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Galanin regulates prolactin release and lactotroph proliferation

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ABSTRACT **The neuropeptide galanin is predominantly expressed by the lactotrophs (the prolactin secreting cell type) in the rodent anterior pituitary and in the median eminence and paraventricular nucleus of the hypothalamus. Prolactin and galanin colocalize in the same secretory granule, the expression of both proteins is extremely sensitive to the estrogen status of the animal. The administration of estradiol-17β induces pituitary hyperplasia followed by adenoma formation and causes a 3,000-fold increase in the galanin mRNA content of the lactotroph. To further study the role of galanin in prolactin release and lactotroph growth we now report the generation of mice carrying a loss-of-function mutation of the endogenous galanin gene. There is no evidence of embryonic lethality and the mutant mice grow normally. The specific endocrine abnormalities identified to date, relate to the expression of prolactin. Pituitary prolactin message levels and protein content of adult female mutant mice are reduced by 30–40% compared with wild-type controls. Mutant females fail to lactate and pups die of starvation/dehydration unless fostered onto wild-type mothers. Prolactin secretion in mutant females is markedly reduced at 7 days postpartum compared with wild-type controls with an associated failure in mammary gland maturation. There is an almost complete abrogation of the proliferative response of the lactotroph to high doses of estrogen, with a failure to up-regulate prolactin release, STAT5 expression or to increase pituitary cell number. These data further support the hypothesis that galanin acts as a paracrine regulator of prolactin expression and as a growth factor to the lactotroph.**

The factors that regulate proliferation of the lactotroph are largely unknown. Further, the relationship of altered prolactin secretion to lactotroph proliferation is also unclear. Pregnancy induces a coordinated increase in prolactin release and the number of lactotrophs, with a marked involution in their number once lactation ceases (1–4). In contrast, a sustained and uncontrolled proliferation of lactotrophs culminates in the development of prolactin-secreting adenomas (prolactinomas) resulting in inappropriate lactation. The prevalence of prolactinomas is an estimated 100 per million (5). However, in autopsy series of elderly females the prevalence is 1,000-fold greater, emphasizing that most prolactinomas are clinically silent and common in the elderly population (6). Prolactinomas are also a common cause of death in female aged rats of a number of strains (7–9). Treatment of prolactinomas with dopamine agonists reduces pituitary prolactin expression and reverses lactotroph hyperplasia, emphasizing the link between prolactin expression and cellular proliferation. Prolactinomas arise as monoclonal neoplasms, indicating that one or more somatic mutations underlie tumor pathogenesis. A large and increasing body of literature has failed to identify mutations in known protooncogenes in human prolactinomas (10–12). In contrast, exogenous estrogen administration to man or rodents, potently induces prolactin gene transcription and secretion (13–16) and stimulates lactotroph proliferation and adenoma formation (17–19). The mechanism by which estrogen-induced proliferation occurs is also unknown, but addition of estradiol-17β to anterior pituitary cultures stimulates the production of a number of growth factors, the most marked increase being in the peptide galanin (20).

Galanin is principally synthesized, stored, and released by a subpopulation of lactotrophs (21) in the rodent pituitary and is extremely sensitive to the estrogen status of the animal. A marked elevation in the expression of pituitary galanin occurs during pregnancy (22), whereas galanin is down-regulated in hypothalamic magnocellular neurons during lactation (23). Exogenous estradiol-17β causes a 6-fold induction in the number of galanin secreting lactotrophs (24) and a 3,000-fold increase in anterior pituitary galanin mRNA content, whereas the peptide levels rise 500-fold (25). In contrast, ovariectomy almost abolishes pituitary galanin content (26). Our previous studies have demonstrated that galanin is a mitogen to the 235–1 clonal lactotroph cell line acting via a novel pituitary-specific galanin receptor (24, 27). We also have shown that immunoneutralization of locally secreted galanin profoundly inhibits prolactin release, particularly in the hyperoestrogenized state (24, 28). A number of studies in humans have confirmed our findings in rodents; human galanin infusion significantly stimulates prolactin secretion in normal female volunteers with an exaggerated response in patients with pituitary tumors (29–32).

Here we report the generation of mice carrying a loss-of-function mutation of the endogenous galanin gene. Prolactin expression is reduced in adult female mice and there is a failure to lactate after pregnancy. There is an almost complete abrogation of the proliferative response of the lactotroph to high doses of estrogen, with a failure to up-regulate prolactin expression or to increase pituitary cell number. Galanin would therefore appear to act as tonic regulator of prolactin release and as a growth factor to the lactotroph.

MATERIALS AND METHODS

Targeting Vector. A mouse 129sv cosmid genomic library (a generous gift of A. M. Frischauf, Imperial Cancer Research Fund, London), was screened by using the full-length rat galanin cDNA as a probe under high stringency. A positive/negative selection targeting vector was constructed (Fig. 1*A*) in

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Abbreviations: LH, luteinizing hormone, GH, growth hormone; TSH, thyroid-stimulating hormone; FSH, follicle-stimulating hormone.

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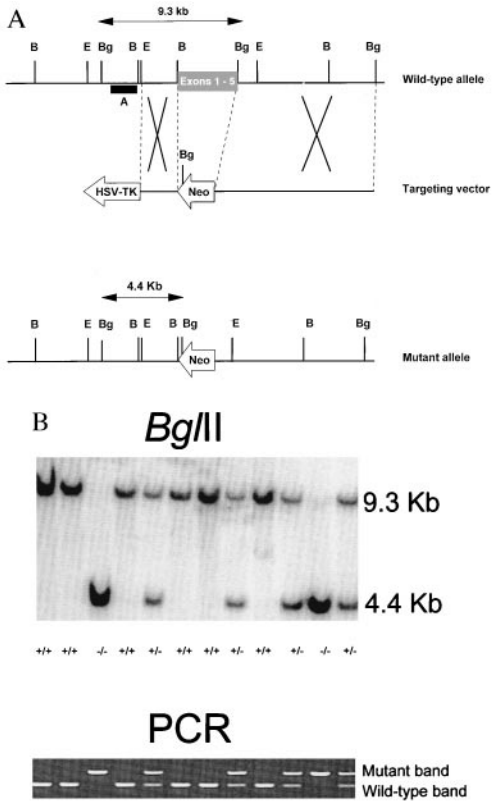

FIG. 1. (*A*) Targeted disruption of the murine galanin gene. The targeting vector replaces the first five exons of the galanin gene with a *Neo* cassette in reverse orientation. HSV-TK denotes the herpes simplex thymidine kinase and *Neo* the neomycin resistance gene. A denotes the 5′ external probe. B, *Bam*HI, Bg, *Bgl*II, E, *Eco*RI., (*B*) Identical results obtained by Southern hybridization (genomic DNA digested with *Bgl*II and probed with the 5′ external probe) and PCR screening on the same litter derived from a mating of two heterozygotes.

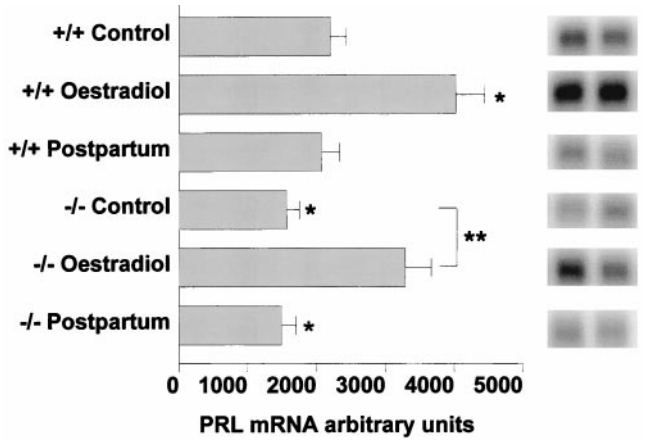

FIG. 2. Pituitary prolactin message levels in arbitrary units, measured by Northern blotting of randomly cycling wild-type and mutant females (denoted control), a second group of randomly cycling wild-type and mutant females treated for 3 weeks with estradiol-17β treatment (estradiol) and thirdly, another group of wild-type and mutant females 7 days postpartum. Values were determined relative to glyceraldehyde-3-phosphate dehydrogenase as a control probe. For all groups, $n = 5$. *, $P < 0.05$ denote levels of significance compared with the wild-type control group. Two representative pituitary samples are illustrated for each group.

which a *PGK-Neo* cassette in reverse orientation was used to replace exons 1–5 of the galanin gene, removing the signal peptide, the coding region for galanin and most of the galanin-associated peptide. A 1.9-kb *Eco*RI/*Bam*HI fragment, 5′ to the gene (the *Bam*HI is 13 bp downstream of the transcriptional start site) was cloned in between the thymidine kinase and *Neo* cassettes. A further 7.8 kb of 3′ homology was added as a *Bgl*II fragment, removing a total of 3.7 kb of the galanin locus (containing the first five exons of the gene). The *Bgl*II fragment was blunted with Klenow and *Xho*I linkers inserted at either end. The 7.8-kb *Xho*I fragment was then cloned into the *Xho*I site, downstream of the *PGK-Neo* cassette. The vector was linearized and electroporated into the previously described E14 embryonic stem cell line (33). Double selection was performed over a 10-day period, G418/gancyclovir-resistant clones were screened by Southern hybridization. Restriction mapping of the wild-type locus with *Bgl*II generates a 9.3-kb fragment when probed with a 5′ external probe (marked A, Fig. 1*A*), whereas the correctly targeted locus generates a 4.4-kb fragment. In total, nine clones were identified in which one allele of the galanin gene was correctly targeted by homologous recombination among 209 double resistant colonies, yielding a targeting frequency of 4.3%. These nine clones were karyotyped, confirming euploidy, and injected into 3.5-day-old blastocysts from C57BL/6 mice. Germ-line transmission of the disrupted galanin locus was obtained from three separate embryonic stem cell clones.

Germ-Line Transmission of the Targeted Allele. Subsequent to germ-line transmission animals were genotyped by both Southern blot analysis and PCR. Fig. 1*B* demonstrates identical results obtained by Southern blot analysis and PCR screening on the same litter derived from a mating of two heterozygotes. Two sets of primers were used in the same PCR. The first set of primers, AACTGGAGGTGGAGGAAAGGAGAC and TAGTGCGGACAATGTTGCTCTCAG, correspond to exons 4 and 5, respectively of the deleted galanin gene and generate a 350-bp fragment. The second set, TGCCGCGCTGTTCTCCTCT and AAGCGGCCATTTTCCACCAT, correspond to the 5′ end of the *Neo* gene and generate a 600-bp fragment.

Mice. All experiments were performed on 8-week-old female randomly cycling animals fed standard chow and water ad libitum. Data are presented as mean ± SEM. Statistics were performed by using ANOVA. Animals were killed by decapitation and the anterior pituitary and hypothalami removed, weighed, and snap frozen in liquid nitrogen. Plasma from trunk blood was collected at the time of death.

Northern Blot Analysis. Total RNA was prepared from pituitaries by using RNAzol-B (34) (Biogenesis). Equal amounts of RNA from mutant and wild-type mice were run on formaldehyde/agarose gels in 10 mM sodium phosphate buffer and blotted onto GeneScreen membrane (DuPont). The previously described prolactin probe (35) was kindly provided by E. Borrelli (Institut National de la Santè et de la Recherche Médicale, Strasbourg, France). A 452-bp probe for the coding

Table 1. Galanin content in various brain and peripheral tissues measured by RIA

Genotype	Cortex	Hypothalamus	Stomach	Duodenum	Ileum
+/+	5.8 ± 0.3	110.3 ± 7.8	27.5 ± 1.9	122.9 ± 11.6	267.4 ± 13.5
+/−	2.9 ± 0.2	53.8 ± 3.8	13.8 ± 0.8	68.4 ± 5.7	125.9 ± 7.6
−/−	UD	UD	UD	UD	UD

All values are mean galanin-LI pmol/g of wet weight tissue ± SEM, $N = 10$ for all groups. Levels in the mutant animals were below the detection limit of the RIA in all cases (UD).

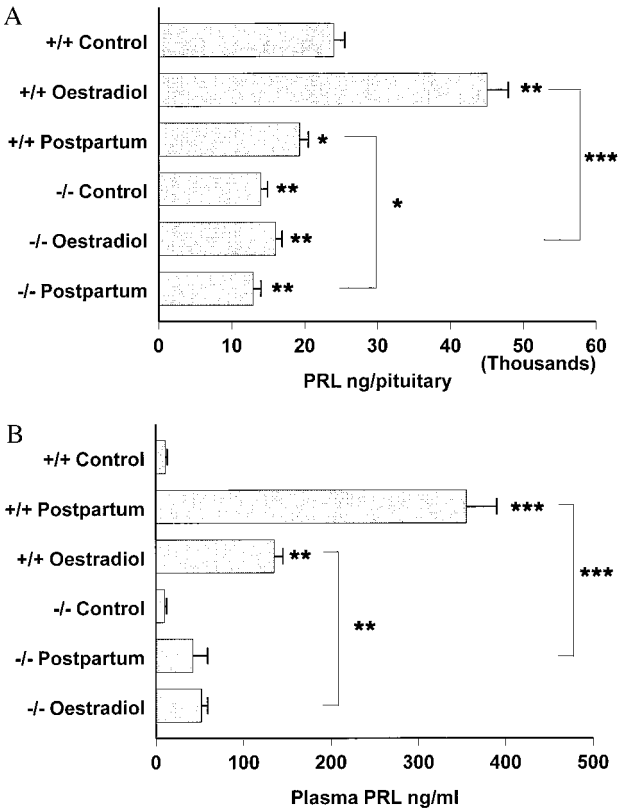

FIG. 3. Pituitary prolactin content in ng/pituitary (*A*) and circulating plasma prolactin in ng/ml (*B*) of randomly cycling wild-type and mutant females (denoted control), a second group of randomly cycling wild-type and mutant females treated for 3 weeks with estradiol-17β treatment (estradiol), and thirdly, another group of wild-type and mutant females seven days postpartum. $n = 10$ for all groups. *, $P < 0.05$, **, $P < 0.01$, ***, $P < 0.001$ denote levels of significance compared with the wild-type control group.

region of mouse glyceraldehyde-3-phosphate dehydrogenase (36) was used as an internal standard for RNA quantitation. Visualization and quantification were performed with a STORM 840 PhosphorImager, Molecular Dynamics.

Radioimmunoassays. RIAs for growth hormone (GH), thyroid-stimulating hormone (TSH), luteinizing hormone (LH), and follicle stimulating hormone (FSH) were performed by using reagent and methods generously donated by P. Smith and A. Parlow of the National Hormone and Pituitary Program and Pituitary Hormones and Antisera Centre, respectively. Assays for murine prolactin (37), gonadotropin-releasing hormone [method and reagents (38) kindly donated by H. M. Fraser, Medical Research Centre Reproductive Biology Unit, Edinburgh], vasoactive intestinal polypeptide (39), neuropeptide Y (39), somatostatin (39), vasopressin (39), oxytocin (41), and thyrotrophin-releasing hormone (42) were performed as described.

Histological Analysis. Immunocytochemistry was carried out on tissues fixed with 4% paraformaldehyde and embedded in paraffin. Two-micrometer sections were stained by using the previously characterized murine prolactin (37) at a dilution of 1/5,000. Labeling was visualized with a standard biotin-streptavidin method, using diaminobenzidine as the substrate. Cell counting was performed on eight separate sections/anterior pituitary. On each section a minimum of 200 cells were counted. Care was taken to ensure that equal representation of all parts of the anterior pituitary was included in the counting.

In the case of mammary gland architecture, the fourth inguinal mammary glands from virgin 8-week animals were dissected free of the skin and spread on a super frost microscope slide, air dried for 5 min then fixed by immersion in 10% neutral buffered formalin overnight. The glands were stained overnight in carmine-potassium aluminium sulfate, dehydrated by passage through a graded ethanol series followed by xylene, and clarified by immersion in methylsalycilate for photography.

Pituitary Cell Counting. Individual anterior pituitaries were enzymatically dispersed and cells counted by using a haemocytometer as described (24).

Western Blotting. Affinity purification of activated STAT5 was performed by using the STAT5 binding sequence of the bovine β-casein promoter (AGATTTCTAGGAATTCAAATC). For each group of animals, two anterior pituitaries were lysed at 4°C in 1 ml of a solution containing 10 mM Tris·HCl (pH 7.6), 5 mM EDTA, 50 mM NaCl, 30 mM disodium pyrophosphate, 50 mM NaF, 100 mM Na3VO4, 1 mM phenylmethylsulfonyl fluoride, and 0.5% Nonidet P-40 (lysis buffer). Lysates were clarified by centrifugation at 15,000 $\times$ *g* for 10 min and activated STAT5 extracted from supernatant fractions (containing a total of 1.3–1.5 mg protein) with 1 μg double-stranded 5′ biotinylated oligonucleotide coupled to 10 μl of a 50% suspension of streptavidin-agarose (Sigma) as described (43). Bound proteins were separated by electrophoresis through an 8% polyacrylamide gel. Proteins were then transferred to Hybond-C membranes (Amersham) and blots were probed with an antibody to STAT5 (Santa Cruz Biotechnology) followed by horseradish peroxidase-conjugated to goat anti-mouse IgG. Bands were revealed by using enhanced chemiluminescence (ECL, Amersham) or ECL Plus. Visualization and quantification were performed with a STORM 840 PhosphorImager (Molecular Dynamics).

RESULTS AND DISCUSSION

The galanin loss-of-function mutation has been bred to homozygosity on the in-bred 129/OlaHsd mouse strain and all data presented are from mice on this background. Galanin levels were measured in a number of brain regions, stomach, and small intestine by RIA (26). Levels in heterozygotes were 50% of wild-type controls, whereas levels in the homozygotes were below the limit of detectability in all cases (Table 1). Results of genotype analysis of live births were in the expected ratio predicted by Mendelian genetics and the sex ratio of homozygote offspring was 1:1.

Prolactin message levels and protein content (Figs. 2 and 3*A*)

Table 2. Pituitary content of GH, TSH, FSH, and LH and hypothalmic neuropeptide content of male and female mice of each genotype

Sex/Genotype	GH	TSH	LH	FSH	GnRH	SRIF	TRH	NPY	GLP-1	AVP
Male +/+	51,781 ± 517	141 ± 8	1,794 ± 98	855 ± 44	55 ± 7	888 ± 85	447 ± 52	510 ± 71	4.4 ± 0.6	544 ± 69
Male +/−	52,161 ± 623	136 ± 8	1,830 ± 119	897 ± 40	62 ± 20	1,111 ± 100	553 ± 52	463 ± 95	4.7 ± 0.7	590 ± 78
Male −/−	50,975 ± 619	154 ± 9	1,980 ± 102	834 ± 38	44 ± 13	1,153 ± 200	472 ± 56	577 ± 82	6.0 ± 1.0	574 ± 82
Female +/+	30,719 ± 380	133 ± 12	344 ± 18	287 ± 13	51 ± 19	500 ± 47	490 ± 40	550 ± 47	6.2 ± 1.2	490 ± 53
Female +/−	31,162 ± 356	145 ± 7	360 ± 16	324 ± 11	62 ± 24	624 ± 50	575 ± 50	614 ± 50	6.7 ± 0.8	516 ± 66
Female −/−	30,883 ± 310	136 ± 9	386 ± 21	315 ± 17	44 ± 14	472 ± 20	467 ± 31	482 ± 20	5.6 ± 0.6	483 ± 71

All hormones are expressed as mean ng hormone/pituitary ±SEM, whilst hypothalamic neuropeptides are expressed as mean neuropeptide-LI fmol/mg of wet weight tissue ±SEM. $N = 10$ for all groups. GnRH, gonadotrophin-releasing hormone; SRIF, somatostatin; TRH, thyrotrophin-releasing hormone; NPY, neuropeptide Y; GLP-1, glucagon-like peptide-1.

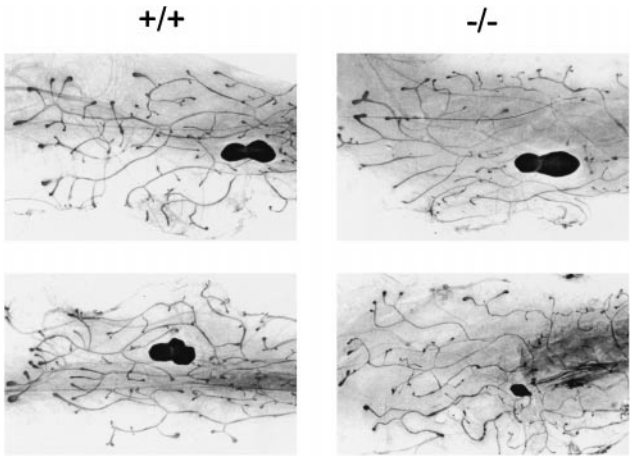

FIG. 4. Carmine-stained whole mounts of the fourth inguinal mammary gland from 8-week-old virgin wild-type (*Left*) and mutant (*Right*) animals. Two representative glands are shown for each genotype. Formation of the ductal tree is in progress, with mitotic terminal end buds clearly visible at the ductal termini; there is reduced ductal branching in the mutants animals. The lymph node is seen as a darkly staining mass at the center of the photographs.

were reduced by 30% and 40%, respectively in randomly cycling adult female mutants, when compared with wild-type controls. Immunocytochemistry confirmed that the number of lactotrophs remained unchanged (46 ± 4% vs. 44 ± 5% adult female wild-type vs. mutants; $n = 5$), as was the distribution of lactotrophs within the gland. Plasma prolactin was unchanged in randomly cycling adult female mutants compared with wild-type controls (10 ± 2.7 vs. 11 ± 2.6 ng/ml prolactin, respectively; $n = 10$). The disparity between the decrease in pituitary prolactin synthesis and content and the unchanged circulating levels may reflect rapid changes in prolactin secretion during the estrous cycle because we studied randomly cycling adult females.

The expression of other factors known to regulate prolactin storage and release were studied. Anterior pituitary content of the neuropeptide vasoactive intestinal polypeptide, previously demonstrated to be a prolactin secretagogue (9), was unchanged in the mutant females (65 ± 9 vs. 71 ± 10 fmol/gland, mutant and wild type, respectively; $n = 8$). Hypothalamic thyrotrophin-releasing hormone content (Table 2) was also unaffected by the mutation. No phenotypic changes have thus far been delineated in animals heterozygote for the mutation.

Mutant females were unable to lactate and all pups died of dehydration/starvation within 48 hr unless fostered by wild-type mothers. This apparent failure of lactation was absolute

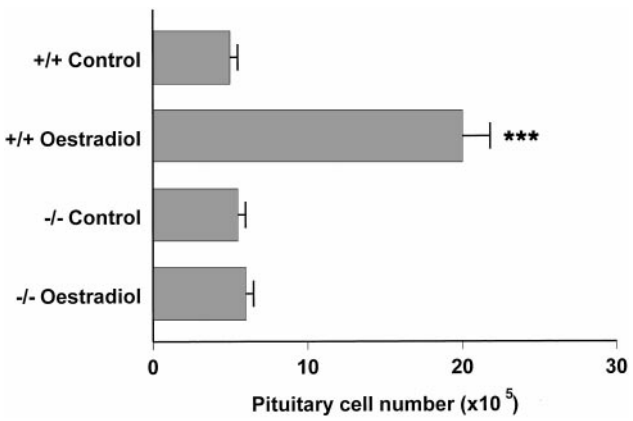

FIG. 5. Dispersed pituitary cell number in randomly cycling wild-type and mutant females (control) and after 3 weeks of estradiol-17β treatment (estradiol). For all groups, $n = 10$. **, $P < 0.01$, ***, $P < 0.001$ denotes levels of significance compared with wild-type controls.

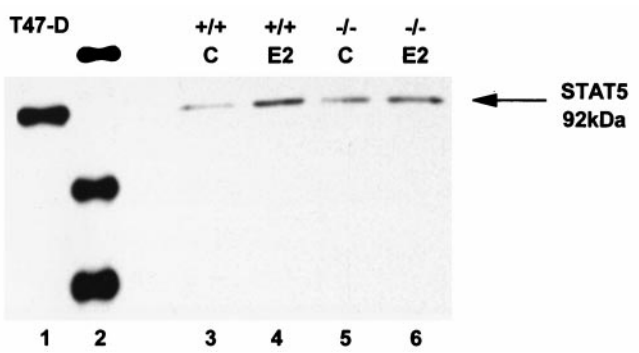

FIG. 6. Representative Western blot of activated STAT5: lane 1, positive control using lysate of T47-D breast cancer cell line; lane 2, size marker; lanes 3 and 4, wild-type anterior pituitary lysates with and without estradiol-17β (E2); lanes 5 and 6, mutant anterior pituitary lysates with and without estradiol-17β; $n = 5$.

during the first two pregnancies. In subsequent pregnancies ≈ 80% of pups died but on some occasions one or two survived to the weaning period. Such pups, once weaned, were indistinguishable in every respect from mutant pups born to heterozygote mothers. Pituitary prolactin message levels (Fig. 2), protein content (Fig. 3*A*), and serum levels (Fig. 3*B*) were significantly reduced by 1.4-, 1.5-, and 8-fold, respectively, in 7-day postpartum (first pregnancy) mutant females when compared with lactating age-matched wild-type controls. It is likely that an 8-fold decrease in circulating prolactin levels in the mutants is sufficient to account for the complete abolition of lactation. Previous human and rat data would indicate that a 50% reduction in circulating prolactin levels using dopamine agonists, halves, or in some cases abolishes, milk production (44–46). The relationship between the levels of prolactin and the production of breast milk is probably nonlinear, once prolactin levels fall below a particular threshold then milk production ceases. The observation of an almost identical phenotype in mice carrying a heterozygous null mutation in the prolactin receptor (47) adds weight to the hypothesis that the actions of galanin on lactation are mediated in part, via the effects of prolactin on the mammary gland.

The initial failure of lactation in the first two pregnancies also may be explained by a delay in the lobulo-alveolar development of the mammary gland caused by the low levels of circulating prolactin in the mutant females. We stained whole mounts of inguinal mammary glands from 8-week-old virgin females with carmine. Mutant females show reduced ductal branching (Fig. 4). These changes in ductal branching are very similar to those observed in mice homozygous for mutations in either prolactin (48) or the prolactin-receptor (47). Lastly, it is also of interest that a number of studies have identified both prolactin (49, 50) and galanin (51, 52) in rat and human mammary tissue, and most recently, an estrogen-inducible increase in galanin mRNA in a number of breast cancer cell lines of epithelial origin (52), implying further paracrine interactions between galanin and prolactin within the mammary gland.

To study galanin-estrogenic interactions and their effects on prolactin expression and lactotroph growth, adult randomly cycling female mice were injected s.c. with 0.5 mg of estradiol-17β dissolved in safflower oil given once a week, for a 3-week period. This dose regime has been previously demonstrated to induce prolactin gene transcription and secretion in both rats and mice, though the increase in the mouse is less robust than in the rat (53). Pituitary prolactin message levels and protein content (Figs. 2 and 3*A*) rose 2-fold in wild-type females after chronic hyperestrogenization, with a 13-fold increase in plasma prolactin levels (Fig. 3*B*). In parallel, galanin content rose 6-fold (7.5 ± 0.5 vs. 1.2 ± 0.1 pmol/gland, $P < 0.001$; $n = 10$, hyperestrogenized vs. control). Mutant females demon-

strated a similar rise in prolactin message levels (Fig. 2) but not in protein content (Fig. 3*A*). Consistent with these findings, there was a marked attenuation in the rise in plasma prolactin levels (Fig. 3*B*). The proliferative effect of estradiol on dispersed pituitary cell number (24) was also studied, demonstrating an almost complete abolition of the increase in cell number observed in the wild-type animals (Fig. 5). The percentage of lactotrophs, as determined by immunocytochemistry, was also significantly lower in mutant animals compared with wild-type estrogen treated controls (51 ± 4% vs. 62 ± 7% mutant vs. wild-type, $P < 0.01$, $n = 5$).

The recent data by Borrelli and colleagues (35) elegantly demonstrate that prolactin itself acts as an autocrine growth factor to the lactotroph through the activation of pituitary prolactin receptors. Signal transduction of the prolactin receptor occurs primarily through JAK2, a tyrosine kinase whose major phosphorylation targets are the STAT5 transcription factors (54). Phosphorylated STAT5 transcription factors are translocated to the nucleus where they are thought to modulate cell cycle and proliferation. We therefore studied the expression by Western blot analysis of activated STAT5 in wild-type and mutant animals before and after chronic hyperestrogenization. Results demonstrate that the 4.2 ± 0.3-fold increase in STAT5 expression after estradiol administration in wild-type females, is marked reduced in mutant animals (1.6 ± 0.2, Fig. 6). These data are therefore compatible with the hypothesis that galanin may in part regulate lactotroph growth by tonically modulating prolactin release (rather than synthesis) and hence the activation of the prolactin receptor signaling pathway.

In addition to the above effects on prolactin expression, galanin has also been demonstrated, under various physiological circumstances, to modulate the release of GH, (55), the gonadotropins (56) (luteinizing hormone and follicle-stimulating hormone), and vasopressin (57) as well as playing a role in food intake and the regulation of body weight (58). Despite these studies, we were unable to demonstrate changes in the time to enter puberty or gestation period in mutant or heterozygote animals compared with wild-type littermate controls. These normal physiological parameters were paralleled by a lack of change in the pituitary content of GH, TSH, luteinizing hormone, and follicle-stimulating hormone in the mutants (Table 2). Further, the hypothalamic content of many of the major releasing factors known to control the secretion of these hormones were also unchanged (Table 2). The growth rates in the first 8 weeks of life and final adult size of the mutant mice were not different from wild-type littermates. Consistent with this observation, the hypothalamic content of neuropeptide Y and glucagon-like-peptide-1, two neuropeptides known to modulate food intake were unchanged in the mutant animals (Table 2). Similarly, hypothalamic content of vasopressin (Table 2) and posterior pituitary oxytocin content (1.6 ± 0.2 vs. 1.4 ± 0.3 μg/pituitary wild-type and mutants respectively, $n = 8$) were also unaltered by the mutation.

The above data provides good evidence for a causal role for galanin as a prolactin-releasing and growth factor to the lactotroph, especially in states of high estrogen exposure. Galanin would appear to principally regulate circulating prolactin levels at the level of storage and release rather than by the regulation of gene transcription, though whether these effects are mediated at the level of the pituitary and/or hypothalamus are, as yet, unknown. Further support for a role for galanin as a growth factor to the pituitary comes from recent data, indicating that targeted over-expression of galanin to the lactotrophs and somatotroph of transgenic mice, induces pituitary hyperplasia and adenoma formation.†† Our recent observation of a long-term impairment in peripheral nerve regeneration after injury in the galanin mutant animals (unpublished data) also indicates a trophic role for galanin in regenerating sensory neurons. Galanin is expressed at relatively low levels under normal physiological conditions in both the lactotroph and dorsal root ganglion. It is only after pathophysiological stimuli (injury to primary sensory neurons and states of high estrogen exposure to the lactotroph) that a marked plasticity in the expression pattern of galanin occurs. Galanin may thus be acting as a trophic factor in a number of tissues in response to injury or pathological change. Recent data indicates that galanin also has proliferative effects on endocrine small cell lung cancer cells acting by the activation of the p42 isoform of the mitogen-activated protein kinase pathway (59).

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