In Vivo and *in Vitro* Prolactin Secretion by Transplanted Rat "Mammotropic" Pituitary Tumors*

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SUMMARY

The functional, transplanted, autonomous rat pituitary "mammotropic" tumor (Furth, MtT.F_4) was compared with normal rat pituitary for capacity to elaborate prolactin *in vivo* and *in vitro*. The prolactin content of transplanted anterior pituitary (AP) and MtT.F_4, as measured by the intradermal pigeon crop technic, was 1.6 and 4.8 per cent, respectively, of that present in the AP *in situ*. Considerable amounts of prolactin were detected in the blood plasma of rats bearing an MtT.F_4, the levels being higher in rats with larger than in rats with smaller tumors. Prolactin activity could not be detected in the blood plasma of normally cycling, estrogen-treated, pregnant, or lactating rats, or in rats bearing a single AP transplant underneath the kidney capsule. The amount of prolactin released into the medium by MtT.F_4 tissue during 3 days of culture was 8–31 times greater than that present initially, indicating active synthesis and release of prolactin by the tumor. These results show that the MtT.F_4, like nontumorous AP transplants removed from hypothalamic inhibition, synthesizes prolactin and releases it at a rapid rate, leaving little in the tissue itself.

The pioneer work of Furth (7) has led to the identification and isolation of a variety of transplantable hormone-secreting rat and mouse pituitary tumors. One of these is a chromophobic, autonomous rat pituitary "mammotropic" tumor, strain F_4 (MtT.F_4), originally induced by estrogen treatment (6, 9). Following transplantation in the rat this tumor appears to elaborate prolactin, STH, and ACTH, but no FSH-LH or TSH (2, 3, 7, 9, 21).

Animal cell cultures rarely perform differentiated functions characteristic of the tissue of origin; this has been particularly true of cultures of endocrine tissue. However, in our laboratory (13, 14) as well as others (11, 15) it has recently been observed that cultures of rat anterior pituitary (AP) actively release prolactin into the medium, whereas release of all other hormones is markedly diminished. The purpose of this study was to test the capacity of the MtT.F_4 to secrete prolactin *in vivo* and *in vitro* as compared with that of normal rat AP. A report of some of the present work has previously been published in abstract form (19).

MATERIALS AND METHODS

Animals.—Mature, virgin female, highly inbred Fischer[1] rats of the CDF strain, 3–4 months old, were used in this study. The rats were maintained in a temperature-controlled (75 ± 1° F.) and artificially illuminated (14 hr/day) room. They were fed ad libitum on Wayne Lab Blox pellets supplemented with canned (Dash) dog food. White King Squabs, 5–8 weeks old, were used for all prolactin assays.

Prolactin assay.—The sensitive intradermal pigeon-crop method was utilized. The test material was injected over one side of the crop-sac, whereas standard NIH prolactin was injected over the other side of the crop-sac in the same pigeons. Prolactin activity was expressed as IU/100 mg of tissue (wet weight) or IU/100 ml plasma. The details of the assay procedure have been described elsewhere (14).

Transplantation and assays of MtT.F_4 and AP.—Serial transplantation of MtT.F_4 was carried out in our laboratory. The tumor transplants were made by giving injections to rats subcutaneously, in the back of the neck, of 0.1 ml. tumor mince in an equal volume of medium 199 (pH 7.4) containing streptomycin[2] (100 μg/ml) and penicillin G potassium[2] (100 U/ml). In some rats injections were made at two sites, resulting in the formation of two tumors. The tumors were palpable within 4–5 weeks. When the tumor reached 1–2 cm. in diameter it was removed, and a portion of it was used for cultures and for histological examination; another part of it was weighed, homogenized with physiological saline, and assayed for prolactin activity. In a total, six tumors (passage 41) from six rats were assayed separately for prolactin activity.

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TABLE 1

PROLACTIN ASSAYS OF FRESH AP, AP TRANSPLANTS, AND MtT.F$_4$ TRANSPLANTS

Tissue	No. donor rats	No. assays	Total no. pigeons	Prolactin IU/100 mg. tissue (wet weight)	Comparative prolactin content (per cent)
Fresh AP	16	4	18	1.2 ± 0.07*	100
AP transplant†	20	2	8	0.02	1.6
MtT.F$_4$ transplant	6	6	30	0.06 ± 0.01	4.8

AP = anterior pituitary; MtT.F$_4$ = mammotropic pituitary tumor, strain F$_4$.

* Mean ± standard error of mean.

† A single anterior pituitary was transplanted underneath the kidney capsule of each rat. Ten days later the transplant was removed and assayed for prolactin activity.

Intact rats were given implants underneath the kidney capsule of one AP from each of the donor rats of the same strain and approximately of the same age group. Ten days later the AP transplants were removed, trimmed of adhering tissue under a dissecting microscope, pooled from ten rats each, weighed, homogenized with saline, and assayed for prolactin activity. Some of the AP transplants were used for histological examination. The AP from normally cycling rats was also removed, homogenized with saline, and assayed for prolactin activity.

Collection of rat blood and assay for prolactin activity.—Rats were anesthetized with ether, and blood was collected in a heparinized syringe from the posterior abdominal artery. After centrifugation the plasma was dialyzed, lyophilized, and assayed for prolactin activity. Rats in different states were used as blood donors, as indicated in Table 2.

Cultures of MtT.F$_4$.—A modification of the watch glass technic (5) was used, in which sterile, plastic (3.5 × 1 cm.) Petri dishes[3] were employed. Platforms were formed from stainless steel mesh rectangles (1 × 2.3 cm.), bent at each end to make a platform 4 mm. high. These were placed in Petri dishes with 3 ml. of culture medium 199[4] containing 2 U insulin,[5] 100 U penicillin G potassium, and 100 μg streptomycin/ml. For some of the cultures calf, rabbit, or horse serum,[6] or calf serum and chicken embryo extract,[7] was added to medium 199, as shown in Table 3.

The MtT.F$_4$ was removed and placed in a Petri dish (10 × 1.5 cm.) containing a few drops of medium. The tumor was cut into small pieces each about 2–3 mm. in diameter, and two such explants were placed on a strip of lens paper (1.5 × 3 cm.), which was then placed on top of the platform inside the Petri dish. The tumor explants were cultured for 3 or 6 days at 35° C. in 95 per cent O_2–5 per cent CO_2 atmosphere. Six MtT.F$_4$ were used to carry out a total of 148 cultures. At the end of the culture period medium from eight to ten culture dishes was pooled, dialyzed, lyophilized, and assayed for prolactin activity. The explants were fixed in Bouins fluid, sectioned at 6 μ, and stained with hematoxylin and eosin. The details of the culture procedure have been described elsewhere (14).

To determine whether prolactin activity could be detected in medium without MtT.F$_4$, medium 199 alone or medium 199 containing 20 per cent calf, rabbit, or horse serum was incubated for 3 days. At termination the medium was dialyzed, lyophilized, and assayed for prolactin activity. Also, pregnant rat mammary gland and pigeon crop gland were cultured separately for 3 days and the medium was similarly treated and assayed for prolactin. In another experiment, standard NIH ovine prolactin was added to medium 199 with or without 20 per cent calf serum, and incubated for 3 days to ascertain the effects of incubation alone on prolactin activity.

RESULTS

The average prolactin content (Table 1) of MtT.F$_4$ and AP transplants was found to be 0.06 and 0.02 IU/100 mg of tissue (wet weight), respectively, as compared with 1.2 IU/100 mg of fresh AP. This represents 4.8 and 1.6 per cent, respectively, of the content of prolactin present in fresh AP.

No prolactin activity was detected in blood plasma (Table 2) of 3- to 4-month-old mature, female, cycling rats, female rats given injections daily of 10 μg. of estradiol for 10 days, 11- to 12-day pregnant rats, 10-day post-partum lactating rats, or rats with a single AP transplant of 10 days' duration. However, considerable amounts of prolactin could be detected in the blood plasma of rats bearing an MtT.F$_4$. The plasma prolactin activity was found to be higher in rats bearing large as compared with rats with smaller tumors.

Explants of MtT.F$_4$ actively released prolactin into the culture medium (Table 3). The amount of prolactin released during 3 days of culture (0.5–1.9 IU/100 mg MtT.F$_4$) was 8–31 times more than the initial content of prolactin in MtT.F$_4$ (0.06 IU/100 mg MtT.F$_4$). Prolactin was also detected in the medium from monolayer cultures of MtT.F$_4$ incubated for 22 days (unpublished). Neither incubation of the medium alone for 3 days without MtT.F$_4$ nor medium from cultures of rat mammary gland or pigeon crop gland contained prolactin. Prolactin potency was not altered when standard NIH ovine prolactin was incubated for 3 days in medium 199 with or without 20 per cent calf serum.

Histological examination of the MtT.F$_4$ explants at the end of culture showed variable degrees of maintenance. The best maintenance of explants, comparable to fresh MtT.F$_4$, was obtained when 20 per cent calf serum was added to medium 199. The histological appearance of the AP transplants underneath the kidney capsule, 10 days after transplantation, indicated that most of the tissue was viable. These AP transplants were actively secreting prolactin, as indicated by mammary growth in the hosts rats.

DISCUSSION

The prolactin content of MtT.F$_4$ and AP transplants is very low as compared with that of the AP *in situ*. The

[3] Falcon Plastics, Los Angeles, Calif.
[4] Difco Laboratories, Detroit, Mich.
[5] Eli Lilly and Co., Indianapolis, Ind.
[6] Microbiological Associates, Bethesda, Md.
[7] Cappel Laboratories, West Chester, Penn.

TABLE 2

PROLACTIN ASSAYS OF BLOOD PLASMA FROM RATS IN DIFFERENT STATES

Type of donor rat	No. donor rats	No. assays	Total no. pigeons	Prolactin (IU/100 ml plasma)	Remarks
Mature, female	4	4	16	0	3–4 months old
Estradiol	4	4	16	0	10 μg., 1 × 10 days
Pregnant	3	3	12	0	Mid-pregnancy
Lactating	5	5	20	0	10 days post-partum
AP transplant	8	2	10	0	10 days after single AP transplant
MtT.F_4 transplant	2	2	8	33	Each rat had 2 tumors (av. diam., 2.5 cm.)
MtT.F_4 transplant	1	1	4	6.2	One tumor (av. diam., 2.4 cm.)
MtT.F_4 transplant	2	2	8	0.4	Each rat had 2 tumors (av. diam., about 1 cm.)

AP = anterior pituitary.
MtT.F_4 = "Mammotropic" pituitary tumor, strain F_4.

TABLE 3

PROLACTIN ASSAYS OF DIFFERENT MEDIA FROM CULTURES CONTAINING MtT.F_4 TRANSPLANTS

Medium	No. cultures	Days of cultures	No. assays	Total no. pigeons	Prolactin (IU/100 mg MtT.F_4)	Prolactin in medium as compared with content in fresh MtT.F_4
"199"	25	1–3	3	12	1.0±0.2*	×16
"199"	10	1–3	1	4	1.9	×31
		4–6	1	4	1.4	×23
"199" + 20% calf serum	47	1–3	5	20	1.0±0.20	×16
	27	1–3	3	12	1.7±0.25	×29
		4–6	3	12	1.2±0.22	×20
"199" + 20% rabbit serum	9	1–3	1	4	0.54	×9
		4–6	1	4	0.51	×8
"199" + 20% horse serum	10	1–3	1	4	1.1	×18
		4–6	1	4	1.0	×16
"199" + 20% calf serum +	10	1–3	1	6	1.3	×21
5% chicken embryo extract	10	1–3	1	6	1.0	×16
		4–6	1	6	0.9	×15

MtT.F_4 = Mammotropic pituitary tumor, strain F_4.
* Mean ± standard error of mean.

MtT.F_4 and AP transplants release considerable amounts of prolactin *in vivo*, as indicated by mammary gland stimulation and by blood assay for prolactin in rats with tumors. Therefore, the MtT.F_4 and AP transplant have less capacity to retain (or store) prolactin than the normal AP *in situ*.

Placement of hypothalamic lesions, transection of the pituitary stalk, transplantation of the AP to noncranial sites, administration of certain depressant drugs, and cultures of AP *in vitro* have all demonstrated that the hypothalamus exerts an inhibitory effect on prolactin secretion (13). We have recently provided evidence that the hypothalamus contains a factor(s) which inhibits prolactin secretion *in vitro* (20). The decreased capacity of the MtT.F_4 or AP transplant to retain prolactin is probably due to removal of hypothalamic inhibition, with a resultant increase in both synthesis and release of this hormone.

Considerable amounts of prolactin were detected in the blood plasma of rats with a MtT.F_4. The high blood prolactin levels may be due in part to the large size of the pituitary tumors (400–1000 times larger than a normal rat AP); it may actually secrete a smaller amount of prolactin per unit weight of pituitary tissue. The MtT.F_4 during 3 days of culture released 0.5–1.9 IU of prolactin per 100 mg. MtT.F_4, whereas under similar conditions

fresh bovine and rat AP released 8–12 IU (Talwalker and Meites, unpublished) and 10–40 IU per 100 mg. of tissue, respectively (13, 14). This indicates that, on a per unit weight basis, the *in vitro* capacity of MtT.F_4 to secrete prolactin is much lower than that of the normal AP. Nevertheless, the MtT.F_4, during 3 days of culture, released 8–31 times more prolactin than was present initially in the tumor, indicating continuous synthesis and release of prolactin. The comparatively lower prolactin release by MtT.F_4 *in vitro*, in contrast to normal AP, cannot be attributed to inactivation of the hormone, since incubation per se of prolactin did not alter its activity. Prolactin was not detectable, by the assay procedure used, in blood plasma from normally cycling, estrogen-treated, pregnant, or lactating rats or in rats with a single AP transplant. Wolthuis (22, 23) reported the presence of prolactin in the blood plasma of rats in similar states by employing a more sensitive prolactin assay involving a luteotropic reaction by the ovaries.

The MtT.F_4 was initially induced by Furth with estrogen (6, 9). It has been observed that direct circulatory connection with the hypothalamus is not necessary for *in vivo* growth of the tumor (6), or for an *in vivo* or *in vitro* (13, 16) prolactin secretory response to estrogen. Estrogen administration *in vivo* has been found to deplete the rat hypothalamus of prolactin-inhibiting activity (Ratner and Meites, in press). Thus, estrogen stimulation may lead to pituitary tumor formation and increased prolactin secretion as a result of direct action of the estrogen on the AP or hypothalamus or on both. It should be noted that transplantation alone of the AP to noncranial sites in certain strains of mice (1, 4, 8) may lead to pituitary tumor formation. Tumorigenesis has also been observed in pituitary grafts in rats which have been subjected to estrogen treatment (6, 10, 12). Thus, removal of hypothalamic inhibition to the AP may lead to increased prolactin secretion and formation of prolactin-secreting tumors under appropriate experimental conditions.

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