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# Gonadotropin and Gonadal Steroid Release in Response to a Gonadotropin-Releasing Hormone Agonist in $G_q\alpha$ and $G_{11}\alpha$ Knockout Mice*

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**ABSTRACT**

In this study, we used mice lacking the $G_{11}\alpha$ [$G_{11}$ knockout (KO)] or $G_q\alpha$ gene ($G_q$ KO) to examine LH release in response to a metabolically stable GnRH agonist (Buserelin). Mice homozygous for the absence of $G_{11}\alpha$ and $G_q\alpha$ appear to breed normally. Treatment of (5 wk old) female KO mice with the GnRH agonist Buserelin (2 $\mu$g/100 $\mu$l, sc) resulted in a rapid increase of serum LH levels (reaching 328 ± 58 pg/25 $\mu$l for $G_{11}$ KO; 739 ± 95 pg/25 $\mu$l for $G_q$ KO) at 75 min. Similar treatment of the control strain, 129SvEvTacfBr for $G_{11}$ KO or the heterozygous mice for $G_q$ KO, resulted in an increase in serum LH levels (428 ± 57 pg/25 $\mu$l for $G_{11}$ KO; 884 ± 31 pg/25 $\mu$l for $G_q$ KO) at 75 min. Both $G_{11}$ KO and $G_q$ KO male mice released LH in response to Buserelin (2 $\mu$g/100 $\mu$l of vehicle; 363 ± 53 pg/25 $\mu$l and 749 ± 50 pg/25 $\mu$l 1 h after treatment, respectively). These values were not significantly different from the control strain. In a long-term experiment, Buserelin was administered every 12 h, and LH release was assayed 1 h later. In female $G_{11}$ KO mice and control strain, serum LH levels reached approximately 500 pg/25 $\mu$l within the first hour, then subsided to a steady level (~100 pg/25 $\mu$l) for 109 h. In male $G_{11}$ KO mice and in control strain, elevated LH release lasted for 13 h; however, LH levels in the $G_{11}$ KO male mice did not reach control levels for approximately 49 h. In a similar experimental protocol, the $G_q$ KO male mice released less LH (531 ± 95 pg/25 $\mu$l) after 13 h from the start of treatment than the heterozygous male mice (865 ± 57 pg/25 $\mu$l), but the female KO mice released more LH (634 ± 56 pg/25 $\mu$l) after 1 h from the start of treatment than the heterozygous female mice (346 ± 63 pg/25 $\mu$l). However, after the initial LH flare, the LH levels in the heterozygous mice never reached the basal levels achieved by the KO mice. $G_{11}$ KO mice were less sensitive to low doses (5 ng/per animal) of Buserelin than the respective control mice. Male $G_{11}$ KO mice produced more testosterone than the control mice after 1 h of stimulation by 2 $\mu$g of Buserelin, whereas there was no significant difference in Buserelin stimulated testosterone levels between $G_q$ KO and heterozygous control mice. There was no significant difference in Buserelin stimulated estradiol production in the female $G_q$ KO mice compared with control groups of mice. However, female $G_{11}$ KO mice produced less estradiol in response to Buserelin (2 $\mu$g) compared with control strain. Although there were differences in the dynamics of LH release and steroid production in response to Buserelin treatment compared with control groups of mice, the lack of complete abolition of these processes, such as stimulated LH release, and steroid production, suggests that these G proteins are either not absolutely required or are able to functionally compensate for each other. (*Endocrinology* **139:** 2710–2717, 1998)

GnRH STIMULATES LH and FSH release from the anterior pituitary. Several pieces of evidence implicated G protein involvement in GnRH receptor mediated actions. First, GTP and its analogs stimulate LH release and inositol phosphate production in permeabilized pituitary cell cultures (1) and provoke the characteristic decrease in affinity of the GnRH receptor for its ligand (2). Second, the GnRH receptor has seven transmembrane segments characteristic of G protein-coupled receptors (3).

Multiple G proteins appear to be involved in mediating the response of the gonadotrope to GnRH (4, 5). GnRH is capable of stimulating IP release through pertussis toxin-sensitive G proteins, whereas a cholera toxin-sensitive G protein appears to provoke gonadotrope sensitization to GnRH and other secretagogues. Immunodepletion studies from $\alpha$T3–1 cell membranes suggest that the GnRH receptor is coupled to $G_{q/11}\alpha$ protein (6), and more recently we have shown that the GnRH receptor regulates $G_{q/11}\alpha$ in rat pituitary cell cultures and in $GGH_3$ cells that stably express the GnRH receptor (7). Stimulation with GnRH also provokes palmitoylation and redistribution of $G_{q/11}\alpha$ in primary pituitary cultures (7, 8).

The $G_q\alpha$ subfamily includes $G_q\alpha$, $G_{11}\alpha$, $G_{14}\alpha$, $G_{15}\alpha$, and $G_{16}\alpha$ (9). These G proteins are capable of activating phospholipase C-$\beta$ (PLC$\beta$) and are unmodified by pertussis toxin (10, 11). $G_q\alpha$ and $G_{11}\alpha$ have 88% amino acid sequence identity (10). In SDS-PAGE, $G_q\alpha$ and $G_{11}\alpha$ migrate at 41–42 kDa (12). Due to the structural similarities between $G_q\alpha$ and $G_{11}\alpha$, it is technically difficult to discriminate between the two G proteins by biochemical approaches.

Very few studies are available to attribute a specific activity to either $G_q\alpha$ or $G_{11}\alpha$, and the likelihood exists that these two proteins functionally compensate for each other. Studies done on Swiss 3T3 cells, for example, indicate that bombesin and vasopressin receptors concurrently activate

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both $G_q\alpha$ and $G_{11}\alpha$ (13), suggesting that, with respect to phospholipase C-$\beta$ activation, these two proteins may function interchangeably. However, when $\alpha_{1A/D}$, $\alpha_{1B}$ and $\alpha_{1C}$ adrenergic receptors are activated by agonists, $G_q\alpha$ and $G_{11}\alpha$ proteins, which are activated by these receptors, are degraded at a similar rate, an observation suggesting that the G proteins may couple to these receptors without any preference (14). Furthermore, in *Xenopus* oocytes, $G_q\alpha$ and $G_{11}\alpha$ show similar modulation of response to TSH releasing hormone (15), suggesting that these two proteins activate similar effectors, although an earlier report in the same system suggests that the response to TSH-releasing hormone is differentially coupled to downstream effectors by $G_q\alpha$ and $G_{11}\alpha$ proteins (16).

All of these studies were performed either in transfected cell lines or in *Xenopus* oocytes. Therefore, little is known about the G protein coupling to the GnRH receptor in intact animals, particularly under endocrine systems with complex feedback mechanisms. To examine this question with respect to G protein-coupling to the GnRH receptor, we examined the role of $G_q\alpha$ and $G_{11}\alpha$ in the mouse gonadotrope by using knockout mice, lacking either the $G_q\alpha$ or the $G_{11}\alpha$ protein. This study provides evidence to suggest that $G_q\alpha$ and $G_{11}\alpha$ proteins can functionally compensate for each other in GnRH analog stimulated LH release and steroidogenesis.

## Materials and Methods

### *Preparation of mice lacking the $G_{11}\alpha$ gene*

$G_{11}\alpha$ knockout mice were prepared from 129/SvEv strain of mice as previously described (Wilkie, T., manuscript submitted). For control experiments 129/SvEvTacfBR strain of mice (Taconic Farms, Germantown, NY) were used. Both control mice and knockout mice were 5 weeks old, and weighed approximately 16–18 g. The breeding pair of $G_{11}\alpha$ knockout mice were homozygous for the knockout gene. The weight to age curves, litter size, and other external characteristics were indistinguishable between 129/SvEvTacfBR mice and knockout mice. Therefore, we used age-matched 129/SvEvTacfBR strain as controls.

### *Preparation of mice lacking the $G_q\alpha$ gene*

$G_q\alpha$ knockout mice were prepared as previously described (18). Male mice homozygous for the $G_q\alpha$ knockout were mated to female mice heterozygous for the $G_q\alpha$ knockout. Offspring that were heterozygous for the $G_q\alpha$ knockout were used as control mice. The experiments were performed at 5 weeks of age, and at this time, both homozygous and heterozygous mice for the knockout gene weighed approximately 15–18 g each. The mice were genotyped at 3 weeks to identify the homozygous knockout mice in the litter. To keep the variations between the animals to a minimum, heterozygous mice from the same litter was used as controls. Heterozygous mice, who have only one functioning $G_q\alpha$ gene, do not show any adverse effects. These mice have weight to age curves similar to the wild-type mice and were indistinguishable from the wild-type mice. Thus, heterozygous mice from the same litter were an appropriate control.

### *Genotyping of $G_q\alpha$ knockout mice*

Approximately 0.5 cm size section of the mouse tails were digested overnight under constant agitation at 50 C in 300 $\mu$l of digestion buffer (100 mM EDTA, 0.5% SDS, 0.5 mg/ml proteinase K and 50 mM Tris-HCl; pH 8). The digested samples were spun at 12,000 $\times$ *g* for 2 min, and 200 $\mu$l of the supernatant was mixed with 100 $\mu$l of 7.5 M ammonium acetate to obtain a final concentration of 2.5 M. Genomic DNA was precipitated by adding 600 $\mu$l of ice cold ethanol and collected at 12,000 $\times$ *g* for 10 min. The DNA pellet was washed once with 70% ethanol and dried. Finally, the DNA pellet was dissolved in 300 $\mu$l of Tris-EDTA buffer (pH 8).

A 150-bp sequence of the disrupted $G_q\alpha$ gene containing the neomycin gene was amplified by PCR with flanking primers NEO4 (5′ GATTCGCAGCGCATCGCCTTCTAT 3′) and QNEO (5′ TTCAAAGTATCACACTCACATCACAG 3′). A 150-bp sequence of the wild-type $G_q\alpha$ gene was amplified with the flanking primers 5EXQ (5′ GAACCGCATGGAGGAGAGCAAAGC 3′) and 3EXQ (5′ CTGGGAAGTAG TCGACTAGGTGGG 3′). The PCR protocol used is as follows: 5 min at 94 C, 35 cycles of 1 min at 94 C, 1 min at 63 C and 3 min at 72 C, and finally 10 min at 72 C.

### *Serum collection and LH RIA*

Each mouse received 2 $\mu$g of Buserelin (GnRH agonist, Hoechst) in 100 $\mu$l of vehicle (PBS/0.3% BSA) or vehicle alone. This is a saturating dose of Buserelin with respect to LH release (19). For the dose-response studies, indicated doses of Buserelin in 100 $\mu$l of vehicle was given. Buserelin was injected sc into the skin behind the neck. Mice were anesthetized with methoxyflurane, and serum collected at the indicated times by intraorbital puncture. In long time course experiments, serum was collected 1 h after injection of Buserelin. Serum was aliquoted and stored at $-20$ C before assay.

The RIA used a highly purified rat LH for iodination (NIDDK; 20) and a mouse reference preparation obtained as a kind gift from Dr. Al Parlow (Harbor-UCLA Hospital, Torrance, CA). LH antisera (C102) was prepared and characterized as previously described (21). Bound and free proteins were separated using the second antibody technique (22). The minimum detectable dose for the RIA was 6 $\pm$ 1 pg (n = 6) and the inter and intraassay variance was less than 10%. The rat and mouse LH standards were approximately identical; the rat $ED_{20}$ never varied more than 9% from the mouse $ED_{20}$.

### *Steroid assays for testosterone and estradiol*

Serum estradiol (23, 24) and testosterone (25) levels were measured by RIA in the ORPRC RIA Laboratory using previously described methods. Antisera for estradiol (23, 24) and testosterone (25) were obtained as previously described. For the estradiol assay, the minimum detectable dose was approximately 0.5 pg. The intra and interassay variance was approximately 7 and 13%, respectively. For the testosterone assay, the minimum detectable dose was 5 pg. The intra and interassay variance was approximately 5 and 8%, respectively.

### *Statistical analyses*

The results are presented as the mean $\pm$ SEM of the indicated number of animals. Data were analyzed by one-way ANOVA, followed by Student's *t* test with Bonferoni correction for multiple comparisons. To examine the overall LH release in mice over time, a two-way ANOVA for repeated measures was performed.

## Results

Figure 1, A and B, shows, respectively, serum LH levels in male and female $G_{11}\alpha$ knockout mice, after an sc injection of 2 $\mu$g of Buserelin or vehicle. Samples were collected at 0, 15, 30, 45, and 75 min after the injection of drug or the vehicle. In male $G_{11}\alpha$ knockout mice, serum LH levels increased from 7 $\pm$ 2 pg/25 $\mu$l (n = 6) to 363 $\pm$ 53 pg/25 $\mu$l (n = 6), when administered with 2 $\mu$g of Buserelin (Fig. 1A). In the control strain, the same treatment of Buserelin resulted in an increase of serum LH from 10 $\pm$ 1 pg/25 $\mu$l (n = 5) to 458 $\pm$ 59 pg/25 $\mu$l (n = 5). When treated with the vehicle (PBS/0.1% BSA), the serum LH levels in male $G_{11}\alpha$ knockout mice and in the male control strain remained between 7–10 pg/25 $\mu$l. In female $G_{11}\alpha$ knockout mice, serum LH levels increased from 6 $\pm$ 1 pg/25 $\mu$l (n = 7) to 328 $\pm$ 58 pg/25 $\mu$l (n = 7), when administered with 2 $\mu$g of Buserelin (Fig. 1B). In the control strain, the same treatment of Buserelin resulted in an increase

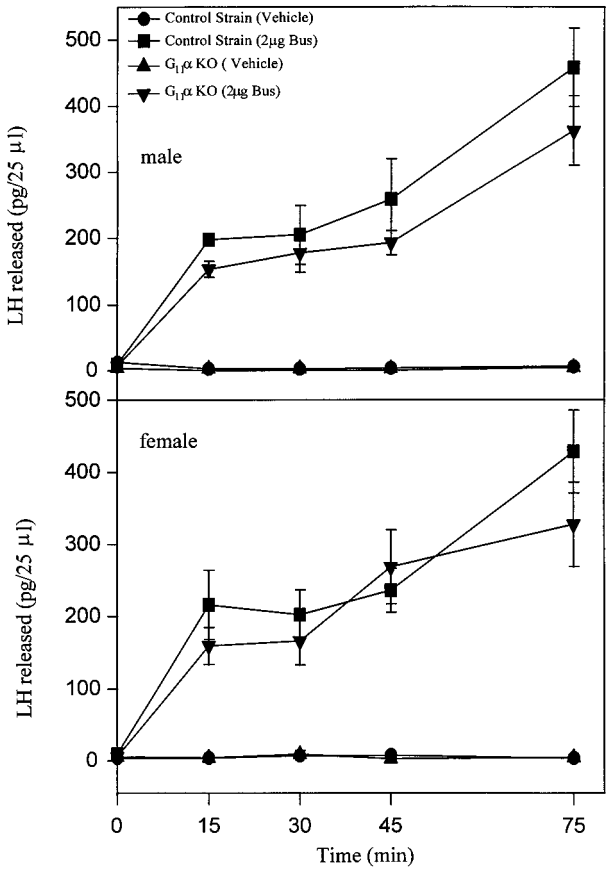


FIG. 1. A and B show, respectively, serum LH levels in male and female $G_{11}\alpha$ knockout mice, after an sc injection of 2 $\mu$g of Buserelin or vehicle. Samples were collected at the indicated times, and LH released was assayed by RIA as mentioned in *Materials and Methods*. Data represent the mean ± SEM.

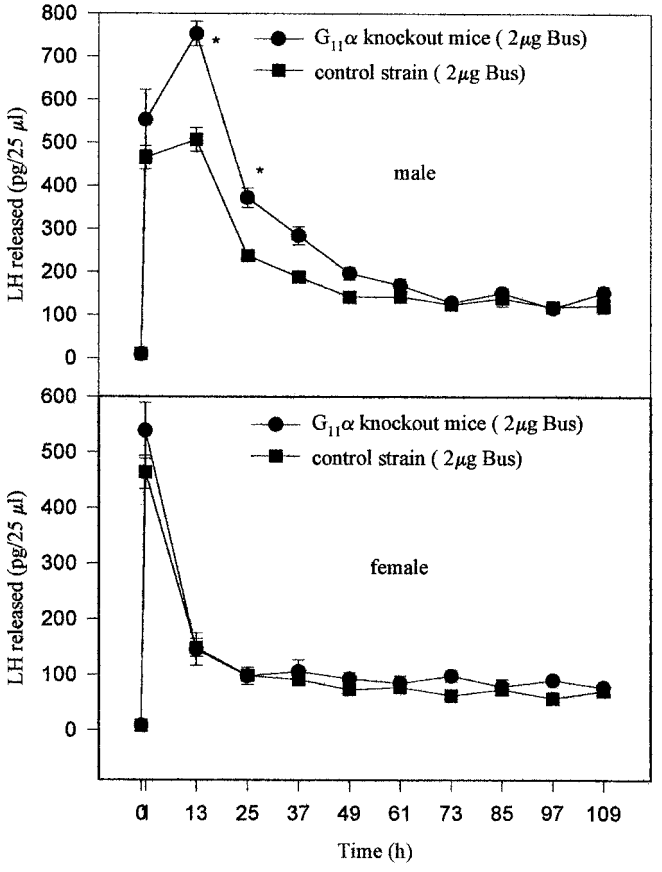


FIG. 2. A and B show serum LH levels in $G_{11}\alpha$ knockout male and female mice, respectively, 1 h after an sc injection of 2 $\mu$g of Buserelin given every 12 h for 108 h. Samples were collected, and LH released was assayed by RIA as mentioned in *Materials and Methods*. The data represent the mean ± SEM. Data points with *asterisks* are significantly different ($P < 0.05$) from the corresponding data points for the control strain.

of serum LH from 10 ± 1 pg/25 $\mu$l (n = 6) to 428 ± 57 pg/25 $\mu$l (n = 6). LH released in response to Buserelin was not significantly different ($P < 0.05$) in both male and female $G_{11}\alpha$ knockout mice compared with control mice. When treated with the vehicle (PBS/0.1% BSA), the serum LH levels in female $G_{11}\alpha$ knockout mice and in female control strain remained between 6–10 pg/25 $\mu$l.

Figure 2, A and B, shows serum LH levels in mice 1 h after an sc injection of 2 $\mu$g of Buserelin given every 12 h for 108 h. In male $G_{11}\alpha$ knockout mice, the serum LH levels reached a maximum of 753 ± 29 pg/25 $\mu$l (n = 7) after 13 h from the start of treatment (Fig. 2A). In the male control strain, a similar treatment resulted in a maximum LH level of 507 ± 27 pg/25 $\mu$l (n = 6) after 13 h from the start of treatment. After 61 h from the start of treatment, the serum LH levels in the male $G_{11}\alpha$ knockout mice reached the serum LH level of the control mice. Male $G_{11}\alpha$ knockout mice released significantly ($P < 0.05$) more LH over time than male control mice. In female $G_{11}\alpha$ knockout mice, the serum LH levels reached a maximum of 579 ± 88 pg/25 $\mu$l (n = 5) after 1 h from the start of treatment (Fig. 2B). In the female control strain, a similar treatment resulted in a maximum LH level of 461 ± 45 pg/25 $\mu$l (n = 7) after 1 h from the start of treatment. The serum LH levels in female $G_{11}\alpha$ knockout mice and control strain were maintained at similar levels after 13 h from start of treatment. There was no significant ($P < 0.05$) differences in LH release over time between the female $G_{11}\alpha$ knockout mice and female control mice.

Figure 3, A and B, shows, respectively, the serum LH level in male and female $G_q\alpha$ knockout mice after an sc injection of 2 $\mu$g of Buserelin or vehicle. Samples were collected at 0, 15, 30, 45, and 75 min after the injection of drug or the vehicle. In male $G_q\alpha$ knockout mice, serum LH levels increased from 8 ± 0 pg/25 $\mu$l to 749 ± 50 pg/25 $\mu$l (n = 5), when administered with 2 $\mu$g of Buserelin (Fig. 3A). In the heterozygous strain, the same treatment of Buserelin resulted in an increase of serum LH from 10 ± 2 pg/25 $\mu$l to 765 ± 85 pg/25 $\mu$l (n = 5). When treated with vehicle (PBS/0.1% BSA), the serum LH levels in male $G_q\alpha$ knockout mice and in male control strain remained between 9–10 pg/25 $\mu$l (data not shown). In female $G_q\alpha$ knockout mice, serum LH levels increased from 7 ± 0 pg/25 $\mu$l to 740 ± 95 pg/25 $\mu$l (n = 9), when administered with 2 $\mu$g of Buserelin (Fig. 3B). In the female heterozygous strain, the same treatment of Buserelin resulted in an increase of serum LH from 9 ± 1 pg/25 $\mu$l to 884 ± 31 pg/25 $\mu$l (n = 6). LH released in response to Buserelin was not significantly different ($P < 0.05$) in both male and female $G_q\alpha$ knockout

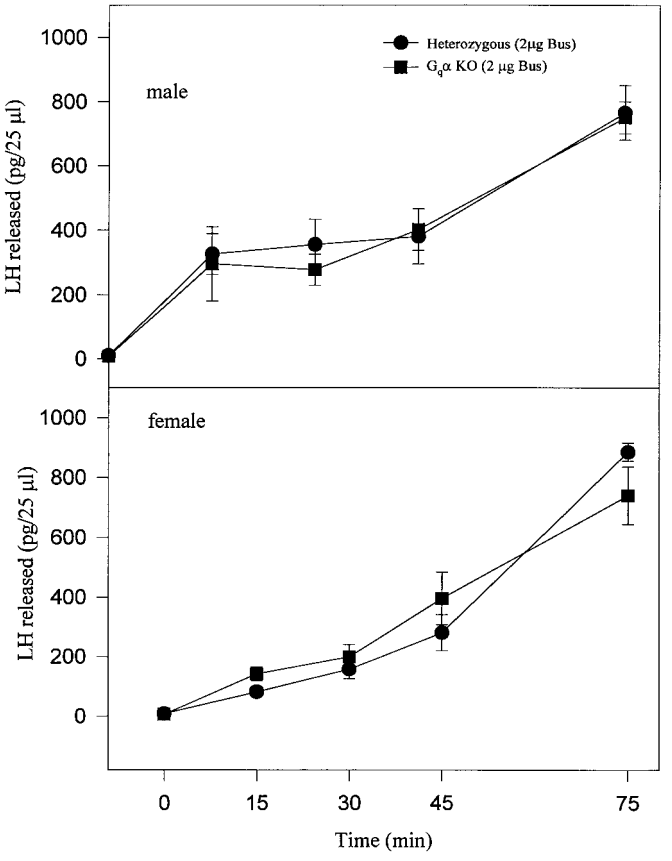


FIG. 3. A and B show, respectively, serum LH levels in male and female $G_q\alpha$ knockout mice, after an sc injection of 2 $\mu$g of Buserelin or vehicle. Samples were collected at the indicated times, and LH released was assayed by RIA as mentioned in *Materials and Methods*. Data represent the mean ± SEM.

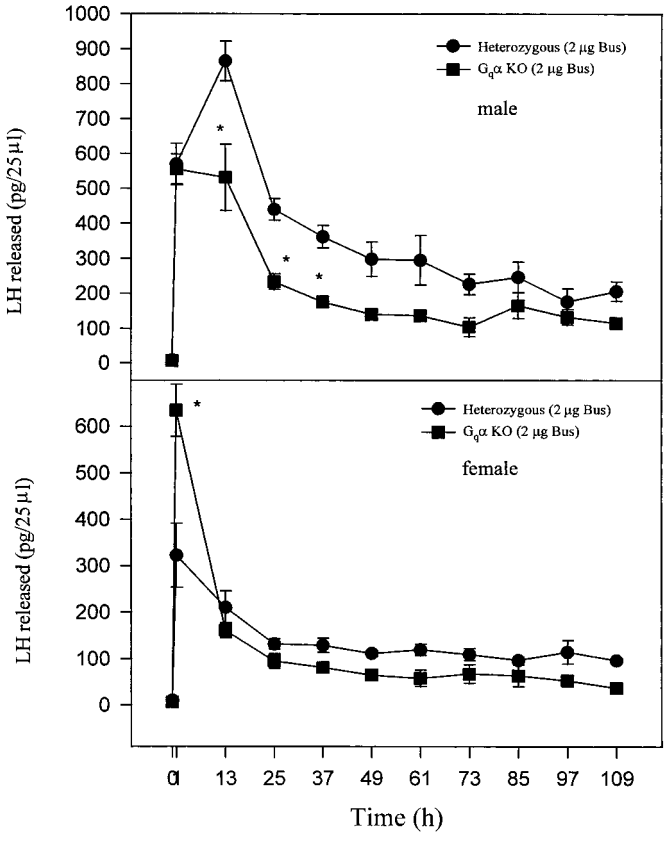


FIG. 4. A and B show serum LH levels in $G_q\alpha$ knockout male and female mice, respectively, 1 h after an sc injection of 2 $\mu$g of Buserelin given every 12 h for 108 h. Samples were collected, and LH released was assayed by RIA as mentioned in *Materials and Methods*. The data represent the mean ± SEM. Data points with *asterisks* are significantly different ($P < 0.05$) from the corresponding data point for the heterozygous mice.

mice compared with heterozygous mice. When treated with the vehicle (PBS/0.1% BSA), the serum LH levels in female $G_q\alpha$ knockout mice and in female heterozygous mice remained between 9–10 pg/25 $\mu$l (data not shown).

Figure 4, A and B, shows serum LH levels in $G_q\alpha$ knockout mice 1 h after an sc injection of 2 $\mu$g of Buserelin given every 12 h for 108 h. In male $G_q\alpha$ knockout mice, the serum LH level reached a maximum of 556 ± 43 pg/25 $\mu$l (n = 7) after 1 h from the start of treatment (Fig. 4A). In the heterozygous male, a similar treatment resulted in LH levels of 570 ± 60 pg/25 $\mu$l (n = 4) after 1 h from the start of treatment, a maximum serum LH level of 865 ± 57 pg/25 $\mu$l was achieved after 13 h from the start of treatment. The serum LH levels of the heterozygous male mice never returned to the serum LH levels of the $G_q\alpha$ knockout mice during the treatment period. In female $G_q\alpha$ knockout mice, the serum LH level reached a maximum of 634 ± 56 pg/25 $\mu$l (n = 4) after 1 h from the start of treatment (Fig. 4B). In the heterozygous strain, a similar treatment resulted in a maximum LH level of 346 ± 63 pg/25 $\mu$l (n = 7) after 1 h from the start of treatment. The serum LH level in female $G_q\alpha$ knockout mice was maintained after the initial LH flare below the heterozygous serum LH levels. There was a significant ($P < 0.05$) difference in LH release over time (both males and females) between $G_q\alpha$ knockout and heterozygous mice.

To examine whether the sensitivity of the gonadotrope is different between the knockout and control mice, we investigated the amount of LH released in response to different doses of Buserelin. Figure 5, A and B, shows the LH released after 1 h in response to the indicated doses of Buserelin in $G_{11}\alpha$ knockout male and female mice, respectively. There is no significant difference between the knockout mice and control mice at doses above 0.05 $\mu$g of Buserelin. The control mice responded more robustly to a 5 ng/per animal dose of Buserelin than the knockout mice; 448 ± 18 pg/25 $\mu$l (n = 5) *vs.* 198 ± 25 pg/25 $\mu$l (n = 6) in male knockout mice, 417 ± 23 pg/25 $\mu$l (n = 5) *vs.* 175 ± 24 pg/25 $\mu$l (n = 7) in female knockout mice.

Figure 6, A and B, shows the LH released after 1 h in response to the indicated doses of Buserelin in $G_q\alpha$ knockout male and female mice, respectively. There was a significant difference in LH release in female $G_q\alpha$ knockout mice when 2 $\mu$g of Buserelin was administered; 634 ± 56 pg/25 $\mu$l (n = 4) for $G_q\alpha$ knockout *vs.* 346 ± 63 pg/25 $\mu$l (n = 4) for control. However, unlike the $G_{11}\alpha$ knockout mice, there was no significant ($P < 0.05$) difference between $G_q\alpha$ knockout mice and heterozygous mice with respect to LH release in response to 0.05 $\mu$g of Buserelin.

To examine whether there is a differential response to

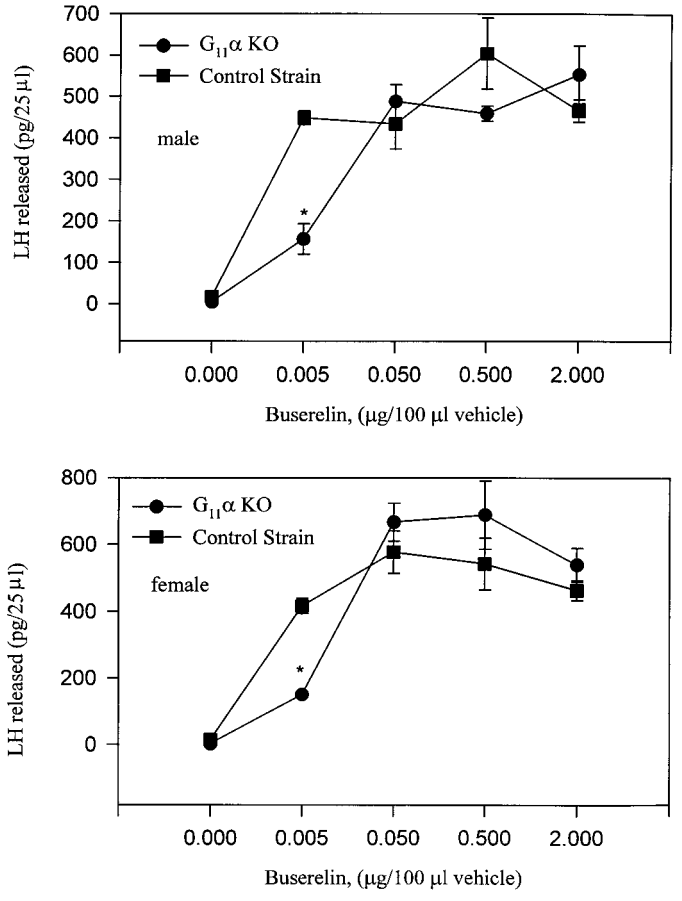


FIG. 5. A and B show the LH released, after 1 h, in response to the indicated doses of Buserelin in $G_{11}\alpha$ knockout male and female mice, respectively. Samples were collected, and LH released was assayed by RIA as mentioned in *Materials and Methods*. The data represent the mean ± SEM. Data points with *asterisks* are significantly different ($P < 0.05$) from the corresponding data points for the control strain.

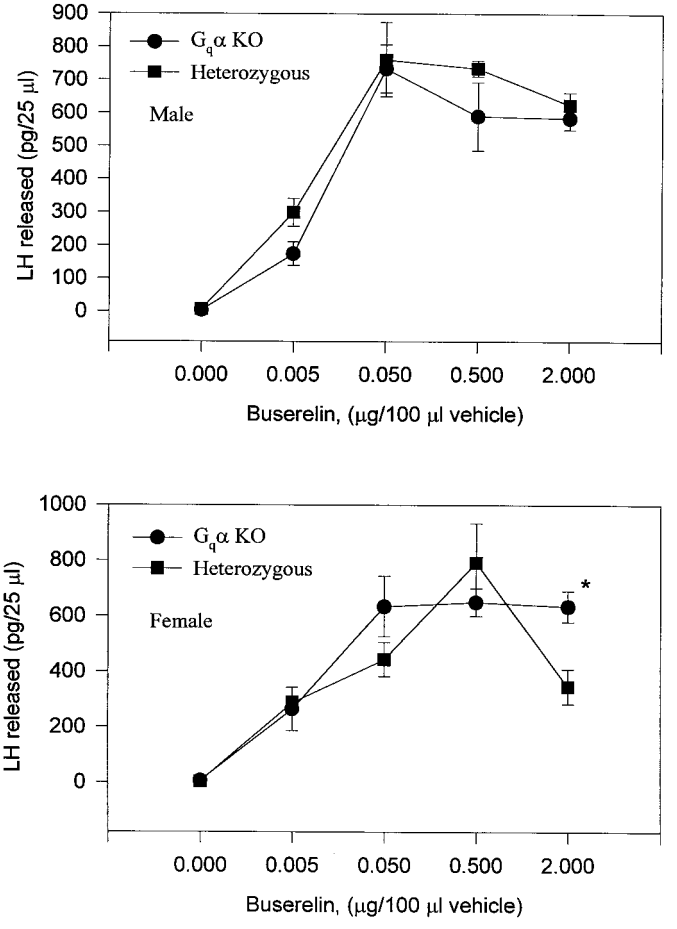


FIG. 6. A and B show the LH released, after 1 h, in response to the indicated doses of Buserelin in $G_q\alpha$ knockout male and female mice, respectively. Samples were collected, and LH released was assayed by RIA as mentioned in *Materials and Methods*. The data represent the mean ± SEM. Data points with *asterisks* are significantly different ($P < 0.05$) from the corresponding data points for the heterozygous mice.

GnRH with respect to steroid production between the knockout mice and the control strain, we examined the production of testosterone and estradiol in mice when injected with 2 $\mu$g of Buserelin. Figure 7, A and B, shows the testosterone and estradiol levels in the serum, respectively, of male and female $G_{11}\alpha$ knockout mice, before and 1 h after an sc injection of 2 $\mu$g of Buserelin. In male $G_{11}\alpha$ knockout mice, serum testosterone levels increased from 2 ± 1 ng/ml to 23 ± 4 ng/ml (n = 10) in response to an sc injection of 2 $\mu$g of Buserelin. In the male control strain, a similar treatment of Buserelin increased serum testosterone levels from 1 ± 1 ng/ml to 14 ± 3 ng/ml (n = 5). In female $G_{11}\alpha$ knockout mice, we were unable to detect a change in serum estradiol levels in response to 2 $\mu$g of Buserelin; 8 ± 1 pg/ml before Buserelin treatment, and 6 ± 1 pg/ml (n = 12) 1 h after Buserelin treatment. In the female control strain, serum estradiol levels increased in response to 2 $\mu$g of Buserelin from 12 ± 1 pg/ml (n = 4) to 17 ± 2 pg/ml (n = 5).

Figure 8, A and B, shows the serum testosterone and estradiol levels, respectively, of male and female mice, before and 1 h after an sc injection of 2 $\mu$g of Buserelin in $G_q\alpha$ knockout mice. In male $G_q\alpha$ knockout mice, serum testosterone levels increased from 1 ± 0 ng/ml to 14 ± 2 ng/ml (n = 7) in response to an sc injection of 2 $\mu$g of Buserelin. In the male heterozygous mice, a similar treatment of Buserelin increased serum testosterone levels from 1 ± 0 ng/ml to 17 ± 2 ng/ml (n = 5). In female $G_q\alpha$ knockout mice there was no significant change in serum estradiol levels in response to 2 $\mu$g of Buserelin; 5 ± 2 pg/ml before Buserelin treatment, and 4 ± 2 pg/ml (n = 3) 1 h after Buserelin treatment. The same was true for female heterozygous mice; 4 ± 1 pg/ml before Buserelin treatment and 7 ± 1 pg/ml (n = 3) after Buserelin treatment. There was no significant difference between $G_q\alpha$ knockout mice and heterozygous mice, with respect to Buserelin stimulated steroid (testosterone and estradiol) release.

## Discussion

In this study, we investigated the role of $G_q\alpha$ and $G_{11}\alpha$ in mouse gonadotrope function using knockout mice lacking the $G_q\alpha$ or the $G_{11}\alpha$ protein. The fact that these knockout mice breed relatively normally immediately suggested that either protein is not essential in the regulation of the gonadotrope; potentially $G_q\alpha$ and $G_{11}\alpha$ can substitute for each other. To

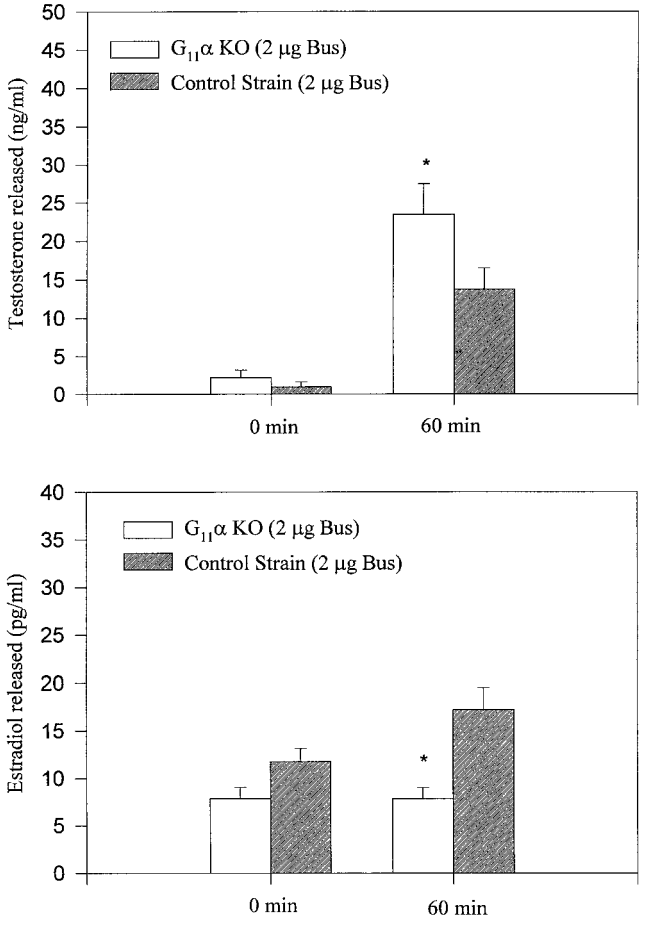


FIG. 7. A and B show the testosterone and estradiol levels in the serum, respectively, of male and female mice, before and 1 h after an sc injection of 2 $\mu$g of Buserelin in $G_{11}\alpha$ knockout mice. Samples were collected and serum testosterone and estradiol levels were assayed as mentioned in *Materials and Methods*. The data represent the mean ± SEM. Data points with *asterisks* are significantly different ($P < 0.05$) from the corresponding data point for the control strain.

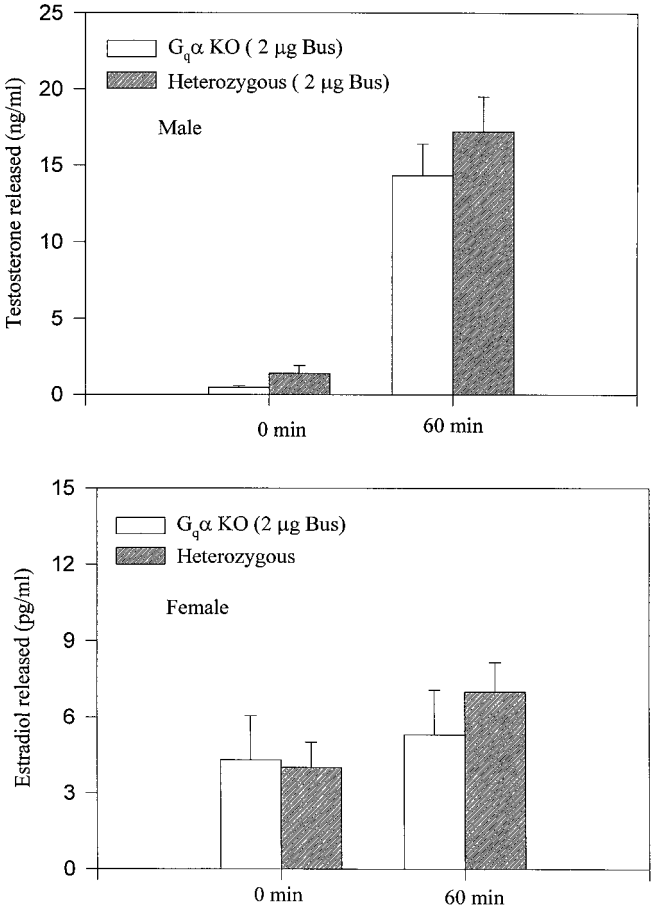


FIG. 8. A and B show the serum testosterone and estradiol levels, respectively, of male and female mice, before and 1 h after an sc injection of 2 $\mu$g of Buserelin in $G_q\alpha$ knockout mice. Samples were collected and serum testosterone and estradiol levels were assayed as mentioned in the *Materials and Methods*. The data represent the mean ± SEM.

examine the role of these two proteins in GnRH-stimulated LH release, we examined the dynamics of GnRH-stimulated LH release in mice. We used two different protocols to address this question. First, we gave each mouse (control and knockout) a single dose of Buserelin and subsequently measured the serum LH levels at the given times. This protocol would show any differences in the release of LH between the knockout mice and control mice in response to acute GnRH analog treatment. Alternatively, we administered Buserelin every 12 h for 108 h, and 1 h after each administration, measured the serum LH levels. The second protocol would address any differences in the LH release between the control mice and knockout mice in response to chronic GnRH analog treatment, and allow assessment of the development of desensitization. To determine the sensitivity of the gonadotrope, we performed an *in vivo* dose-response of Buserelin-stimulated LH release in the knockout and control mice. Furthermore, to determine whether any differences in Buserelin-stimulated LH release between knockout mice and control mice are due to different levels of gonadal steroids, producing varying levels of negative feedback, we assayed the serum testosterone and estradiol levels in male and female mice, respectively.

In the short time course study, in both sexes, we did not observe any significant differences between the control strain and the $G_{11}\alpha$ knockout mice. In the long time course study, the male $G_{11}\alpha$ knockout mice had a rapid increase in serum LH levels after Buserelin treatment compared with the control strain. Furthermore, in the male $G_{11}\alpha$ knockout mice, the serum LH levels were elevated above those of the control mice up to 61 h after the start of treatment, indicating that the knockout mice did not become refractory to the GnRH analog as rapidly as did the control strain. Another possible interpretation would be that LH is elevated to such an extent, that it takes a longer time to return to control plasma levels.

The short time course studies, in both sexes of the $G_q\alpha$ knockout mice, showed no significant differences in LH release in response to a GnRH analog when compared with the control heterozygous strain. In the long time course studies, both sexes showed a significant difference in the LH release compared with heterozygous mice. The $G_q\alpha$ knockout male mice released less LH than the heterozygous mice, and after the initial LH flare, the LH levels in the heterozygous mice

never reached the basal levels achieved by the knockout mice. Paradoxically, in the female $G_q\alpha$ knockout mice, Buserelin stimulated a higher level of LH release compared with the control heterozygous strain.

Only $G_{11}\alpha$ knockout mice and not $G_q\alpha$ knockout mice were less sensitive to low doses of Buserelin with respect to LH release. Stimulation of knockout mice with 5 ng of Buserelin resulted in substantially less LH release than in the control mice in $G_{11}\alpha$ knockout mice. This may indicate different roles for $G_{11}\alpha$ and $G_q\alpha$ in the gonadotrope.

To determine whether the differential responses to Buserelin, with respect to LH release, was due to different levels of gonadal steroids being released resulting in differing levels of negative feedback on the gonadotrope, we examined the serum testosterone and estradiol levels in male and female mice, respectively. Buserelin-stimulated serum testosterone levels in male $G_{11}\alpha$ knockout mice was significantly higher than in control mice, although Buserelin stimulated estradiol levels in female $G_{11}\alpha$ knockout mice were lower than in control mice. In male and female $G_q\alpha$ knockout mice, there were no significant differences in Buserelin-stimulated gonadal steroid levels compared with control heterozygous strain.

The fact that there was a difference in Buserelin-stimulated LH release between the short and long time course studies suggests that there is a differential response to Buserelin under different treatment protocols. It is possible that mice in the short time course protocol may be stressed, resulting in a retarded response to Buserelin.

In the gonadotrope, LH release is under the negative feedback control of estrogen and testosterone. Therefore, if gonadal steroid production was affected in the knockout mice, this could produce different intensities of negative feedback on the gonadotrope resulting in differential levels of LH release in response to Buserelin. The steroid data suggest that negative feedback cannot account for the high LH release in male $G_{11}\alpha$ knockout mice in the long time course study, because in the $G_{11}\alpha$ knockout mice, the testosterone levels are higher than in control mice. In $G_q\alpha$ knockout male mice, Buserelin-stimulated LH release is lower than in the control mice in the long time course study, although there is no significant difference in testosterone production compared with the control strain, at 1 h or 13 h after the start of Buserelin treatment. Therefore the differences in the Buserelin-stimulated LH release in the knockout mice compared with the control mice cannot be clearly explained with the differing steroid levels. In the female $G_{11}\alpha$ knockout mice we could not detect an increase in the serum estradiol production after Buserelin stimulation, although in the control wild-type mice we measured a Buserelin-stimulated increase in serum estradiol production. The absence of a Buserelin-stimulated estradiol increase in female $G_{11}\alpha$ knockout mice may be a physiological manifestation of $G_{11}\alpha$ absence, or may be a technical artifact due to serum estradiol levels too low to detect with our assay. In the female $G_q\alpha$ knockout mice, we could not detect an increase in serum estradiol in response to Buserelin. However, there were no significant differences in the serum estradiol production between the knockout mice and the control heterozygous mice. This may indicate that $G_q\alpha$ does not play a role in Buserelin-stimulated estradiol production in these mice, although, as mentioned earlier, this observation may be due to low serum estradiol levels compared with the sensitivity of our assay.

The fact that in the long time course study, Buserelin-stimulated LH release is higher in male $G_{11}\alpha$ knockout mice than in control (Fig. 2), whereas in male $G_q\alpha$ knockout mice, Buserelin-stimulated LH release is lower than in the control strain (Fig. 4), suggests that these proteins may play a different role in LH synthesis. A disruption in LH synthesis, manifested as a difference in Buserelin-stimulated LH release in the long time course experiments cannot be discounted in these knockout mice.

The differences between male control mice and male $G_{11}\alpha$ knockout mice, with regard to Buserelin-stimulated LH release and testosterone production, may be due to the fact that the control mice and the knockout mice were from two different breeding groups. However, this is unlikely, as the strain of the two groups of mice were the same (129/SvEvTacfBR). Similarly, the differences between the heterozygous mice and $G_q\alpha$ knockout mice, with respect to LH release, are not likely due to differences in strain, age or pubertal status, as we tried to use mice from the same litter group.

The reason for a sex based difference in Buserelin-stimulated LH release in $G_q\alpha$ and $G_{11}\alpha$ knockout mice is unclear. One possibility is that there may be differences in the sexual maturation time between males and females, although how this may affect Buserelin-stimulated LH release is not known. It would be interesting to see whether there are other sex based differences in these G protein knockout mice.

The changes in serum LH levels observed between the knockout mice and the control mice is not due to differences in degradation, because serum LH clearance is dependent on sialation of the protein. Changes in sialation is observed in different aged animals, and we used mice that were of similar age (26).

This study shows that there are differences in the dynamics of LH release in response to chronic GnRH analog treatment. The lack of complete abolition of processes, such as stimulated LH release and steroid production, suggest that these G proteins are either not required or are able to functionally compensate for each other. Experiments similar to this study will have to be performed with conditional double knockouts of $G_{11}\alpha$ and $G_q\alpha$ to determine whether these proteins are required for GnRH activity in the pituitary.

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