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Research Article

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Primate Mammary Development

Effects of Hypophysectomy, Prolactin Inhibition, and Growth Hormone Administration

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Abstract

The pituitary gland has been found to be an important factor in mammary development in primates. Hypophysectomy in 12 sexually immature monkeys caused significant inhibition of estradiol (E_2)-induced mammary growth and development. A histological index of mammary development in sexually immature hypophysectomized animals was lower (0.82) than in intact E_2-treated controls (3.4; $P < 0.008$). Hypophysectomy also inhibited growth of the mammary gland as judged by a size index. Despite the hypophysectomy, E_2 stimulated some, albeit blunted, mammary growth and development, which may have been due to incomplete hypophysectomy.

Selective inhibition of prolactin by ergot drugs in intact animals did not prevent full mammary development, suggesting that there may be pituitary mammogens other than prolactin, or that very low or unmeasurable concentrations of prolactin were sufficient to synergize with E_2 to cause full acinar development. The mean histological index was 3.08 in E_2-treated animals and 3.16 in animals treated with E_2 plus pergolide. There was also no difference in the size of the glands.

We evaluated the effect of growth hormone on mammary development by treating three hypophysectomized animals with pure 22,000 mol wt human growth hormone (hGH) (Genentech, Inc., South San Francisco, CA). We found that physiological or slightly supraphysiological concentrations of hGH in animals with unmeasurable prolactin were incapable of restoring the capacity of E_2 to induce full mammary growth. These findings suggest that, if growth hormone is a mammary mitogen, that physiological concentrations are insufficient to synergize with E_2 to induce full mammary growth or that other forms of hGH are mammogenic.

Our studies suggest that the role of the pituitary gland in mammary mitogenesis in primates is more complicated than previously thought. They also raise the possibility that heretofore unidentified pituitary substances may be mammogenic.

Introduction

In recent years the medical community has focused attention away from the pituitary gland as a potential source of growth factors influencing the course of breast cancer. One reason may have been that prolactin-lowering ergots have not been effective in the treatment of metastatic breast cancer in humans (1), as they are in rats with dimethylbenzanthracene-induced tumors (2), and another is that tamoxifen therapy has virtually replaced hypophysectomy in treating metastatic breast cancer. Several convincing lines of evidence, however, indicate that the pituitary gland does play a role in the growth and maintenance of breast cancer in humans (3–9). Doubts about the relationship of the pituitary to human breast cancer have raised questions about the relationship of the pituitary gland to normal mammary mitogenesis in primates, a subject which has not been addressed. Previous well-designed studies in lower mammals have led many to assume that the process of mammary development in primates is a relatively uncomplicated one in which prolactin and growth hormone synergize with estrogen and other hormones to induce full mammary development (10–18).

In retrospect, however, those studies, in which prolactin and growth hormone were found to be important mammogens were potentially flawed because they employed huge concentrations of relatively impure hormones to make their determinations. Not only might one hormone have been contaminated with enough of the other to provide physiological concentrations of the contaminant, but the pituitary extract might have contained other unidentified hormones of potential importance. In our view, a reevaluation of the individual and combined effects of prolactin and growth hormone in mammary development and the importance of the pituitary gland itself in mammary development in primates was essential before accepting the premise that mammary development was as uncomplicated as it was once thought to be and that studies in rodents were applicable to humans.

We chose the subhuman primate to study the importance of the pituitary gland in mammary growth and the role of physiological concentrations of prolactin and growth hormone in estradiol (E_2)[1]-induced mammary development for three reasons: (*a*) to mimic the human situation as closely as possible and to determine whether there were species differences between rodents and primates, (*b*) to utilize an animal model in which serum concentrations of hormones could be regularly measured in individual samples and during 24-h periods, and (*c*) to utilize a species in which growth hormone was highly lactogenic. Our initial findings are presented in this paper.

Methods

Studies were carried out in 23 sexually immature monkeys (17 males and 6 females) weighing 2–2.5 kg (*Macaca nemestrina* or *Macaca mulatta*), in 4 sexually mature and regularly cycling adult female monkeys (*M. mulatta*), and in 6 sexually immature male dogface

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1. *Abbreviations used in this paper:* E_2, estradiol; hGH, human growth hormone; TRH, thyrotropin-releasing hormone.

baboons. Animals were housed singly in cages and fed monkey chow, oranges, apples, and water ad lib. Blood samples were drawn weekly under ketamine tranquilization. In addition to these weekly bloods, some animals had 24-h sampling of growth hormone and prolactin; bloods were drawn every 2–4 h. The results in individual animals are expressed as a mean 24-h value. The weekly hormone concentrations are expressed as a mean of all samples during the test period for each animal excluding 24-h studies and values during stimulation tests. Pituitary prolactin reserve was evaluated using 500 μg of thyrotropin-releasing hormone (TRH) intravenously with bloods drawn at 0, 15, 30, and 60 min. No adverse effects were noted. Insulin tolerance tests were done using 1.5 U of regular insulin/kg intravenously with bloods drawn at 0, 30, 60, and 90 min to evaluate growth hormone reserve. In all but two animals, this dose of insulin was sufficient to reduce blood glucose by at least 50% to below 40 mg/dl. These large doses of insulin were used because the animals were resistant to the hypoglycemic effects of lower doses. In hypophysectomized animals, both tests were done before the hypophysectomy, ~2 wk after the surgery, and again before the end of the experiment to determine whether regeneration of pituitary tissue had occurred.

17-B E_2 was administered by implanting silastic capsules (4 cm of Dow Corning Medical grade tubing [Dow Corning Corp., Midland, MI], 0.132 in. i.d. and 0.185 in. o.d., with 0.5-cm plugs of silatic medical grade elastomer at either end) subcutaneously in the abdominal wall (19). The capsules were designed to deliver an E_2 load similar to that found in the follicular phase of the menstrual cycle and enough to inhibit high levels of luteinizing hormone in menopausal monkeys (20). Animals were observed daily for evidence of E_2 treatment which causes reddening and swelling of perineal tissues. These changes disappear rapidly if capsules are lost. Serum E_2 was also measured using a Pantex immuno-direct E_2 ^{125}I kit (Pantex, Inc., Santa Monica, CA). Mean serum E_2 before therapy was 24.3±4.3 pg/ml (SEM) and 202.1±33.5 pg/ml during therapy. Female animals were oophorectomized before the experiments.

Unilateral mastectomies were done in intubated anesthetized animals. Under sterile conditions a midline chest incision was made and the skin reflected back. The mammary gland was separated from the skin. The boundaries of the gland were determined by visualization and transillumination. Fully developed glands were more readily identifiable than rudimentary ones, and for that reason the surgeon may, in some cases, have extended the boundaries of the undeveloped glands to insure complete removal. A unilateral mastectomy was done immediately before implantation of E_2-containing capsules at the start of the experiment. The second one was done ~2 mo later at the end of the experiment. Once the mammary glands were removed, they were placed in sterile petri dishes and photographed against a rule. Size estimates were made by measuring the mammary glands horizontally and vertically, and then, multiplying the two numbers to obtain a "size index."

The mammary tissues were fixed in formalin and then carefully imbedded so that coronal sections could be made. They were stained with hematoxylin eosin. A histological scale ranging from 0 to 4+ was constructed to analyze the histological changes as follows: 0, loosely arranged connective tissue with or without occasional ducts; ½+, ducts occupying <20% of mammary gland; 1+, ducts occupying >20% of mammary gland; 2+, ducts, ductules with changes in connective tissue supporting them; 3+, same as 2 but with acini; and 4+, acini occupying >50% of the gland. They were rated independently and randomly by two of us. The correlation coefficient between readers was high (r = 0.956).

Hypophysectomies were done using the transorbital approach of Carmel et al. (21). Nembutal and ketamine were used for anesthesia. During surgery, hydrocortisone (100 mg) was given intravenously by slow drip. During the test period, control and hypophysectomized animals were treated chronically with daily injections of hydrocortisone and levothyroxine. Diabetes insipidus was an unusual complication and was transient when it occurred. Injectable vasopressin was only required on one occasion for a short period.

Pure 22,000 mol wt human growth hormone (hGH) produced in vitro in *Escherichia coli* using recombinant DNA techniques and kindly supplied by Dr. Robert Swift of Genentech, Inc., was administered to determine if growth hormone in hypophysectomized animals could restore the capacity of E_2 to induce mammary development. The lactogenic activity of this genetically engineered material was determined by comparing it with hGH, human prolactin, and ovine prolactin prepared by the National Pituitary Agency (NPA). It was found to be roughly equipotent with the NPA hGH and human prolactin in an NB2 cell bioassay (22, 23) but ~40% less potent than the hGH of the NPA in the primate gland bioassay developed in our laboratories (24), which utilizes α-lactalbumin production as the endpoint.

Pergolide mesylate (kindly supplied by Eli Lilly Laboratories, Indianapolis, IN), a long-acting dopamine agonist which has a duration of action of greater than 24 h (25), was used to inhibit prolactin in animals with intact pituitaries. It was given in doses from 200 to 500 μg daily by injection. After it became apparent that it was not always possible to do a complete hypophysectomy and that low but detectable concentrations of prolactin were found in some animals, we treated all remaining hypophysectomized animals with 500 μg of pergolide daily to insure that prolactin would be as completely inhibited as possible. We did not find that chronic treatment with pergolide raised growth hormone in either intact or hypophysectomized animals.

Prolactin and growth hormone were measured by previously described radioimmunoassays (26, 27). The sensitivity of both assays for serum samples was 1 ng/ml. Values of <1 ng/ml were assumed to be 0 in calculating means. Statistical analyses were done using the Wilcoxon two-sample rank sum test. Two-tailed paired t test was also used to determine correlation coefficients.

Results

Effect of E_2

Treatment of sexually immature subhuman primates (*M. nemestrina* and *M. mulatta*) with E_2 led to full mammary development resembling that of adult female animals within a 2-mo period (Table I). Two were females and three were males. Both prolactin and growth hormone were higher during E_2 treatment than before. Mean serum prolactin before E_2 treatment was 3.96 ng/ml in these five control animals. During E_2 therapy, the mean serum prolactin during 2 mo was increased to a mean of 10.6 ng/ml. Mean growth hormone for the entire group was 4.81 ng/ml before any treatment and 15.75 ng/ml during E_2 therapy.

During treatment the mammary glands matured both in size and in histological development (Figs. 1 and 2). Gland size increased in four of the five animals. There was a mean increase of 272.6% of control. Histological examination revealed that initially the immature mammary glands were composed

Table I. Effect of E_2 on Mammary Development in Intact Sexually Immature Primates

				Mean histologic grade	
No.	Sex	Mean prolactin*	Size index	Before	After
		ng/ml	*% of base line*		
1	F	12.8	532	0.5	4.0
2	F	7.4	296	0	3.75
3	M	9.5	142	0.25	3.75
4	M	17.8	87	0.25	2.5
5	M	5.6	306	0.25	3.0
Mean (±SEM)		10.6 (±2.2)	272.6 (±77.6)	0.25 (±.08)	3.4 (±.28)

* Mean prolactin during 2-mo test period.

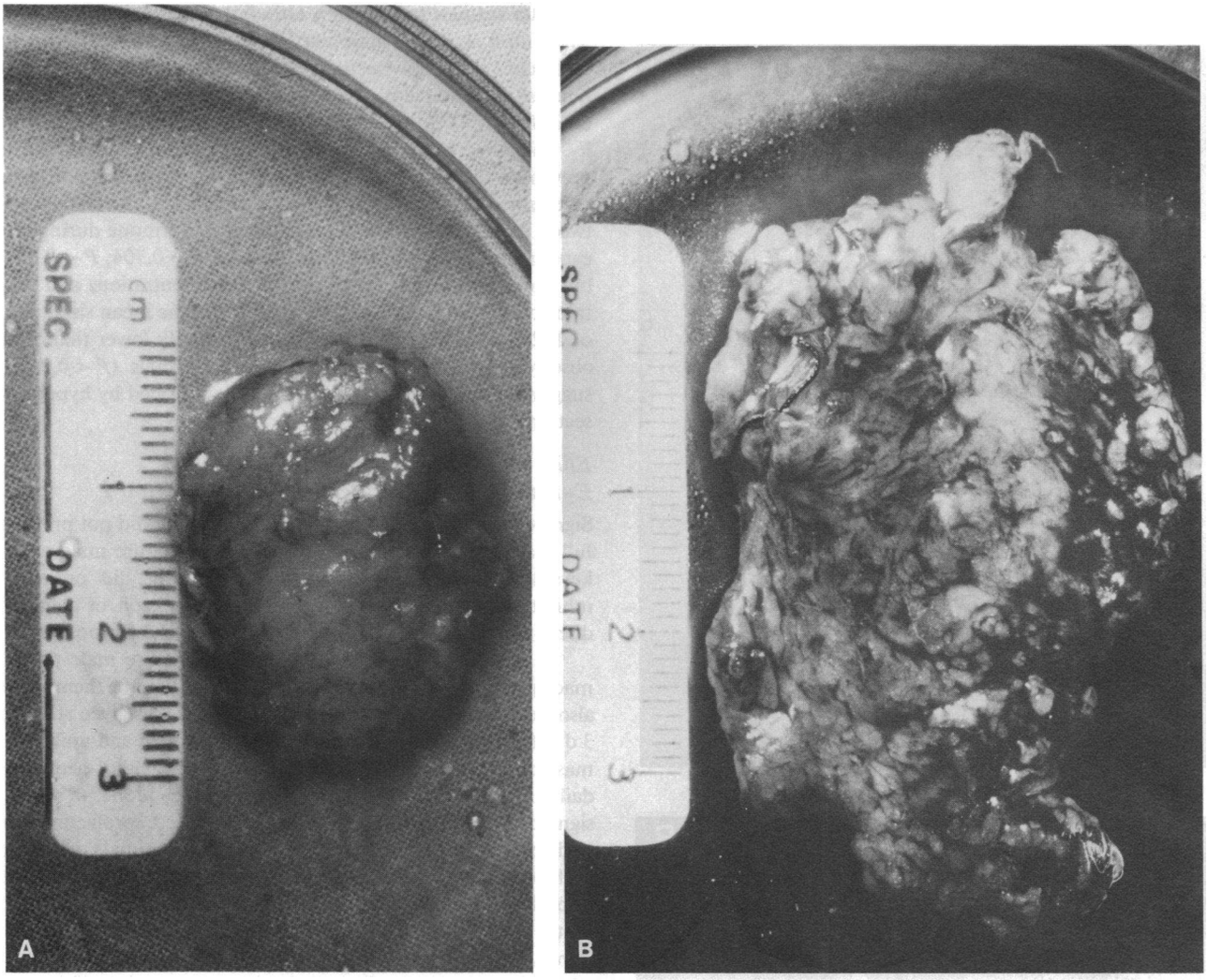

Figure 1. Photographs of whole mammary glands (*A*) before and (*B*) after treatment with E_2 from an intact sexually immature monkey.

of loosely organized connective tissue with small areas of ducts under the nipple. Glandular elements when found in sections of the rudimentary glands occupied only a small area (<20%) of the tissue identified by the surgeon as mammary gland. During E_2 therapy, the glandular elements branched out and proliferated to occupy most of the mammary gland and consisted of ducts, ductules, and acini. There were also changes in the connective tissues supporting the glands from loosely organized fibrous tissue to more densely packed swirls. The histological index was significantly increased from a mean base line in the control mammary glands of 0.25 to 3.4 in the contralateral mammary glands after treatment ($P < 0.008$).

Effect of hypophysectomy on E_2-induced mammary development

Hypophysectomy was found to inhibit E_2-induced mammary development in all 12 animals studied (Table II). The completeness of hypophysectomy was judged by stimulation of prolactin by TRH and of growth hormone by insulin-induced hypoglycemia. These tests were done before and after hypophysectomy. Peak responses of prolactin and growth hormone to TRH and insulin, respectively, before and after hypophysectomy in individual animals are shown in Fig. 3. The postoperative peaks were blunted in all cases. Mean reduction in peak growth hormone responses to hypoglycemia was 77.3% (range, 36–98%) and mean reduction in peak prolactin responses to TRH was 95.4% (range, 67–100%).

Concentrations of serum prolactin and growth hormone were also measured before the hypophysectomy and weekly during the 2-mo course of E_2 treatment. Before hypophysectomy, mean serum prolactin was 4.87 ng/ml and growth hormone was 3.51 ng/ml. Mean weekly prolactin and growth hormone concentrations during the experimental period are listed individually in Table II. Mean prolactin and growth hormone values for the entire group during therapy (assuming 0 for values of <1) was 0.89 and 0.85 ng/ml, respectively, representing reductions of 82 and 76%. The significance of these reductions in prolactin and growth hormone due to hypophysectomy is even greater when one considers the fact that E_2 would have led to a greater than 2.5-fold increase in growth hormone and prolactin had the animals been intact (*vide supra*). After it became obvious that a total hypophysectomy was not always possible, we began to treat animals with a dopamine agonist, pergolide, to prevent increases in prolactin. These animals are identified in Table II.

Hypophysectomy prevented full E_2-induced mammary development. The mean histological grade in the E_2-treated, hypophysectomized animals (0.82) was significantly lower than

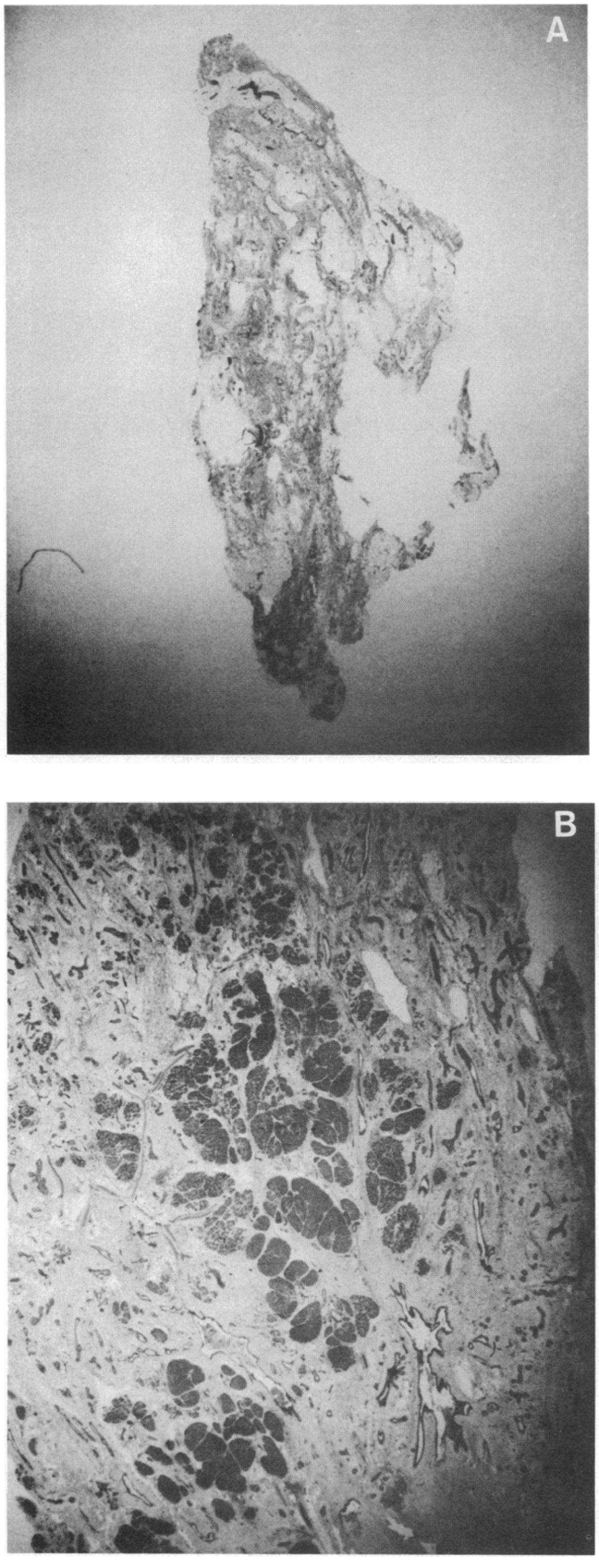

Figure 2. (*A*) Histological section through a rudimentary mammary gland in a sexually immature monkey before therapy, × 1.5–3. (*B*) Histological section of contralateral mammary gland removed 2 mo after therapy with E_2 in an intact animal.

in intact animals treated with estradiol alone (3.4; $P < 0.008$). That full E_2-induced mammary development was not entirely inhibited by hypophysectomy was evidenced by a significant increase in histological development (control 0.083 vs. treated 0.82; $P < 0.0003$) in hypophysectomized animals treated with E_2. Whether more complete hypophysectomy would have prevented the mammary development observed has not been ascertained. There was, however, a significant correlation between the concentration of serum growth hormone during the experiment and the histological score ($R = +0.704$; $P < 0.02$), but the correlation between prolactin concentrations and histological development was not significant. The mean size index of 112.75% of base line was also significantly lower than that observed with E_2 treatment in intact animals ($P < 0.015$), suggesting that total mammary size was inhibited by hypophysectomy.

Effect of selective inhibition of prolactin on E_2-induced mammary development

Significant inhibition of prolactin by pergolide did not prevent full mammary development in sexually immature male monkeys treated with E_2 for 2-mo periods, nor did prolactin inhibition for periods of 5-mo lead to regression of acini or ductal structures in sexually mature female rhesus monkeys.

M. nemestrina. Six intact sexually immature male pigtail macaques were treated with E_2 for 2 mo. Three of them were also treated chronically with pergolide, which was given starting 3 d before E_2-containing capsules were implanted and unilateral mastectomies done. They were started on 200 μg of pergolide daily. In contrast to the animals receiving E_2 alone, in which significant increases in prolactin were noted, prolactin was undetectable or barely detectable in the three animals receiving both pergolide and E_2 (Table III). After 6 wk, a 24-h sampling of prolactin was carried out in all the animals. Mean prolactin in the three animals receiving both drugs was 1.74 ng/ml (range, 0.58–2.8 ng/ml); control animals receiving E_2 without pergolide had a mean 24-h prolactin concentration of 11 ng/ml (range, 2.9–15.5 ng/ml). Before any treatment, the mean 24-h prolactin in the three untreated animals which later received pergolide was 5.8 ng/ml (range, 4.3–8.2 ng/ml). Thus, in the 24-h studies, prolactin in animals receiving E_2 and pergolide when compared with untreated controls was reduced by a mean of 72%. Prolactin inhibition was 85% or more in random weekly bloods.

Treatment with E_2 caused an increase in serum growth hormone in all animals whether or not they received pergolide (Table IV). The mean of six individual 24-h studies for growth hormone before taking E_2 was 5.7±1.6 ng/ml. It was 16.8±3 ng/ml in the six E_2-treated animals. Pergolide had no significant stimulatory or inhibitory effect on serum growth hormone. Mean 24-h growth hormone was 14.1 ng/ml in the three animals treated with pergolide and E_2, and 19.4 ng/ml in the animals receiving E_2 alone.

Examination of the mammary glands before and after treatment revealed that full mammary development was not inhibited by selective inhibition of prolactin using both the mean histological grade and the size index (Table III).

Dogface baboons. Similar experiments were carried out in a group of six sexually immature male dogface baboons. Unfortunately, full histological data were available in only four (one E_2-treated control and three animals treated with E_2 and pergolide). Prolactin was significantly inhibited in all four animals receiving pergolide both in 24-h studies during treat-

Table II. Effect of Hypophysectomy on E_2-induced Mammary Development

No.	Sex	Mean serum prolactin*	Mean serum growth hormone*	Size index	Mean histologic grade	
					Before E_2	After E_2
		ng/ml	*ng/ml*	*% of base line*		
6	M	<1	2.0	84	0	2.0
7	M	2.27	1.9	122	0.5	2.0
8	M	4.4	2.5	116	0	2.0
9	M	<1	2.1	115	0	0.5
10	M	<1	1.8	—	0	0.5
11**	M	1.2	<1	93	0	0.5
12	M	<1	<1	—	0	0.5
13**	M	<1	<1	189	0.5	0.375
14**	M	<1	<1	186	0	0
15**	M	1.1	<1	69	0	0.5
16**	M	1.8	<1	63.5	0	0
17**	F	<1	<1	90	0	1.0
Mean (±SEM)				112.75% (±13.9)	0.083 (±.05)	0.82 (±0.2)

* Mean serum prolactin and growth hormone concentrations during 2-mo test period. ** Animals received pergolide daily during experimental period.

ment and in random weekly samples (Table V and Fig. 4). No significant change in serum growth hormone was noted in these dogface baboons.

The mean histological grade in the one E_2 control in which we had full histological data was 2.0. It was also 2.0 in each of three animals treated with E_2 and pergolide. Size indices were not done in these animals. Mammary gland weight was 2 g in the control and 2.1, 2.1, and 1.5 g in the animals treated with pergolide.

M. mulatta. In a different set of experiments, four adult regularly cycling female rhesus monkeys were treated with 200 μg of pergolide daily for 5 mo. As previously reported (25), 24-h prolactin concentrations were inhibited from a pretreatment mean of 30.9±5 ng/ml (±SEM) to 2.03±0.69 ng/ml. The mean histological index before treatment was 3.75 and after treatment was 4.0.

Effect of hGH on mammary development in hypophysectomized monkeys

Administration of pure hGH in physiological or slightly supraphysiological concentrations did not completely restore the capacity of E_2 to stimulate full mammary development in 2 mo. Three animals were hypophysectomized. Growth hormone was undetectable in all of them postoperatively and prolactin was either undetectable or just barely measurable during TRH tests. It was undetectable in all serum samples analyzed during the experimental period. In addition to E_2, animals received two daily intramuscular injections of growth hormone. One animal was given 100 μg of Genentech 22,000 mol wt hGH at 9 a.m. and 200 μg at 5–6 p.m. (Fig. 5). The other two received 50 μg in the morning and 100 μg in the evening. Mean 24-h hGH was 14.8 ng/ml in the animal receiving the higher dose and 10.1 ng/ml in one of the animals receiving the lower dose. In the other, a 24-h study was not done because the animal expired suddenly 1 wk before the end of the experiment. Therefore, in that animal, only random morning specimens before the daily injection of growth hormone were available for testing.

As in the other animals, a unilateral mastectomy was done at the time of insertion of the E_2 capsule, at which time the growth hormone was also started. After 2 mo (7 wk in one animal), the other gland was removed. There was some mammary development in each of three animals during the 2-mo test period (Table VI). In no case, however, was the mean histological grade > 2.0. In each case there was proliferation of intraductal epithelial cells, suggesting heightened stimulation of the gland but no acinar development was evident.

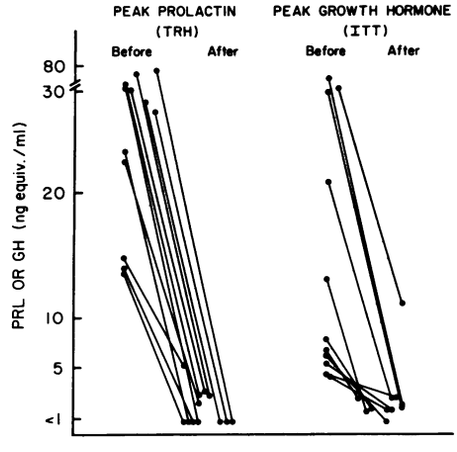

Figure 3. Peak responses of prolactin to TRH (500 μg) before and after hypophysectomy in individual animals (left) and peak responses of growth hormone to insulin (1.5 U/kg body weight) (right).

Discussion

Our results provide evidence that the pituitary gland plays a vital role in mammary development in subhuman primates. When the pituitary glands of 12 sexually immature monkeys were removed, E_2-induced mammary development was significantly blunted. Thus it appears that primates resemble lower mammals (10–18) in which pituitary hormones are also necessary for growth and development.

That physiological concentrations of E_2 caused some, albeit blunted, glandular proliferation in 9 of 12 hypophysectomized animals raises the possibility that E_2 may have some independent mitogenic action on the mammary gland. However, the fact that detectable concentrations of prolactin or growth

Table III. Effect of Prolactin Inhibition on E_2-induced Mammary Development in Six Male Monkeys

No.	Control prolactin*	Mean prolactin**	Mean histological grade		Size index
			Before	After	
	ng/ml	*ng/ml*			*% of base line*
E_2 alone					
3	4.8	9.5	0.25	3.75	532
4	4.1	17.9	0.25	2.5	296
5	0.63	5.6	0.13	3.0	142
Mean	3.17	11.0	0.21	3.08	323.3
E_2 + pergolide					
18	4.3	<1	0	2.5	124
19	4.9	<1	0.13	4.0	718
20	8.2	<1	0	3.0	302
Mean	5.8	<1	0.04	3.17	381

* Before receiving drugs. ** Mean of samples drawn weekly during drug administration.

hormone were found in seven of the nine animals in which mammary growth occurred despite hypophysectomy may mean that minute concentrations of prolactin and growth hormone or other unidentified pituitary substances are sufficient to synergize with E_2 to induce partial mammary development.

Our studies in which, despite chronic inhibition of circulating prolactin, full mammary development occurred in E_2-treated animals raise new and important questions about the role of prolactin as a mammary mitogen in primates. On one hand, it is possible that prolactin is not a mammary mitogen in primates at all and that other pituitary substances are the active factors. If that were the case, it would indicate species differences between ruminants and primates; in goats, treatment with bromocriptine has been found to inhibit udder growth (28–30). On the other hand, if prolactin is mammogenic, the function of the pituitary gland in mammary mitogenesis must be more complicated than previously thought. It is possible that minute concentrations of prolactin are sufficient to synergize with estrogens to induce mammary growth or that there are other forms of prolactin not recognized by presently utilized antisera to prolactin. Other substances of pituitary origin might also be capable of acting alone or in concert with prolactin to induce mammary growth. A list of these might include low molecular weight substances similar to those in rodents described by Mittra (30, 31) or Mayer and Russell (32), or heretofore unidentified pituitary mammogens or growth hormone.

Several lines of direct or indirect evidence indicate that growth hormone is an important participant in mammary growth and development. In the first place, when given to rodents in pharmacological concentrations, growth hormone synergized with estrogen to induce growth and differentiation of the mammary gland (10–18). Secondly, primate growth hormones are more lactogenic than rodent ones (33), inferring an even greater role for growth hormone in mammary development in primates than in lower animals. Lastly, the positive correlation we observed between the presence of growth hor-

Table IV. Effect of E_2 (± Pergolide) on Mean Serum Growth Hormone during 24-h Studies

Animal no.	24-h growth hormone before treatment*	24-h growth hormone during treatment*
	ng/ml	*ng/ml*
E_2 alone		
3	7.5	24.5
4	5.2	13.3
5	4.9	20.5
E_2 + pergolide		
18	3.3	15.0
19	12.2	22.5
20	1.1	4.8

* Mean of six blood samples drawn every 4 h for 24 h in individual animals.

Table V. 24-h Prolactin and Growth Hormone Concentrations in Sexually Immature Male Dogface Baboons

	Mean 24 h prolactin*		Mean random prolactin**	Mean 24 h GH*	
	Before	After		Before	After
	ng/ml	*ng/ml*	*ng/ml*	*ng/ml*	*ng/ml*
E_2 alone					
	2.3	5.3	2.8	4.1	6.8
	2.1	3.6	2.6	6.0	6.1
Mean	2.2	4.5	2.7	5.0	6.5
E_2 + pergolide					
	2.5	0.7	1.0	5.3	5.9
	3.2	0.99	0.6	3.7	4.7
	2.3	0.93	0.7	5.9	4.4
	4.9	0.8	0.5	4.1	5.2
Mean	3.22	0.85	0.7	4.75	5.05

* Mean of 13 samples drawn every 2 h over a 24-h period.
** Mean of weekly samples.

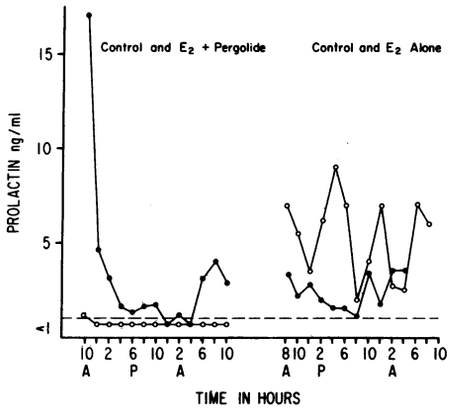

Figure 4. 24-h prolactin patterns in two representative sexually immature male dogface baboons before (— ● —) and after (— ○ —) treatment with pergolide + E_2 (left) or E_2 alone (right).

mone and mammary growth in hypophysectomized animals and the fact that E_2 treatment raised growth hormone concentrations even in animals receiving pergolide provides indirect evidence for growth hormone being a mammogen.

In sharp contrast, our studies raise major questions about the action of growth hormone in mammary development. They provide direct evidence that physiologic concentrations of pure human growth hormone did not substitute for the normal pituitary gland in synergizing with E_2 to induce full mammary development. It is possible that other forms of growth hormone might be potent mammogens; only the 22,000 mol wt form was tested. It is also possible that this form of growth hormone might be more effective in higher concentrations or require synergy with other known or unknown pituitary substances or growth factors to induce normal growth of the mammary gland. Thus, these studies weaken but do not rule out an independent role for growth hormone in mammary mitogenesis.

In summary, in our search for factors involved in mammary growth and development, we have found that the pituitary

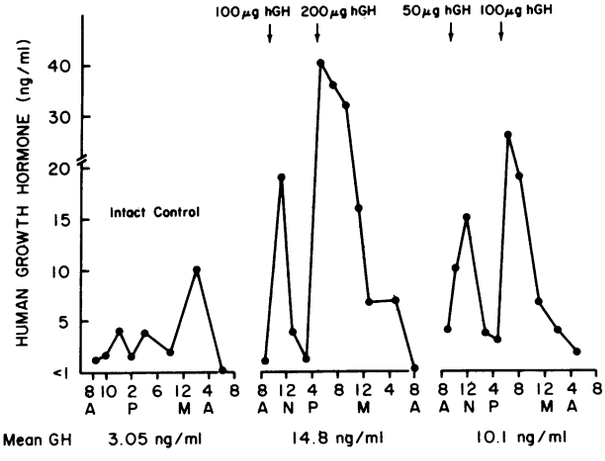

Figure 5. 24-h growth hormone patterns in three rhesus monkeys. A control intact animal (left) and two completely hypophysectomized animals being treated with two injections of pure 22,000 mol wt hGH daily (middle and right).

Table VI. Effect of Growth Hormone on E_2-induced Mammary Development in Hypophysectomized Primates

No.	Mean 24-h GH	Mean prolactin	Mean histologic grade	
			Before	After
	ng/ml	*ng/ml*		
21	14.8	<1	0.25	1.25
22	10.1	<1	0	1.75
23	3.4*	<1	0.37	2.0
Mean (±SEM)			0.167 (±0.08)	1.67 (±0.27)

* Mean of seven morning growth hormones before injection.

gland is an important factor, but that prolactin does not have to be present in physiological concentrations, as we know them, for full mammary development to take place. Our studies further suggest that the process of mammary growth and development is more complex than expected. Human growth hormone in physiological concentrations did not restore full mammary development when given to hypophysectomized animals. Whether there are pituitary mammogens other than prolactin and growth hormone, or whether pure prolactin in physiologic concentrations, or a combination of prolactin and growth hormone are capable of substituting for the place of the pituitary gland in mammogenesis is still to be determined.

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