of stimulation of GH production by HC (Fig. 5 and reference 3). Likewise, the magnitude of the stimulation (6- to 9-fold) was also similar. In other experiments, in which shorter incubation times were used, no inhibition of prolacting production was detected in cells treated with hypothalamic extract for 2, 4, or 7 hr. In fact, a stimulation of prolactin production could be detected as early as 7 hr after adding medium containing hypothalamic extract.

The specificity of the effect of hypothalamic extract was then examined. Similar extracts were prepared from bovine cerebral cortex, liver, and kidney. The results, shown in Table VI, revealed that stimulation of prolactin production was not a specific effect of hypothalamic tissue. Extracts of

[3] It is important to note that the morphological change observed does not appear to be a manifestation of a toxic effect of the extract on the cells. On the contrary, the cells appeared very healthy in extract-containing medium for as long as 2 wk. This subjective observation was confirmed by the finding at the end of this time that treated cultures did not contain less protein than controls; if anything, they contained more.

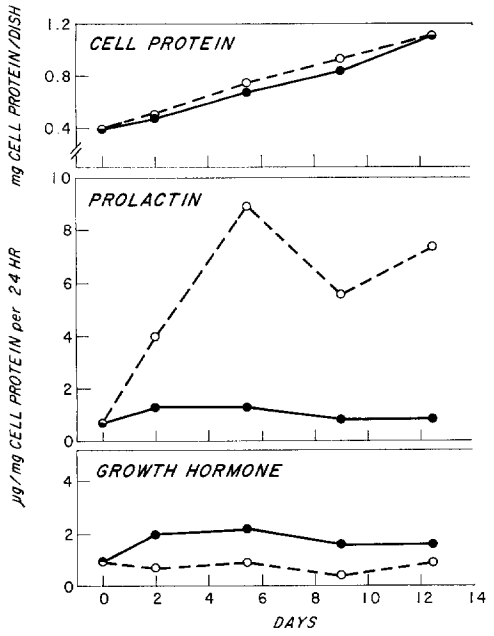

FIGURE 7 Effects of bovine hypothalamic extract on cell protein, and on prolactin and GH production by cultures of GH_3 cells. Duplicate dishes were used for each point. At zero time, medium either containing hypothalamic extract (0.14 ml extract/dish, 1.5 mg extract protein/dish) or lacking extract was added to each dish. Medium was collected at intervals from experimental (○) and control (●) dishes and was frozen for hormone assays. These dishes were washed and frozen for determination of cell protein. Medium containing hypothalamic extract did not fix C′ with anti-prolactin or anti-GH, nor did it interfere with the assay of prolactin or GH in medium.

TABLE VI

Effects of Extracts of Several Bovine Tissues on the Production of Prolactin and Growth Hormone by GH_3 Cells

Extract	Dose	Cell protein	Prolactin	Growth hormone
	mg protein/ml	*mg/dish*	*μg/mg cell protein per 24 hr*	
None	—	0.56	2.0	1.6
Hypothalamus	0.4	0.62	4.6	1.7
Cerebral cortex	0.4	0.72	5.0	1.2
Liver	1.0	0.82	9.3	1.1
Kidney	0.9	0.78	8.8	1.2

Cells in replicate dishes were incubated without tissue extracts or with tissue extracts at the dose levels indicated. At the end of 96 hr the medium was collected and frozen for hormone assays. The dishes were washed and the cells were frozen for protein determination. The results given are mean values of duplicate determinations. The total protein concentration of control medium (medium without extracts added) was 12 mg/ml. Thus, the increase in total protein content of the medium due to tissue extracts was approximately 3–7%.

the three other tissues tested contained material that was able to stimulate the production of prolactin. These three extracts had the same effect as hypothalamic material on the morphology of the cells.

DISCUSSION

The validity of our conclusion that several clonal strains of rat pituitary cells synthesize prolactin and secrete the protein into the culture medium depends on the specificity of the assay method for prolactin. Four lines of evidence support the specificity of the immune system:

First, only one sharp peak of C′ fixation was seen with antiserum to prolactin and crude rat pituitary extract. If more than one antigen-antibody system were being measured, more than one peak of C′ fixation would probably have been seen (20).

Second, medium from pituitary cells and rat prolactin fixed the same maximum amounts of C′ with anti-prolactin (Fig. 2). Since small qualitative differences in the structures of proteins are often revealed by differences in the heights of their C′-fixation curves at equivalence (16, 17), these results are strong evidence in favor of the conclusion that the material secreted by GH_3 cells into culture medium is rat prolactin.

Third, fresh uninoculated medium or medium from six other strains of cells did not fix C′ with anti-prolactin (Fig. 2 and Table I).

Fourth, undiluted antiserum against rat prolactin gave only one band of precipitation in double diffusion with prolactin, crude pituitary extract, and medium from GH_3 cells (Fig. 3). These three bands merged without spur formation.

The rate of production (as defined in footnote 2) of either GH or prolactin by pituitary cells in culture can be measured directly. This quantity is the end result of three processes whose relative effects on the rate of production of either hormone are at present difficult to quantitate: synthesis, intracellular turnover, and secretion. Indirect measurements indicate that the rate of GH production by GH_3

cells accurately reflects the rate of its synthesis (3). The findings that inhibitors of protein synthesis suppress prolactin production (Fig. 4) indicates that this may be true for prolactin as well. However, the intracellular level of prolactin in the GH_3 cells is equal to the amount produced in about 1–2 hr (see Results), while the comparable figure for GH is about ¼ hr (3). Thus it may be that the production and synthesis of prolactin are not so closely linked to each other as these two processes are in the case of GH.

In previous studies of pituitary explants, Nicoll and Meites (23) examined the effects of hydrocortisone on prolactin secretion in vitro. At concentrations of hydrocortisone of 0.5 μg/ml they noted no effect on prolactin secretion. At 10 μg/ml (2.8×10^{-5} M) there was a decrease in prolactin secretion. Our observation that prolactin production was suppressed dramatically by 5×10^{-6} M hydrocortisone (Fig. 6) may be due to the greater sensitivity of the dispersed cell culture method.

Since each of the strains of pituitary cells used in these experiments was derived from a single cell, our results suggest that both prolactin and GH can be synthesized and secreted by the same type of pituitary cell. However, these results do not prove this point since all measurements to date have been made on mass cultures. It will not be possible to demonstrate this conclusively until it is shown that a single cell can synthesize both prolactin and GH. Likewise, since the cells used here are neoplastic, it is not possible to conclude that in the normal pituitary gland both hormones are produced by the same cell type.

Most evidence in mammals suggests that hypothalamic factors stimulate the release of GH from the pituitary gland and inhibit the release of prolactin (22). However, one laboratory has presented evidence that there may also be a hypothalamic GH-inhibiting factor (22). Our results with crude hypothalamic extracts, repeated in a number of independent experiments, revealed somewhat unexpected findings. We regularly observed a large stimulation of the production of prolactin and either no stimulation or a concomitant suppression of GH production. Since these effects were observed with extracts of several tissues (hypothalamus, cerebral cortex, liver, and kidney), they are clearly not due to the classical hypothalamic factors (22). The nature of the prolactin-stimulating and GH-inhibiting materials in tissue extracts remains to be defined. Furthermore, knowledge about their physiological significance must await further studies in the living animal. Nevertheless, the observations that prolactin and GH production can be altered differentially by HC and tissue extracts indicate that these substances are useful reagents for further studies of the control of the biosynthesis and secretion of prolactin and GH in functional pituitary cells. These results also indicate that caution should be used in ascribing effects of crude brain extracts on pituitary function to specific hypothalamic factors.

The usefulness of the functional pituitary cell systems is greatly enhanced by the findings that the cells produce more than one organ-specific product. That these functional cell systems can be modulated or controlled selectively by the experimenter adds further to their use as model systems in which to study the integration of multiple differentiated activities in mammalian cells. Since HC and tissue extracts have distinct and opposite effects on the production of exportable proteins in pituitary cells, further studies of how these effects take place may suggest how integrated activities are controlled in complex, multipotent animal cells.

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