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Social suppression of reproduction in male naked mole-rats, *Heterocephalus glaber*

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Summary. To investigate possible anatomical and endocrine differences between breeding and non-breeding male naked mole-rats, 113 animals from 24 captive and 4 wild colonies were studied.

While breeding males had larger reproductive tract masses compared to non-breeders relative to body mass ($P < 0{\cdot}01$), spermatogenesis was active in all of the non-breeding males examined histologically ($n = 9$) and spermatozoa were present in the epididymides. Compared with non-breeders, breeding males had significantly higher urinary testosterone concentrations (mean ± s.e.m.: 23·8 ± 2·3 *vs* 5·2 ± 1·4 ng/mg Cr respectively; $P < 0{\cdot}001$), and plasma LH (10·7 ± 1·7 *vs* 5·0 ± 0·8 mi.u./ml respectively; $P < 0{\cdot}01$). 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 males at all doses ($P < 0{\cdot}001$). However, there were differences in the magnitude of the LH response following administration of GnRH between breeding and non-breeding males, with non-breeding males showing a dose–response and having lower plasma LH concentrations 20 min after a single injection of 0·1 or 0·5 μg ($P < 0{\cdot}05$), but not 1·0 μg, GnRH. This apparent lack of pituitary sensitivity of non-breeding males to single doses of exogenous GnRH was reversed by 4 consecutive injections of 0·5 μg GnRH at hourly intervals, suggesting that the reduced sensitivity may be the result of insufficient priming of the pituitary by endogenous GnRH.

These results indicate that, despite the fact that non-breeding males were apparently producing mature gametes, clear endocrine deficiencies existed in male naked mole-rats.

Keywords: reproductive suppression; naked mole-rats; hystricomorph rodent; testosterone; LH

Introduction

Colonies of naked mole-rats, *Heterocephalus glaber*, a subterranean hystricomorph rodent inhabiting the arid regions of East Africa, including Kenya, Ethiopia and Somalia, commonly contain 40–90 individuals (Brett, 1986, 1991; Jarvis, 1985). Living entirely underground, their burrow systems contain communal nest and toilet chambers, and an extensive network of foraging tunnels, which may total 2–3 km in length (Brett, 1986, 1991). Apart from having a behavioural division of labour similar to that of the eusocial insects (Jarvis, 1981; Lacey & Sherman, 1991; Faulkes *et al.*, 1991), naked mole-rats exhibit perhaps the most extreme example of socially-induced suppression of reproduction so far discovered in mammals. Within both captive and wild colonies, reproduction is restricted to a single breeding female, the 'queen', while in the remaining non-breeding females ovulation is blocked (Faulkes *et al.*, 1990a), possibly as a result of inadequate plasma LH concentrations arising from impaired hypothalamic GnRH secretion (Faulkes *et al.*, 1990b). Behavioural observations have also shown that reproduction is restricted to 1, 2 or sometimes 3

male naked mole-rats (Jarvis, 1981; Brett, 1986; Lacey & Sherman, 1991; Faulkes *et al.*, 1991). Behaviourally, differences between breeding and non-breeding males are clear-cut in that only the breeding males are solicited by, and mate with, the queen (Jarvis, 1991). However, anatomical differences in the reproductive tract of males are apparently less distinct, because Jarvis (1991) found that, out of 84 wild-caught males, 76% had spermatozoa in their vas deferens. This suggests that most non-breeding male naked mole-rats may potentially be capable of fertilizing a female, should they mate. This is different from non-breeding female naked mole-rats, in which the development of mature gametes does not appear to occur because ovulation is blocked (Faulkes, 1990; Faulkes *et al.*, 1990a).

The following study was undertaken to confirm that spermatogenesis was occurring in non-breeding males, by histological examination, and to examine whether differences in breeding status in males were reflected by differences in concentrations of reproductive hormones. As reduced pituitary LH secretion was implicated in reproductive suppression in female naked mole-rats (Faulkes *et al.*, 1990a, b), plasma LH concentrations and LH response to exogenous GnRH administration were investigated in breeding and non-breeding males, together with determinations of urinary testosterone values as a measure of testicular function.

Materials and Methods

Animals and sampling

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, the details of which have been described previously (e.g. Faulkes *et al.*, 1990a, b). The total tunnel length of these artificial burrows varied from 2 to 15 m, according to the number of animals in the colony, which ranged from pairs up to 72 individuals. In captivity, animals were numbered and identified by a system of toe clipping and tattoos. Breeding males were distinguished from non-breeders by observations of mating. Altogether 61 males from 24 captive colonies were used in this study.

Naked mole-rats from wild colonies were captured near Mtito Andei, Kenya, approximately 230 km south-east of Nairobi, as described by Faulkes *et al.* (1990c). A total of 52 males from 4 wild colonies were used in this study.

Collection and fixation of reproductive tract tissue. From captive colony males, reproductive tracts were either removed at *post mortem*, no more than 12 h after the death of the animal, or removed within 30 min of euthanasia. The reproductive tract was cut just below the junction of the left and right vas deferens, and the abdominal testes, together with the attached vasa deferentia were then removed. Tissue was fixed by immersion in 10% formal saline. Tissue samples collected from colonies in the wild were fixed by immersion in 4% paraformaldehyde in saline within 15 min of killing the animal.

All samples were fixed for 7 days to 24 months (captive and wild-caught animals) before measurement of testicular mass (testes plus epididymis and vasa deferentia, as detailed above) and histology. Because an accurate balance was not available in the field, it was not possible to weigh the reproductive tracts and gonads of wild-caught animals before fixation. Therefore, all measurements of testicular mass were made on fixed material, and the results are based on the assumption that any size changes in the tissues examined, resulting from the process of fixation, were constant across all the samples.

Urine sampling. Urine was chosen for routine testosterone determination in captive colonies in preference to blood because it is a non-invasive technique and disturbance to the animals was minimized. Urine is also widely used as a medium for hormonal analysis in other species (e.g. Lasley, 1985; Hodges, 1986), and the female naked mole-rat (Faulkes *et al.*, 1990a), and the analytical methods have been well validated.

Urine sampling involved the removal of all the shavings from the toilet chamber in each colony and wiping the chamber clean with tissue paper. Immediately after each urination, the sample was collected in a glass pipette. After collection of each sample, the toilet chamber was wiped clean with tissue paper. Samples were placed in a freezer within 1 h of collection, and stored at −20°C until hormone determination. Sampling was carried out between 08:00 and 18:00 h.

Blood sampling. Animals were hand-held, the tip of the tail was cut with a sterile scalpel blade and blood (approximately 200 µl) was collected by capillary action using heparinized micro-haematocrit tubes. Blood samples were collected within 2–4 min of animal capture from the captive colonies, 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 800 µl, and after the last blood sample had been collected, the wound was treated with 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 for 5 min at 500 *g*, and the plasma was stored at −20°C before LH determination.

Hormone determinations

Radioimmunoassay of testosterone. Before testosterone assay all urine samples were subjected to a determination of urinary creatinine as described by Bonney *et al.* (1982). All urinary testosterone concentrations were expressed as mass per mg creatinine (mg/Cr) to correct for dilution of urine.

Chromatographic separation of testosterone from the samples were required before radioimmunoassay, because there was a significant difference between urinary testosterone concentrations in samples assayed with and without chromatography (32·4 ± 12·3 and 108·8 ± 37·1 respectively; paired *t* test, $t = 2{\cdot}95$, d.f. = 8, $P < 0{\cdot}02$).

The method for separating testosterone using Celite column chromatography has been previously described by Hodges *et al.* (1981) for primates, and by Faulkes (1990) for naked mole-rats. Briefly, this involved washing columns of Celite:ethylene glycol (2:1 w/v) with 8·0 ml iso-octane, followed by addition of a further 3·5 and 5·0 ml iso-octane which eluted progesterone and dihydrotestosterone from the column, respectively. These fractions were discarded. After rinsing the column with 2·0 ml iso-octane, the testosterone fraction was eluted and collected after addition of 5·0 ml cyclohexane:benzene (95:5, v/v). After evaporation of solvent, the samples were reconstituted in 1·0 ml buffer and subjected to testosterone radioimmunoassay as described below.

Testosterone concentrations were determined in diethyl ether-extracted urine (50–100 µl) by radioimmunoassay following Celite chromatography, using sheep anti-testosterone antibody no. 505 (MRC Reproductive Physiology Unit, Edinburgh, UK; see Hodges *et al.*, 1987).

The sensitivity of the assay (determined as 90% binding) was 2·0 pg/tube. At an average dilution of urine this was equivalent to < 1·0 ng/mg Cr. Inter-assay precision, expressed as the coefficient of variation for repeated determinations of a quality control (2·22 ng/mg Cr), was 15·1% ($n = 4$) for quality controls subjected to column chromatography and 16·9% ($n = 4$) for quality controls extracted only. Intra-assay variation was 7·5% ($n = 7$).

The assay was validated for use in the naked mole-rat by tests of accuracy and parallelism. Accuracy was assessed by addition of urine to the reference preparation. The mean ± s.e.m. recovery of unlabelled testosterone added to a naked mole-rat urine pool was 92·6 ± 10·0% ($n = 4$) over the standard curve range of 2·5–160 pg/tube. Parallelism was demonstrated by an absence of a significant interaction between preparation (testosterone standard *vs* urine containing high levels of testosterone) and dilution, when using a two-way ANOVA repeated-measures design ($F(5,10) = 0{\cdot}78$; $P > 0{\cdot}58$) (Sokal & Rohlf, 1981).

Luteinizing hormone 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 the radioimmunoassay described by Hodges *et al.* (1987).

To validate the LH bioassay for the naked mole-rat, dilutions of plasma samples taken before or after GnRH treatment, and of a pituitary homogenate containing high concentrations of LH, were shown to be parallel to, and not significantly different from, the reference preparation (see Faulkes *et al.*, 1990b, for further details).

The sensitivity of the assay (determined at 90% binding) was 0·1 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.

Experimental procedures

Measurement of reproductive tract masses. Testes, including epididymides and vasa deferentia, from captive breeding (4 males from 4 colonies), captive non-breeding (6 males from 3 colonies), and wild-caught (52 males from 4 colonies) male naked mole-rats were individually weighed. Although the reproductive status of the wild-caught males was not known, they were assumed to be non-breeders because the mass of their testes resembled those of captive non-breeders, and because in both wild and captive colonies, only 1–3 males are of breeding status (Jarvis, 1981; Brett, 1986; Lacey & Sherman, 1991).

Histology. Testes from captive breeding ($n = 4$), captive non-breeding ($n = 6$) and wild-caught males ($n = 3$, assumed to be non-breeders due to the low body mass of the animals, and the small testes masses which were comparable to those of other non-breeding males), were examined. Sections 0·5–0·8 µm thick were cut from tissue embedded in paraffin wax, and stained for light microscopy with haematoxylin–eosin. Full details of the method are described by Faulkes (1990). Photomicrography was carried out with a Zeiss Ultraphot 2 photomicroscope, using 35 mm Kodak Pan-F black and white film.

Urinary testosterone in breeding and non-breeding males. To investigate differences in urinary testosterone concentrations between breeding and non-breeding males as a possible reflection of differences in testicular function, 142 samples were collected from 9 breeding males from 9 colonies, and 72 samples were collected from 12 non-breeding males from 8 colonies.

Plasma LH in breeding and non-breeding males. To investigate possible differences in pituitary function in breeding and non-breeding males, 27 plasma samples were collected from 14 breeding males from 13 colonies, while 37 plasma samples were collected from 24 non-breeding males from 8 colonies. Bioactive LH concentrations in these samples were then measured.

GnRH administration. To investigate possible differential LH responses of the pituitary to stimulation by GnRH, the effects of administration of exogenous GnRH were investigated in breeding (N = 17) and non-breeding (N = 30) male naked mole-rats. 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 subcutaneously as a 200 μl injection, giving doses of 0·1, 0·5 and 1·0 μg GnRH/200 μl saline. In Exp. 1, blood samples were taken immediately before, then 20 min after, a single subcutaneous injection of 0·1, 0·5 or 1·0 μg GnRH in 200 μl saline (4–6 animals), or of saline alone (N = 3), in breeding and non-breeding male naked mole-rats. In Exp. 2, blood samples were taken from non-breeding males before, then 20 min after, 4 and 8 subcutaneous injections of 0·1 (N = 5), 0·5 (N = 6) or 1·0 μg (N = 6) GnRH in 200 μl saline, or of saline alone (N = 3), administered at 1-h intervals.

Statistical analysis. Reproductive tract masses, urinary testosterone and basal plasma LH data were analysed using Student's *t* test, while GnRH challenge data were subjected to analysis of variance for repeated measures following log transformation. Plasma LH concentrations after single GnRH administration were analysed by two-way analysis of variance for repeated measures. Due to a significant interaction at the 0·5 μg dose, results from this dose were subjected to one-way analysis of variance. Plasma LH concentrations after multiple GnRH administration were analysed by one-way analysis of variance for repeated measures. Log transformation of plasma LH concentrations was carried out as a standard procedure, to increase the linearity of the data and to reduce the heterogeneity of variance (Sokal & Rohlf, 1981). Results quoted in the text are means ± s.e.m. for the non-transformed data, while the figures reflect the data as the antilog of the means transformed for statistical analysis, together with their 95% confidence limits. Comparisons of individual transformed means were made *post hoc* using Duncan's multiple-range test with a level of significance of $P = 0·05$ (Helwig & Council, 1979).

Results

Reproductive tract masses

The absolute and relative to body mass testicular masses (testes, epididymides and vasa deferentia) for captive breeding and non-breeding males, and wild-caught males of assumed non-breeder status are given in Table 1.

Table 1. Mean ± s.e.m. testicular mass in captive breeding, non-breeding and wild caught male naked mole-rats

Male status	No. of males	Total testicular mass (mg)	Total testicular mass/ body mass (mg/g)
Captive breeder	4	79·0 ± 14·7*	2·0 ± 0·2†
Captive non-breeder	6	34·0 ± 2·9	1·2 ± 0·1
Wild caught	52	33·9 ± 2·2	1·5 ± 0·3

*$P < 0·01$ compared with values for the other two groups.
†$P < 0·01$ compared with values for captive non-breeders.

Relative to body mass, captive breeding males had a significantly higher total testicular mass ($P < 0·01$, $t = 3·70$, d.f. = 8), than did captive non-breeding males. The absolute testicular masses were also significantly different between captive breeding and captive non-breeding males ($P < 0·01$, $t = 3·59$, d.f. = 8), and between captive breeding and wild-caught males ($P < 0·01$, $t = 3·03$, d.f. = 54). The mass of the reproductive tracts of wild-caught males resemble those of captive non-breeders, and not of captive breeders. It was therefore likely that the reproductive tracts of the wild-caught males were taken from non-breeders.

Histology

Representative sections from the testis and epididymis of one breeding and one non-breeding male are illustrated in Fig. 1. There was evidence of spermatogenesis in all the breeding and non-

breeding males examined in this study. Various numbers of spermatozoa were present in the seminiferous tubules of all the animals, e.g. Figs 1 (a) and (b), indicating active spermatogenesis. In addition, large numbers of spermatozoa were evident in the lumen of the epididymis of non-breeding males (Fig. 1d). This indicates that, as well as undergoing spermatogenesis, these non-breeding males were also apparently producing mature spermatozoa. A striking feature of the testes of breeding and non-breeding male naked mole-rats was the presence of large amounts of interstitial tissue, when compared with laboratory rodents such as mice and rats (e.g. Fawcett *et al.*, 1973). Although no quantitative measurements were made, visual examination of the histological sections suggested that, in breeding males, there were greater numbers of interstitial cells than in non-breeders. This may have contributed, at least in part, to the larger testicular mass of breeding males.

Urinary testosterone

Urinary testosterone concentrations showed high individual variation (Table 2), ranging from 0·3 to 42·4 ng/mg Cr in non-breeding male naked mole-rats, and from 1·0 to 176·4 ng/mg Cr in breeding males.

Despite this individual variation, the grand means of these individual means revealed differences in the urinary testosterone concentrations between breeding and non-breeding males, with breeders having significantly higher values (23·8 ± 2·3 *vs* 5·2 ± 1·4 ng/mg Cr respectively; $P < 0·001$; $t = 6·96$, d.f. = 20).

The overall mean value of urinary testosterone in the 9 breeding males was 24 ng/mg Cr. Of the 12 non-breeders listed in Table 2, only 4 of these males had urinary testosterone concentrations that were comparable to or exceeded 24 ng/mg Cr. However, these instances of high urinary testosterone in non-breeding males occurred relatively infrequently. Only 8% of values from non-breeders exceeded 24 ng/mg Cr (the mean value for breeding males), and the highest value obtained was 42·4 ng/mg Cr. In breeding males, 30% of samples had urinary testosterone concentrations that exceeded 24 ng/mg Cr, with the highest value reaching 176 ng/mg Cr. These results show that overlap between the highest values of urinary testosterone in non-breeding males and the mean value for breeding males does occur in some individuals, but that most of the time breeding males maintained higher urinary testosterone concentrations which were mostly greatly in excess of those found in non-breeding males (Table 2).

Plasma LH

The differences in urinary testosterone concentrations between breeding and non-breeding male naked mole-rats were reflected in the circulating concentrations of LH. Breeding males had significantly higher plasma LH concentrations compared with non-breeders (10·7 ± 1·7 and 5·0 ± 0·8 mi.u./ml respectively; $P < 0·01$, $t = 3·05$, d.f. = 63).

GnRH treatment

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 and non-breeding males (Fig. 2, $F(3,31) = 26·21$; $P < 0·001$). There was no response to the saline control injections in breeding or non-breeding male naked mole-rats ($F(1,4) = 1·83$; $P > 0·25$).

This experiment revealed differences in LH responses to GnRH in breeding males compared with non-breeding males. While there was no significant difference in LH response at any of the three doses of GnRH in breeding males ($P > 0·05$), non-breeding males showed a dose–response which gave rise to lower plasma LH concentrations 20 min after a single injection of 0·1 or 0·5 μg GnRH, compared with breeding males (Fig. 2), suggesting that the pituitaries of non-breeding males were less sensitive to the lower doses of GnRH.

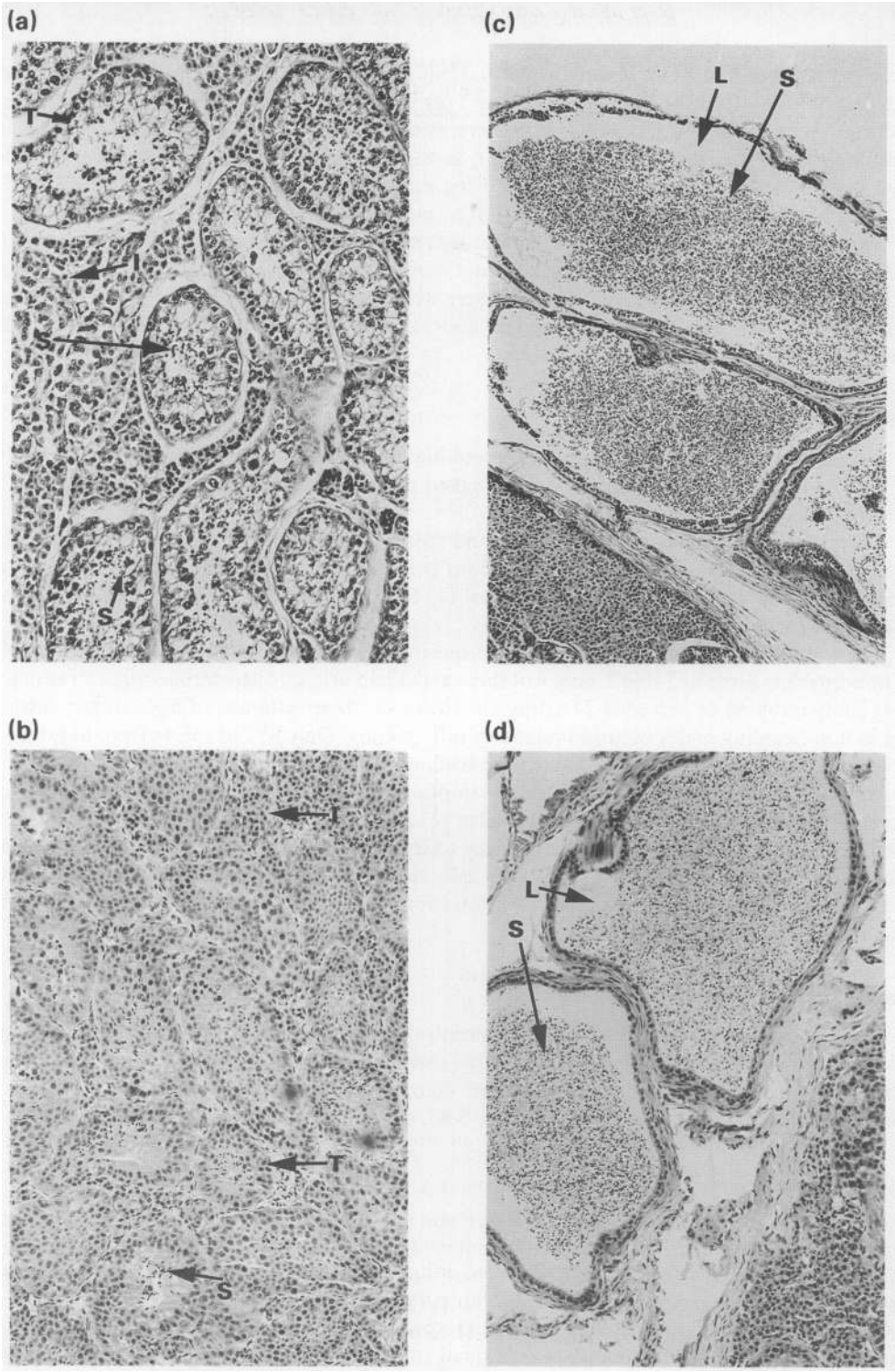

Fig. 1. Representative transverse section through the testes (a, b) and epididymides (c, d) of a breeding (a, c) and a non-breeding (b, d) naked mole-rat. I = interstitial (Leydig) cells, S = spermatozoa; T = seminiferous tubule; L = lumen of epididymis. a, × 62·5; b, × 100; c, × 100; d, × 100.

Table 2. Mean and range of urinary testosterone values (ng/mg Cr) taken from 9 breeding and 12 non-breeding naked mole-rats

Non-breeding males				Breeding males			
Animal	Mean	Range	No. of samples	Animal	Mean	Range	No. of samples
B9*	6·4	1·0–29·2	9	K9	18·7	1·0–112·3	26
B16	4·8	1·0–36·0	20	J16	23·9	1·0–114·0	28
B22	3·6	1·0–5·4	3	D22	24·8	1·0–118·0	14
D88	6·4	1·0–11·3	7	H88	32·7	1·0–176·4	13
A10	1·8	1·2–2·3	2	N98	30·1	3·6–154·7	18
A14	1·4	—	1	O8	29·6	1·0–71·5	10
A27	0·7	0·3–1·0	2	P3	17·6	1·7–42·6	9
B27	1·8	1·0–2·6	2	Q1	24·7	1·0–95·5	19
2208	17·7	3·4–42·4	5	R40	11·7	2·7–24·1	5
2203	10·2	3·4–26·4	12				
2201	2·8	1·0–7·0	7				
2240	4·5	1·0–8·8	3				
Grand mean ± s.e.m.		5·2–1·4				23·8–2·3	

*The letter prefix to the animal number identifies the colony to which the animal belonged.

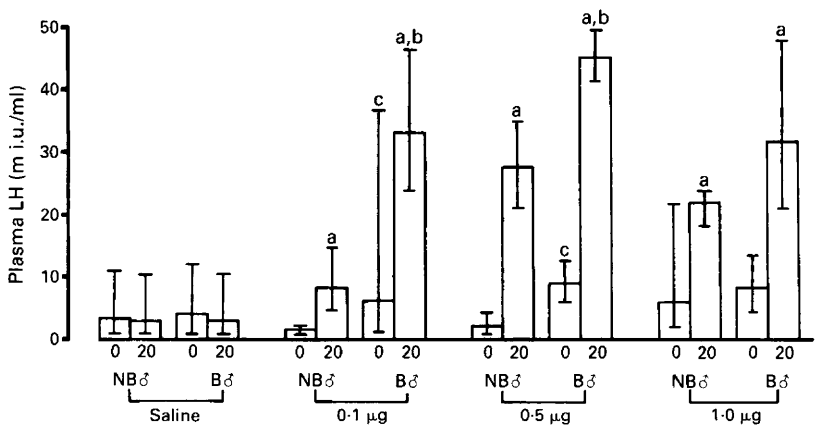

Fig. 2. Concentrations of plasma LH (antilog of the transformed mean ± 95% confidence limits) in breeding (B♂) and non-breeding (NB♂) male naked mole-rats before (0), and 20 min after (20), a single s.c. injection of 0·1, 0·5 or 1·0 μg GnRH, or saline. a, $P < 0·05$ *vs* 0 time; b, $P < 0·05$ *vs* non-breeding males at 20 min; c, $P < 0·05$ *vs* non-breeding males at 0 time (Duncan's multiple range test following ANOVA for repeated measures).

Administration of 1·0 μg GnRH produced an increase in plasma LH in breeders that was not significantly different from that of non-breeding males (8·3 ± 1·7 to 32·8 ± 5·0 mi.u./ml, N = 5, and 8·0 ± 2·0 to 21·6 ± 1·3 mi.u./ml, N = 5 respectively; $F(1,8) = 1·53$, $P > 0·25$). With 0·5 μg GnRH, maximum LH responses were obtained in breeding (N = 5) and non-breeding (N = 5) males, although at this dose breeding males produced significantly greater plasma concentrations of LH 20 min after injection (8·9 ± 1·2 to 45·1 ± 1·4 mi.u./ml, N = 5, and 2·1 ± 0·7 to 27·6 ± 2·6 mi.u./ml, N = 5, respectively; $F(1,8) = 25·95$, $P < 0·001$). At the lowest dose of 0·1 μg GnRH, the difference in LH response between breeding and non-breeding males was greatest

($F(1,7) = 17{\cdot}14$; $P < 0{\cdot}005$). Non-breeders (N = 5) produced only a small increase in plasma LH concentrations from $1{\cdot}5 \pm 0{\cdot}2$ to $8{\cdot}9 \pm 0{\cdot}5$ mi.u./ml, compared with breeding males (N = 4), whose plasma LH rose from $10{\cdot}0 \pm 6{\cdot}4$ to $33{\cdot}7 \pm 3{\cdot}7$ mi.u./ml.

Basal concentrations of plasma LH (0 time values in this study) were significantly lower in non-breeding males compared with breeding males in the 0·1 and 0·5 μg treatment groups ($P < 0{\cdot}05$), reflecting the results described in the previous section. There was no difference in basal plasma LH concentrations between breeding and non-breeding males in the 1·0 μg treatment group, probably because of the relatively small group sizes and the relatively high intra-group variability in concentrations of plasma LH (Fig. 2).

Experiment 2: multiple injections of GnRH. GnRH produced a significant increase in concentrations of plasma LH in non-breeding males after 4 ($2{\cdot}8 \pm 0{\cdot}7$ to $10{\cdot}6 \pm 1{\cdot}6$ mi.u./ml, N = 5) or 8 ($2{\cdot}8 \pm 0{\cdot}7$ to $11{\cdot}1 \pm 2{\cdot}4$ mi.u./ml, N = 5) 0·1 μg injections of GnRH, comparable with the basal concentration of LH (0 time) in breeding males ($10{\cdot}3 \pm 6{\cdot}3$ mi.u./ml, N = 5; Fig. 3a). At this dose, repeated GnRH injections to non-breeding males did not reverse the apparent lack of pituitary sensitivity to exogenous GnRH. Plasma LH concentrations after a single injection of 0·1 μg GnRH in breeding males were significantly greater than LH responses to GnRH in non-breeding males given 4 or 8 0·1 μg injections ($F(7,24) = 17{\cdot}86$; $P < 0{\cdot}001$; Fig. 3a). There was no response to repeated saline injections.

Figure 3(b) summarizes the results obtained after administering multiple doses of 0·5 μg GnRH to non-breeding males (N = 5). At this dose, plasma LH concentrations increased from $3{\cdot}9 \pm 1{\cdot}5$ to $29{\cdot}5 \pm 2{\cdot}8$ mi.u./ml after 4 injections, and to $18{\cdot}2 \pm 2{\cdot}3$ mi.u./ml 20 min after 8 consecutive hourly injections. Plasma concentrations of LH in non-breeding males after 4 injections were not significantly different from those of breeding males given a single 0·5 μg injection (Fig. 3b). At this dose, therefore, 4 repeated injections of GnRH were sufficient to reverse the lack of sensitivity to single injections of exogenous GnRH at this dose in non-breeding males.

Although there was no statistical difference in LH response between breeding and non-breeding males given a single injection of the highest GnRH dose of 1·0 μg, the effects of multiple GnRH injections as this dose were also investigated in non-breeders (Fig. 3c). The LH response in non-breeding males following multiple injections of GnRH was greatest at this dose: plasma LH concentrations increased from $3{\cdot}3 \pm 1{\cdot}1$ to $40{\cdot}3 \pm 7{\cdot}8$ mi.u./ml after 4 injections and decreased to $27{\cdot}0 \pm 5{\cdot}0$ mi.u./ml after 8 injections. Both values were comparable to those of breeding males after a single injection of 1·0 μg GnRH ($32{\cdot}8 \pm 5{\cdot}1$ mi.u./ml; Fig. 3c).

Discussion

While breeding naked mole-rat males had larger testes, both in terms of absolute size and relative to their body mass, microscopic examination revealed the presence of spermatozoa in the seminiferous tubules and epididymides of breeding and non-breeding males, suggesting that active spermatogenesis was occurring in these animals, and confirming the observations of Jarvis (1991). Breeding and non-breeding naked mole-rats exhibited a sparse distribution of seminiferous tubules, and the presence of large quantities of interstitial cells, as reported by Fawcett *et al.* (1973) and Jarvis (1991). Histological examination suggested that the greater testis size of breeding males may have been due to the presence of greater numbers of interstitial cells than in non-breeders. The functional significance of this remains unknown, but large accumulations of testicular interstitial tissue do not appear to be a characteristic of hystricomorph rodents *per se*, because it was not observed in chinchilla, *Chinchilla laniger*, or agouti, *Dasyprocta aguti* (Weir, 1967). Fawcett *et al.* (1973) estimated that interstitial cells made up approximately 60% of the testicular mass in naked mole-rats, although the breeding status of the animals which were investigated was not noted. This was markedly different from the guinea-pig, in which interstitial cells were found to represent only about 2% of the volume of the testis (Fawcett *et al.*, 1973).

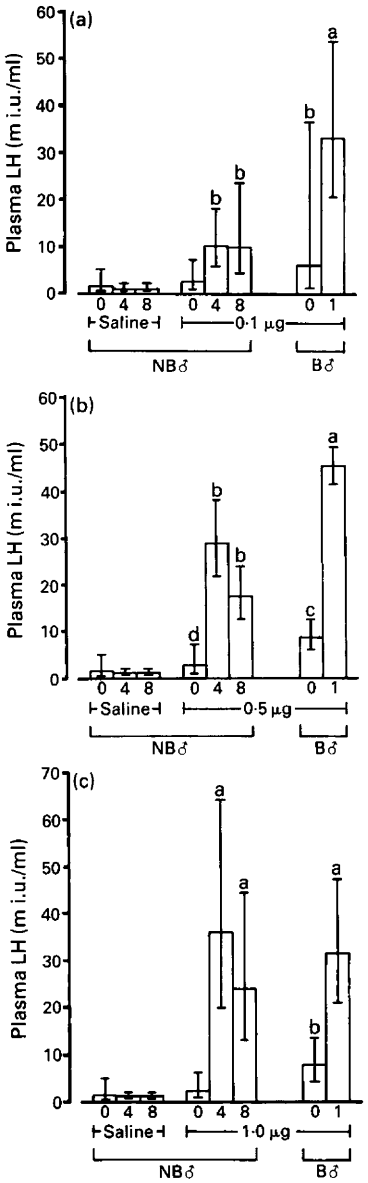

Fig. 3. Concentrations of plasma LH (antilog of the transformed mean ± 95% confidence limits) in breeding (B♂) and non-breeding (NB♂) male naked mole-rats before (0), and 20 min after, a single s.c. injection (1), or 20 min after the last of 4 or 8 s.c. injections of GnRH given at hourly intervals, or an equivalent saline control. (**a**) 0·1 μg GnRH dose: a, $P < 0{\cdot}05$ *vs* breeding males at time 0 and all non-breeding male values; b, $P < 0{\cdot}05$ *vs* non-breeding males at 0 time and saline controls. (**b**) 0·5 μg GnRH dose: a, $P < 0{\cdot}05$ *vs* 0 time values (B♂ and NB♂), saline controls and NB♂ 20 min after 8 injections; b, $P < 0{\cdot}05$ *vs* 0 time values (B♂ and NB♂) and saline controls; c, $P < 0{\cdot}05$ *vs* NB♂ 0 time values and saline controls; d = $P < 0{\cdot}05$ *vs* saline controls. (**c**) 1·0 μg GnRH dose: a, $P < 0{\cdot}05$ *vs* 0 time values (B♂ and NB♂), saline controls; b, $P < 0{\cdot}05$ *vs* NB♂ 0 time values and saline controls. (Duncan's multiple-range test following 1-way ANOVA for repeated measures.)

Despite the fact that spermatogenesis was occurring in all the males examined, the endocrine results show that, as with female naked mole-rats, there were clear physiological differences between breeding and non-breeding males. Breeding males had significantly higher basal concentrations of plasma LH, and greater LH responses to single injections of GnRH at lower doses (0·1 and 0·5 μg), compared with non-breeding males. The lower concentrations of plasma LH in non-breeding males were reflected in lower concentrations of urinary testosterone in these individuals.

While basal concentrations of plasma LH in non-breeding males were less than in breeding males, they were, however, higher than those measured in non-breeding females (5·0 ± 0·8 and 1·6 ± 0·1 mi.u./ml respectively; Faulkes *et al.*, 1990b). It is not known whether these differences in plasma LH concentrations are due to changes in LH pulse frequency or amplitude of LH secretion from the pituitary. While a physiological suppression mechanism appears to be in operation in non-breeding male naked mole-rats, the hypothalamic–gonadal axis may be more active in non-breeding males than in non-breeding females. This hypothesis is consistent with anatomical and histological investigations mentioned above and reported by Jarvis (1991), which suggest that most non-breeding males produce mature gametes. Conversely, production of mature gametes does not normally occur in non-breeding females because ovulation is blocked (Faulkes *et al.*, 1990a), and the ovaries of these females are under-developed and lack preovulatory follicles and corpora lutea (Kayanja & Jarvis, 1971; Faulkes, 1990). Therefore, in physiological and anatomical terms, the socially-induced suppression of reproduction in non-breeding male naked mole-rats does not appear to be as complete as in non-breeding females, and these non-breeding males may be capable of fertilization should they mate.

The socially-induced block to ovulation in non-breeding female naked mole-rats appears to be due to reduced plasma LH concentrations arising from an inhibition of hypothalamic GnRH secretion (Faulkes *et al.*, 1990b). Results from the present GnRH experiments suggest that some degree of suppressed hypothalamic GnRH secretion may also occur in non-breeding males. Like females, male naked mole-rats showed clear differences in their LH responses to different doses of exogenous GnRH. While breeders produced greater LH responses which did not decline at lower doses, non-breeding males showed a reduced LH response to 0·1 and 0·5 μg doses of GnRH, giving rise to significantly lower plasma LH concentrations 20 min after injection, compared to breeders (Fig. 2), and suggesting a lack of pituitary sensitivity to GnRH in the non-breeding males at these doses. Assuming that clearance rates of plasma LH do not differ between breeding and non-breeding males, then, as with females (Faulkes *et al.*, 1990b), this apparent lack of pituitary sensitivity may result from reduced concentrations of pituitary LH receptors, as a consequence of a lack of endogenous GnRH priming. In female rats, changes in sensitivity to GnRH are reflected in changes of pituitary GnRH receptor concentrations (Sandow, 1983; Clayton & Catt, 1987). The fact that the higher dose of 1·0 μg GnRH stimulated an LH response in non-breeders that was equivalent to that of breeding males, suggests that the pituitaries of non-breeders contained a similarly sized releasable pool of bioactive LH.

Non-breeding males responded to repeated injections of GnRH in a similar way to non-breeding females (Faulkes *et al.*, 1990b). Although 4 or 8 consecutive doses of 0·1 μg GnRH failed to reverse the lack of pituitary sensitivity to single injections at this dose (Fig. 3a), 4 consecutive hourly injections of 0·5 μg GnRH were sufficient to reverse the reduced pituitary sensitivity to a single injection of this dose (Fig. 3b). The ability of 4 priming injections of GnRH to overcome reduced pituitary sensitivity to GnRH in non-breeding males therefore suggests that reduced or impaired secretion of hypothalamic GnRH may result in reduced endogenous GnRH priming of the pituitary, compared with breeding males. Repeated administration of GnRH has been shown to increase pituitary LH content in hypogonadal male mice, a mutant which lacks endogenous hypothalamic GnRH (Charlton *et al.*, 1983), and increase plasma LH levels in men with hypothalamic hypogonadism (Snyder *et al.*, 1979).

Examples of the social suppression of reproduction in males of other species are less well documented than for females. Among rodents, perhaps the best example is the prairie deermouse,

Peromyscus maniculatus bairdii, in which juvenile males are thought to be inhibited in their maturation by pheromones from adult males (Lawton & Whitsett, 1979). Subordinate males in social groups of marmoset monkeys have their sexual behaviour disrupted by dominant males, although they are occasionally seen copulating with the dominant breeding female. Like non-breeding male naked mole-rats, these subordinate 'non-breeding' male marmosets had lower concentrations of plasma LH and testosterone, and reduced LH responses to GnRH, although spermatogenesis was not inhibited (Abbott, 1986). In the subordinate male marmoset, lower plasma testosterone concentrations may arise as a result of an alteration in the steroidogenic pathway by which androgens are synthesized (Sheffield *et al.*, 1989).

These examples of reproductive suppression in male mammals are not so extreme as in the male naked mole-rat, in which most non-breeding males may never breed, despite having a lifespan which can exceed 15 years in captivity (Jarvis, 1991). Among other African mole-rats (Family Bathyergidae), the genus *Cryptomys* contains socially-living species which exhibit a behavioural and reproductive division of labour (Bennett, 1988). Colony sizes are considerably smaller than those of the naked mole-rat and may only number up to 12–22 individuals, in the case of *C. hottentotus damerensis* (Bennett & Jarvis, 1988). Behavioural studies in captivity of *C. h. hottentotus* and *C. h. damarensis* have shown that, in these species, reproduction is limited to 1 male (Bennett, 1988, 1989). Histological investigations of non-breeding male *C. h. damarensis* have revealed that, as with non-breeding male naked mole-rats, spermatogenesis occurred in non-breeders, again making it difficult to relate the observed behavioural differences to functional changes, detrimental to fertility, in the reproductive tract.

While the present study showed that there were definite differences in the reproductive physiology of breeding and non-breeding male naked mole-rats, these differences were less clear-cut than in female naked mole-rats (Faulkes *et al.*, 1990a, b). It is difficult to relate these differences in physiology in the male naked mole-rat to differences in fertility between breeders and non-breeders because spermatogenesis is maintained in both types of males. Because of the reduced plasma LH and testosterone concentrations, the development and secretion of accessory sexual glands may be reduced in non-breeding male naked mole-rats, which may, in turn, have a detrimental effect on sperm viability in the ejaculate. A characteristic of the male hypogonadal mouse is a failure of accessory sexual tissue growth (Charlton *et al.*, 1983).

The endocrine differences between breeding and non-breeding male naked mole-rats may be brought about by a suppression of reproductive function in the non-breeders, or may simply reflect that only breeding males are solicited for mating by the queen during oestrus and show active sexual behaviour.

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