Secretion of Prolactin and Growth Hormone by Cultures of Adult Simian Pituitaries[1] (35090)

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The identity of growth hormone and prolactin in primates, as distinct chemical entities, remains an unsettled question. The fact that neither hormone has been isolated in a biologically pure form in the human or monkey (1–5) lends support to the concept that prolactin and growth hormone activity reside in the same molecule. On the other hand the experiments of Nicoll *et al.* (6) in which addition of crude hypothalamic extract to organ cultures of monkey pituitaries promoted selective secretion of growth hormone compared to prolactin activity, as well as the culture experiments of Pasteels *et al.* (7) in the human, suggest that primate prolactin and growth hormone are separate molecular species.

In the present study explants of rhesus monkey pituitary glands were cultured and the secretion of both growth hormone and prolactin activity was estimated after various time periods in an attempt to determine if a differential secretion of the two hormones would be demonstrated.

Methods. Pituitary glands were removed from normal 4.5–6.1-kg adult female rhesus monkeys 10–20 min after sacrifice by rapid injection of an overdose of Nembutal (60 mg/kg). The posterior lobe of the pituitaries was removed and the glands were placed in ice-cold tissue culture medium, stored at 4°, and cultured within about 1 hr after removal from the animal. The pituitaries were cut into small pieces, approximately 1 mm^3, and placed in Leighton tubes under coverslips 10.5 × 35 mm in size. One gland was used for 6 or 7 cultures, each in 1.5 ml of culture medium containing 15% fetal calf serum, 85% medium 199 plus Earles Salts, 2.2 g/liter of $NaHCO_3$, 0.75 mg/ml of fungizone (Amphoterecin B); 60 IU/ml of penicillin; and 60 μg/ml of streptomycin. All the medium ingredients were purchased from the Grand Island Biological Co. The cultures were gassed with 5% CO_2 in air and incubated at 37.5°. The medium was changed every other day, frozen immediately, and stored at −20° prior to assay.

The cultures were observed periodically and at the end of the experiment the coverslips were fixed in 10% formalin and stained with hematoxylin and eosin.

Growth hormone concentrations in the culture medium were determined in duplicate by a radioimmunoassay previously described in detail (9). Either highly purified human growth hormone or highly purified simian growth hormone was used as standard in the assay (10). The sensitivity of the assay was about 1 ng/ml.

Prolactin activity in the medium was estimated by the local pigeon crop sac assay as previously described (11). In all cases the unknowns were tested concurrently with two doses of NIH ovine prolactin (P-S-8; 28.6 IU/mg). Most of the medium samples were assayed at two-dose levels. The bioassay data

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TABLE I. Effect of Culture Time on Prolactin and Growth Hormone Secretion by Rhesus Monkey Pituitary Cultures.[a]

Expt.	Days in culture	GH (μg/ml)	Prolactin (mU/ml)	Prolactin/ GH ratio
1	0–5	29.9	624 ±100[b]	21.4
	6–15	2.2	168 ± 24	76.2
	16–25	0.07	72 ± 4	1030.
2	0–5	9.7	156 ± 24	16.1
	6–15	2.1	120 ± 20	57.0
3	0–2	36.49	458 ± 64	12.5
	2–4	13.78	278 ± 42	20.3
	4–6	23.16	243 ± 25	10.4
	6–8	4.57	73 ± 12	16.7
	8–10	2.03	46 ± 8	23.0
	12–14	0.807	32 ± 6	39.6
	34–36	0.187	9 ± 2	48.2
4	0–2	29.83	120 ± 15	4.0
	4–6	9.81	40 ± 5	4.0
	6–8	2.94	55 ± 7	18.7
	10–12	0.968	25 ± 4	25.8
	14–16	0.224	25 ± 3	112.0
	18–20	0.103	13 ± 2	126.0
5	4–6	22.32	63 ± 8	2.8
	6–8	18.60	45 ± 5	4.1
	10–12	3.39	18 ± 3	5.3
	14–16	1.40	13 ± 2	9.3
	18–20	1.14	12 ± 2	10.5

[a] Each experiment was carried out using a pool of 2–3 simian pituitaries.

[b] Mean ± SE.

were analyzed using standard statistical procedures (12).

Results. Pituitary explants attached to the glass within 2–4 days and growth of cells outward from the explants could be observed after about 6 days. After 11 days the Leighton tube bottoms were 20–90% covered with a monolayer of heterogeneous fibroblastic and epithelioid cells. Some of the cells contained numerous small and large dark granules. After about 30 days, the bottoms of the Leighton tubes were completely covered and some cells migrated to form a second layer of cells on top of the first.

In each experiment there was a variable decline in the secretion rate of both hormones but the rate of decline of growth hormone secretion was always greater than that of prolactin. This is reflected in the ratio of medium prolactin concentration (mU/ml) to growth hormone concentration (μg/ml) which increased as culture time progressed in each experiment (Table I).

Discussion. The present findings agree with those of Pasteels *et al.* (7, 8) using fetal human pituitary tissue cultures and Nicoll *et al.* (6) using organ cultures of adult monkeys. In each instance the ratio of prolactin activity to immunoreactive growth hormone increased as culture time progressed. The findings of Solomon *et al.* (13), who demonstrated that tissue cultures of fetal monkey and human pituitaries stopped secreting prolactin in the presence of a slow decrease in growth hormone secretion, are in disagreement with those of Pasteels and the present observations. However, the prolactin bioassays of Soloman *et al.* (13) were not quantitative and their culture procedure may have selected for somatotrophs and against lactotrophs.

The present results suggest that growth hormone and prolactin activities secreted by the monkey pituitary gland reside in separate molecules. Other physiological observations in primates support the concept that they are separate hormones. Ateliotic dwarfs who have no detectable growth hormone in plasma (14) can lactate normally (15). Section of the pituitary stalk can initiate lactation in some women whereas it does not elevate plasma growth hormone levels (16, 17). Growth hormone and prolactin activity can also be disassociated in lactating women or subjects with galactorrhea who have no elevation of plasma growth hormone (18). Acromegalic patients who have high plasma levels of immunoreactive growth hormone, do not show elevated prolactin activity as determined by the pigeon crop sac assay (19).

Alternatively, however, it is conceivable that growth hormone and prolactin activities reside in the same molecule with a ratio of about 2–4 mU prolactin/μg of growth hormone and that, with the passage of time in culture, a conformational change could occur in the molecule with a resultant loss in immunoreactivity and an increase in the ratio

of prolactin to growth hormone. The final resolution of this problem must await the chemical separation and identification of primate prolactin and growth hormone.

Summary. Rhesus monkey pituitaries were grown in tissue culture; and growth hormone and prolactin secretion were measured by radioimmunoassay and bioassay, respectively. As culture time progressed the secretion of growth hormone and prolactin declined but the ratio of prolactin to growth hormone increased. These results support the concept that simian growth hormone and prolactin are separate hormones.

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