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Modulation (Feminization) of Hepatic Enzymes by an Ectopic Pituitary Tumor*

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ABSTRACT. The ontogeny and endocrine regulation of sex-differentiated hepatic metabolism is mediated via the hypothalamic-pituitary axis. Using *in vitro-in vivo* systems, we demonstrate alterations in activity levels of six sex-differentiated enzyme systems in male rats bearing ectopic pituitary tumors after the injection of a pituitary cell line, $C_8$11RAP. Activity levels of hepatic glutathione *S*-transferase, UDP-glucuronyltransferase, and aryl hydrocarbon hydroxylase are reduced to activity levels of control females, while histidase, 5α-reductase, and serum cholinesterase levels are increased to levels of control females, *i.e.* feminization of all of these enzymes. RIAs of testosterone, estrogen, FSH, and PRL are similar in tumor-bearing and control animals, but GH levels are significantly higher in tumor-bearing animals than in the controls. It is suggested that GH may be the pituitary factor responsible for the expression of sex-differentiated hepatic metabolism. (*Endocrinology* **117**: 523–526, 1985)

SEX-DIFFERENTIATED hepatic enzymology in rats is characterized by higher activities in adults of one sex over the other, while prepubertal males and females have similar activities. The ontogeny of sex-differentiated enzyme patterns has been linked to imprinting in the brain by estrogen derived from testicular androgen during the late fetal and/or early neonatal period of development (1–6). The expression and regulation of these enzyme levels are mediated via the hypothalamic-pituitary axis. Efforts to identify the specific hormones involved in modulating these enzymes have centered around endocrine-organ ablation and appropriate hormone replacement. The pituitary plays a major role in regulating the expression of these enzymes. Hypophysectomy can abolish sex differences, and in some cases, this effect can be reversed by the implantation of an ectopic pituitary gland (2, 5, 6). Furthermore, the action of the gonadal steroid hormones requires an *in situ* pituitary (2, 6–10).

The aim of this study was to investigate, in an intact animal, the effect of an ectopic pituitary tumor derived from the injection of a pituitary cell line ($C_8$11RAP) on three sex-differentiated hepatic enzyme systems that are higher in adult male rats (glutathione *S*-transferase, UDP-glucuronyltransferase, and aryl hydrocarbon hydroxylase) and three that are higher in adult female rats (hepatic histidase and 5α-reductase, and serum cholinesterase). Changes in enzyme activities were correlated with changes in endocrine parameters.

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Materials and Methods

Cell culture and tumor propagation

Pituitary tumor cells ($C_8$11RAP) were obtained from C. Sonnenschein (11) and maintained in Ham's F-12 medium plus 10% horse serum and 5% fetal calf serum (Grand Island Biological Co., Grand Island, NY) at 37 C in the presence of 5% CO_2 in air. The cells were grown to 80% confluency, collected by centrifugation (1000 × *g*; 20 min), washed once with 20 mM potassium phosphate buffer, pH 7.0, containing 0.85% NaCl (PBS), and subsequently resuspended in PBS. Cells (5×10^6; ~0.5 ml) or heat-treated cells (100 C for 5 min) were subsequently injected sc in the thigh of 35-day-old male Sprague-Dawley CD rats (Charles River Breeding Laboratories, Willmington, MA). To facilitate growth of the cells, Silastic implants of estrogen were placed sc at the nape of the neck on the same day. These implants consisted of 2.5 mm packed estradiol benzoate in 0.058 in. (id) × 0.077 in. (od) Silastic medical grade tubing sealed with silicone type A adhesive (Dow-Corning Co., Midland, MI). The animals were housed four per cage in a control environment (21 C; 12-h light-dark cycle) with free access to food (Purina Lab Chow 5001, Ralston-Purina, St. Louis, MO) and water. Tumors were evident by 21 days postinjection, at which time the estrogen implants were removed. The animals were killed 14 days later. Preliminary experiments with serum cholinesterase and hepatic histidase as sensitive enzyme markers of estrogen action (7, 8) revealed

that 10 and 14 days after the removal of the estrogen implants, these enzyme activities were similar to those in animals that never received estrogen.

Serum preparation

Animals were decapitated, trunk blood was collected, and the blood samples were placed in ice. After 1 h, the blood was allowed to clot at room temperature for 30 min before centrifugation (1200 × *g* for 20 min at 4 C). Supernatant serum aliquots were removed for immediate assay of cholinesterase activity, and the rest was stored at −80 C for later assay of hormones by RIA.

Enzyme assays

Livers were rapidly excised and placed on ice. Hepatic cytosol and microsomes were prepared as previously described (12). UDP-glucuronyltransferase was assayed from microsomes on the same day using *p*-nitrophenol as the substrate (4, 13). 5α-Reductase was assayed from freshly prepared microsomes using ^{3}H-labeled testosterone as the substrate and NADPH as cofactor (14). Aryl hydrocarbon hydroxylase activity was determined from microsomes prepared in 50 mM Tris (pH 7.4) containing 0.25 M sucrose, frozen at −80 C, and later assayed according to the method of Nebert and Gelboin (15) as modified by Yang *et al.* (16) using 3-hydroxybenzo(a)pyrene as the product standard. Cytosolic glutathione *S*-transferase activity was assayed from samples frozen at −80 C by measuring the conjugation of glutathione with the substrates 1,2-dichloro-4-nitrobenzene and 1-chloro-2,4-dinitrobenzene (5). Histidase was assayed spectrophotometrically, as previously described (17). Danner-Rabovsky and Groseclose (18) have shown that microsomal monooxygenase activities are stable for 1–2 months when frozen under these conditions, and we have confirmed this for aryl hydrocarbon hydroxylase, glutathione *S*-transferase, and histidase for a period of at least 8 weeks. Serum cholinesterase activity was determined using acetylthiocholine iodine and butyrylthiocholine iodide as substrates (7). The results on glutathione *S*-transferase using 1-chloro-2,4-dinitrobenzene as substrate and serum cholinesterase with acetylthiocholine iodine as substrate are not presented in the text, but similar patterns of alterations from the presence of the tumors were seen using 1,2-dichloro-4-nitrobenzene and butyrylthiocholine iodide as substrates, respectively.

Serum hormone levels

RIA of testosterone and estrogen was carried out by Dr. G. A. Mason, University of North Carolina (Chapel Hill, NC). Antiserum employed for testosterone exhibited 27.7% cross-reactivity with dihydrotestosterone, 1.3% cross-reactivity with androstenediol, and negligible (<0.06%) cross-reactivity with other androgens, estrogens, or corticosteroids (Serono Laboratories, Braintree, MA). The antiserum for RIA of 17β-estradiol (Radioassay Systems Laboratories, Carson, CA) exhibited 6.5% cross-reactivity with estriol, 5.2% cross-reactivity with 17α-estradiol, and negligible (0.55%) cross-reactivity with other estrogens, androgens, or corticosteroids. Serum FSH, PRL, and GH concentrations were determined by RIA by Dr. A. F. Parlow, Harbor General Hospital (Torrance, CA) using NIAMDD rat FSH RP-1, NIAMDD rat PRL RP-2, and NIAMDD rat GH I-4 as reference standards.

Other determinations

At the time of death, wet weights of liver, testes, seminal vesicles, ventral prostate, whole pituitary, and adrenals were recorded in both control and tumor animals. Protein determinations were performed by the method of Lowry *et al.* (19), with BSA as the standard. Statistical comparisons between groups were performed using Student's *t* test (two-tailed).

Results

Three weeks after injection of the $C_8$11RAP cells, palpable tumors were present at the site of injection. The tumors increased in size (1–5 cm in diameter) during the next 2 weeks even in the absence of exogenously administered estrogen. Whole body, testicular, pituitary, and adrenal weights were similar in the two experimental groups (Table 1). Liver weights were, however, increased in the tumor animals, while seminal vesicle and ventral prostate weights were decreased in the tumor-bearing animals compared to those in the controls.

As seen in Fig. 1, hepatic glutathione *S*-transferase, UDP-glucuronyltransferase, and aryl hydrocarbon hydroxylase activities were higher in adult males than in adult females (controls). The activity levels of these three enzyme systems were, however, significantly reduced in tumor-bearing males compared to the control males. Hepatic histidase and 5α-reductase, and serum cholinesterase activities were higher in control females than in control males. The presence of an ectopic pituitary tumor resulted in adult male rats having increased levels of the latter three enzyme systems, levels similar to those in adult females.

We subsequently determined certain circulating sex

TABLE 1. Body and organ weights of male rats with ectopic pituitary tumors

	Control males[a]	Tumor males[b]
BW (g)	285 ± 6	281 ± 8
Liver wt (g)	11.6 ± 0.3	14.7 ± 0.8[c]
Testes wt (g)	1.67 ± 0.10	1.65 ± 0.11
Seminal vesicles wt (mg)	464 ± 62	262 ± 41[c]
Ventral prostate wt (mg)	268 ± 34	100 ± 22[d]
Pituitary wt (mg)	15 ± 1	12 ± 3
Adrenal wt (mg)	74 ± 3	78 ± 7

The capsules were removed on day 56, and the animals were killed 14 days later. Values are the mean ± SEM (n = 8).

[a] Control males received heat-treated pituitary cells and Silastic implants of estradiol benzoate.

[b] Tumor-bearing males received 5 × 10^6 $C_8$11RAP cells and Silastic implants of estradiol benzoate at 35 days of age.

[c] $P < 0.05$ compared to control males.

[d] $P < 0.01$ compared to control males.

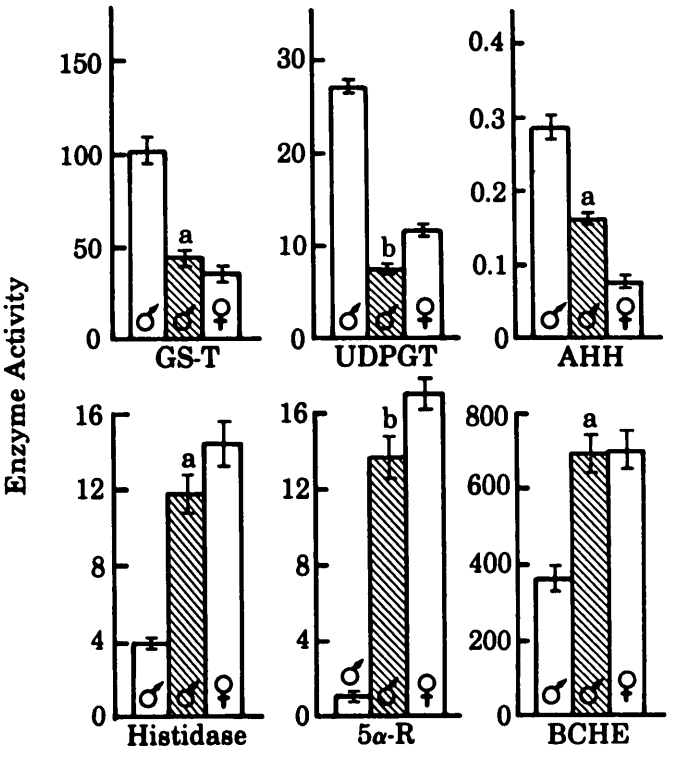

FIG. 1. Feminization of hepatic enzyme levels by an ectopic pituitary tumor. Control males received heat-treated pituitary cells and Silastic implants of estrogen. Tumor-bearing males (▨) received 5×10^6 $C_8$11RAP cells and Silastic implants of estradiol benzoate at 35 days of age. The capsules were removed after 21 days, and the animals were killed 14 days later. The values for the control females are representations of previous work. Enzyme activity levels (*ordinate*) of glutathione *S*-transferase (GS-T), UDP-glucuronyltransferase (UDPGT), aryl hydrocarbon hydroxylase (AHH), histidase, and 5α-reductase (5α-R) are expressed as nanomoles per min/mg protein, while butyryl cholinesterase (BCHE) is expressed as nanomoles per min/ml serum. Values are the mean ± SEM for at least eight animals per group. Statistical comparisons are made between control males and tumor-bearing males: a) $P < 0.01$; b) $P < 0.001$.

steroid and anterior pituitary peptide hormone levels. Testosterone, 17β-estradiol, FSH, and PRL concentrations were similar in both groups, while GH levels were significantly higher in the tumor-bearing animals than in the control animals (Table 2).

TABLE 2. Serum hormone levels in rats with ectopic pituitary tumors

Hormone	Control males[a]	Tumor males[b]
Testosterone (ng/ml)	4.1 ± 0.8	3.9 ± 0.7
Estrogen (pg/ml)	190 ± 43	171 ± 16
FSH (ng/ml)	389 ± 63	450 ± 28
PRL (ng/ml)	29 ± 7	29 ± 3
GH (ng/ml)	34 ± 8	200 ± 21[c]

[a] Control males received heat-treated pituitary cells and Silastic implants of estradiol benzoate.

[b] Tumor-bearing males received 5×10^6 $C_8$11RAP cells and Silastic implants of estradiol benzoate at 35 days of age. The capsules were removed 21 days postimplantation, and then the animals were killed 14 days later. Values are the mean ± SEM (n = 4 for each set of values, with each sample being equivolume from two animals within the treatment group).

[c] $P < 0.001$ compared to the control males.

Discussion

The enzyme systems that we measured are characterized by being sex differentiated in adult rats. Adult male rats have higher hepatic glutathione *S*-transferase, UDP-glucuronyltransferase, and aryl hydrocarbon hydroxylase, but lower hepatic histidase and 5α-reductase, and serum cholinesterase activity levels. These sex differences have been attributed to perinatal imprinting and endocrine regulation via the hypothalamic-pituitary axis (1–8, 14, 17, 20).

Hypophysectomy of male rats has been shown to result in increased activity levels of hepatic glutathione *S*-transferase (5), histidase (8), and serum cholinesterase (7), but decreased activity levels of hepatic UDP-glucuronyltransferase (4), aryl hydrocarbon hydroxylase (9), and 5α-reductase (2, 10). A pituitary implanted under the kidney capsule of a hypophysectomized male rat can reverse the effect of hypophysectomy on activity levels of glutathione *S*-transferase (5) and 5α-reductase (2). The sex steroids play a prominent role in the endocrine regulation of UDP-glucuronyltransferase (4), aryl hydrocarbon hydroxylase (9), histidase (21), 5α-reductase (2, 10, 14), and serum cholinesterase (7), but require an *in situ* pituitary for their action. The role of the pituitary in the regulation of these enzymes is obviously complex, and no common effector has been attributed to account for all of these postpubertal sex differences.

Our work demonstrates that an ectopic pituitary tumor in male rats modulates the activity levels of glutathione *S*-transferase, UDP-glucuronyltransferase, and aryl hydrocarbon hydroxylase toward female levels (a decrease in activity) and results in activity levels of histidase, 5α-reductase, and serum cholinesterase similar to female levels (an increase in activity). The ectopic pituitary has, therefore, caused a feminization of all of these sex-differentiated enzyme systems.

An assessment of organ weights revealed an increase in whole liver weights in tumor-bearing animals, suggesting increased protein synthesis, an observation that was confirmed from protein assays of cytosolic and microsomal fractions (results not shown). Seminal vesicle and ventral prostate weights were decreased in the tumor-bearing animals, but testes and whole body weights were not significantly altered. Circulating testosterone and FSH levels were, likewise, unchanged. While Silastic implants of estradiol benzoate had been given to promote growth of the pituitary cells (controls also received estrogen), the implants were removed 2 weeks before death to allow the estrogen to be completely metabolized and

disposed before the determinations. No significant differences in estrogen concentrations were noted between groups. Since estrogen, testosterone, and FSH levels are unchanged between experimental groups, it is doubtful that the gonadal steroids alone are directly responsible for the alterations in expression of these sex-differentiated enzyme levels and in the reduction of accessory sex organ weights.

GH was the only measured pituitary peptide concentration that changed as a consequence of the tumors; it was dramatically higher in tumor-bearing males than in control males. In contrast to reports by Sonnenschein and co-workers (11, 22), PRL levels in our tumor-bearing animals were not altered. In our experiments, exogenous estrogen had been discontinued, and androgen levels were normal, while their studies were carried out in tumor-bearing animals castrated 3 days before death (11). We have previously shown that exogenously administered GH, but not PRL, can reverse the effect of hypophysectomy on hepatic glutathione *S*-transferase (5). Feigelson (8) has previously demonstrated that GH exerts an inhibitory effect on histidase. Mode and coworkers (23) and Rumbaugh and Colby (24) have shown that GH modulates several hepatic steroid-metabolizing enzymes. Our work supports the concept of GH playing a major role in the expression and regulation of all of these sex-differentiated enzymes. Since there is no convincing evidence for GH sex differences to account for sex-differentiated enzymology, GH may or may not be the feminizing factor. This leaves open the possibilities of GH having differential effects in males and females and in imprinted animals (2, 5, 6) or GH being a mediator of a yet unidentified feminizing factor.

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