

LH responses of female naked mole-rats, *Heterocephalus glaber*, to single and multiple doses of exogenous GnRH

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Summary. To investigate possible differential pituitary secretion of LH in breeding and non-breeding female naked mole-rats, the LH responses to administration of exogenous GnRH were measured in 55 females from 20 captive colonies. Single doses of 0.1, 0.5 or 1.0 µg GnRH produced a significant rise in plasma LH concentrations 20 min after s.c. injection in breeding and non-breeding females at all doses ($P < 0.001$).

While at the highest dose of 1.0 µg there was no difference in the LH response between breeding and non-breeding females, as the dose was lowered there was a progressive decline in the LH response in non-breeding females such that, at the 0.1 µg dose, GnRH produced only a small, but significant, increase in plasma LH (1.3 ± 0.2 to 2.9 ± 0.5 mi.u./ml, $N = 5$) compared with breeding females (3.4 ± 0.8 to 9.6 ± 2.0 mi.u./ml, $N = 6$). The LH responses of the latter were not significantly reduced at the lower doses of GnRH.

The apparent lack of sensitivity to low doses of exogenous GnRH in non-breeding females was reversed by 4 consecutive 1-h injections of 0.1 µg, which produced a rise in LH from 1.2 ± 0.2 to 9.0 ± 0.2 mi.u./ml ($N = 4$), comparable to that of breeding females given a single injection of 0.1 µg GnRH.

These results suggest that the anterior pituitary in non-breeding female naked mole-rats is less sensitive to low doses of exogenous GnRH than in breeding females, possibly due to a lack of priming by endogenous GnRH. Therefore, the socially-induced block to ovulation in non-breeding female naked mole-rats may be due to inhibition of hypothalamic GnRH secretion.

Keywords: naked mole-rat; reproductive suppression; LH; pituitary; GnRH

Introduction

Naked mole-rats exhibit the most extreme example of socially-induced infertility so far discovered in mammals. These highly social hystricomorph rodents inhabit large subterranean burrow systems in the semi-arid regions of East Africa (Jarvis, 1978; Brett, 1986). Within colonies which commonly contain 40–90 individuals in the wild, reproduction is restricted to one female, the 'queen', and 1–2 males in wild (Jarvis, 1978; Brett, 1986) and captive (Jarvis, 1981) colonies. The remaining colony members are reproductively quiescent and adopt a behavioural role related to their body mass. Small animals function as 'workers' and carry out such tasks as tunnel digging, and foraging for food in the form of underground roots and tubers. As individuals become larger they perform these behaviours progressively less and adopt a defensive role within the colony (Jarvis, 1981; Brett, 1986).

In captive and wild colonies, the block to reproduction in non-breeding females appears to result from an inhibition of ovulation. In addition, plasma LH concentrations are low in comparison to those in breeding females (Faulkes *et al.*, 1990). These results suggest that the block to ovulation in non-breeding female naked mole-rats may be due to inadequate circulating concentrations of LH. This may, in turn, be due to suppressed hypothalamic GnRH, or an impairment of pituitary function without suppressed GnRH secretion, resulting in inadequate synthesis and release of LH.

The aim of this study was to investigate whether pituitary sensitivity to GnRH was affected by breeding status by (i) determination of the time course and dose-response relationship between GnRH and LH in breeding and non-breeding female naked mole-rats, and (ii) investigating the effect of repeated injections of a low dose of GnRH on the LH responses of non-breeding females.

Materials and Methods

Animals. Captive colonies of naked mole-rats were maintained at the Institute of Zoology, London, and at the University of Cape Town, South Africa, using artificial burrow systems (Brett, 1985) in rooms heated to 28–30°C, with a relative humidity of 40–60%. The burrow systems consisted of a series of interconnecting Perspex tubes with a nest chamber, a toilet chamber, and a chamber where food was introduced. The nest chambers were heated to 32–34°C with a thermostatically controlled lamp to provide additional heating in the London colonies. Cape Town colonies were provided with 40-W lamps positioned at several points of the colony. Fresh food was given daily *ad libitum* and included sweet corn, sweet potatoes, carrot, potato, apple, and banana. A cereal supplement containing vitamins and minerals was also provided once per week (London colonies) or daily (Cape Town colonies). The total tunnel length varied from 2 to 15 m, according to the number of animals in the colony, which ranged from pairs up to 72 individuals. Animals were numbered and identified by a system of toe clipping and tattoos. Breeding females were readily distinguished from non-breeders by their large body size, and well developed teats and external genitalia. Altogether 55 females from 20 captive colonies were used in this study.

Blood sampling. Animals were hand-held, the tip of the tail was cut with a sterile scalpel blade and approximately 200 µl blood were collected by capillary action using heparinized micro-haematocrit tubes. Blood samples were collected within 2–4 min of animal capture, and subsequent blood samples were collected from the same wound, after removing the clot by washing with sterile saline. The total amount of blood taken from each animal after serial sampling did not exceed a maximum of 800 µl, and after the last blood sample had been collected, the wound was treated with an antibiotic powder (Aureomycin), and the animal returned to its colony. After collection the samples were stored on ice for a maximum of 2 h before being centrifuged at 500 g, and the plasma was stored at –20°C before LH determination.

LH bioassay. LH was measured using an in-vitro bioassay based on the production of testosterone by dispersed mouse Leydig cells (Van Damme *et al.*, 1974). Details of the method have been described previously (Harlow *et al.*, 1984; Hodges *et al.*, 1987; Abbott *et al.*, 1988). Plasma samples were assayed in duplicate at two dilutions of 1:10 and 1:20, or 1:20 and 1:40, as a routine check for parallelism, and compared with a rat LH standard (the rLH antigen preparation: rLH-I-7) over the range 2–0.0625 mi.u./ml. The testosterone produced was measured by radioimmunoassay, also described by Hodges *et al.* (1987).

To validate the LH bioassay for plasma taken from animals after GnRH treatment, checks for parallelism were carried out. Dilutions of naked mole-rat plasma samples taken before or after GnRH treatment, and of a pituitary homogenate containing high concentrations of LH, were parallel to, and not significantly different from the reference preparation (2-way analysis of variance with replication).

The sensitivity of the assay (determined at 90% binding) was 0.01 mi.u. per tube. Intra- and inter-assay precision for the whole assay, expressed as the mean coefficients of variation for repeated determinations of an LH quality control (1.53 mi.u./ml), were 10% ($n = 15$) and 16% ($n = 9$) respectively.

GnRH administration. Three solutions of 0.5, 2.5 and 5.0 µg GnRH (NIDDKD) per ml sterile saline were divided into 1 ml samples and stored at –20°C until required. In all experiments the GnRH was administered s.c. as a 200 µl injection. It was not possible to standardize the stage of the reproductive cycle at which breeding females were treated with GnRH.

To establish the time course of LH responses to GnRH blood samples were taken from 4 breeding and 3 non-breeding females at 5, 20 and 40 min after a single s.c. injection of 0.1 µg GnRH in 200 µl saline. Plasma LH concentrations were maximal 20 min after 0.1 µg GnRH administration, although this was only statistically significant in breeding females ($F(2,10) = 9.6$, $P < 0.005$). The LH values in non-breeding females were significantly lower than those in breeding females at all three time points ($F(1,5) = 7.3$, $P < 0.05$). The 20 min time point was therefore adopted in the following experiments to monitor pituitary LH responses to GnRH administration.

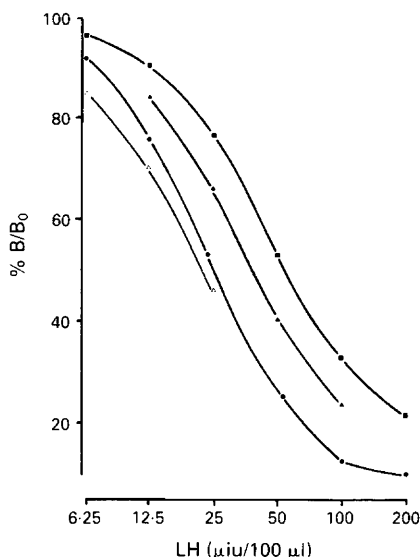


Fig. 1. A standard curve of rat LH (rLH-I-7) concentrations (●) plotted on a logarithmic scale against % binding (%B/B₀), compared with dilution curves for naked mole-rat plasma before (△) and 20 min after (▲) a 1.0 µg injection of GnRH, and naked mole-rat pituitary homogenate (■), showing parallelism.

Experiment 1: LH responses to a single injection of GnRH. Blood samples were taken from breeding and non-breeding females before, then 20 min after a single subcutaneous injection of 0.1, 0.5 or 1.0 µg GnRH in 200 µl saline (N = 5–9 animals), or of saline alone (N = 3).

Experiment 2: multiple injections of GnRH. Blood samples were taken from non-breeding females before, then 20 min after 4 and 8 subcutaneous injections of 0.1 µg GnRH in 200 µl saline (N = 4), or of saline alone (N = 3), administered at 1-h intervals.

Statistical analysis. Log transformation of plasma LH concentrations was carried out to increase the linearity of the data and to reduce the heterogeneity of variance (Helwig & Council, 1979; Sokal & Rohlf, 1981). Plasma LH concentrations after single injections of GnRH were analysed by two-way analysis of variance for repeated measures. Due to a significant interaction between breeding status and time at the 1.0 µg dose, one-way analysis of variance for repeated measures was carried out on each female breeding status group for this GnRH dose. Plasma LH concentrations after multiple GnRH administration were analysed by one-way analysis of variance for repeated measures. In all cases, comparisons of individual transformed means were made *post hoc* using Duncan's multiple range test (Helwig & Council, 1979).

Results

Experiment 1: LH responses to a single injection of GnRH

Administration of GnRH produced significant increases in circulating LH concentrations at all doses in breeding (F(3,17) = 13.4; $P < 0.001$) and non-breeding females (F(3,19) = 17.5; $P < 0.001$; Fig. 2). There was no response to the saline control injections in breeding (F(3,17) = 0.61; $P > 0.6$) or non-breeding (F(3,19) = 0.47; $P > 0.6$) females. Basal plasma LH concentrations (0 min values in this study) were significantly lower in non-breeding ($P < 0.05$) than in breeding females in all treatment groups except saline controls, in which the sample size was small (N = 3).

This experiment revealed differences in LH responses to GnRH in breeding females compared with non-breeding females. At the 1.0 µg dose, there was no significant difference in LH responses between the two groups of females (F(1,14) = 0.94; $P > 0.3$). However, with the 0.5 µg GnRH dose there was a significant difference in the LH response between breeding (N = 5) and non-

breeding ($N = 6$) ($F(1,9) = 1.03$; $P < 0.05$) females, with plasma LH concentrations rising from 3.5 ± 1.1 to 12.6 ± 2.1 mi.u./ml in the former and from 1.2 ± 0.2 to 6.3 ± 0.6 mi.u./ml in the latter. At the lowest GnRH dose of $0.1 \mu\text{g}$, the difference in LH response was even greater ($F(1,9) = 21.4$; $P < 0.01$), with non-breeders ($N = 5$) producing only a small increase in plasma LH from 1.3 ± 0.2 to 2.9 ± 0.5 mi.u./ml. However, in breeding females ($N = 6$) the magnitude of the response was not significantly different from the 0.5 or $1.0 \mu\text{g}$ GnRH doses, with plasma LH concentrations rising from 3.4 ± 0.8 to 9.6 ± 2.0 mi.u./ml.

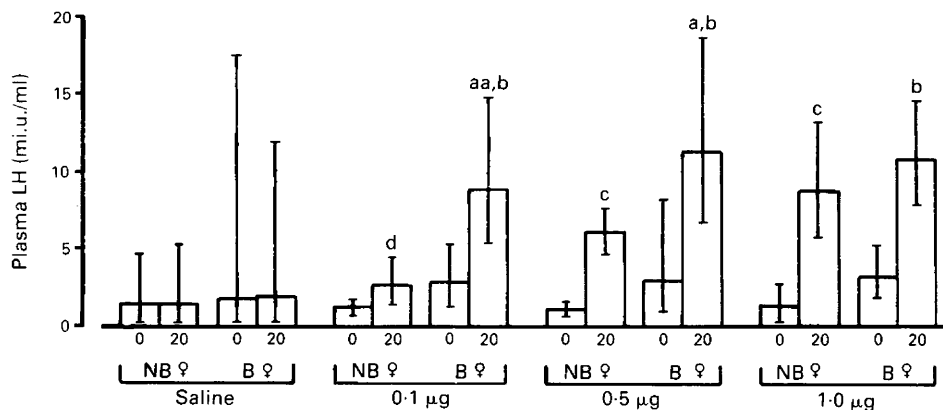


Fig. 2. Plasma concentrations of LH (mean \pm 95% confidence limits) in breeding (B♀) and non-breeding (NB♀) female naked mole-rats before (○) and 20 min after (20) a single s.c. injection of 0.1, 0.5 or 1.0 μg GnRH, or saline. The data are expressed as the antilog of the transformed means. a: $P < 0.05$ vs non-breeding females, 0.5 μg dose; aa: $P < 0.01$ vs non-breeding females, 0.1 μg dose; b: $P < 0.01$ vs saline treated breeding females; c: $P < 0.01$ vs saline treated and 0.1 μg dose non-breeding females; d: $P < 0.01$ vs 0 min, non-breeding females, 0.1 μg dose (Duncan's multiple range test following ANOVA for repeated measures).

Experiment 2: multiple injections of GnRH

As shown in Fig. 3, GnRH produced a significant increase in plasma LH concentrations in non-breeding females after 4 (1.2 ± 0.2 to 9.0 ± 0.2 mi.u./ml, $N = 4$) or 8 (1.2 ± 0.2 to 7.9 ± 1.7 mi.u./ml, $N = 4$) injections of $0.1 \mu\text{g}$ GnRH, or after a single injection of $0.1 \mu\text{g}$ GnRH in breeding females (3.4 ± 0.8 to 9.6 ± 2.0 mi.u./ml, $N = 6$) ($F(13,4) = 10.36$; $P < 0.001$). There was no difference in the magnitude of the LH responses in these three groups of females, and the values were significantly greater than those obtained with non-breeding females given a single injection of $0.1 \mu\text{g}$ GnRH ($F(13,4) = 10.36$; $P < 0.001$). There was no response to saline injection.

Discussion

Previous studies have provided circumstantial evidence that the socially-induced block to ovulation in non-breeding female naked mole-rats may be the result of reduced plasma LH concentrations, and that this may be due to either a suppression of hypothalamic GnRH secretion or an unrelated characteristic of pituitary function (Faulkes *et al.*, 1990). The results of the present investigation strongly suggest that the former hypothesis is the most likely, and are consistent with earlier findings (Faulkes *et al.*, 1990) in showing that basal plasma LH values are undetectable or very low in non-breeding females, compared with breeding females.

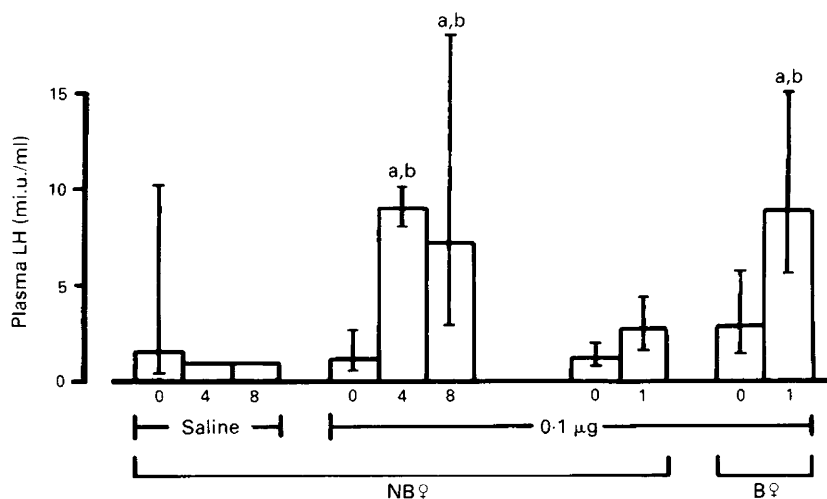


Fig. 3. Plasma concentrations of LH (mean \pm 95% confidence limits) in breeding (B♀) and non-breeding (NB♀) female naked mole-rats before (0) and 20 min after a single 0.1 μ g s.c. injection (1), or 20 min after the last of 4 or 8 s.c. injections of 0.1 μ g GnRH given at 1-h intervals, or a saline control. The data are expressed as the antilog of the transformed means. a: $P < 0.01$ vs 0 min, b: $P < 0.01$ vs non-breeding females after a single 0.1 μ g GnRH dose (Duncan's multiple range test following ANOVA for repeated measures).

In Exp. 1, breeding and non-breeding female naked mole-rats responded with equal magnitude to the highest single dose of 1.0 μ g GnRH, with an increase in circulating LH by 20 min after injection. This indicates that the pituitary of non-breeders contains a releasable pool of bioactive LH which can be secreted if the animal is given a sufficiently large GnRH stimulus. However, while lowering the dose of GnRH resulted in an undiminished LH response in breeding females, non-breeding females were less responsive at the 0.5 and 0.1 μ g doses, suggesting that the pituitaries of non-breeding females were less sensitive to these lower concentrations of GnRH.

The lack of sensitivity to exogenous GnRH in non-breeding females may be the result of a reduction in pituitary GnRH receptors or an alteration in post-receptor metabolic events. In rats, changes in the pituitary responsiveness to exogenous GnRH during the oestrous cycle are paralleled by changes in pituitary GnRH receptor concentrations (Smith, 1984; Fox & Smith, 1985). If, in the non-breeding female naked mole-rat, the anterior pituitary has reduced concentrations of GnRH receptors, then this in turn may reflect a lack of endogenous GnRH priming of the pituitary in these females, and an absence of receptor self-induction. The ability of GnRH to autoregulate its receptors is well documented (for review see Sandow, 1983; Clayton & Catt, 1987). The results from Exp. 2 are consistent with this hypothesis, because the priming effect of 4 consecutive injections of a low dose of GnRH (0.1 μ g) was sufficient for non-breeding females to produce an LH response equivalent to that of breeding females given a single injection of that GnRH dose. No further enhancement of response was obtained after 8 consecutive injections.

In man, GnRH has been shown to alter the biological potency of LH due to alterations in carbohydrate content of the molecule (Montanini *et al.*, 1984), but it is not known whether or not administration of GnRH produces these effects in the naked mole-rat, or if such changes would be detected by the mouse Leydig cell bioassay. Parallelism of naked mole-rat plasma with the rat LH standard was not significantly different in samples taken before and after injection of GnRH, thus if structural changes in the LH molecule were occurring in this study, they did not affect the validity of the assay. Because a bioassay rather than an immunoassay was used, it is possible that the changes in bioactive LH resulting from administration of GnRH were due to a combination of changes in LH potency, and in absolute concentration.

The results from these experiments therefore suggest that the socially-induced block to ovulation in non-breeding female naked mole-rats may be due to reduced plasma LH concentrations resulting from impaired endogenous hypothalamic GnRH secretion. The consequences of this GnRH inhibition on LH pulse frequency and amplitude in the naked mole-rat are not known, but plasma LH values in non-breeding females are consistently low, or below the sensitivity limit of the assay.

The reversible nature of the lack of responsiveness to a low dose of exogenous GnRH (0.1 µg) in non-breeding female naked mole-rats reflects the rapidity with which the block to ovulation can be reversed in non-breeding females if their social environment is changed. For example, if a non-breeding female is removed from her parent colony and housed singly or paired with a male, sustained elevations of urinary progesterone (indicative of the luteal phase of an ovarian cycle) occur for the first time 8.0 ± 1.9 days after separation (Faulkes *et al.*, 1990). As the follicular phase of the cycle is 6.0 ± 0.6 days, this suggests that the hypothalamic-pituitary axis must commence normal activity within the first 2 days of separation.

In respect of the response of the pituitary to GnRH, the non-breeding female naked mole-rat resembles the hypogonadal (*hpg*) mouse, a mutant strain in which hypothalamic GnRH is deficient or absent (Young *et al.*, 1983). These animals have low levels of pituitary LH, and like the non-breeding naked mole-rat have low plasma LH concentrations (Charlton *et al.*, 1983). The *hpg* mouse also responds to administration of exogenous GnRH, with plasma LH rising significantly 5 min after a single intravenous GnRH injection (Iddon *et al.*, 1980), while multiple daily injections of GnRH restore pituitary LH content (Charlton *et al.*, 1983). This illustrates that even in animals which are genetically deficient in GnRH, the pituitaries contain sufficient GnRH receptors and stores of releasable LH to produce an immediate LH response when given their first GnRH stimulus.

The central role of hypothalamic GnRH secretion in integrating environmental cues with reproductive function is well documented in other examples of natural suppression of fertility, for example the seasonally anoestrous ewe (Legan & Karsch, 1979), during lactational amenorrhoea in humans (Glasier *et al.*, 1986), and in the socially-induced suppression of reproduction in female common marmoset monkeys (Abbott *et al.*, 1988). The naked mole-rat is perhaps the most extreme example of how environmental cues might modulate GnRH and LH release, and further investigation of this species could provide an insight into the detailed mechanisms of the control of GnRH and LH secretion.

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