Refractoriness to Short Day Lengths Augments Tonic and Gonadotrophin-Releasing Hormone-Stimulated Lutenising Hormone Secretion

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Abstract

Siberian hamsters (Phodopus sungorus) undergo reproductive involution following exposure to short winter day lengths. Following approximately 20 weeks of exposure to short day (SD) lengths, hamsters become refractory to the inhibitory effects of SD, and reproductive competence is restored in anticipation of spring. The extent to which changes in gonadal steroid-dependent and -independent regulation of gonadotrophin secretion participate in this vernal reactivation of the gonads is not known. This experiment tested whether tonic and gonadotrophin-releasing hormone (GnRH)-stimulated regulation of lutenising hormone (LH) secretion differs between photoresponsive and photorefractory Siberian hamsters. Male hamsters born into long day (LD) lengths were castrated or subjected to a sham-castration surgery at 17 days of age, implanted s.c. with blank or testosterone-filled capsules, and housed in LD or SD thereafter. Baseline LH and LH responses to GnRH (200 ng/kg, s.c) were measured at 14 (photoresponsive) and 40 (photorefractory) weeks of age. Despite lower circulating testosterone concentrations in gonadally regressed SD hamsters on week 14, tonic LH concentrations were comparable among all groups of gonad-intact hamsters on weeks 14 and 40; however, week 14 SD hamsters exhibited significantly higher GnRH-stimulated LH responses. Tonic LH concentrations were indistinguishable among all groups of castrated hamsters bearing empty implants on week 14, but prolonged exposure to LD led to a decrease in resting LH, whereas prolonged exposure to SD resulted in an increase in LH. In castrated hamsters bearing testosterone implants, baseline LH concentrations were comparable in all groups, but GnRH treatment resulted in significantly higher LH concentrations in photorefractory (week 40, SD) hamsters relative to all other groups. The data suggest that the development of photorefractoriness in Siberian hamsters is characterised by enhanced gonadal hormone-independent stimulation of LH secretion, and diminished sensitivity to inhibitory negative-feedback effects of testosterone on LH secretion. Decreases in responsiveness of gonadotrophin secretion to gonadal hormone negative feedback may contribute to the process of photorefractoriness and assist in maintaining the growth of reproductive organs during the process of gonadal recrudescence.

Most seasonally breeding mammals time their reproductive efforts such that the production of offspring coincides with favourable environmental conditions, principally those of food, water, and ambient temperature (1, 2). Small mammals use a variety of timing mechanisms to inhibit reproductive function during the time of year when energetic resources are relatively scarce, and to reactivate reproductive physiology during times when such resources are relatively plentiful. At least two distinct classes of timing mechanisms have been identified as participating in the generation of rodent seasonal neuroendocrine rhythms: (i) endogenous circannual clocks [e.g. ground squirrels (3), woodchucks (4)] and (ii) semiannual interval timers [e.g. Syrian hamsters (5), Siberian hamsters (6)]. Seasonal changes in day length (photoperiod) provide relatively accurate indicators of phase in the annual geophysical cycle and are required either to entrain (circannual clocks) or to initiate (semiannual interval timers) seasonal timekeeping mechanisms.

Siberian hamsters (Phodopus sungorus) exhibit reproductive involution in response to short photoperiods typical of late
summer/early autumn (≤ 13 h light/day; 13L) (7). Regression of the gonads, a 10–30% decrease in body mass (8) and molt to a white winter pelage reflect changes in neuroendocrine activity and metabolism typical of adaptation to short days (SD). Prolonged exposure to SD is not compatible with indefinite maintenance of the winter reproductive and somatic phenotype. Rather, hamsters use an endogenous interval timing mechanism to initiate somatic and gonadal regeneration after approximately 20 weeks of exposure to SD (9). Referred to as ‘spontaneous recrudescence’, the recovery of long-day (LD)-like body mass and reproductive capacity thus occurs despite continued exposure to photoperiods that were previously inadequate to sustain such traits. After recrudescence has occurred, hamsters are considered photorefractory to SD and do not undergo another transition to the SD reproductive phenotype unless first exposed to ≥ 2 weeks of LD, which restore neuroendocrine responsiveness to SD (9, 10). Photorefractoriness is a defining feature of one class of seasonality mechanisms (so-called, Type I rhythms) in which one phase of the seasonal cycle (somatic and gonadal regression) relies on an environmental cue, whereas another phase (somatic and gonadal re-growth) is dependent on an endogenous interval timer. Unlike animals exhibiting Type II rhythms, hamsters with Type I rhythms do not exhibit repeated seasonal cycles independent of environmental input (11, 12).

Photorefractoriness arises from a loss of responsiveness to melatonin by neural tissues after 5–6 months of exposure to SD (13, 14). Although the neuroendocrine mechanisms by which hamsters lose responsiveness to inhibitory melatonin signals are not completely understood, altered responsiveness to gonadal steroid feedback concurrent with photorefractoriness has been described previously. In Syrian hamsters, the gonadotrophin response to castration is initially attenuated in SD, but restored coincident with refractoriness to SD (15). Syrian hamsters also exhibit gradual changes in gonadal steroid-dependent and -independent regulation of the hypothalamic-pituitary-gonadal (HPG) axis (16, 17). Transfer to SD suppresses circulating luteinising hormone (LH) concentrations in male Syrian hamsters but, after approximately 20 weeks of exposure to SD, castrated hamsters exhibit ‘spontaneous’ increases in LH; around the same time, physiological testosterone treatments that were previously sufficient to inhibit pituitary gland follicle-stimulating hormone (FSH) and LH secretion no longer do so (16). However, it is not known whether refractoriness-associated changes in tonic LH secretion and in steroid-dependent negative feedback regulation of LH reflect changes in hypothalamic gonadotrophin-releasing hormone (GnRH) stimulation of the pituitary gland, pituitary gland responsiveness to GnRH, or an interaction of the two. The present study aimed to test the hypothesis that the development of SD photorefractoriness is associated with changes in pituitary responsiveness to GnRH. Tonic LH concentrations and LH responses to GnRH were assessed in photoresponsive and photorefractory male Siberian hamsters. To control for fluctuations in testosterone-negative-feedback which likely accompany the photoperiodic gonadal cycle, experiments were also conducted in castrated hamsters and in castrated, steroid-clamped hamsters to determine whether refractoriness-induced changes in tonic LH secretion and in LH responsiveness to GnRH were a result of gonadal hormone-dependent or -independent mechanisms.

Materials and methods

Animals

Male Siberian hamsters (Phodopus sungorus) from a breeding colony maintained at The Ohio State University were used in all experiments. Breeding pairs were maintained under illumination conditions that provided a long-duration (16 : 8 h light/dark photoperiod, onset of darkness at 15:00 h EST) LD photoperiod. Pairs were inspected daily for the presence of pups, and the day of birth was designated as day 0. Hamster pups were weaned at 17 days of age (day 17), and male offspring were housed singly in polypropylene cages (27.8 x 7.5 x 13 cm) under conditions of constant temperature and humidity of 21 ± 4 °C and 50 ± 10%, respectively, with ad lib access to food (Harlan Teklad 8640 rodent diet, Indianapolis, IN, USA) and filtered tap water. All procedures in these experiments were conducted in accordance with the National Institutes of Health guidelines for the use of experimental animals, and the protocols were approved by the local Institutional Animal Care and Use Committee.

Castration and hormone replacement protocol

On day 17, male hamsters were either surgically castrated or subjected to a sham castration surgery (externalisation of each testicle without ligation or incision) under sodium pentobarbital anaesthesia. Castrated hamsters were implanted s.c. with either a 5-mm section of silastic tubing (inner diameter 1.47 mm, outer diameter 1.96 mm) containing testosterone (LD: n = 11; SD: n = 12) or a 5-mm section of empty silastic tubing (LD: n = 11; SD: n = 11). Silastic implants were prepared over the 3-day interval immediately preceding use; implants were allowed to soak in 0.9% saline at 37 °C for 24–36 h before implantation. The 5-mm silastic implants were removed on week 4.5 and replaced with matching (testosterone-filled or empty, as appropriate) implants that were 10 mm in length, to accommodate developmental increases in body size. Testosterone-filled silastic implants of this diameter and length result in circulating concentrations of testosterone that are comparable to those observed in gonad-intact LD housed male Siberian hamsters (18, and present data). Sham-castrated hamsters were implanted s.c. with an empty section of silastic tubing (LD: n = 12; SD: n = 11) of appropriate length. After surgery, hamsters either remained in LD or were transferred to a SD photoperiod (8 : 16 h light/dark photoperiod, onset of darkness at 15:00 hours EST).

Two separate cohorts of hamsters were subjected to identical surgical (sham-surgery/castration), implant (empty/testosterone-filled) and photoperiod (LD/SD) treatments, but were delayed by approximately 26 weeks to permit synchronous GnRH challenges in the laboratory. In one cohort of hamsters (‘week 40’, group abbreviations: ‘LD-40’ and ‘SD-40’), experimental treatments and measurements continued until all animals were 40 weeks of age; this interval of exposure to SD is sufficient to render all SD-housed hamsters refractory to the SD photoperiod. In these animals, testosterone-filled implants were removed and replaced under isoflurane anaesthesia on weeks 4.5, 7, 18 and 30. In a separate cohort of animals (‘week 14’, ‘LD-14’ and ‘SD-14’), treatments and photoperiod manipulations were continued until week 14. Photoresponsive hamsters housed in SD for 14 weeks have not initiated spontaneous recrudescence and are not considered refractory to SD. In these animals, implants were replaced on weeks 4.5 and 7. Under isoflurane anaesthesia (2% mixture with medical oxygen; flow rate = 2 (min), blood samples (270 μl) were obtained via the retroorbital sinus in all hamsters at the time of each capsule replacement, and again on the day of GnRH challenge (see below), to determine circulating testosterone concentrations. Blood was centrifuged at 2500 r.p.m. for 30 min and the supernatant was collected and frozen at −70 °C. Serum testosterone concentrations were determined in a single radioimmunoassay (RIA) (see below).

Somatic and reproductive measurements

Body mass was determined by weighing (± 0.1 g) hamsters on day 32 (week 4.5), week 7 and at weekly intervals thereafter. Reproductive condition in
Induction of anaesthesia (2% mixture with medical oxygen; flow rate
about 1.5 l/min) was performed in this manner to minimise any effects of handling-stress
on gonad-intact hamsters. Anaesthetised hamsters (24, T. Horton, unpublished data). Hamsters were anaesthetised within 2 min
of initiating delivery of the isoflurane/oxygen mixture. Anaesthetised hamsters were
removed from their cages, weighed (± 0.1 g), and their testes were
manually palpated to verify gonadal responsiveness to the SD photoperiod. A
challenge to assess gonadotrophin responsiveness, week 14 hamsters were injected s.c.
with GnRH on week 14–15 (hereafter ‘week 14’), and week 40 hamsters were
injected with GnRH on week 40–42 (hereafter ‘week 40’). On the day of GnRH
treatment, hamster cages were individually moved from the LD/SD colony
rooms into an acoustically isolated room for anaesthetisation, injections and
blood sampling. GnRH injections and blood sampling were performed during a
4-h interval surrounding the midpoint of each groups’ subjective day; this interval was
between 05.00 h and 09.00 h EST for LD hamsters and 09.00 h and 13.00 h EST for SD hamsters. Without handling the hamster, the entire
cage was placed into a large plastic chamber which was then sealed and
and 13.00 h EST for SD hamsters. Without handling the hamster, the entire
cage was placed into a large plastic chamber which was then sealed and
and connected via plastic tubing to an isoflurane/oxygen anaesthesia machine. Induction of anaesthesia (2% mixture with medical oxygen; flow rate = 2 l/
min) was performed in this manner to minimise any effects of handling-stress
on gonadotrophin and gonadal hormone secretion (20). Although some halogenated anaesthetics suppress LH surges in ungulates (21, 22), they are
generally preferable for determination of LH concentrations in rodents (23). In
Siberian hamsters, induction of anaesthesia with isoflurane does not alter
baseline concentrations of circulating LH, nor does it interfere with LH
responses to chemosensory and environmental stimuli in Siberian hamsters
(24, T. Horton, unpublished data). Hamsters were anaesthetised within 2 min
of initiating delivery of the isoflurane/oxygen mixture. Anaesthetised hamsters were
removed from their cages, weighed (± 0.1 g), and their testes were
manually palpated to verify gonadal responsiveness to the SD photoperiod. A
270-μl blood sample (baseline, t0) was then obtained via the right retro-orbital
sinus using heparinised Natleson collection tubes. Immediately following t0
t0 blood collection, hamsters were injected s.c. with GnRH (200 ng/kg, in sterile
0.9% saline; injection volumes in the range 114–314 μl, depending on body
mass) (25). During bleeding and injection, the anaesthesia machine was turned
off, and the chamber and home cage were purged of isoflurane using an electric
fan. Following GnRH injection, hamsters received a 500-μl s.c. injection of
sterile saline to replace fluids and were returned to their home cages inside the
anaesthesia chamber. Ten minutes after GnRH injection (t15), an additional
270-μl blood sample was obtained and fluid was replaced in the same manner
as described above. Blood samples were transferred from Natleson tubes into
microcentrifuge tubes and kept on ice for 1–3 h, centrifuged at 2500 r.p.m. for
30 min, and the supernatant was collected and frozen at −70 °C until assayed
for LH (see below).

Radioimmunoassays
Testosterone was measured using a double-antibody 125I RIA kit (DSL-4100;
Diagnostic Systems Laboratories, Webster, TX, USA). Cross-reaction of the
testosterone antibody to 5α-dihydrotestosterone for this kit is 6.6% as reported
by the manufacturer, and the upper and lower limits of detectability are 25 and
0.1 ng/ml, respectively. This kit has previously been validated for use in
Siberian hamsters (18). Serum samples were aliquoted in 25-μl amounts into
duplicate assay tubes and incubated with 125I-labelled testosterone tracer
and rabbit antitestosterone antibody for 1 h at 23 °C. Pellets were precipitated
with goat antirabbit gamma globulin serum and polyethylene glycol, and were
centrifuged at room temperature for 20 min at 15000 g. The supernatant was
then decanted and aspirated, and gamma emissions from each tube were
recorded for 1 min using an automated gamma counter (Packard Instrument
Company, Meriden, CT, USA). Testosterone values were determined in three
separate RIAs, each of which included aliquots from the same pool of hamster
serum. The upper and lower limits of detectability for the testosterone assay
were 25 and 0.1 ng/ml, respectively. Intra-assay coefficients of variation ranged
from 0.7% to 4.2%, and the interassay coefficient of variation was 8.6%.
The LH assay was performed at the Ligand Assay Core of Northwestern
University. This assay has been validated previously for use in this species
(26, 27). LH was measured in duplicate from 35-μl aliquots of serum. The LH
standard used was rat LH RP-3, and the antibody used was rat LH-S-11. The
average intra-assay coefficient of variation for LH was 3.5%, and the
interassay coefficient of variation was 3.1%. Serial dilutions of male Siberian
hamster sera were included with each LH assay, and the concentration curves
were parallel to the standard LH curves in the RIAs. A subset of the samples
(n = 130; composed primarily of samples from the blank-implanted and
testosterone-implanted castrated hamsters) exceeded the lower or upper limits
of detectability the initial LH assay (0.28 and 14.3 ng/ml, respectively). To
more accurately quantify LH concentrations in these samples, these samples were
assayed in a second LH RIA using larger (70 μl) or smaller (20 μl) serum aliquots,
as appropriate. The respective upper and lower limits of detectability in this assay were 106.1 and 0.15 ng/ml. This second LH RIA also included 30
samples that had generated LH values within the limits of detectability of the
first LH RIA. An interassay linear regression correlation of these samples in
the consecutive assays yielded a significant (P = 0.0001) R 2 value of 0.92 (data
not shown).

Statistical analysis
Body masses for week 40 hamsters were compared within surgical/implant
treatments using repeated-measures ANOVA to determine whether the pattern
of change in body mass differed in response to photoperiod treatments;
Fisher’s PLSD tests were used to determine the week on which somatic
growth and development was completed. SD hamsters were considered to have attained
a LD-like phenotype on the first week that their body masses were statistically
comparable to those of LD hamsters (P > 0.05) and remained so on three
consecutive weekly measurements. Week 14 and week 40 body masses were
compared using between-subjects ANOVA followed by Fisher’s PLSD tests for
pairwise comparisons between means.

Plasma testosterone and LH concentrations were compared separately at
each sampling interval using 2×2 [photoperiod (LD, SD) and time (week 14, week
40)] between-subjects ANOVA followed by Fisher’s PLSD tests for
pairwise comparisons between means.

Testosterone and LH samples were used to determine whether a change in LH relative to
baseline values occurred in response to GnRH treatments. Within each
photoperiod condition, repeated measures ANOVA were conducted using time
(week 14 versus week 40) as a between-groups factor to determine whether
SD-refractoriness or prolonged exposure to LD affected the pattern of change
in LH following GnRH treatment. Because groups differed significantly in
baseline LH concentrations, values for LH samples obtained following GnRH
treatment were also expressed as a percentage of the baseline LH value for
each animal to permit meaningful comparisons of the magnitude of LH
responses among groups. For each of the three surgical/implant conditions,
the percent change in LH value (from baseline) was compared across time
(week 14, week 40) and photoperiod (LD, SD) groups using between-subjects
ANOVA followed by Fisher’s PLSD tests for pairwise comparisons between means.

Statistical analyses were performed using Statview 5.0 statistical software
(SAS Institute, Cary, NC, USA). Sample sizes for each group are shown in
Fig. 1. Differences between means were considered statistically significant at
P ≤ 0.05.

Results
Refractoriness to SD in week 40 hamsters
Body mass and reproductive physiology
Among week 40 hamsters, within each surgical/implant
treatment group, exposure to SD inhibited the accrual of
body mass relative to LD treatments. Intact SD hamsters
weighed less than intact LD hamsters at every measurement
between week 4.5 and week 24 (P = 0.05, all comparisons),
but did not differ in mass from LD-intact hamsters thereafter
(P > 0.05, all comparisons) (Fig. 1A). LD-castrated hamsters did not differ on or after


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week 29 (P > 0.05, all comparisons) (Fig. 1B). Castrated hamsters implanted with testosterone-filled capsules and housed in SD weighed significantly less than their LD-housed counterparts on weeks 7–13, inclusive (P < 0.05, all comparisons) but, beginning on week 14, body masses were comparable between SD- and LD-exposed T-treated castrated hamsters (P > 0.05, all comparisons) (Fig. 1C). Testes of SD-intact hamsters were significantly smaller than those of LD-intact hamsters from weeks 4.5–32, after which they did not differ statistically (data not shown).

Testosterone concentrations
Among gonad-intact hamsters, serum testosterone concentrations were significantly lower in SD relative to LD hamsters on weeks 4.5, 7 and 18 (P ≤ 0.05, all comparisons) (Fig. 1A). Testosterone concentrations of SD-intact hamsters were comparable to those of LD-intact hamsters in blood samples obtained on weeks 30 and 40. In castrated hamsters implanted with empty capsules, testosterone concentrations remained low (< 0.5 ng/ml) on all weeks of the experiment, and photoperiod had no effect on testosterone concentrations of these hamsters (P > 0.30, all comparisons) (Fig. 1C). Five-millimeter implants filled with testosterone yielded circulating testosterone concentrations that exceeded values observed in LD gonad-intact hamsters on week 4.5 (P ≤ 0.01, both comparisons) (Fig. 1C); treatment with 10-mm testosterone-filled implants, initiated following week 4.5, yielded circulating testosterone concentrations that were comparable to...
values observed in LD gonad-intact hamsters (P > 0.05, all comparisons). Photoperiod did not affect testosterone concentrations of castrated hamsters bearing testosterone-filled implants on any week (P > 0.05, all comparisons).

**Week 14 relative to week 40 hamsters**

**Somatic responses in week 14 hamsters**
Somatic responses of week 14 hamsters to photoperiod, surgical, and implant manipulations were comparable to those observed in the initial responses of week 40 hamsters. Briefly, at the time of GnRH treatments, both intact and castrated hamsters implanted with empty silastic tubing weighed significantly less in SD relative to LD counterparts (P ≤ 0.05) (Fig. 2a). Castrated hamsters bearing testosterone-filled implants did not differ in body mass as a function of photoperiod (P > 0.05) (Fig. 2a).

**Somatic responses in week 40 hamsters**
Immediately prior to week 40 GnRH treatments, body mass did not differ as a function of photoperiod within any of the three surgery/implant treatment groups among week 40 hamsters (P > 0.05, all comparisons) (Fig. 2a).

**Testosterone concentrations**
Among gonad-intact hamsters, testosterone concentrations were significantly higher in LD relative to SD on week 14 (P ≤ 0.05) but not on week 40 (P > 0.05) (Fig. 2a). Castrated hamsters bearing blank implants exhibited low (< 0.5 ng/ml) circulating concentrations of testosterone in blood samples obtained immediately prior to GnRH injections. Neither photoperiod (LD versus SD; P > 0.05) nor time (week 14 versus week 40; P > 0.05) had a significant effect on testosterone concentrations among castrated hamsters implanted with empty sections of silastic tubing (Fig. 2a). Photoperiod (P > 0.05) and time (P > 0.05) were also without effect on testosterone concentrations in castrated hamsters bearing testosterone implants (Fig. 2a). Across all castrated groups, testosterone-implanted hamsters had significantly higher concentrations of testosterone in the circulation relative to hamsters implanted with empty capsules (P ≤ 0.001). Five-millimeter testosterone implants (day 17 to week 4.5) yielded circulating testosterone concentrations that were significantly higher than those of intact hamsters on week 4.5 (P ≤ 0.005, all comparisons); 10-mm testosterone implants (weeks 4.5–40) yielded testosterone concentrations that were comparable to those of gonad-intact hamsters housed in LD (P > 0.05, all comparisons). At the time of GnRH treatment, testosterone concentrations of testosterone-implanted week 40 hamsters in LD and SD were 73% and 85% of values of age-matched gonad-intact LD and SD animals, respectively; in testosterone-implanted hamsters injected with GnRH on week 14, testosterone

![Graph](image-url)

**Fig. 2.** (A) Mean ± SEM body masses and (B) serum testosterone concentrations determined 14 or 40 weeks following surgical, implant, and photoperiod manipulations described in Fig. 1 and the materials and methods section. ‘Week 14’ and ‘Week 40’ designations on the abscissa identify data collected from separate cohorts of male Siberian hamsters that were subjected to comparable experimental manipulations. *P ≤ 0.05, **P ≤ 0.005. LD, long day; SD, short day.
concentrations of LD hamsters were 113% of those of age-matched LD hamsters.

**Baseline LH concentrations**

Overall, baseline concentrations of LH were higher in castrated-blank-implanted hamsters (P ≤ 0.0001) and lower in castrated-testosterone-implanted hamsters (P ≤ 0.05), relative to gonad-intact controls (Fig. 3). Among gonad-intact hamsters, LH concentrations prior to GnRH treatment were statistically comparable among all four treatment groups. Neither photoperiod (F1,33 = 0.52, P > 0.05) nor time (F1,33 = 0.03, P > 0.05) affected baseline LH (Fig. 3).

Among castrated hamsters implanted with empty silastic tubing, photoperiod treatments significantly affected baseline LH values (F1,35 = 4.87, P ≤ 0.05). No main effect of time was evident (F1,35 = 0.01, P > 0.05), but photoperiod and time interacted to affect LH (F1,35 = 8.85, P ≤ 0.01) (Fig. 3). LD-housed hamsters had significantly lower LH concentrations on week 40 relative to week 14 (P ≤ 0.05) whereas, in SD-housed hamsters, LH values were higher on week 40 relative to week 14 (P ≤ 0.05). In addition, among week 40 hamsters, tonic LH concentrations were significantly higher in SD relative to LD animals (P ≤ 0.0005), but this relation was not observed among week 14 hamsters (P > 0.05) (Fig. 3). In castrated hamsters bearing testosterone-filled implants, LH values were relatively low, and neither photoperiod (F1,34 = 0.15, P > 0.05) nor time (F1,34 = 2.19, P > 0.05) significantly affected baseline LH concentrations (Fig. 3).

**GnRH-stimulated LH secretion**

**Gonad-intact, blank implants**

In all four gonad-intact groups, significant increases in LH were evident 10 min after GnRH injection (paired t-tests, P ≤ 0.05, all comparisons) (Fig. 4A). Repeated measures ANOVA indicated that the pattern of change in LH following GnRH treatment differed significantly between SD-14 and SD-40 hamsters (F1,17 = 7.6; P ≤ 0.05) (Fig. 4A); SD-14 hamsters exhibited significantly greater increases in LH relative to SD-40 hamsters (F1,17 = 8.9; P ≤ 0.01). By contrast, increases in LH were comparable between LD-14 and LD-40 hamsters (F1,16 = 2.9; P > 0.05) (Fig. 4A).

Expressed as a percentage of baseline LH values, increases in LH were greatest in SD-14 hamsters; these hamsters exhibited LH responses that exceeded those of all other gonad-intact groups (P ≤ 0.01, all comparisons) (Fig. 4B).

**Castrated, blank implants**

Among castrated, blank-implanted hamsters, only LD-40 hamsters exhibited significant increases in LH following GnRH treatment (P ≤ 0.01) (Fig. 4A). The pattern of change in LH following GnRH treatment differed significantly between LD-14 and LD-40 hamsters (F1,18 = 5.0; P ≤ 0.05) (Fig. 4B), but not between SD-14 and SD-40 hamsters (F1,17 = 0.17; P > 0.05) (Fig. 4B). The percentage increase in LH elicited by GnRH treatment was significantly greater in LD-40 relative to LD-14 hamsters (P ≤ 0.05) (Fig. 4E).

**Castrated, testosterone implants**

Among castrated testosterone-treated hamsters, within-groups analyses did not reveal significant increases in LH after GnRH injections (P > 0.05, all comparisons) (Fig. 4C). However, in SD hamsters, the change in LH following GnRH treatment differed between week 14 and week 40 cohorts: SD-14 hamsters exhibited modest decreases in LH, whereas SD-40 hamsters manifested modest increases in LH, resulting in LH concentrations that differed significantly at t10 and yielding a significant interaction between time and pattern of change in LH (F1,18 = 4.4, P ≤ 0.05) (Fig. 4C). By contrast, LD-housed testosterone-implanted hamsters exhibited comparable patterns of change in LH on weeks 14 and 40 (F1,16 = 0.19; P > 0.05) (Fig. 4C). Ten minutes after GnRH treatment, LH concentrations of SD-40 hamsters were significantly higher than those of LD-40 hamsters (P ≤ 0.05). Expressed as a percentage of baseline LH values, GnRH elicited a greater LH response in SD hamsters on week 40 relative to week 14 (P ≤ 0.05) (Fig. 4F).
Across the three surgical/implant conditions, hamster body masses spanned a substantial range (23–52 g) at the time of GnRH treatment; however, in no treatment group did body mass significantly predict LH responses to GnRH treatment ($R^2 = 0.029$, $P < 0.05$; data not shown).

**Discussion**

Gonad-intact and castrated Siberian hamsters exhibited somatic and reproductive inhibition when weaned into inhibitory SD, but prolonged (approximately 40 weeks) exposure to SD resulted in photorefractoriness and spontaneous somatic development. By contrast, maintenance in LD accelerated somatic and reproductive development (6). The induction of photorefractoriness was associated with marked changes in regulation of LH secretion. Between weeks 14 and 40, castrated hamsters housed in LD exhibited an age-related decline in tonic LH secretion; over the same interval, castrated hamsters housed in SD increased resting LH concentrations, suggesting that SD exposure sufficient to induce photorefractoriness enhances production of LH in the absence of gonadal hormone negative-feedback. Testosterone implants suppressed tonic LH production comparably in photoresponsive and photorefractory hamsters. However, when challenged with GnRH, greater LH responses were observed in SD-photorefractory hamsters, suggesting that photorefractoriness is associated with a reduced ability of testosterone to inhibit gonadotrophin secretion. In common with mechanisms operant in Syrian hamsters, SD photorefractoriness in Siberian hamsters is associated with changes in gonadal steroid-independent and gonadal steroid-dependent regulation of LH secretion.

LH concentrations were measured at single time points, either during a specific phase of the circadian cycle (for tonic measurements) or 10 min following (t10), GnRH treatments. ($A$–$C$) *$P \leq 0.05$, **$P \leq 0.005$ versus SD14 t10 value; †$P \leq 0.05$ versus LD-40 value. ($A$–$C$) *$P \leq 0.05$, **$P \leq 0.005$. Note the differences in the scales (absolute and relative) of the ordinate axes. LD, long day; SD, short day.

**Fig. 4.** ($A$–$C$) Mean ± SEM tonic and gonadotrophin-releasing hormone (GnRH)-stimulated lutenising hormone (LH) values and ($D$–$F$) mean ± SEM percent increases in LH (relative to baseline, t0), of male Siberian hamsters subjected to surgical, implant and photoperiod manipulations as described in Fig. 1 and the materials and methods section, and injected on either week 14 or week 40 with GnRH (200 ng/kg, s.c) at the mid-point of the light cycle. Blood samples were obtained immediately before (baseline, t0), and 10 min following (t10), GnRH treatments. ($A$–$C$) *$P \leq 0.05$, **$P \leq 0.005$ versus SD14 t10 value; †$P \leq 0.05$ versus LD-40 value. ($D$–$F$) *$P \leq 0.05$, **$P \leq 0.005$. Note the differences in the scales (absolute and relative) of the ordinate axes. LD, long day; SD, short day.
photoperiodic regulation of LH in small rodents, which have likewise used a single-sample approach.

**Gonad-intact, blank-implanted hamsters**

Consistent with previous reports (25, 28), exposure to SD for 14 weeks did not affect tonic LH concentrations in gonad-intact hamsters. In Siberian hamsters, transfer from SD to LD induces increases in FSH within a few days, whereas increases in LH typically require ≥3 weeks (25, 29), and additional sociosexual stimuli (25), to manifest. After reproductive development in LD is completed, LH declines, despite continued exposure to LD, to values indistinguishable from those of SD-housed hamsters, whereas FSH concentrations remain elevated in LD relative to SD (25). In the present study, the absence of differences across gonad-intact groups in baseline LH values on week 40 may reflect analogous dynamic changes in LH during and after spontaneous recrudescence in SD (17). However, despite comparable baseline concentrations of LH on week 14, pituitary gland responses to GnRH stimulation differed markedly in SD relative to LD hamsters. GnRH treatments elicited robust pituitary gland LH responses within 10 min, and these responses were of greater magnitude in SD relative to LD hamsters on week 14, which is an anticipated outcome of the withdrawal of inhibitory gonadal hormone feedback in hamsters exhibiting SD-induced gonadal regression (30). Diminished LH responses to GnRH in LD-40 relative to SD-40 hamsters presumably reflected the influence of increased steroid negative feedback upon restoration of elevated serum testosterone following gonadal recrudescence. LD-40 and SD-40 hamsters exhibited comparable LH responses to GnRH treatment. Because these groups were similar in age and circulating testosterone concentrations, and differed primarily in the respect that SD-40 hamsters were refractory to SD, these data support the hypothesis that, in gonad-intact hamsters, responsiveness to GnRH is unaltered following the induction of photorefractoriness. These data are similar to those described previously in photoperiodic birds (white-crowned sparrows), in which LH responses to LHRH treatments were comparable among reproductively photosensitive and photorefractory individuals (31), an observation which supported the conclusion that 'the physiological basis underlying photorefractoriness resides in the hypothalamus or other levels of the central nervous system' (31).

**Castrated, testosterone-implanted hamsters**

In castrated hamsters bearing testosterone implants, no differences in baseline LH secretion were observed associated with either photoperiod or photorefractoriness. At week 14, 10-mm testosterone implants were equally effective in both LD and SD hamsters in suppressing castration-induced increases in LH secretion. In Syrian hamsters, another LD breeding photoperiodic rodent, substantially lower concentrations of testosterone were sufficient to inhibit tonic LH secretion in SD relative to LD animals, indicating Syrian hamsters are more responsive to steroid negative feedback in SD (16). The present experiment did not titrate the minimally sufficient amount of testosterone adequate to suppress tonic LH secretion in week 14 hamsters; therefore, quantitative specification of photoperiodic changes in thresholds for steroid negative feedback efficacy are not possible (16). These data permit only the conclusion that implants which mimic LD-like physiological concentrations of testosterone are equally effective in inhibiting tonic LH secretion under both LD and SD photoperiods. On week 40, tonic LH values in LD and SD hamsters were likewise relatively low, and were statistically indistinguishable, suggesting that chronic exogenous testosterone is sufficient to inhibit baseline LH secretion despite the development of photorefractoriness. However, photorefractoriness-induced changes in steroid negative feedback sensitivity were evident when SD hamsters were challenged with GnRH. Ten minutes after GnRH treatment, LH concentrations of SD-40 hamsters were significantly higher than those of LD-40 and SD-14 hamsters and, when expressed as a percentage of baseline LH values, LH responses of SD-40 hamsters significantly exceeded those of SD-14 hamsters. Together, these data are consistent with the conclusion that negative feedback sensitivity is significantly diminished following an interval of exposure to SD sufficient to induce photorefractoriness.
Conclusions
The present data provide novel basic insights into changes in gonadal hormone-dependent and -independent hypothalamic-pituitary gland interactions associated with the development of photorefractoriness in Siberian hamsters. Artificially elevated testosterone concentrations were sufficient to inhibit tonic LH secretion, despite the development of photorefractoriness. However, when additional gonadotrophic drive was provided (either by direct challenge with GnRH injections in testosterone-implanted hamsters, or by the elimination of inhibitory gonadal hormone feedback in castrated hamsters) enhanced pituitary gland gonadotropin secretion was evident in SD photorefractory hamsters relative to SD-14 and LD-40 hamsters. Greater LH production in SD-40 hamsters relative to SD-14 hamsters suggests that intervals of exposure to SD that are inadequate to induce the photorefractory state are likewise not sufficient to yield enhanced LH responsiveness to GnRH; greater LH secretion in SD-40 hamsters relative to LD-40 hamsters indicates that neither advanced age nor the extended duration of exposure to gonadal hormone manipulations are responsible for the observed increases in gonadotrophin secretion. Thus, the photorefractory state in Siberian hamsters is characterised by increases in gonadal hormone-independent LH production, and decreases in inhibitory feedback sensitivity to gonadal hormones.

Previous work in Syrian hamsters established similar changes in gonadal hormone-dependent and -independent regulation of the HPG axis (15–17). In Syrian hamsters, testosterone implants that yielded circulating testosterone concentrations equal to 80% of normal LD values were effective in inhibiting LH secretion during the early weeks of exposure to SD, but the efficacy of such implants waned with the development of refractoriness, indicating that a decrease in sensitivity to steroid negative feedback on tonic LH secretion occurs as refractoriness develops (16). In the present study, 10-mm implants yielded circulating testosterone concentrations equal to approximately 90% of values observed in age-matched gonad-intact LD hamsters (Fig. 2). Following the induction of photorefractoriness, these implants remained effective in suppressing tonic LH secretion, but were significantly less effective in inhibiting LH secretion in the face of substantial gonadotrophic drive (i.e. GnRH injection). Taken together, these results are consistent with the hypothesis that, in common with photorefractory Syrian hamsters, LH responsiveness to steroid negative feedback is diminished in photorefractory Siberian hamsters.

The neural and endocrine mechanisms that render LD breeders photorefractory to SD are not completely known. Reproductive refractoriness to SD is mediated by a loss of responsiveness to long-duration, inhibitory patterns of melatonin secretion, acquired after many weeks of exposure to SD (13, 14). Because the melatonin receptors necessary and sufficient to mediate the effects of SD melatonin signals on gonadotrophin secretion are located in the hypothalamus and thalamus (33), it is very likely that the neuroendocrine events which initiate reactivation of the quiescent HPG axis occur in the brain. The present study suggests that, following the induction of the refractory state, constitutive gonadotrophin production is increased, and the ability of gonadal hormone secretion to inhibit gonadotrophin secretion is diminished. In terms of functional significance, the extent to which the observed gonadal steroid-independent increase in tonic LH production participates in the process of vernal gonadal recrudescence is difficult to infer, in light of the contrived circumstances (castration) required to reveal it. In contrast, provided that sufficient central gonadotrophic drive is present, the attenuated responsiveness of gonadotrophin secretion to the negative feedback effects of testosterone may be of functional importance to overcoming the otherwise inhibitory influence of testicular hormone secretion and maintaining gonadal growth in the spring.

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