Testosterone influences hibernation in golden-mantled ground squirrels

THERESA M. LEE, KIMBERLY PELZ, PAUL LICHT, AND IRVING ZUCKER
Departments of Psychology and Integrative Biology, University of California, Berkeley, California 94720

At different phases of the hibernation season, castrated male golden-mantled ground squirrels were implanted with capsules that either were filled with testosterone (T) or left empty (blank). Blank-treated animals hibernated normally when housed at 5°C. Entry into hibernation was prevented in the majority of squirrels treated with T several days before the initial cold challenge. T concentrations that inhibited torpor (>1.2 ng/ml) were comparable with those of intact males at the end of the hibernation season. In some squirrels, moderate T concentrations were compatible with hibernation, but torpor bout duration was shorter than normal. The inhibitory effect of T on hibernation did not appear to require aromatization of T to estradiol. We suggest that a steroid-independent mechanism triggers arousal from hibernation and that T-dependent processes determine whether hibernation is resumed at the end of an arousal period.

PRECEDING THE ERA of modern endocrine investigation, hibernation and reproduction were conceived as mutually antagonistic processes, temporally dissociated in the course of evolution (16). This conjecture was reinforced by subsequent demonstrations that gonadal hormone secretion (e.g., Refs. 2, 4) and target tissue responsiveness to gonadotropins (3) were inhibited at low body temperatures. Furthermore, involution of the reproductive apparatus, and a decline in steroid hormone production, appear to be preconditions for entry into hibernation by male mammals (reviewed in Refs. 6, 14), and treatment of castrated males with testosterone before the onset of hibernation inhibited torpor in several hamster species (7, 9), hedgehogs (14), and Belding's ground squirrels (5).

The relation between recrudescence of the reproductive system and termination of dormancy in the spring remains unclear. Wimsatt (16) and Goldman et al. (6) suggested that increased testosterone secretion at the end of the hibernation season may cause termination of hibernation in males; however, Darrow et al. (4) failed to detect significant increases in endogenous blood testosterone concentrations of Turkish hamsters before the terminal arousal from hibernation in the spring. Also, in the edible dormouse (Glis glis), plasma testosterone levels remained low during hibernation and did not increase before the terminal arousal in March (8). Similar data have been reported for chipmunks (15) and bats (13). In contrast, testosterone titers increase in the weeks preceding the end of the hibernation season in hedgehogs (14) and reach their maximum at the time of the terminal arousal. In the golden-mantled ground squirrel, Spermophilus lateralis, substantial reproductive development does not occur during hibernation but begins shortly after the terminal arousal from hibernation (1). Plasma testosterone concentrations at the time of the terminal arousal are not elevated above levels measured during the preceding month (2). These observations do not support the hypothesis that increased androgen secretion is instrumental in terminating hibernation. It remains possible, however, that target tissue responsiveness to testosterone increases in the later stages of the hibernation season and that low androgen levels compatible with torpor early in winter act to terminate hibernation in the spring, either by inducing arousal or preventing reentry to torpor after an arousal.

To address the role of testosterone in termination of torpor, we assessed the frequency and duration of torpor bouts in castrated male squirrels treated with testosterone at different phases of the hibernation season. Telemetric monitoring of body temperature permitted definitive tests of the compatibility of high androgen levels and torpor in this species.

MATERIALS AND METHODS
Animals and General Care

Male golden-mantled ground squirrels (Spermophilus lateralis) used in experiment 1 were trapped near Lake Almanor and Susanville, CA (n = 14) or were born in the laboratory (n = 6) in May, 1987, to females trapped at these sites. Squirrels for experiment 2 were trapped near the Sagehen Creek Field Station, Nevada County, CA (n = 13), or were born in our laboratory in May, 1988 to females trapped at the same site (n = 17). Animals were housed individually in plastic cages (45 x 24 x 20 cm) with wood shavings for bedding material. All animals were bilaterally castrated under deep anesthesia induced by intraperitoneal injection of pentobarbital sodium (12.5 mg/initial 100 g body wt + 0.5 mg/each additional 10 g body wt). Food (Simonsen Rat Pellets, Maintenance Diet) and tap water were provided ad libitum, except as indicated below. Before the hibernation phase of the study, and several times during intervals between cold challenges, squirrels were maintained at an ambient temperature (T_a) of 23°C. At all other times, T_a was 5°C. Animal rooms were illuminated with fluorescent lights...
from 0700 to 2100 h daily (Pacific Standard time) throughout the experiments as a matter of convenience.

**Hibernation**

Small pieces of paper toweling were placed on the dorsal surface of torpid animals to assess hibernation. Towel fragments are displaced during an arousal from hibernation; presence of towel on the animal’s dorsum each day indicated it was continuously torpid during the preceding 24 h (cf. Ref. 1, 11). All males in experiment 1 also were fitted with abdominal radiotransmitters (model VM-FH, Minimitter, Sunriver, OR) that broadcast frequency-encoded body temperature (Tb) to an automated data-collection apparatus that continuously sampled Tb at 10-min intervals.

**Hormone Determinations**

Plasma testosterone was measured in ether-extracted samples by radioimmunoassay (RIA) using an antiserum cospecific for testosterone and dihydrotestosterone supplied by Dr. Gordon Niswender. Dihydrotestosterone concentrations are <20% of testosterone levels in S. lateralis (10). The intra-assay coefficient of variation (CV) was 6%.

**TABLE 1. Experimental sequence for experiment 1**

<table>
<thead>
<tr>
<th>Date</th>
<th>Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nov. 17, 1987</td>
<td>Squirrels gonadectomized</td>
</tr>
<tr>
<td>Dec. 3</td>
<td>Transmitters implanted</td>
</tr>
<tr>
<td>Dec. 12</td>
<td>Capsules implanted</td>
</tr>
<tr>
<td>Dec. 21-Feb. 4</td>
<td><em>Hibernation test I:</em> Tb lowered 2–3°C/day till 5°C reached on Dec 28; food removed Jan. 11</td>
</tr>
<tr>
<td>Feb. 4</td>
<td>Animals rewarmed for 7 h at 23°C, blood withdrawn, capsules removed, and squirrels returned to 5°C</td>
</tr>
<tr>
<td>Feb. 18–25</td>
<td><em>Hibernation test II:</em> torpid squirrels implanted with capsules</td>
</tr>
<tr>
<td>March 17</td>
<td>Blood withdrawn; capsules removed</td>
</tr>
<tr>
<td>March 20</td>
<td>Food removed from all animals</td>
</tr>
<tr>
<td>March 24</td>
<td>Capsules implanted. <em>Hibernation test III</em></td>
</tr>
<tr>
<td>April 5</td>
<td>Blood withdrawn; capsules removed</td>
</tr>
</tbody>
</table>

Tb, ambient temperature.

**TABLE 2. Sequence of experimental manipulations in experiment 2**

<table>
<thead>
<tr>
<th>Date</th>
<th>Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nov. 4–11, 1988</td>
<td>Gonadectomy</td>
</tr>
<tr>
<td>Nov. 29-Dec. 15</td>
<td>Screening test for hibernation</td>
</tr>
<tr>
<td>Dec. 20</td>
<td>Capsules implanted</td>
</tr>
<tr>
<td>Dec. 28</td>
<td>Blood withdrawn</td>
</tr>
<tr>
<td>Dec. 28 Jan. 13</td>
<td><em>Hibernation test I:</em> Tb = 5°C</td>
</tr>
<tr>
<td>Jan. 13</td>
<td>Blood withdrawn; capsules removed</td>
</tr>
<tr>
<td>Jan. 18–March 7</td>
<td>Tb = 5°C</td>
</tr>
<tr>
<td>March 8</td>
<td>Capsules implanted</td>
</tr>
<tr>
<td>March 20</td>
<td>Blood withdrawn</td>
</tr>
<tr>
<td>March 20–April 5</td>
<td><em>Hibernation test II</em></td>
</tr>
<tr>
<td>April 5</td>
<td>Blood withdrawn; capsules removed</td>
</tr>
<tr>
<td>May 3</td>
<td>Capsules implanted</td>
</tr>
<tr>
<td>May 3–15</td>
<td><em>Hibernation test III</em></td>
</tr>
<tr>
<td>May 15</td>
<td>Blood withdrawn</td>
</tr>
</tbody>
</table>

Tb = 23°C except during screening test, hibernation tests I–III, and from Jan. 18 to March 7, when Tb = 5°C. See text for feeding schedules.

Estrogen was measured in plasma with an antiserum that is cospecific for 17β-estradiol and estrone. Plasma was extracted with ether (5:0.1 ml plasma), and dried extracts were diluted in 50 mM phosphate-buffered saline (0.1 M NaCl). Tracer was [2,4,6,7-3H(N)]estradiol from New England Nuclear (NET-317; 95.4 Ci/mmol); bound and free hormone were separated by use of dextran-coated charcoal before supernatants were counted by liquid scintillation. The minimal level of detectability is 7.5 pg. Intra- and interassay CV values were 8 and 11%, respectively.

**Capsule Preparation and Implantation**

Silastic capsules (Dow Corning; 1.56 mm ID, 3.15 mm OD) were filled to an effective length of 10 mm with crystalline testosterone (Sigma) or 5 mm with crystalline 17β-estradiol (Sigma). Blank (empty) capsules were either 10 or 5 mm long. Capsules were sealed with epoxy cement and soaked for 24 h in isotonic saline and implanted under anesthesia induced by methoxyflurane (Metofane, Pitman-Moore) vapors.

**Experimental Treatments and Sequence**

Treatment sequences are summarized in Tables 1 and 2. In experiment 1, squirrels were initially assigned at random for implantation with blank or testosterone-filled capsules. After capsules were removed, groups were reconstituted with one-half the animals previously implanted with testosterone-filled or blank capsules randomly selected and implanted with blank capsules, and the remainder with testosterone-filled capsules.

**Feeding Schedules**

*Experiment 1.* Food was withheld beginning Jan. 11, 1988, for the duration of hibernation test I. Squirrel no. 2739 was fed ad libitum after Jan. 29, because its body weight fell below 110 g.

At the end of the first hibernation test (Feb. 4), food was restored to all animals. Squirrels that subsequently hibernated (n = 9) or remained euthermic (n = 8) were...
deprived of food on Feb. 18; on Feb. 22, three animals were fed ad libitum after they had failed to hibernate, and their body weights declined below 130 g (Table 1).

**Experiment 2.** To promote hibernation, food was withheld during the first 3 days of the screening test for hibernation; thereafter, one food pellet (mean weight = 7.8 g) was provided and consumed each day that a squirrel was not hibernating. In a preliminary study euthermic squirrels not provided with food lost weight rapidly and had to be removed from the experiment. During hibernation tests I and II, the same feeding schedule was in force (Table 2). Throughout hibernation test III, one food pellet was provided during each day squirrels were not torpid.

From Jan. 18 to 24, each nontorpid squirrel was provided with 1 food pellet/day. Thereafter, until March 20, animals were fed ad libitum.

**Bleeding Schedules**

**Experiment 1.** Blood (0.05 ml) was withdrawn via retroorbital puncture. On Feb. 2, 1988, while squirrels were maintained at 5°C, three torpid animals were bled by cardiac puncture, and seven animals that were not hibernating were bled from the ocular orbit (Table 1). Two days later, animals were transferred from 5 to 23°C at 0700 h, and blood was collected between 1330 and 1500 h. All animals regained the euthermic state by 0830 h. On March 17 and April 5, blood was withdrawn from all remaining animals (T<sub>e</sub> = 5°C). Samples were stored frozen until assayed for testosterone.

**Experiment 2.** Blood was withdrawn via retroorbital puncture on Dec. 28, Jan. 13, March 20, April 5, and May 15 (Table 2). Samples were stored frozen until assayed for testosterone or estradiol. Animals either had been euthermic (T<sub>e</sub> = 37°C) for several days before blood withdrawal or, if sampled at the end of a hibernation test, rewarmed for 3–6 h before bleeding.

**RESULTS**

**Experiment 1**

Effects of testosterone on entry into hibernation. Entry into hibernation was prevented in squirrels implanted with testosterone (T) capsules several weeks before the initial cold challenge. With one exception, animals with T titers >1.24 ng/ml never displayed torpor, and all squirrels with T levels <1.0 ng/ml hibernated (Fig. 1). The mean T levels of nonhibernators and hibernators were 2.1 ± 0.3 and 0.7 ± 0.2 ng/ml (P < 0.002, t test), respectively.

Overall, only three of nine T-treated, as compared with seven of seven blank-implanted, squirrels hibernated (x<sup>2</sup> = 7.5, P < 0.01); two of the three hormone-
Testosterone and hibernation

Fig. 3. T<sub>2</sub> records of 2 squirrels implanted with testosterone-filled capsules while hibernating. Animal no. 2709 was typical of animals that exhibited terminal arousal after testosterone implantation. Animal no. 2893 (testosterone = 2.27 ng/ml) was the only hormone-treated squirrel that repeatedly displayed normal torpor bouts. First arrow depicts time of capsule implantation, and 2nd arrow denotes capsule removal and blood withdrawal.

Hormone treatment initiated Feb. 18. Seven of eight torpid animals implanted with empty capsules hibernated normally during the succeeding 4 wk. A one-day arousal apparently was induced by the implantation procedure in three squirrels. As a group, hibernating squirrels implanted with blank capsules were torpid on 77 ± 2% of test days (torpor bout length = 4.4 ± 0.4 days).

By contrast, only one of seven torpid animals treated with T continued to hibernate normally (χ² = 8.03; P < 0.005 for blank vs. T-treated group) and was torpid on 23 of 28 days (no. 2893, Fig. 3). The T<sub>2</sub> record confirms that from Feb. 16 through March 19 this squirrel had several extended hibernation bouts (bout length = 7 ± 0.5 days); its T level was 2.27 ng/ml on March 17. The remaining animals either ceased hibernating concurrently with T^-capsule implantation (n = 3) or hibernated for 1, 4, and 4 days, then aroused and never resumed hibernation (Fig. 3, no. 2769). The six squirrels whose torpor was suppressed after implantation of T capsules resumed hibernation within 2.1 ± 0.6 days of capsule removal. T levels were 1.8 ± 0.3 and 0.1 ng/ml for animals implanted with T-filled and blank capsules, respectively (P < 0.001).

Hormone treatment initiated March 24. Usable data were obtained from 11 torpid squirrels implanted with T capsules. Representative T<sub>2</sub> data extend and confirm patterns obtained with the "towel" method. Four animals ceased hibernating coincident with capsule implantation (no. 2810, Fig. 4); two animals hibernated for 5 and 6 days, respectively, after capsule implantation, aroused, and never again displayed torpor (no. 2869). The remaining five animals hibernated for 2, 3, 6, 6, and 9 days, respectively, in each case reinitiating hibernation after achieving complete arousals and euthermia (no. 2729). The mean T concentration for the group of 11 squirrels was 2.8 ± 0.6 ng/ml. The five animals that reinitiated hibernation had T concentrations that did not differ from those of squirrels that immediately ceased hibernating (4.3 ± 0.9 vs. 3.9 ± 0.4 ng/ml, P > 0.05).

Some animals (nos. 2726, 2754, 2893, and 2769) were torpid at the time of blood withdrawal and the remainder euthermic. Surviving animals (n = 9) resumed hibernation in 5.8 ± 0.8 days after capsules were removed.

Thus T administered at different stages of the hibernation season terminated hibernation in most squirrels,
with a substantial minority continuing or resuming hibernation despite the T treatment.

**Experiment 2**

*Initial phase of hibernation season (Dec./Jan.)*. Nineteen of the 30 squirrels examined during the screening test were selected for further study based on consistent hibernation (3.7 ± 0.2 days of torpor) during the cold challenge. The experimental and control groups contained approximately equal numbers of field-caught and laboratory-born individuals.

Hibernation was completely suppressed in 9 of 13 squirrels implanted with T capsules. The remaining four animals hibernated for 1, 2, 2, and 3 days, respectively, during the 14 day test (Fig. 5). By contrast, all six animals bearing blank implants hibernated consistently (Fig. 5; torpor on 9.8 ± 0.8 days). T-treated squirrels were less likely to hibernate than control animals ($\chi^2 = 7.9, P < 0.005$), and when they did hibernate, they were torpid on fewer test days ($t = 10.1, P < 0.001$).

T concentrations, measured before the onset of hibernation test I were 3.5 ± 0.3 and 0.6 ± 0.1 ng/ml for squirrels implanted with T capsules and blank capsules, respectively ($P < 0.001$). Similar values were recorded in the first few hours after the end of test I (3.7 ± 0.5 vs. 0.5 ± 0.1 ng/ml). T values of the four hormone-treated squirrels that hibernated (3.7 ± 0.7 ng/ml) did not differ significantly from those of the nine animals that never displayed torpor (3.4 ± 0.3 ng/ml, $t = 0.5, P > 0.05$).

In the 48-day interval after T capsules were removed and animals returned to the cold, 8 of 12 surviving animals hibernated consistently, as defined by torpor on at least 33% of days (torpid on 32.4 ± 3.3 days; bout length of 4.3 ± 0.2 days). Latency for resumption of torpor was 2.3 ± 0.3 days for 7 squirrels and 18 days for one animal (group value of 4.3 ± 1.9 days). The remaining four animals first displayed torpor 2, 4, 17, and 24 days after capsule removal, but hibernated on fewer than one third of test days.

**During the last third of hibernation season (March/April).** Twelve animals that consistently hibernated were treated with T-filled or blank capsules. Results were similar to those obtained in hibernation test I. All four squirrels implanted with empty capsules hibernated consistently (11.0 ± 0.4 days of torpidity during the 15-day cold challenge), and no animal was torpid for fewer than 10 days. Four of eight T-treated squirrels never hibernated, and each of the remaining animals was torpid on only 1 of the 15 test days. T-treated and control groups again differed significantly with respect to percent
TESTOSTERONE CAPSULES

BLANK CAPSULES

FIG. 5. Hibernation scores during early phase of hibernation season for squirrels implanted with testosterone-filled capsules (shown for the only 4 of 13 animals that displayed torpor) or blank capsules. Hibernation scores were based on towel method; 0 = euthermic, active animal, 1 = continuously torpid for <24 h, 2 = continuously torpid for entire preceding 24 h.

TESTOSTERONE AND HIBERNATION

FIG. 6. Hibernation scores obtained late in hibernation season from squirrels implanted with estradiol-filled or blank capsules.

DISCUSSION

Most castrated male golden mantled ground squirrels treated with testosterone were unable either to initiate or maintain hibernation. Experimentally elevated testosterone concentrations of 2–4 ng/ml that inhibited torpor were comparable with those measured in intact males at the end of the hibernation season (2). A minority of testosterone-treated squirrels initiated and maintained torpor despite elevated testosterone concentrations, but even in these males suppressive effects of testosterone were evident in shorter than normal torpor bouts. Because estradiol did not affect hibernation in castrated male squirrels (preliminary data), it is likely that testosterone inhibits torpor by acting on androgen receptors and does not require aromatization to estradiol. Estradiol also was less effective than testosterone in inhibiting torpor in Turkish hamsters (6).

All but one squirrel that entered torpor when treated with testosterone before the initial cold challenge had testosterone concentrations below 1 ng/ml. Plasma testosterone levels of intact squirrels are basal (<1 ng/ml) during most of the hibernation season; beginning 3 wk
before hibernation is terminated, testosterone levels increase from \( \sim 0.7 \) ng/ml during torpor to 2.1 ng at 6 h after initiation of an arousal. This increase is not sustained and testosterone levels decline to basal levels 18 h after initiation of arousal, and squirrels resume hibernation (2). Only in the days after the terminal arousal, when squirrels chronically maintained body temperatures of \( \sim 37^\circ C \), do testosterone concentrations consistently rise to \( \geq 2 \) ng/ml (2). It appears that as long as testosterone levels are sustained at \( \geq 1.2 \) ng, a return to hibernation is precluded in the majority of aroused squirrels. Additional experiments are needed to delineate the mechanisms that regulate the duration of testosterone secretion during arousals, as this may be a significant determinant of arousal duration.

There were no differences in responsiveness of squirrels challenged with testosterone at three different phases of the hibernation season. In the absence of dose-response studies, we cannot definitively exclude the possibility that increased sensitivity to testosterone as spring approaches contributes to terminating torpor. We favor the alternative hypothesis that testosterone does not actively provoke arousals from hibernation but instead determines whether the animal will reenter the torpid state at the end of an arousal bout. In this scheme, a steroid-independent mechanism triggers arousals from hibernation, and a testosterone-dependent mechanism controls whether hibernation is resumed. Because untreated castrated males of several species do not hibernate indefinitely (6, 12, 14), the resumption of torpor after an arousal eventually becomes impossible even in the absence of testicular hormones.

Among squirrels challenged with testosterone after hibernation had been initiated (i.e., in torpid animals), there was no significant correlation between testosterone concentrations and latency to terminal arousal in late February or late March. In the latter test, only 36% of squirrels terminated hibernation within 24 h of capsule implantation, 18% delayed terminal arousal for 5-6 days, and 45% of squirrels aroused and reentered torpor one or more times before terminating hibernation.

One animal treated in February continued to hibernate normally despite a testosterone concentration of 2.27 ng/ml. Testosterone treatment resulted in terminal arousal within 24 h of implantation for 43% of the animals, whereas terminal arousal was delayed 1-4 days for 43% of squirrels. There was no correlation between testosterone concentrations and time until terminal arousal. During the late hibernation period, when one might expect increased sensitivity to testosterone, 45% of the animals aroused and reentered hibernation one or more times, 18% delayed terminal arousal for 5-6 days, and only 36% terminated hibernation within 24 h of testosterone capsule implantation.

Separate experiments, conducted 1 yr apart on different populations of squirrels, are consistent in identifying a minority of animals for whom hibernation is compatible with testosterone concentrations \( >1 \) ng/ml. These squirrels were not completely insensitive to testosterone, since their torpor bouts were shorter than normal during hormone treatment; they may require higher testosterone levels to inhibit torpor. Such squirrels may represent individuals in natural populations who delay termination of hibernation relative to the majority of animals. Effects of testosterone on torpor apparently are more varied in this species than in several others and range from outright prevention of torpor to shortening of torpor bouts.

Moderate testosterone levels are more compatible with hibernation in golden-mantled ground squirrels than in Turkish hamsters (7) or Belding's ground squirrels (5). In the latter species, hibernation was uniformly prevented in castrated males treated with testosterone. These studies did not, however, monitor hibernation telemetrically, and it remains possible that torpor bouts less than 24 h long, characteristic of hormone-treated squirrels in experiment 1, may have gone undetected. In European hedgehogs, as in golden-mantled ground squirrels, a minority of castrated males treated with testosterone displays torpor (14).

We are grateful for the expert assistance of Kimberly Kelly, Susan Pawli, Norman Ruby, Christiana Tuthill, and Joan Wallace. This research was supported by National Institute of Child Health and Human Development Grants HD-14595 and HD-24575 and by National Science Foundation Grant DCR-88-48099.

Present address of T. M. Lee: University of Michigan, Neuroscience Laboratory Building, 1103 E. Huron St., Ann Arbor, MI 48104-1687.

Address for reprint requests: I. Zucker, Psychology Dept., University of California, Berkeley, CA 94720.

Received 12 February 1990; accepted in final form 22 May 1990.

REFERENCES


