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REVIEW

Estrogen Action in the Regulation of Cell Proliferation, Cell Survival, and Tumorigenesis in the Rat Anterior Pituitary Gland

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Estrogens act as important regulators of cell proliferation, cell survival, and differentiation in a variety of organ systems and tissues and have been implicated in the etiology of a variety of malignant cancers and benign tumors. The anterior pituitary gland of the rat provides an excellent model for the study of estrogen action in the regulation of cell proliferation and survival. Estrogens stimulate proliferation of the prolactin (PRL)-producing lactotroph and enhance lactotroph survival. Through these actions on lactotroph proliferation and survival, estrogens induce or contribute to the development of PRL-producing pituitary tumors in several rat strains. Data from our laboratory and others indicate that estrogen-induced pituitary growth is rat strain specific and segregates as a quantitative genetic trait in crosses between different rat strains. The purpose of this review is to summarize current knowledge pertaining to estrogen action in the regulation of cell proliferation, cell survival, and tumorigenesis in the anterior pituitary gland of the rat species, *Rattus norvegicus*, and to illustrate the advantages of the rat pituitary gland as a model for elucidating the mechanisms through which estrogens regulate these processes.

Key Words: Estrogen; anterior pituitary; lactotroph; pituitary tumor; prolactin; rat.

Introduction

Estrogens, a class of steroid hormones produced primarily in the gonads, act as important regulators of cell proliferation, cell survival, and differentiation in a variety of organ systems and tissues, including the reproductive tract and the skeletal, cardiovascular, and central nervous systems, as well as a number of endocrine glands, including the adrenal, thyroid, and pituitary. Estrogens have been implicated in the etiology of a variety of malignant cancers, including those of the breast and uterus, and benign tumors of the anterior pituitary gland. The purpose of this review is to summarize current knowledge pertaining to estrogen action in the regulation of cell proliferation, cell survival, and tumorigenesis in the anterior pituitary gland of the rat species, *Rattus norvegicus*, and to illustrate the advantages of the rat pituitary gland as a model for elucidating the mechanisms through which estrogens regulate these processes.

Rat Anterior Pituitary Gland as a Model for the Study of Estrogen Action in the Regulation of Cellular Proliferation and Survival

The anterior pituitary gland of the rat provides an excellent model for the study of estrogen action in the regulation of cell proliferation and survival. Estrogens stimulate proliferation primarily within a single pituitary cell population, the prolactin (PRL)-producing lactotroph, and this cell population can be identified using antibodies specific to PRL. Estrogens also enhance lactotroph survival. Through these actions on lactotroph proliferation and survival, estrogens induce or contribute to the development of PRL-producing pituitary tumors in several rat strains. Data from our laboratory *(1)* and others *(2–7)* indicate that estrogen-induced pituitary growth (i.e., pituitary tumorigenesis) is rat strain specific and segregates as a quantitative genetic trait in crosses between different rat strains. This allows genetics- and genomics-based experimental approaches to be used to identify the genes that function in the regulation by estrogen of lactotroph proliferation and survival. This knowledge will lead to the elucidation of the molecular mechanisms through which estrogens regulate proliferation and survival in the lactotroph and perhaps other estrogen-responsive cell populations.

Estrogens and PRL-Producing Pituitary Tumors in Humans

Tumors within the anterior pituitary gland are common in humans; the incidence of subclinical microadenomas

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observed in unselected autopsies is as high as 27% *(8,9)*. Pituitary adenomas are usually comprised primarily of a single secretory cell type and the tumor-associated pathologies often result from a mass effect or an overproduction of the hormone product of that cell type. PRL-producing tumors, prolactinomas, comprise the most common type of pituitary tumor in humans *(8,9)*. Estrogens are implicated in the etiology of the development of prolactinoma. The incidence of prolactinoma. has been reported to be two-fold higher in females than males, when asymptomatic individuals dying of unrelated causes were examined at autopsy *(10)*. Furthermore, several case reports suggest that estrogens may act as a causative factor in prolactinoma development. The development of prolactinoma has been reported in male-to-female transsexuals who were treated with estrogen to induce breast development *(11,12)* and in a young woman with tall stature who was treated with estrogen to retard growth *(13)*. Development of prolactinoma has also been reported in a man with industrial exposure to mestranol, a semisynthetic estrogen used in oral contraceptives *(14)*. The dopamine agonist bromocriptine provides an effective treatment for prolactinoma and associated hyperprolactinemia *(15,16)*. These reports *(11–16)* and others illustrate many similarities in the manner in which the lactotrophs of the human and rat species respond to estrogens and suggest that knowledge generated in the study of estrogen action in the regulation of lactotroph proliferation and survival in the rat will prove relevant to human pituitary gland biology. The literature on the etiology, pathology, and treatment of human pituitary tumors was recently reviewed *(17–19)*.

Physiological Roles of Estrogens in the Anterior Pituitary Gland

The anterior pituitary is composed of five distinct secretory cell types, which are defined by the peptide hormones they produce. These cell types are the adrenocorticotrophic hormone–producing corticotroph, the thyroid-stimulating hormone–producing thyrotroph, the luteinizing-hormone– and/or follicle-stimulating hormone–producing gonadotroph, the growth hormone (GH)–producing somatotroph, and the PRL-producing lactotroph. The lactotroph and somatotroph are derived from a common stem cell and are the most abundant cell types within the anterior pituitary gland, comprising 20–50% and 20–35%, respectively, of the total anterior pituitary cells in mature rats *(20–22)*. In comparison, the gonadotrophs, corticotrophs and thyrotrophs each comprise only 2–10% of the total anterior pituitary cells *(23,24)*. The lactotroph, gonadotroph and corticotroph each express estrogen receptor (ER) alpha (ERα) and ER beta (ERβ) *(25)*. The actions of ERα have best been established in the lactotroph and gonadotroph *(26)*. The roles of estrogens in the regulation of lactotroph physiology are discussed next.

Estrogen Regulation of PRL *Gene Expression*

Estrogens regulate *PRL* gene expression at the levels of transcription and secretion. The stimulatory effects of estrogens on *PRL* gene transcription are rapid, independent of protein synthesis, and appear to parallel occupancy of the ER by ligand *(27–31)*. The *cis*-acting element through which the ER enhances *PRL* gene transcription has been localized upstream of the *PRL* promoter *(32-34)*. Estrogens also exert indirect stimulatory effects on the *PRL* gene *(28–30,35,36)*. One indirect mechanism involves a rapid inhibitory effect of estrogens on production by the hypothalamic tuberoinfundibular neurons of dopamine, a potent inhibitor of *PRL* gene transcription *(30,36,37)*. Estrogens stimulate PRL secretion through several direct and indirect mechanisms, including modulation of release and/or responsiveness of the lactotroph to a variety of neuropeptides and hypothalamic factors *(38–41)*.

Estrogen Regulation of Lactotroph Proliferation and Survival

The steady-state number of lactotrophs within the anterior pituitary gland is impacted by the rates at which lactotrophs proliferate and die, and estrogens play a major role in regulating this homeostasis in lactotroph number *(42,43)*. The stimulatory effect of administered estrogens on lactotroph proliferation has long been known *(42–47)*. The lactotroph population undergoes a marked expansion during pregnancy and lactation, which is followed by a rapid decline in lactotroph number on cessation of lactation *(48)*. The lactotroph population in rodents also oscillates over the course of each reproductive cycle; both the mitotic index *(49)* and S-phase fraction *(50,51)* within the lactotroph population are highest during the afternoon of estrus. Furthermore, the number of lactotrophs within the anterior pituitary of the mature female rat is markedly higher than in the male rat, and this difference is abrogated by peripubertal ovariectomy *(52)*. Together, these reports strongly suggest that the lactotroph population is remarkably dynamic in response to changes in endogenous estrogen levels.

Estrogens also regulate lactotroph survival. Withdrawal of administered estrogen allows regression of PRL-producing pituitary tumors induced in F344 rats by chronic treatment with hormone *(2,53)*. More recently, it has been demonstrated that regression of these estrogen induced pituitary tumors is associated with an increase in the number of pituitary cells undergoing apoptosis *(53)*. Data from our laboratory indicate that chronic treatment with 17β-estradiol (E_2) results in marked downregulation of the steady-state level of *t*estosterone *r*epressed *p*rostate *m*essage-2 (*TRPM-2*); also known as clusterin or sulfated glycoprotein-2) mRNA in the anterior pituitary of ovariectomized F344 and ACI rats *(42,54,55)*. Because the level of *TRPM-2* mRNA correlates with apoptotic activity in hormone-dependent tissues such as the prostate and breast *(56–58)*,

these data suggest that administered E_2 inhibits apoptosis within one or more anterior pituitary cell populations.

Estrogen Induction of Pituitary Tumors

Estrogen-Induced Pituitary Tumors: Historic and Histological Definitions

Chronic administration of estrogens, such as the synthetic nonsteroidal, diethylstilbestrol (DES) or the naturally occurring E2, has long been known to induce development of pituitary tumors in certain strains of rats *(59–61)* and mice *(61,62)*. Clifton and Meyer *(61)* and Clifton et al. *(63)*, using the pigeon crop sac assay, demonstrated that pituitary tumors induced in rats by estrogens produce abundant biologically active PRL *(61,63)*. Immunohistochemical techniques have been used to demonstrate that the lactotroph comprises the predominate cell type in estrogen-induced, PRL-producing pituitary tumors *(64,65)*.

The grossly enlarged pituitary masses that arise in rats in response to administered estrogen have historically been referred to as tumors by virtue of their markedly increased wet weight. Clifton and Meyer *(61)* defined a pituitary tumor induced by estrogens in rats as a pituitary mass that exceeds 30 mg in wet wt, a mass approximately three-fold greater than normal. Welsch et al. *(66)* defined a tumor as a gland with a mass exceeding 50 mg. It has been demonstrated in many studies that pituitary mass correlates with total pituitary cell number and pituitary gland DNA content *(2,43,46,67)*. Consequently, pituitary mass is a useful quantitative indicator of estrogen-induced pituitary growth. Moreover, pituitary mass correlates with the level of circulating PRL (Fig. 1), suggesting that both pituitary tumor mass and associated hyperprolactinemia are proportional to the total number of lactotrophs within the estrogen-induced tumor *(1,68)*.

The pituitary tumors that develop in rats in response to chronic treatment with estrogens are most accurately defined as diffuse lactotroph hyperplasias that exhibit vascular lakes and focal regions of hemorrhage, but generally lack adenomatous foci *(42,43,46,47,69)*. Mitotic figures are numerous in these tumors relative to that observed in the pituitary gland of the unstimulated female rat *(42,43,46,47,69)*. The lactotrophs in these tumors are hypertrophic, exhibiting abundant and well-developed rough endoplasmic reticulum and prominent Golgi complexes, consistent with stimulated PRL-synthesizing activity *(46,47,69)*.

Estrogen-induced pituitary tumors are generally estrogen dependent and regress on withdrawal of administered hormone *(2,53,70)*. It has been demonstrated that these pituitary tumors can, on long-term treatment with estrogens, progress to a hormone-independent state *(70)*. From our personal experience, it is apparent that chronic treatment with estrogens can occasionally lead to the development of pituitary carcinoma, but only after many months of treatment.

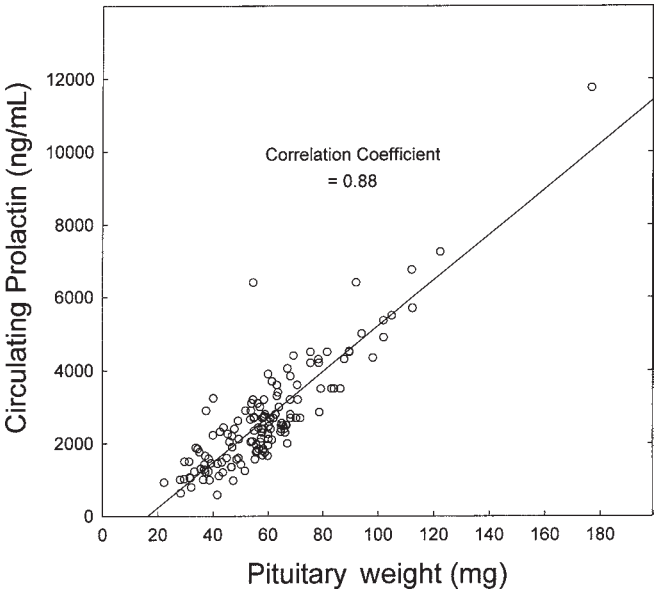

Fig. 1. Circulating PRL correlates with pituitary mass in DES-treated rats. Male progeny from an intercross between the ACI and Copenhagen rat strains were treated with DES for 12 wk. Pituitary wet weight was plotted against the concentration of PRL in serum prepared from trunk blood. ○, data from an individual animal. (Adapted with permission from ref. *1*.)

Rat Strain–Specific Effects of Estrogens on Pituitary Tumor Development

Fischer 344

The Fischer 344 (F344) rat strain, developed in 1920 by Maynie Curtis *(71,72)*, appears to be the most sensitive of the inbred rat strains to the pituitary tumor–inducing actions of administered estrogens (Fig. 2). Consequently, the actions of estrogens in stimulating pituitary growth have been most extensively studied in this strain. Continuous treatment of F344 rats, both females and males, with estrogens induces rapid pituitary growth, resulting in significant increases in pituitary weight within a few days of initiation of estrogen treatment *(67,73,74)* and 5- to 11-fold increases in pituitary weight following 8–12 wk of estrogen treatment *(2,42,43,46,54,67,70,75–78)*. The naturally occurring estrogens E2 *(42,54,74)* and, to a lesser extent, estrone *(79)* and the synthetic estrogens DES *(43,70,75,76)*, DES-dipropionate *(53)*, and estradiol valerate *(80)* are all effective in inducing pituitary tumors in the F344 strain. Overproduction of PRL by these estrogen-induced tumors results in gross hyperprolactinemia *(42,46 47,69,78)*. For example, data from our laboratory *(42)* indicate that circulating PRL in ovariectomized female F344 rats was increased 220-fold following 10 wk of E_2 treatment (Fig. 3). The lactotroph populations within these estrogen-induced tumors exhibit significant heterogeneity with respect to cell size as well as PRL-secreting properties *(81,82)*. As we have already noted, the pituitary tumors induced in F344 rats by chronic treatment with estrogens are most accurately defined as

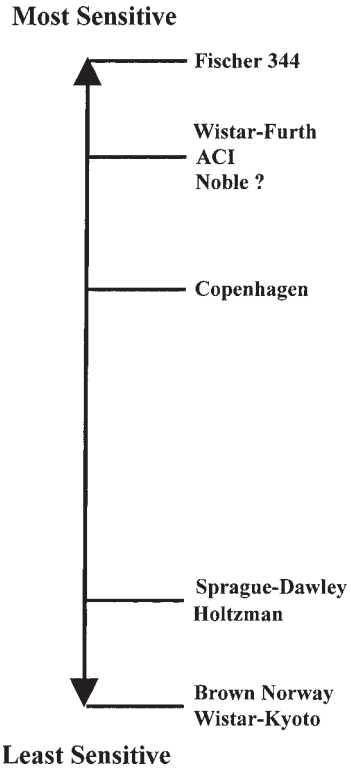

Fig. 2. Sensitivity of different inbred rat strains to estrogen-induced pituitary growth. Relative sensitivity to estrogen-induced pituitary growth is represented as a continuum from most sensitive (**top**) to least sensitive (**bottom**). (Adapted from published studies cited within the text.)

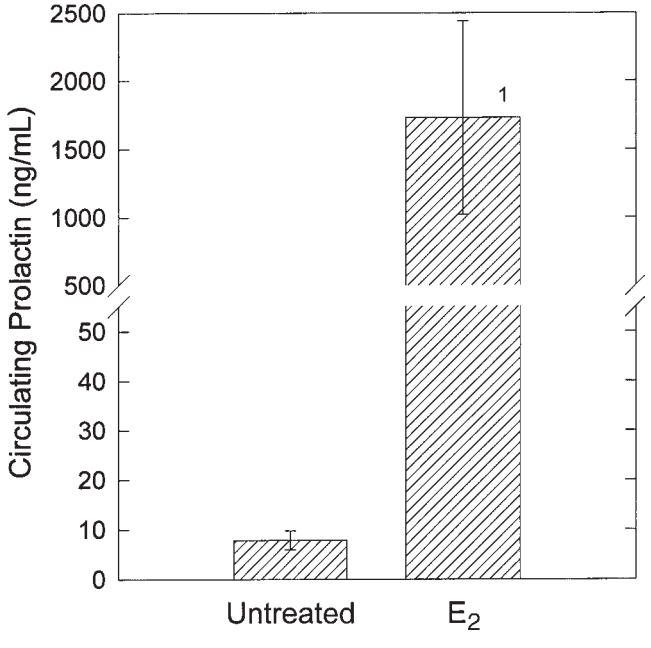

Fig. 3. Estradiol induces hyperprolactinemia in F344 rats. Ovariectomized F344 rats were treated with E_2, administered from sc Silastic implants, for 10 wk, and the concentration of PRL in trunk blood serum was measured by radioimmunoassay. Each data bar represents the mean (± SE). 1, a statistically significant difference ($p \leq 0.05$) between untreated and E_2-treated animals. (Adapted from ref. *42*. Reprinted by permission of Wiley-Liss, Inc., a division of John Wiley & Sons, Inc.)

diffuse lactotroph hyperplasias that lack adenomatous foci *(42,43,46,47,54)*.

The PRL-producing pituitary tumors that develop in F344 rats in response to estrogen treatment are estrogen dependent and regress when estrogen is withdrawn *(2,53)*. On withdrawal of hormone, the capacity of these estrogen-induced tumors to produce PRL declines more rapidly than does the mass of the tumorous gland, suggesting that regulation of PRL gene expression may occur through a mechanism(s) that is distinct from that which regulates lactotroph proliferation and/or survival *(47,69)*. Although regulation of cell death in the anterior pituitary has been investigated in only a few studies, it appears that regression of estrogen-induced pituitary tumors following withdrawal of hormone occurs, at least in part, through apoptosis *(53)*.

Dunning et al. *(83)* were the first to demonstrate that pituitary tumors induced by estrogens in F344 rats could be transplanted into estrogen treated, but not untreated, syngeneic hosts. This finding was subsequently confirmed by Furth et al. *(76)*, who further demonstrated that these estrogen-induced pituitary tumors could, on successive transplantation into estrogen-treated hosts, achieve an autonomous state capable of growing as grafts in untreated female and male hosts. The transition from the estrogen-dependent to the autonomous state was associated with changes in histological features consistent with neoplastic transformation *(76)*. Transplantable pituitary tumor lines have been established from these autonomous tumors *(70)*. One of these transplantable tumor lines, MtTF4, expresses the ER *(84)* and, in contrast to the lactotroph of the F344 pituitary gland, is growth inhibited by administered E2 *(84–86)*. Interestingly, E_2 enhances expression of PRL and GH mRNA by this transplantable pituitary tumor *(86)*.

It is well established that the lactotroph of the F344 rat proliferates in response to estrogens. Gersten and Baker *(44)* were the first to demonstrate that administered estradiol benzoate induces lactotroph hyperplasia in the F344 pituitary gland *(44)*, and this observation has been confirmed in numerous studies *(42,43,46,47,74)*. Consequently, the number of lactotrophs, when expressed as a percentage of total anterior pituitary cells, is approximately two-fold greater in female and male F344 rats treated chronically with estrogens than in untreated ovariectomized females and males *(42,43,46)*. Administered E_2 increases significantly the fraction of lactotrophs staining positive for proliferating cell nuclear antigen *(74)*. Data from our laboratory *(42)* indicate that the number of lactotrophs incorporating the thymidine analog 5-bromo-2'-deoxyuridine (BrdU), is increased approximately three-fold in ovariectomized F344 rats treated with E_2 for 10 wk relative to untreated controls (Fig. 4). The stimulatory actions of administered estrogens on lactotroph proliferation are rapid. Wiklund and Gorski *(4)* observed a significant stimulation of anterior pituitary DNA synthesis in the male F344 rat within 24 h of initiation of treatment with E_2 or DES.

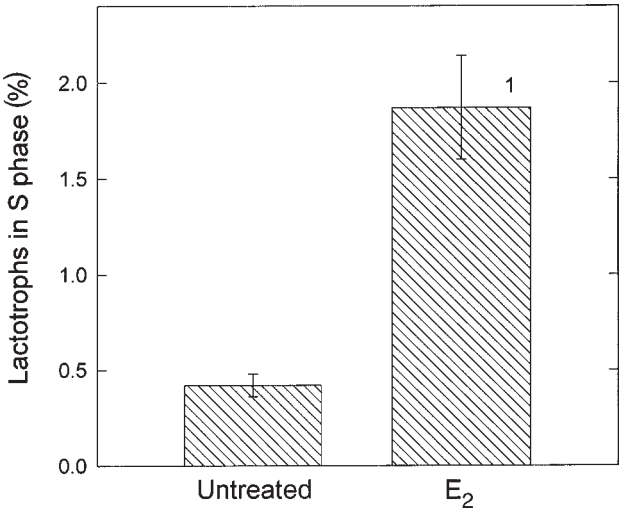

Fig. 4. Estradiol induces lactotroph proliferation in F344 rats. Ovariectomized F344 rats were treated with E_2, administered from sc Silastic implants, for 10 wk. Lactotrophs incorporating BrdU were identified immunohistochemically and quantified using a computer-assisted imaging system. Each data bar represents the mean (± SE) number of PRL/BrdU-positive cells expressed as a percentage of total PRL-positive cells. 1, a statistically significant difference ($p \leq 0.05$) between untreated and E_2-treated animals. (Adapted from ref. *42*. Reprinted by permission of Wiley-Liss, Inc., a division of John Wiley & Sons, Inc.)

Minami and Sarkar *(87)* demonstrated an increase in the number of lactotrophs incorporating BrdU in ovariectomized F344 rats that were treated with E_2 for 6 d. Significant increases in pituitary mass have been observed within 8–21 d of initiation of estrogen treatment *(2,67,88)*, and this increase in mass is associated with increases in pituitary DNA content *(2)* and cell number *(46,67)*. Together, these data indicate that the lactotroph of the F344 rat proliferates in response to administered estrogens, resulting in lactotroph hyperplasia and most probably contributing to development of PRL-producing pituitary tumors.

Data from our laboratory suggest that in addition to stimulating lactotroph proliferation, estrogens may also enhance lactotroph survival. Treatment of ovariectomized F344 rats for 10 wk with E_2 down-regulates the level of *TRPM-2* mRNA in the anterior pituitary gland *(42)*. Because the level of *TRPM-2* mRNA is upregulated in association with an induction of apoptosis in a variety of hormone-dependent tissues and cell lines following hormone withdrawal *(56-58)*, this mRNA is considered to be a surrogate marker of apoptosis. Consequently, our data suggest that estrogens enhance survival within one or more pituitary cell populations, most likely the lactotroph population. Supporting this assertion is the observation of Drewett *et al. (53)* indicating that the number of apoptotic bodies in estrogen-induced pituitary tumors in F344 rats is increased following hormone withdrawal. From these data, it appears that enhancement of lactotroph survival may contribute in a significant manner to the development of PRL-producing pituitary tumors in the F344 rat strain.

The development of PRL-producing pituitary tumors in the F344 rat appears to be associated with an excessive proliferative response within the lactotroph population to administered estrogen. Wiklund et al. *(2)* and Wiklund and Gorski *(4)* were the first to demonstrate a marked difference in the manner in which estrogens stimulate pituitary DNA synthesis and induce pituitary growth between the F344 and the Holtzman rat strains; the latter does not develop a PRL-producing pituitary tumor in response to chronic estrogen treatment. E_2 and DES rapidly induced pituitary DNA synthesis, expressed as picomoles of thymidine incorporated per microgram of DNA, in both the F344 and Holtzman strains. However, the proliferative response in the Holtzman strain was attenuated following 7–10 d of estrogen treatment, whereas the proliferative response in the F344 strain was not *(2,4)*. These observations have been confirmed and extended in our laboratory (*[43]*, Shull et al., unpublished data). These data indicate that administered estrogens rapidly stimulate lactotroph proliferation in males and ovariectomized females of both the Holtzman and F344 rat strains, leading to lactotroph hyperplasia and a significant increase in pituitary mass in both strains. The attenuation of stimulated DNA synthesis in the Holtzman strain, first reported by Wiklund et al. *(2)* and Wiklund and Gorski *(4)* and confirmed in our laboratory, allows the lactotrophs of the Holtzman pituitary gland to remain in the hyperplastic/hypertrophic state while pituitary mass is maintained at a new steady-state value 2.0- to 2.5-fold greater than that observed in untreated animals. By contrast, the unattenuated proliferative response of the F344 lactotroph results in a hyperplastic/hypertrophic state and a continuous increase in pituitary mass, total lactotroph number, and circulating PRL level (*[43]*; Shull et al., unpublished data). Figure 5 illustrates these strain differences. Studies summarized below indicate that this excessive proliferative response of the F344 lactotroph is genetically conferred.

It is clear that estrogens are capable of acting directly on the anterior pituitary gland of the F344 rat to stimulate lactotroph proliferation. Insertion of estrogen-containing pellets directly into the F344 anterior pituitary gland induces lactotroph hyperplasia in the ipsilateral, but not contralateral, lobe *(44)*. Studies by Clifton and Furth *(70)* and Wiklund et al. *(2)* have demonstrated estrogen-induced growth of F344 pituitary glands transplanted under the kidney capsule. Moreover, Wiklund et al. *(2)* demonstrated that DES induces marked growth of F344, but not Holtzman, pituitary glands following transplantation of these glands under the kidney capsule of F344 x Holtzman F1 hosts, indicating that the genetically conferred growth response of the F344 pituitary gland is inherent to the gland itself. The lactotroph-like cell line, PR1, was derived from a pituitary tumor induced in an F344 rat by DES, and exhibits a proliferative response to estrogens in vitro, suggesting

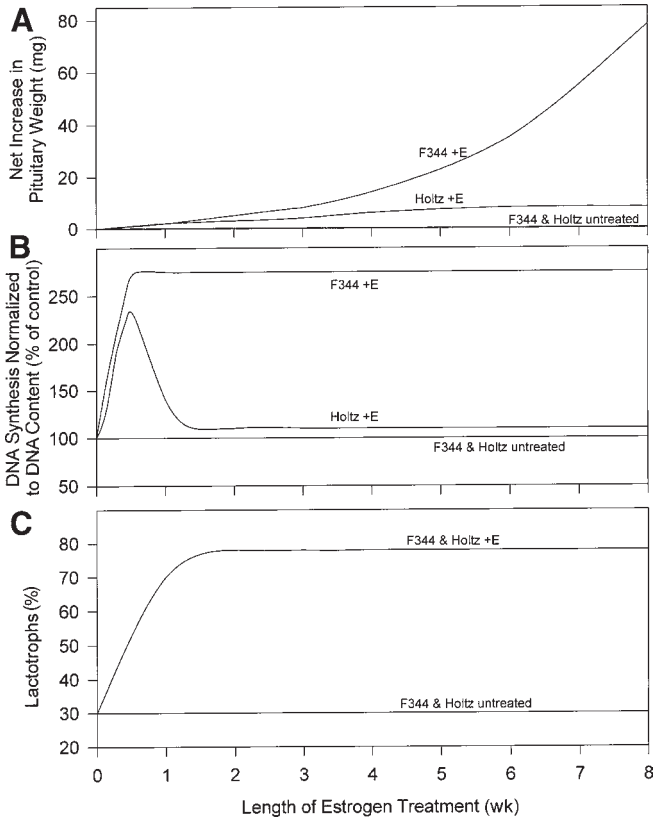

Fig. 5. Summary of rat–strain specific actions of estrogens in stimulating pituitary growth. (**A**) Chronic treatment with estrogens induces continuous pituitary growth (i.e., pituitary tumor development) in F344, but not Holtzman rats (**B**). In the F344 rat strain, administered estrogens induce excessive lactotroph proliferation, as evidenced by continuous stimulation of pituitary DNA synthesis, and the rate of lactotroph proliferation exceeds the rate of lactotroph death, resulting in continuous pituitary growth (**A**). (**C**) The lactotroph population in the estrogen-treated F344 rat is in a hyperplastic and hypertrophic state, resulting in marked hyperprolactinemia. By contrast, in the Holtzman rat strain, the induction of lactotroph proliferation is attenuated following 7–10 d of estrogen treatment (**B**). Thereafter, the rate of lactotroph proliferation and the rate of lactotroph death are in equilibrium, the lactotroph population is maintained in a hyperplastic state (**C**), and pituitary mass (**A**) and total lactotroph number are maintained at a new steady-state level approximately two-fold greater than that observed in an untreated animal. (Data adopted with permission from ref. *2*, *4*, and *43*, as well as from the Shull laboratory.)

that estrogens act, at least in part, directly on the lactotroph to stimulate proliferation *(89,90)*.

One potential autocrine mediator of the actions of estrogens on lactotroph proliferation is transforming growth factor-β1 (TGF-β1). The lactotroph of the F344 rat expresses TGF-1 and the type II TGF-β receptor (TβR-II) *(89,91–93)*. TGF-β1 inhibits proliferation of and secretion of PRL by cultured lactotrophs isolated from E2-treated F344 rats *(91)*. Moreover, intrapituitary administration of TGF-β1 reverses in part the stimulatory effect of administered E_2 on lactotroph proliferation in ovariectomized F344 rats treated with E_2 *(87)*. Pastorcic et al. *(89)* demonstrated that continuous treatment of F344 rats with E_2 results in a significant inhibition of expression of the genes encoding TGF-β1 and TβR-II. Together, these data suggest that estrogens may stimulate lactotroph proliferation and contribute to pituitary tumor development in the F344 rat by abrogating the ability of TGF-β1 to act as an autocrine/paracrine inhibitor of lactotroph proliferation.

The neuropeptide galanin also appears to be a mediator of the actions of estrogens on lactotroph function. Numerous reports indicate that the expression of galanin in the rat anterior pituitary gland (various rat strains have been examined in this regard) is stimulated by estrogens *(94–99)*. Vrontakis et al. *(100)* identified galanin as a gene that is upregulated during estrogen-induced pituitary tumor development in the F344 rat, by differential screening of a cDNA library prepared from mRNA isolated from a pituitary tumor induced by DES. Rat anterior pituitary cells express high affinity receptors for galanin *(101)*, and this peptide is a potent stimulator of PRL secretion from cultured rat lactotrophs and 235-1 rat pituitary tumor cells *(39,101)*. A neutralizing antibody to galanin blocks the ability of E2 to stimulate proliferation of 235-1 cells *(39)*. Recently it has been demonstrated that E_2, when administered to mice that are homozygous for null mutant galanin alleles, appears unable to stimulate lactotroph proliferation *(40)*. Although existing data strongly suggest that galanin may be an important autocrine regulator of estrogen action on the lactotroph, a role for galanin in the development of estrogen-induced pituitary tumors has not been established.

Numerous studies suggest that estrogens may also act through the hypothalamus to stimulate lactotroph proliferation and induce development of PRL-producing pituitary tumors in the F344 rat. Three nonmutually exclusive mechanisms have been proposed in this regard: (1) estrogens downregulate production of hypothalamic dopamine, a potent inhibitor of lactotroph proliferation; (2) chronic estrogen treatment results in damage to the dopamine-producing neurons of the hypothalamus; and (3) estrogen treatment induces development of a direct arterial blood supply to the anterior pituitary gland, thereby bypassing negative regulation of lactotroph proliferation by hypothalamic dopamine.

The tuberoinfundibular dopaminergic neurons of the mediobasal hypothalamus, with cell bodies in the arcuate nucleus and termini in the median eminence, synthesize and release dopamine into the hypophysial portal blood for transport to the anterior pituitary *(102)*. There, dopamine interacts with D2 dopamine receptors present on the lactotrophs *(103–106)* and inhibits PRL gene transcription *(36,107–110)*, PRL secretion *(111–115)*, and lactotroph proliferation *(111)*. Production of dopamine is controlled in large part through regulation of expression of the gene encoding tyrosine hydroxylase (TH), the enzyme that catalyzes the rate-limiting step in the biosynthetic pathway

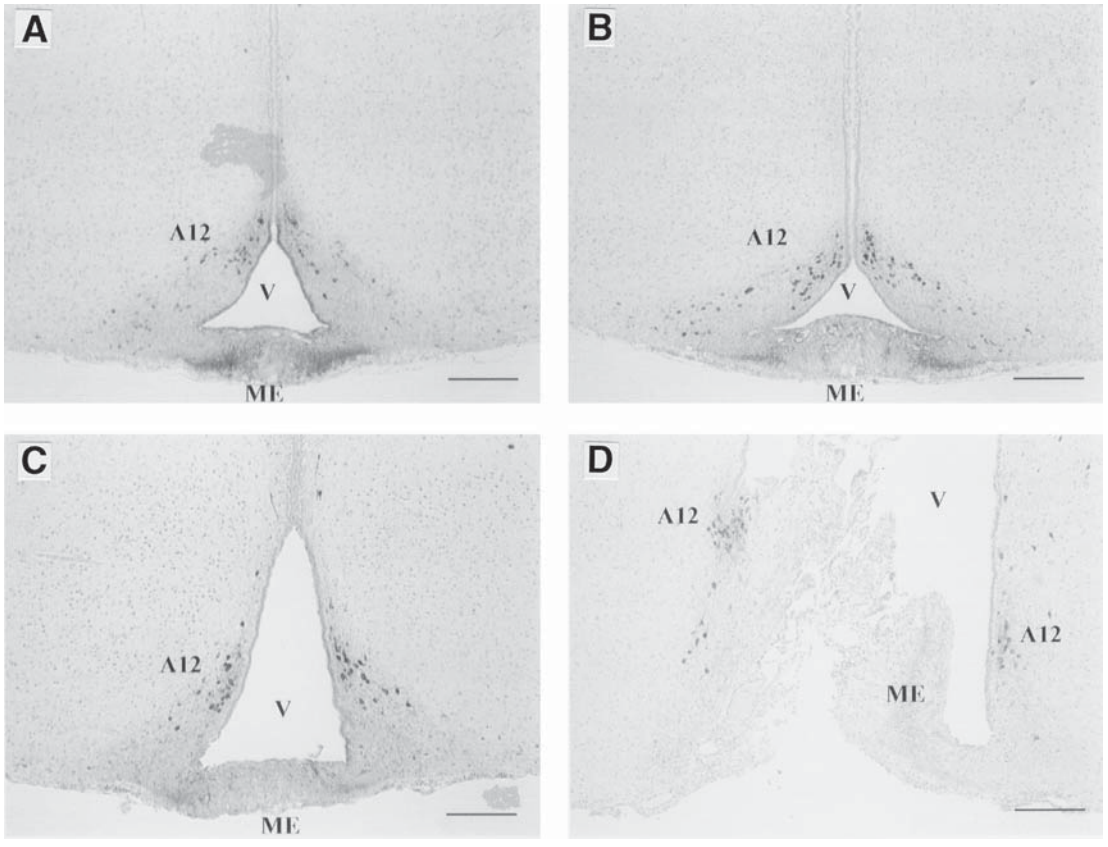

Fig. 6. Hypothalamic function and estrogen-induced pituitary tumorigenesis. (**A**) Mediobasal hypothalamus from an untreated, ovariectomized, F344 rat. TH staining was observed in the arcuate nucleus (A12) and median eminence (ME). (**B**) Hypothalamus of an ovariectomized F344 rat following 8 wk of treatment with E_2. This animal exhibited a moderately sized pituitary tumor and hyperprolactinemia. TH staining was clearly apparent, although perhaps slightly reduced relative to that observed in an untreated ovariectomized rat. (**C**) Hypothalamus of an ovariectomized F344 rat following 8 wk of DES treatment. This animal exhibited a moderately sized pituitary tumor and hyperprolactinemia. TH staining was slightly reduced relative to control. The third ventricle was enlarged, perhaps owing to hydrostatic pressure generated on decapitation. (**D**) Hypothalamus from a second ovariectomized F344 rat treated with DES for 8 wk. This animal exhibited a markedly enlarged pituitary tumor and hyperprolactinemia. The hypothalamus was severely distorted and the ME was displaced rostrally. TH staining in the arcuate nucleus remained apparent, but these neurons were displaced from their normal position. Because both E_2 and DES are capable of inducing significant pituitary growth in the absence of a loss of TH immunoreactivity, it is suggested that hypothalamic distortion resulting from the presence of a markedly enlarged pituitary tumor may contribute to loss of hypothalamic function, which in turn may allow rapid pituitary growth during the latter stage of tumor development.

leading to dopamine. Estrogens inhibit TH gene expression and thereby inhibit production and release of dopamine *(37,116–118)*. Because of the well documented inhibitory actions of dopamine agonists on lactotroph proliferation *(53,78,111)*, it is generally believed that this reduction in dopamine output may relieve a tonic inhibition of lactotroph proliferation.

It has been demonstrated that chronic treatment of female F344 rats with DES virtually abolishes TH immunoreactivity in the arcuate nucleus within 30 d of initiation of treatment ***(73)***. Withdrawal of hormone following 30 d of treatment resulted in restoration of TH immunoreactivity to control levels, whereas TH immunoreactivity remained depressed when DES was withdrawn after 60 d (73). We too have examined the effects of chronic estrogen treatment on TH immunoreactivity in the perikarya of neurons in the arcuate nucleus and in the axonal processes in the median eminence (Fig. 6). Treatment of ovariectomized female F344 rats with E_2 for 8 wk induced pituitary tumors that exceeded normal mass by up to seven-fold. Immunoreactivity to TH was similar or only slightly reduced in animals treated with E_2 for 8 wk relative to the intensity of TH staining in untreated ovariectomized female rats. Treatment of six ovariectomized female F344 rats for 8 wk with DES resulted in pituitary tumors that exceeded by 4- to 20-fold the mass of a normal gland. One DES-treated animal harbored a pituitary tumor weighing 42 mg and exhibited normal TH immunoreactivity and no apparent hypothalamic distortion. A second DES-treated animal harbored a pituitary tumor weighing 69 mg; TH immunoreactivity in this animal was reduced relative to that of control animals, but the degree of hypothalamic deformation was slight.

Pituitary tumors exceeding 99 mg were observed in the four remaining DES-treated animals. In these animals, the hypothalamus was compressed and severely distorted, the arcuate nucleus and median eminence were displaced rostrally, and TH immunoreactivity was clearly reduced relative to that observed in untreated control animals (Fig. 6).

Although the data of El-Azouzi et al. *(73)* and our laboratory illustrate a correlation among marked loss of TH protein, hypothalamic distortion, and development of markedly enlarged pituitary tumors in DES-treated F344 rats, cause-and-effect relationships cannot be concluded. Because our data indicate that both E_2 and DES are capable of inducing significant pituitary growth in the absence of loss of TH immunoreactivity, we suggest that hypothalamic distortion resulting from the presence of a markedly enlarged tumor may in fact contribute to loss of hypothalamic function and in turn allow for more rapid pituitary growth during the latter stage of tumor development.

It has long been appreciated that the development of estrogen-induced pituitary tumors in the F344 rat is associated with marked hypervascularity and hemorrhage *(46,78,119)*. Elias and Weiner *(120)* demonstrated that administration of E_2 for 9 wk to ovariectomized F344 rats resulted in the formation of a direct arterial blood supply to the anterior pituitary gland. Interestingly, a direct arterial blood supply to the pituitary gland did not develop, or developed to a much lesser extent, in E_2-treated rats of the Sprague-Dawley strain, which, relative to F344 rats, are resistant to development of estrogen-induced pituitary tumors. The F344 and Sprague-Dawley rat strains also differ with respect to the vascular changes that occur within the pituitary gland in response to chronic treatment with estradiol benzoate *(121)*. Whereas the vasculature within the F344 pituitary gland was disrupted, resulting in the formation of hemorrhagic lakes, the vasculature of the Sprague-Dawley pituitary remained essentially normal *(121)*. Simultaneous treatment of ovariectomized F344 rats with the dopamine agonist bromocriptine and E_2 partially abrogated the estrogen-mediated effects on the pituitary vasculature, pituitary growth, and circulating PRL *(122)*. More recently, the antiangiogenesis agents TNP-470 and fumagillin have been demonstrated to inhibit development of estrogen-induced pituitary tumors in the F344 rat *(88,123)*. Based on these data, researchers have suggested that estrogen-induced vascular changes, such as the development of an extraportal blood supply that would subvert dopamine-mediated inhibition of lactotroph proliferation, may be important contributing factors in the development of estrogen-induced pituitary tumors. In this regard, it is interesting to note that estrogen-induced pituitary growth and the development of the hemorrhagic appearance of the induced tumor appear to be genetically separable phenotypes *(6)*. Therefore, the use of genetics-based approaches may prove useful for establishing the role of es trogen-induced vascular changes in the development of pituitary tumors.

ACI

The inbred ACI (also known as AxC, Irish) rat strain was derived by Maynie Curtis and Wilhelmina Dunning in 1926 from the mating of a male of the August (AUG) strain with a female of the Copenhagen (COP) strain *(72)*. The ACI rat is unique among rat strains in that it is highly susceptible to development of estrogen-induced mammary cancers, but is highly resistant to spontaneously arising and carcinogen-induced mammary cancers ([124] and references cited therein).

Segaloff and Dunning *(75)* were the first to report that continuous treatment with estrogens induces development of pituitary tumors in ACI rats, and this observation was confirmed and extended in subsequent studies by Dunning and various colleagues *(75,83,125–127)* which indicated that both female and male ACI rats develop pituitary tumors when treated with DES, E2, or estrone. A direct comparison of the ACI and F344 strains indicated that the ACI strain is somewhat less sensitive to the pituitary growth promoting actions of estrogens than is the F344 strain *(75)*. Holtzman et al. *(128)* biochemically and histologically characterized the pituitary tumors that develop in female ACI rats on chronic treatment with DES. Lactotroph hypertrophy was observed within 10 days of initiation of DES treatment, and diffuse lactotroph hyperplasia was observed within 28 d. Friable and hemorrhagic tumors exhibiting adenomatous foci were observed in animals treated with DES for 130 d and longer. The level of circulating PRL was increased within 2 d of initiation of treatment, and the degree of hyperprolactinemia increased with the duration of DES treatment. In the same study, it was demonstrated that the Sprague-Dawley rat strain is much less sensitive than the ACI strain to the pituitary tumor–inducing actions of DES *(128)*, leading these investigators to conclude that pituitary tumor–associated hyperprolactinemia may play a causative role in development of mammary cancers in DES-treated ACI rats *(129)*. However, recently published *(124)* and unpublished data from our laboratory indicate that hyperprolactinemia is not the causative factor in the development of estrogen-induced mammary cancers in the ACI rat. The development of pituitary tumors in male ACI rats treated with E_2 appears to be mediated through the ER, as evidenced by inhibition of E_2-induced pituitary growth by the antiestrogen tamoxifen *(130)*.

Recent studies from our laboratory indicate that administered estrogens induce development of PRL-pituitary tumors in both ovary intact and ovariectomized female as well as male ACI rats *(1,54,55,124)*. Treatment with E_2 resulted in a five-fold stimulation of lactotroph proliferation, as indicated by immunohistochemical detection of lactotrophs incorporating the BrdU, and inhibition of apoptosis, as evidenced by reduced expression of *TRPM-2* mRNA (55). The data summarized under "Genetic Control of Estrogen-Induced Pituitary Growth" indicate that the genetic bases of estrogen-induced pituitary tumor develop-

ment in the ACI rat differ in many important respects from those exhibited by the F344 rat *(1)*. These data indicate that multiple molecular pathways contribute to the development of estrogen-induced, PRL-producing pituitary tumors in the rat species.

Copenhagen

The Copenhagen 2331 (COP) inbred rat strain was developed by Curtis in 1926 *(72)*. The COP rat is noted among inbred rat strains for its high degree of resistance to spontaneously arising, carcinogen- and estrogen-induced mammary cancers *(125–127,131–136)*. Dunning et al. *(125,127)* and Dumming and Curtis *(126)* were the first to report the development of pituitary tumors in COP rats treated chronically with estrogens. Data from our laboratory indicate that DES and E_2 induce pituitary tumors and associated hyperprolactinemia in COP rats, both female and male *(1,136)*. Like estrogen-induced pituitary tumors in the F344 and ACI strains, these tumors in the COP strain are grossly enlarged, highly vascularized masses exhibiting diffuse lactotroph hyperplasia *(136)*. Both DES *(136)* and E_2 (Shull et al., unpublished data) stimulate lactotroph proliferation as evidenced by immunohistochemical identification of lactotrophs incorporating BrdU. The observation that estrogens induce PRL-producing pituitary tumors and associated hyperprolactinemia in both the COP and ACI strains, whereas only the ACI strain is highly susceptible to the development of estrogen-induced mammary cancers, strongly suggests that hyperprolactinemia is not the causative factor of the development of mammary cancer in the ACI strain *(136)*. Direct comparison of the COP and ACI strains indicates that the pituitary growth response of the COP strain is significantly less than that of the genetically related ACI strain *(1)*. Genetic studies summarized next indicate that this quantitative difference in the pituitary growth response of the COP and ACI strains is genetically conferred.

Wistar Furth

The Wistar Furth inbred rat strain was developed by Jacob Furth from outbred Wistar stock from the Wistar Institute *(71,137)*. A noteworthy characteristic of the Wistar Furth rat strain is its propensity to develop PRL-producing pituitary tumors in an age-dependent manner *(137–140)*. Because the incidence of these spontaneous pituitary tumors is much higher in females than males, it is assumed that estrogens may play a role in the etiology of these tumors. Numerous studies indicate that prolonged treatment of Wistar Furth rats, both female and male, with estrogens induces PRL-producing pituitary tumors *(141–149)*. Histologically, these tumors exhibit diffuse lactotroph hyperplasia, lactotroph hypertrophy, vascular lakes, and hemorrhagic foci *(141–148)*, similar to those induced by estrogens in F344 and ACI rats. Pituitary tumors induced by estrogens in Wistar Furth rats regress when the exogenous hormonal stimulus is withdrawn *(142)*. Although the rate of estrogen-induced pituitary growth in the Wistar Furth strain has not, to our knowledge, been compared directly to that of other strains, it appears from published data that the pituitary growth response of the Wistar Furth rat is less than that of the F344 rat (Fig. 2). The role of the hypothalamus in the development of estrogen-induced pituitary tumors in Wistar Furth rats is not clear. Brawer and Sonnenschein *(150)* have reported estrogen-induced neuronal degeneration in the arcuate nucleus, whereas data of Fujimoto et al. *(147)* and Arita et al. *(149)* suggest that dopamine-producing capacity is not permanently impacted by long-term estrogen treatment.

Numerous transplantable tumor lines have been developed from spontaneously arising pituitary tumors in the Wistar Furth strain *(137,140,151)*, and administered estrogens inhibit the growth of these tumor lines in syngeneic hosts *(143,145,146,148)*. The widely used pituitary tumor cell lines GH3 and GH4C1 were established from a pituitary tumor induced in Wistar Furth rats by radiation *(152,153)*. Estrogens stimulate proliferation of these cell lines in vitro in a population density–dependent manner *(154–157)*.

Pei and Melmed *(158)* recently identified, by differential display comparative analysis of mRNA from the GH4C1 and normal rat pituitary gland, a gene, *PTTG*, that appears to be associated with pituitary tumor development. Expression of *PTTG* is increased in a large fraction of human pituitary tumors, and both the rat and human homologs of *PTTG* exhibit transforming activity in mouse 3T3 cells *(158–160)*. To our knowledge, a role for *PTTG* in the development of estrogen-induced pituitary tumors has not been established at this time.

Noble

The Noble (Nb) inbred rat strain was developed by Robert Noble from an outbred colony maintained in J. B. Collip's laboratory at McGill University that was probably originally derived from the Long-Evans strain *(161)*. The Nb rat strain exhibits two traits that make it unique among rat strains. First, the Nb strain has a propensity to develop mammary cancers when treated with estrogen alone *(161–165)* and in combination with androgens *(166–168)*. Second, long-term treatment of Nb rats with estrogens in combination with androgens leads to development of cancers of the prostate *(169)*, whereas shorter periods of treatment with estrogens and androgens induce atypical prostatic hyperplasia *(170,171)*. It has long been known that estrogens induce development of pituitary tumors in Nb rats *(161,172,173)*. Estrone, estriol, and DES were each capable of inducing the development of pituitary tumor *(161)*. Direct comparison of the Nb and F344 strains indicated that the Nb strain is less sensitive to the pituitary tumor–inducing actions of estrogens than the F344 strain *(161)*. Although it is documented that pituitary tumors induced in Nb rats with estrogens overproduce PRL *(171)*, the events associated with estrogen-induced pituitary tumorigenesis in the Nb rat strain are

less well characterized than in the other rat strains we have just discussed.

Brown Norway

The Brown Norway (BN) rat strain was developed by Slivers and Billingham in 1958 from stock originally derived from wild rats by Helen King at the Wistar Institute *(72)*. Compared to the rat strains we have already discussed, the BN rat is insensitive to the pituitary growth-inducing actions of estrogens (Fig. 2). Wendell et al. *(6)* directly compared the ability of DES to induce pituitary growth in male rats of six different inbred strains and observed that the BN is among the least sensitive of the examined strains in that it exhibited insignificant pituitary growth in response to 10 wk of DES treatment. In our laboratory, we have observed a modest 1.5-fold increase in pituitary weight in male BN rats treated with DES for 12 wk *(1)*, and a similar modest induction of pituitary growth and circulating PRL in female BN rats treated with E2 for varying durations (unpublished data). Similarly, Blankenstein et al. *(174)* observed only a modest induction of hyperprolactinemia in BN rats treated with E_2 for 52–104 wk. Because of this insensitivity to estrogens, the BN strain is being used in genetic studies (summarized next) to identify genes that confer and/or modulate the actions of estrogens in regulating pituitary growth.

Genetic Control of Estrogen-Induced Pituitary Growth

The data we have summarized clearly indicate that sensitivity to induction of pituitary growth by estrogens is rat strain specific. Gorski and various colleagues *(2–7)* were first to examine the genetic bases of estrogen-induced pituitary growth in the highly sensitive F344 strain. We have focused our attention on establishing the genetic bases of estrogen-induced pituitary growth in the ACI strain *(1)*. The data from these studies indicate that multiple genetic loci modulate the pituitary growth response to estrogens and illustrate that the mechanisms leading to development of estrogen-induced pituitary tumors in the different inbred rat strains are not identical. Ongoing research in our laboratory and that of Wendell *(6,7)* is directed toward identification of the genes residing at these loci in order to identify the molecular mechanisms through which estrogens regulate lactotroph proliferation and survival.

F344 x Holtzman Intercross

Wiklund et al. *(3)* quantified DES-induced pituitary growth in the inbred F344 and outbred Holtzman rat strains as well as first generation (F1), second generation (F2), and backcross progeny produced by crossing these strains. Pituitary weight was increased 5.5- and 7.8-fold in male and female F344 rats, respectively, following 8 wk of DES treatment. By contrast, pituitary weight was increased 1.3-fold in DES-treated male Holtzman rats, whereas no significant induction of pituitary growth was observed in female Holtzman rats. DES induced pituitary growth in male and female F1 progeny approximately two-fold, suggesting that sensitivity to the pituitary growth inducing actions of DES was inherited as a recessive genetic trait. Pituitary weight was increased by DES approximately 3-fold in the F2 progeny, approximately 3.5-fold in F1 x F344 backcross progeny, and less than 2-fold in F1 x Holtzman backcross progeny. Genetic models derived from these data suggested that the pituitary growth response of the F344 rat to administered DES is conferred by three or more genetic loci that act and segregate independently *(3)*. In a subsequent study, Shepel and Gorski *(5)* presented data suggesting that a locus linked to the *PRL* gene may contribute to the growth response of the F344 pituitary gland to administered DES.

F344 x BN Intercross

The genetic bases of estrogen-induced pituitary growth has been further refined by Wendell et al. *(6)* and Wendell and Gurski *(7)* through analysis of an F344 x BN intercross. Ten weeks of treatment with DES induced pituitary growth 9.7-fold in female F344 rats, whereas no induction of pituitary growth was observed in female BN rats. Pituitary growth was induced 3.1-fold by DES in F1 progeny, again suggesting that the sensitivity of the F344 rat to the pituitary growth inducing actions of estrogens behaved as a recessive or incompletely dominant genetic trait *(6)*. By correlating DES-induced pituitary growth in F344 x BN F2 progeny with genotype at loci distributed across the rat genome, Wendell and Gorski *(7)* have identified five loci that confer or modulate the pituitary growth response to estrogens. These estrogen-dependent pituitary mass (Edpm) loci reside on rat chromosomes 2 (two loci), 3, 5, and 9. Each of these *Edpm* loci was observed to confer a portion of the total pituitary growth response to administered DES; for example, *Edpm3*, residing on rat chromosome 3, accounted for approx 17% of the phenotypic variance exhibited by the DES-treated F2 population. From these data it was concluded that the estrogen-induced pituitary growth is a complex genetic trait that is manifested through the actions of multiple genes, some of which act independently and some of which act epistatically to modulate the actions of other pituitary growth-conferring genes *(7)*.

ACI x COP Intercross

The data we have summarized indicate that the genetically related ACI and COP rat strains exhibit quantitatively different pituitary growth responses to administered estrogens *(1)*. To identify the genetic bases for this difference, we have examined the ability of DES to stimulate pituitary growth in F1, F2, and backcross progeny produced by crossing these strains and are currently mapping the genetic loci that confer the pituitary growth response to DES. Following 12 wk of treatment with DES, pituitary weight was

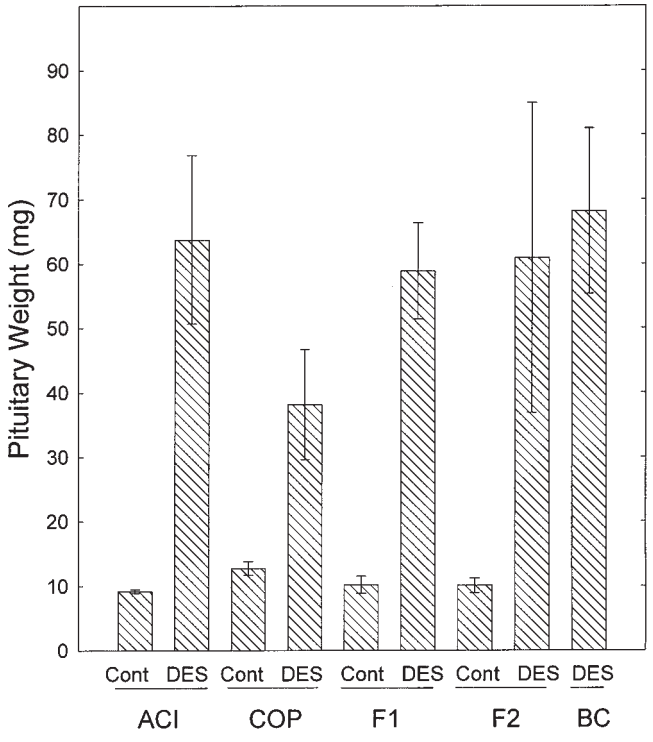

Fig. 7. Genetic analysis of estrogen-induced pituitary growth in an ACI x Copenhagen intercross: dominant inheritance of the ACI phenotype. The pituitary growth response to DES was approximately two-fold greater in the ACI than in the COP rat strain. F1 progeny exhibited a pituitary growth response similar to that of the ACI strain, indicating that the ACI phenotype was inherited as a dominant genetic trait. Analyses of F2 and backcross (BC) progeny suggest that this strain difference was conferred through the actions of a minimum of two genetic loci. Cont, control. (Data adapted with permission from ref. *1*.)

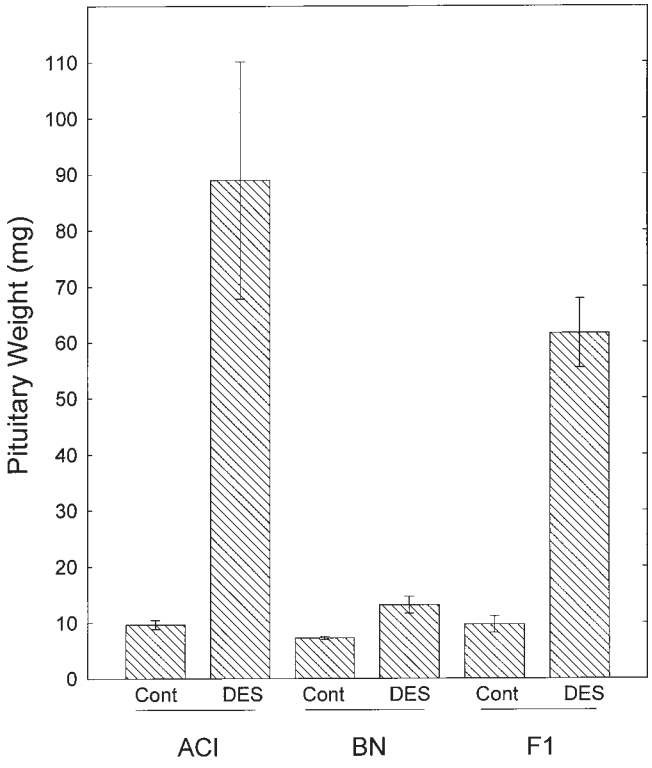

Fig. 8. Genetic analysis of estrogen-induced pituitary growth in an ACI x Brown Norway intercross: dominant inheritance of the ACI phenotype. ACI x BN F1 progeny exhibited a pituitary growth response that was similar to that of the ACI strain. By contrast, data from Wendell and Gorski *(6,7)* indicate that F344 × BN F1 progeny more closely resemble the BN strain in their sensitivity to the pituitary growth-inducing actions of DES. These data strongly suggest that the genetic bases of estrogen-induced pituitary growth in the ACI and F344 strains differ. Cont, control. (Data adapted with permission from ref. *1*.)

increased 6.9-fold in male ACI rats compared to 3-fold in male COP rats (Fig. 7). DES increased pituitary weight 5.8-fold in F1 progeny, an induction similar to that observed in ACI rats, indicating that the ACI phenotype in this genetic cross was inherited as a dominant trait *(1)*. At this time, we have scanned approx 70% of the rat genome and have localized two loci that confer pituitary growth responsiveness to DES (unpublished data). Estrogen-induced pituitary tumor *(Ept1)* and *Ept2* have been localized to rat chromosomes 6 and 3, respectively. *Ept2* may represent the same locus as *Edpm3* identified in the analysis of an F344 x BN intercross by Wendell and Gorski *(7)*. From these data we conclude that development of estrogen-induced pituitary tumors in the ACI and F344 rat strains probably occurs through both shared and rat strain-specific mechanisms.

ACI x BN Intercross

We have also examined the ability of DES to induce pituitary tumor development in F1, F2, and backcross progeny derived from a cross between the ACI and BN strains. Following 12 wk of DES treatment, pituitary weight was increased 9-fold in male ACI and 1.8-fold in male BN rats *(1)*. DES increased pituitary weight 6.4-fold in male ACI x BN F1 progeny, suggesting that the ACI phenotype was inherited as a dominant or incompletely dominant trait (Fig. 8). By contrast, data from Wendell and Gorski *(7)*, indicated that the F1 progeny in an F344 x BN intercross more closely resembled the insensitive BN strain than the sensitive F344 strain. To date, we have identified three loci that confer estrogen-induced pituitary growth in the ACI x BN intercross (data not shown). None of these three loci were observed by Wendell and Gorski in their analysis of an F344 x BN intercross *(7)*. Together, these data further indicate that the ACI and F344 rat strains differ significantly with respect to their genetic predispositions to development of estrogen-induced pituitary tumors.

In a parallel line of investigation, we are mapping the loci that confer or modulate susceptibility to estrogen-induced mammary cancers in ACI x COP and ACI x BN intercrosses. To date, three loci have been identified that confer susceptibility to estrogen-induced mammary cancers. Only one of these three loci appears to contribute to pituitary tumor development, suggesting that, for the most part, development of estrogen-induced pituitary tumors and

estrogen-induced mammary cancers may be genetically distinct traits (unpublished data).

Modulation of Estrogen-Induced Pituitary Growth by Dietary Energy Consumption

Diet has long been known to play an important role in the etiology of many types of cancer in humans, and the amount of energy consumed in the diet appears to modulate the development of several cancer types in humans *(175)*. For many years it has been appreciated that diet restriction, in which intake of all nutrients is restricted, and energy restriction, in which caloric intake is restricted through a reduction in consumption of specific diet components such as carbohydrate and/or fat, markedly inhibit the development of a variety of spontaneous and carcinogen-induced cancers in experimental animals *(176,177)*. The mechanisms through which energy restriction inhibits carcinogenesis are not well understood. We are testing the hypothesis that dietary energy consumption may modulate development of estrogen-dependent tumors by altering the manner in which target cell populations respond to estrogens. Using the rat anterior pituitary gland as a model, we have demonstrated that dietary energy restriction exerts rat strain–specific inhibitory effects on development of estrogen-induced pituitary tumors by modulating the manner in which specific pituitary cell populations respond to administered hormone.

Dietary Energy Restriction Virtually Abolishes Estrogen-Induced Pituitary Tumorigenesis in the F344 Rat

Data from our laboratory indicate that a 40% restriction of dietary energy consumption markedly inhibits the development of PRL-producing pituitary tumors in F344 rats treated with estrogens *(42,43,54)*. In a study by Spady et al. *(42)*, 10 wk of E_2 treatment increased pituitary weight and pituitary weight to body weight ratio and induced marked hyperprolactinemia in ovariectomized female F344 rats allowed to consume a control diet ad libitum. By contrast, the ability of E_2 to increase pituitary weight, pituitary weight to body weight ratio, and circulating PRL was greatly attenuated in ovariectomized F344 rats that consumed 40% less energy than the animals fed the control diet (Fig. 9). Dietary energy restriction was similarly demonstrated to inhibit development of PRL-producing pituitary tumors in male F344 rats treated with DES for 8 wk *(43)*, and in male F344 rats treated with E_2 for either 10 or 26 wk (unpublished data).

To elucidate the mechanisms through which dietary energy restriction inhibits estrogen-induced pituitary tumorigenesis, we have examined whether or not dietary energy consumption modulates the ability of administered estrogens to regulate lactotroph proliferation and/or survival. Dietary energy restriction did not significantly inhibit the ability of E_2 *(42)* or DES *(43)* to induce lactotroph hyperplasia or stimulate lactotroph proliferation, as evidenced by histological and biochemical criteria including quantification of anterior pituitary DNA synthesis *(43)* and the

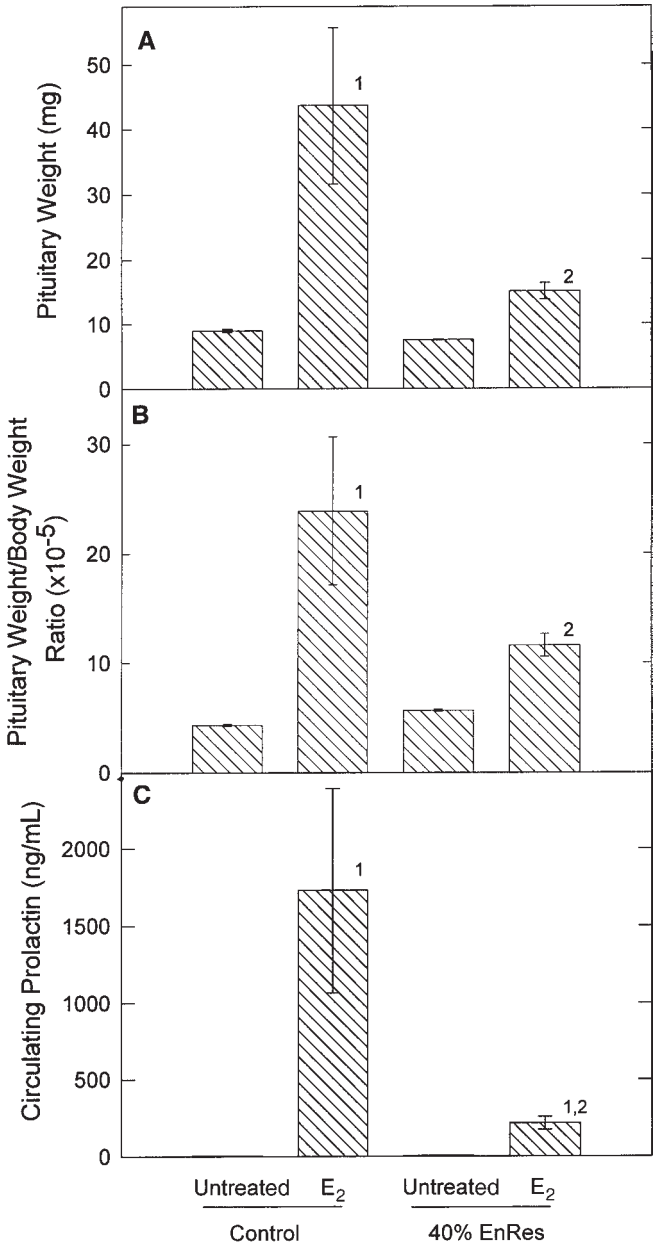

Fig. 9. Dietary energy restriction virtually abolishes estrogen-induced pituitary tumor development and hyperprolactinemia in F344 rats. Ovariectomized female F344 rats were allowed to feed ad libitum (control) or were subjected to a 40% restriction in energy consumption (40% EnRes). Pituitary weight (**A**), pituitary weight to body weight ratio (**B**) and circulating PRL (**C**) were dramatically increased in response to 10 wk of E_2 treatment in animals fed the control diet, whereas induction of these indicators of pituitary growth by E_2 were markedly inhibited in animals fed the energy-restricted diet. 1, a statistically significant difference ($p \leq 0.05$) between untreated and E_2-treated animals fed the same diet; 2, a statistically significant difference ($p \leq 0.05$) between E_2-treated animals fed the 40% energy restricted and control diets. (Data adapted from ref. *42*. Reprinted by permission of Wiley-Liss, Inc., a division of John Wiley & Sons, Inc.)

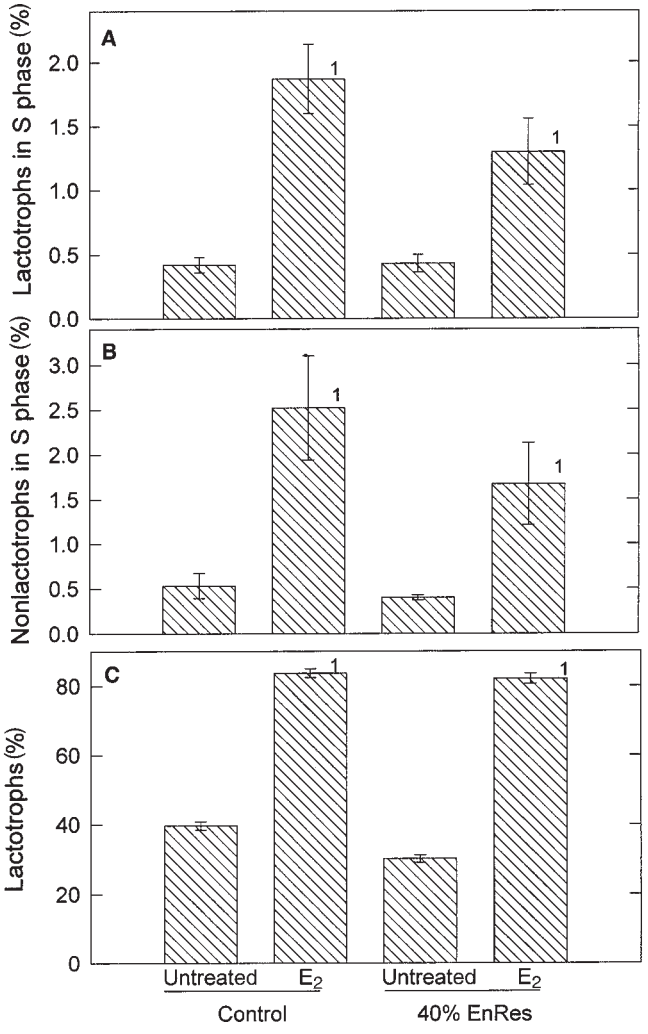

Fig. 10. Dietary energy restriction does not inhibit estrogen induction of lactotroph proliferation. Ovariectomized female F344 rats were allowed to feed ad libitum (Control) or were subjected to a 40% restriction in energy consumption (40% EnRes). Cell proliferation in the lactotroph and nonlactotroph populations was assayed by immunohistochemical detection of BrdU and PRL. Administered E_2 increased the fraction of lactotrophs (**A**) and PRL-negative nonlactotrophs (**B**) incorporating BrdU, and induced lactotroph hyperplasia (**C**) regardless of whether the rats were fed the control or energy-restricted diet. These data, together with those presented in Fig. 9, indicate that energy restriction acts at a point subsequent to induction of lactotroph proliferation to inhibit estrogen-induced pituitary tumorigenesis in the F344 rat. 1, a statistically significant difference ($p \leq 0.05$) between untreated and E_2=treated animals fed the same diet. (Data adapted from ref. *42*. Reprinted by permission of Wiley-Liss, Inc., a division of John Wiley & Sons, Inc.)

number of lactotrophs incorporating BrdU (Fig. 10). Similar results were observed in male F344 rats treated with E_2 for 10 and 26 wk (unpublished data). These data indicate that dietary energy restriction inhibits estrogen-induced pituitary growth and development of PRL-producing pituitary tumors in the F344 rat by acting at a step subsequent to stimulation of lactotroph proliferation.

Based on these data, we hypothesize that energy restriction modulates lactotroph survival. To test this hypothesis, we utilized the TUNEL method *(178)* to identify pituitary cells undergoing apoptosis. This method proved unsatisfactory because apoptotic cells in the anterior pituitary gland are rapidly phagocytized and are consequently rarely observed histologically *(42)*. Therefore, we examined expression of *TRPM-2* mRNA, a surrogate marker of apoptosis *(56-58)*. Following 10 wk of E_2 treatment, the steady-state level of *TRPM-2* mRNA was reduced by approx 65% in ovariectomized female F344 rats allowed to feed ad libitum, relative to the level observed in untreated rats (Fig. 11). These data suggest that administered E_2 enhances cell survival in the anterior pituitary gland of the ovariectomized rat by inhibiting apoptosis. By contrast, administered E_2 did not significantly reduce the steady-state level of *TRPM-2* mRNA in ovariectomized F344 rats subjected to a 40% restriction of dietary energy consumption (Fig. 11), suggesting that dietary energy restriction abrogates the ability of E_2 to enhance pituitary cell survival. Moreover, the number of folliculo-stellate cells in the anterior pituitary gland of F344 rats allowed to feed ad libitum, but not in the pituitary gland of energy-restricted rats, was significantly reduced in response to estrogen treatment *(55)*. Because the pituitary folliculo-stellate cells function in the phagocytosis of cells undergoing apoptosis *(53)* and increase in number in the anterior pituitary gland of estrogen-treated F344 rats following withdrawal of hormone *(53,179)*, we interpret these data to support the conclusion that dietary energy restriction abrogates the ability of administered estrogens to enhance lactotroph survival. Figure 12 illustrates this model of estrogen/diet interactions in the regulation of pituitary growth and pituitary tumor development.

Inhibitory Effect of Dietary Energy Restriction on Estrogen-Induced Pituitary Tumorigenesis Is Rat Strain Specific

In a parallel line of investigation, we are examining the effects of dietary energy restriction on the development of estrogen-induced pituitary tumors in the ACI rat. Data from these experiments indicate that energy restriction does not inhibit estrogen-induced pituitary tumorigenesis in female ACI rats *(54,55)*. Treatment of ovariectomized ACI rats with E_2 for 20 wk resulted in similarly increased pituitary weight to body weight ratio, increased circulating PRL, stimulated lactotroph proliferation and lactotroph hyperplasia, reduced *TRPM-2* mRNA, and reduced the number of pituitary folliculo-stellate cells, regardless of whether the animals were allowed to feed ad libitum or were energy restricted. Together, the data from the study of the F344 and ACI rat strains indicate that the inhibitory actions of dietary energy restriction on the development of estrogen-induced pituitary tumors is rat strain specific. This is, to our knowledge, the first demonstration that an antitumorigenic action of energy restriction is impacted by genetic background. In addition, these data imply that it should be feasible to use

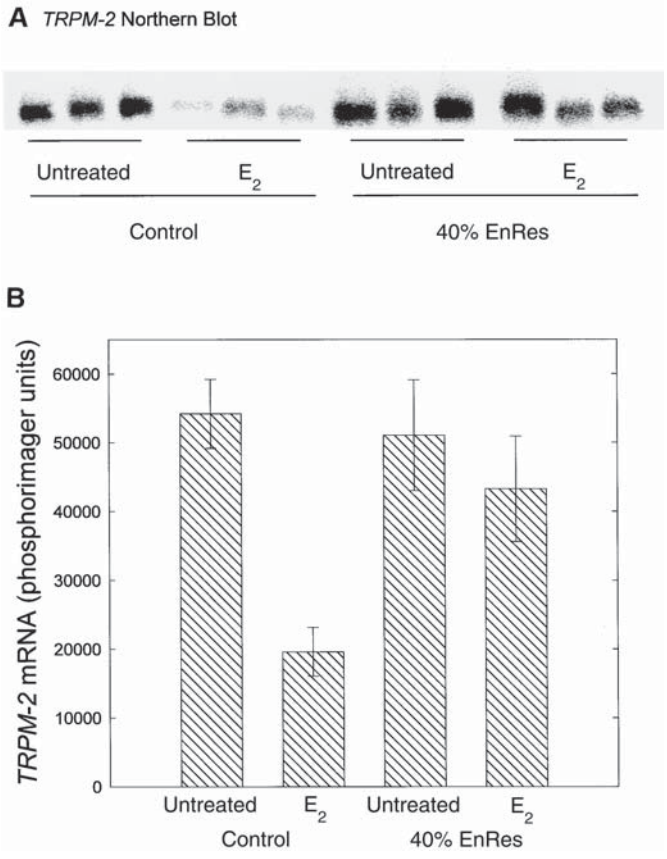

Fig. 11. Expression of *TRPM-2* mRNA as a surrogate marker of apoptosis: modulation of estrogen action by dietary energy restriction. Ovariectomized female F344 rats were allowed to feed ad libitum (Control) or were subjected to a 40% restriction in energy consumption (40% EnRes). *TRPM-2* mRNA was quantified in individual anterior pituitary glands following 10 wk of E_2 treatment. Administered E_2 significantly downregulated *TRPM-2* expression in animals fed the control diet, suggesting that this hormone enhanced pituitary cell survival by inhibiting apoptosis. Energy restriction blocked the ability of E_2 to downregulate expression of *TRPM-2*, suggesting that pituitary cell survival was not enhanced in the restricted animals. The Northern blot (**A**) and graphical representation of the data (**B**) are illustrated. (Data adapted from ref. *42*. Reprinted by permission of Wiley-Liss, Inc., a division of John Wiley & Sons, Inc.)

genetics-based approaches, similar to those we described in under "Genetic Control of Estrogen-Induced Pituitary Growth," to identify the genes that confer sensitivity to this antitumorigenic action of energy restriction.

Concluding Summary

The data summarized herein indicate that estrogens act through both direct and indirect mechanisms to stimulate pituitary growth and induce the development of pituitary tumors. Estrogen-induced pituitary growth is achieved through stimulation of lactotroph proliferation and enhancement of lactotroph survival. Different rat strains exhibit marked differences in the responsiveness of their pituitary

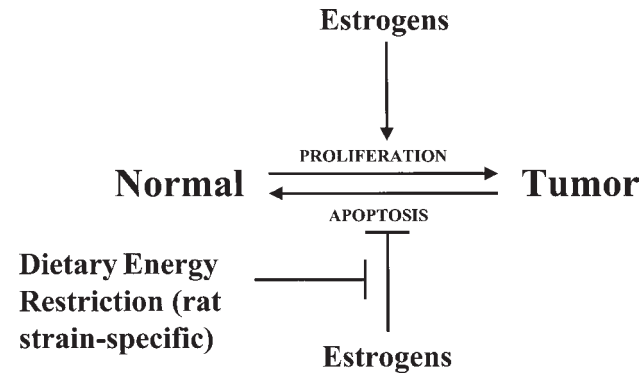

Fig. 12. Proposed mechanism for modulation of pituitary growth by estrogens and dietary energy restriction. Estrogens stimulate lactotroph proliferation and inhibit apoptosis. In certain rat strains, such as F344 and ACI, the rate of lactotroph proliferation exceeds the rate of lactotroph death and PRL-producing pituitary tumors develop. In the F344 rat strain, dietary energy restriction abrogates the ability of estrogens to enhance lactotroph survival. Consequently, total lactotroph number and pituitary mass in energy-restricted F344 rats treated with estrogens are constrained despite continued stimulation of lactotroph proliferation. By contrast, energy restriction does not abrogate the ability of estrogens to enhance lactotroph survival in the ACI rat strain, and total lactotroph number and pituitary mass are not constrained. (This model is based on data from refs. *42* and *55*.)

lactotroph population to estrogens. These strain differences are heritable and are conferred through the actions of multiple genes. Genetic characterization of the inbred F344 and ACI rat strains indicates that both strain-specific and shared loci confer the pituitary growth response to estrogens in these strains. An environmental factor, dietary energy consumption, acts in a rat–strain specific manner to modulate the responsiveness of the lactotroph population to estrogens, and thereby dramatically impacts estrogen-induced pituitary tumorigenesis in certain rat strains but not others. The data we have summarized in this review indicate that the anterior pituitary lactotroph provides a well-defined and physiologically relevant model for studying the interactions among hormonal, genetic, and environmental factors in the regulation of cell proliferation, cell survival, and tumorigenesis.

Acknowledgments

We dedicate this review to Professor Jack Gorski, who for nearly four decades has provided leadership and inspiration to the field of estrogen action. Our research was supported by grants R01CA68529 and R01CA77876 from the NIH, grant 97A146 from the American Institute for Cancer Research, and grant DAMD17-98-1-8217 from the U.S. Army Breast Cancer Research Program. Shared facilities were supported by Cancer Center Support Grant P30CA36727 from the NIH. Thomas Spady was supported by a Bukey Presidential Fellowship from the University of Nebraska.

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