Effects of GnRH analogues on pituitary–testicular function in free-ranging African elephants (*Loxodonta africana*)

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We tested the ability of several GnRH analogues to suppress pituitary–testicular activity and potentially musth in free-ranging African elephants (*Loxodonta africana*). In Study 1, adult bulls were given 4 or 12 mg GnRH antagonist (Detirelix) or saline i.m. on day 0 ($n = 3$ bulls per treatment). Animals were then recaptured on day 2 (about 48 h later) and given 300 µg GnRH i.v. to assess the ability of the antagonist to block pituitary activity. Detirelix reduced ($P < 0.05$) basal concentrations of serum LH and testosterone on day 2 compared with day 0, with no effect of dose. Similarly, LH and testosterone release induced by GnRH were also reduced ($P < 0.05$) in the Detirelix-treated bulls (50–70% reduction in peak concentrations). In Study 2, elephants were given 30 mg of a structurally similar GnRH antagonist (103-201-40; $n = 6$), 22.5 mg of a long-acting GnRH agonist (Lupron Depot; $n = 4$) or D-mannitol carrier ($n = 4$) i.m. on day 0. All bulls were recaptured and given GnRH on day 2 (103-201-40 treatment) or on days 2 and 20 (Lupron Depot treatment) after the initial injection. In contrast to Detirelix, 103-201-40 did not inhibit basal or GnRH-induced LH or testosterone secretion. Pituitary–testicular responses to Lupron Depot were initially stimulatory, as evidenced by increased ($P < 0.05$) LH and testosterone secretion on days 0 and 2. By day 20, basal LH concentrations had returned to baseline values and the response to GnRH was markedly reduced ($P < 0.05$), indicating that the pituitary was at least partially desensitized. Basal testosterone concentrations had also returned to baseline values by day 20 after Lupron Depot treatment. However, despite the attenuated LH response to GnRH, subsequent testosterone secretion was increased ($P < 0.05$) compared with controls, suggesting the testes of agonist-treated bulls had, instead, become hyper-responsive to small increases in LH secretion. These results suggest that GnRH analogues can suppress the pituitary–gonadal axis in African elephants; however, longer treatment periods, more frequent injection intervals or higher doses are probably needed to inhibit testosterone secretion completely and, thus, musth.

Introduction

The phenomenon of musth has been recognized for centuries to occur in the Asian elephant (*Elephas maximus*) and, more recently in the African elephant (*Loxodonta africana*) (Poole and Moss, 1981; Hall-Martin and van der Walt, 1984). Musth occurs annually or biannually in most adult bulls and, although not absolutely necessary for breeding, it is regarded as an important reproductive strategy (Eisenberg *et al.*, 1971; Jainudeen *et al.*, 1972a; Poole and Moss, 1981; Poole, 1987, 1989a, b; Hall-Martin, 1987). In general, males in musth experience a temporary rise in dominance rank, are more successful at courting and mating oestrous females, and travel long distances for breeding, thus ensuring outbreeding and the transmission of genetic material. From a management standpoint, however,

musth bulls create serious problems because of associated increases in aggressive and unpredictable behaviour (Eisenberg *et al.*, 1971; Jainudeen *et al.*, 1972a). Captive elephants in musth have severely injured and even killed handlers, whereas, free-living musth bulls often threaten human life and property.

Present methods for controlling musth in captive elephants include isolation and reducing food and water intake, actions that could elicit animal welfare concerns. Although castration offers a permanent solution, the surgery is both difficult and often unacceptable since some bulls may eventually be needed for breeding. Because musth appears to be related to high circulating testosterone concentrations (Jainudeen *et al.*, 1972b; Hall-Martin and van der Walt, 1984; Howard *et al.*, 1984; Rasmussen *et al.*, 1984; Hall-Martin, 1987; Cooper *et al.*, 1990), any therapy that temporarily suppresses pituitary LH release and subsequent testosterone secretion might alleviate behaviour problems until the musth cycle ends. Several analogues

of GnRH have been shown to suppress LH and testosterone secretion in domestic and laboratory animals (Vickery *et al.*, 1984; Mann *et al.*, 1985; Lincoln *et al.*, 1986) and humans (Labrie *et al.*, 1980), and for this reason are being considered as possible male contraceptives (Bremner *et al.*, 1991; Pavlou *et al.*, 1991). Because of their antagonodotrophic activity, it is possible that these compounds might similarly inhibit endocrine function during periods of musth in the bull elephant. As a first step to considering this approach as a means of controlling musth, our objectives were to evaluate the effectiveness of two GnRH antagonists and a GnRH agonist to reduce LH and testosterone secretion acutely in mature bull elephants. We chose to conduct these studies using free-ranging males living in the Kruger National Park because of the scarcity of adult bull elephants in captivity.

Materials and Methods

GnRH analogues

In Study 1, a GnRH antagonist provided by B. Vickery (Syntex Research, Palo Alto, CA) (Detirelix; RS-68439) was used. In Study 2, Detirelix was unavailable; therefore, a second antagonist (103-201-40), synthesized by the Salk Institute (San Diego, CA) (NIH contract N01-HD-2-2824), was made available by M. Karten of the Contraceptive Development Branch, Center for Population Research, National Institutes of Health, Bethesda, MD. The two antagonist peptides are similar in structure except that Detirelix [Ac-D-Nal¹, D-4-Cl-Phe², D-Trp³, D-hArg(Et)₄⁶, D-Ala¹⁰-GnRH] has a D-diethylhomoarginine in the sixth position, whereas 103-201-40 (Ac-D-Nal¹, D-4-Cl-Phe², D-Trp³, D-Arg⁶, D-Ala¹⁰-GnRH) has a D-arginine. Detirelix and 103-201-40 may have similar potencies (M. Karten, personal communication). For injection, the antagonists were suspended in saline (Detirelix) or 2.5% D-mannitol (103-201-40). The GnRH agonist, Lupron Depot (leuprolide acetate; D-Leu⁶-Des Gly¹⁰-NHET-GnRH), was provided by J. Seely, Takeda-Abbott, Abbott Park, IL, and supplied as sterile, lyophilized microspheres (leuprolide acetate incorporated in a biodegradable copolymer of lactic and glycolic acids) that formed a suspension when mixed with diluent (carboxymethylcellulose sodium, D-mannitol, polysorbate 80 and water).

Animals and blood collection

Study 1 was conducted in the Kruger National Park in October–November and Study 2 in April–May. Although it was not possible to obtain individual body weights, all bulls were considered adult. Mean ($\pm$ SEM) body measurements were as follows: shoulder height (312 ± 4 cm; range, 260–334 cm), chest girth (427 ± 7 cm; range, 364–452 cm) and body length (304 ± 7 cm; range, 222–334 cm). There were no differences ($P > 0.05$) among body measurements between studies.

Bull elephants, free-ranging in bachelor herds of one to four animals, were approached by helicopter and anaesthetized with a remotely delivered dart injection of 15 mg etorphine–HCl (M99; Reckitt and Coleman, Hull). A surgical plane of anaesthesia was maintained with supplemental i.v. injections of this

same drug. All animals were fitted with radiocollars (Telonics, Inc., Mesa, AZ) to facilitate relocation and capture. Blood samples were collected at intervals of 5 min for 90 min via an indwelling catheter inserted into an ear vein. All treatments were administered after the third blood sample. GnRH analogues were administered i.m. by first inserting a 14-gauge, 4 cm long needle through the hide into the muscle and then inserting a 16-gauge, 10 cm long injection needle through the 14-gauge needle. To ensure complete delivery, injections were followed by a 3 ml saline flush through the 16-gauge needle. After the last blood sample, anaesthesia was reversed with an i.v. injection of 40 mg diprenorphine–HCl (Revivon; Reckitt and Coleman), and animals were observed until they stood up and walked away. Blood samples were kept cool on ice until serum was separated from cells by centrifugation (1000 g, 15 min) about 6 h after collection. Serum was frozen and stored on dry ice until transported to the United States in full compliance with US Fish and Wildlife and CITES regulations.

Musth ($n = 2$) and non-musth ($n = 23$) bulls were evaluated. Animals in musth were identified as those displaying temporal gland secretions, urine dribbling (with associated staining on the medial aspect of the rear legs) and having a strong odour (Poole and Moss, 1981).

Experimental protocol

Study 1. Nine elephants were evaluated. On day 0, each animal was injected i.m. with 4 or 12 mg Detirelix (in 6 ml saline) or saline (6 ml) ($n = 3$ bulls per treatment). On day 2 (about 48 h later), all animals were recaptured and challenged with GnRH (300 μ g, i.v.; 6 ml) to assess the ability of the antagonist to block pituitary function. One musth bull was found and included in the treatment group receiving 12 mg Detirelix.

Study 2. In this study, 14 bull elephants were given 6 ml D-mannitol carrier ($n = 4$), 30 mg GnRH antagonist 103-201-40 ($n = 6$) or Lupron Depot (equivalent to 22.5 mg leuprolide acetate; $n = 4$) i.m. on day 0. The results of Study 1 showed that Detirelix had not completely suppressed pituitary–testicular function; a higher dose of antagonist (103-201-40) was therefore chosen for this study. All antagonist-treated bulls were recaptured on day 2 and administered a GnRH challenge (300 μ g; 6 ml i.v.). Because of its different mechanism of action (initial pituitary stimulation followed by desensitization), Lupron Depot-treated bulls were recaptured and given GnRH challenges on days 2 and 20. For comparative purposes, four additional non-musth elephants were anaesthetized once and given a GnRH challenge on day 0 (day 0 GnRH control), and the results compared with animals challenged with GnRH on day 2 to determine whether repeated capture or anaesthesia affected pituitary–testicular responsiveness. One musth bull was found and given D-mannitol carrier on day 0 and challenged with GnRH on day 2. This animal was further evaluated by administering Lupron Depot after the last blood sample on day 2 and given GnRH challenges on days 2 and 20 after Lupron Depot treatment.

Radioimmunoassays

All assays were validated for use with elephant serum by demonstrating significant recovery of mass and parallelism

Fig. 1. Mean ($\pm$ SEM) serum (a,b) LH and (c,d) testosterone concentrations in non-musth bull elephants administered ($\circ$) saline ($n = 3$) or ($\bullet$) GnRH antagonist (Detirelix; $n = 6$), i.m. on day 0 (a,c) and given GnRH (300 μ g i.v.) on day 2 (b,d). Blood samples were collected at intervals of 5 min for 90 min and all treatments administered after the third sample (arrow).

between serial dilutions of pooled elephant serum and the standard curve. Serum LH was measured using a heterologous radioimmunoassay, with an anti-bovine LH antiserum (JJR 5; provided by J. Reeves, Washington State University, Pullman, WA), [¹²⁵I]-labelled ovine LH (LER-1056-C2; provided by L. Reichert Jr, Albany Medical College, Albany, NY) and ovine LH standard (NIADDK-LH-S18; provided by the National Hormone and Pituitary Program, Baltimore, MD). After adding 0.025, 0.05, 0.1, 0.2, 0.4, 0.8 and 1.6 ng ovine LH to 50 μ l serum and subtracting endogenous ligand, 0.021, 0.06, 0.13, 0.18, 0.43, 0.77 and 1.58 ng were recovered ($y = 0.98x + 0.009$; $r = 0.99$). Assay sensitivity (at 90% binding) was 0.1 ng ml⁻¹ for 200 μ l serum. Intra- and interassay coefficients of variation were 7.6 and 9.1%, respectively.

Serum testosterone was measured in unextracted serum using antisera and [¹²⁵I]-labelled testosterone purchased from ICN Biomedicals (Carson, CA). Upon adding 0.1, 0.25, 0.5, 1.0, 2.5 and 5.0 ng testosterone ml⁻¹ to 25 μ l serum, and after subtracting endogenous hormone, 0.12, 0.26, 0.47, 1.22, 2.88 and 4.95 ng ml⁻¹ were recovered ($y = 1.00x + 0.09$; $r = 0.99$). Because of high testosterone concentrations in serum from musth bulls, samples were diluted 1:10 with phosphate buffer (0.01 mol l⁻¹, pH 7.4) before analysis. Diluted samples of pooled musth bull serum also produced displacement curves parallel to the standard curve. The assay sensitivity was 0.05 ng ml⁻¹ for 50 μ l of serum, and the intra- and interassay coefficients of variation were 6.4 and 7.8%, respectively.

Statistical analysis

Data were analysed by analysis of variance. LH and testosterone responses to treatment were evaluated as peak height, net peak height (greatest post-treatment concentration minus pre-treatment concentration) and net area under the response curves as previously described (Brown *et al.*, 1988). Basal hormone concentrations were calculated as the mean of all samples (saline treatment on day 0) or as the mean of the initial three pre-treatment samples (GnRH or GnRH analogue treatments). Upon detection of a significant effect, differences among treatment groups were determined using Duncan's new multiple range tests or Student's *t* tests. Differences for hormonal data between days 0 and 2 were determined using paired *t* tests, allowing each animal to serve as its own control. Data are presented as means $\pm$ SEM.

Results

Study 1

Hormonal responses to both doses of Detirelix in non-musth bulls were similar ($P > 0.05$); data were therefore pooled for presentation (Fig. 1). On day 0, Detirelix administration had no effect ($P > 0.05$) on LH or testosterone secretion over the period when blood samples were taken. Concentrations of LH

Fig. 2. Serum (○) LH and (●) testosterone concentrations in a musth bull elephant administered 12 mg Detirelix i.m. on (a) day 0 and (b) given GnRH (300 µg i.v.) on day 2. Blood samples were collected at intervals of 5 min for 90 min and all treatments administered after the third sample (arrow).

in Detirelix-treated bulls were similar ($P > 0.05$) to those observed in saline-treated animals (Fig. 1a), whereas concentrations of testosterone were inexplicably higher ($P < 0.05$) before and after Detirelix treatment (Fig. 1c). There were no differences ($P > 0.05$) in basal testosterone concentrations between day 0 (1.38 ± 0.26 ng ml⁻¹) and day 2 (1.46 ± 0.59 ng ml⁻¹) in saline-treated bulls. In contrast, basal testosterone concentrations in Detirelix-treated animals were lower ($P < 0.05$) on day 2 (0.59 ± 0.16 ng ml⁻¹) compared with values measured on day 0 (3.18 ± 0.41 ng ml⁻¹) (Fig. 1c, d). Similarly, on day 2, LH (peak height, 3.08 ± 0.81 versus 6.68 ± 1.57 ng ml⁻¹) and testosterone (peak height, 3.31 ± 0.62 versus 10.69 ± 4.37 ng ml⁻¹) responses to GnRH were less ($P < 0.05$) in Detirelix-treated than in saline-treated bulls (Fig. 1b, d).

The one bull suspected of being in musth based on the criteria described had high testosterone concentrations (approximately 50 ng ml⁻¹; Fig. 2a) compared with non-musth bulls (overall mean, 2.25 ± 0.59 ng ml⁻¹; Fig. 1c). These high concentrations were apparently unrelated to increased basal gonadotrophin secretion, because LH concentrations (Fig. 2a) were similar to those observed in non-musth bulls (Fig. 1a). Treatment of the musth bull with Detirelix had no effect on basal LH or testosterone secretion on day 0. However, by day 2, testosterone concentrations were markedly reduced and the animal no longer appeared to be in musth (Fig. 2b). After the GnRH challenge, LH concentrations increased to a peak of 2.95 ng ml⁻¹ (Fig. 2b), within the range of that observed for the Detirelix-treated non-musth bulls, but less than the GnRH-

induced LH surge in saline-treated males (Fig. 1b). Although the LH response was attenuated in the antagonist-treated musth bull, testosterone secretion increased markedly (more than 30 ng ml⁻¹; Fig. 2b), exceeding the response observed in saline-treated non-musth bulls (Fig. 1d).

Study 2

Results of the GnRH challenge on day 0 (data not graphically presented) demonstrated that peak concentrations of LH (3.51 ± 0.19 ng ml⁻¹) and testosterone (8.55 ± 0.84 ng ml⁻¹) were similar ($P > 0.05$) to those observed in animals recaptured and given GnRH on day 2 (LH, 3.49 ± 0.56 ; testosterone, 8.60 ± 2.15 ng ml⁻¹; Fig. 3b, e), indicating that repeated capture or anaesthesia did not affect pituitary responsiveness.

In contrast to the results obtained for Detirelix in Study 1, basal and GnRH-stimulated LH and testosterone profiles were not reduced 48 h after administering 30 mg of the GnRH antagonist 103-201-40 (Fig. 3b, e). However, the GnRH agonist Lupron Depot markedly altered pituitary-testicular function beginning on day 0 (Fig. 3a, d). Within approximately 35 min of injection, LH concentrations in serum increased above baseline values, reaching 7.19 ± 1.08 ng ml⁻¹ by the end of the sampling period. On day 2, serum LH was still higher ($P < 0.05$) in the treatment than the control group, but the net response to native GnRH was attenuated compared to mannitol- and antagonist-treated bulls (Fig. 3b). By day 20 after Lupron Depot injection, serum LH had returned to baseline concentrations, and the response to GnRH was negligible (Fig. 3c). On day 0, testosterone concentrations increased within approximately 10 min of the Lupron Depot-induced rise in LH, reaching a maximum of 10.39 ± 4.63 ng ml⁻¹ (Fig. 3d). Forty-eight hours later, basal and post-GnRH testosterone concentrations were higher ($P < 0.05$) than in mannitol-treated controls (Fig. 3e). By day 20, basal testosterone concentrations had declined to values before Lupron Depot treatment; however, despite the small increase in GnRH-stimulated LH release, subsequent testosterone secretion increased 15-fold (Fig. 3f).

In the one musth bull of Study 2, basal concentrations of serum LH and testosterone (Fig. 4a) were similar to those observed for the musth bull in Study 1 (Fig. 2a). An unexpected finding was that LH and testosterone concentrations were markedly reduced 48 h after D-mannitol control treatment (Fig. 4b), similar to that observed for the Detirelix-treated musth bull (Fig. 2b). After the GnRH challenge, LH increased to a maximum of 5.23 ng ml⁻¹, slightly higher than the average response observed for non-musth control bulls (3.51 ± 0.19 ng ml⁻¹; Fig. 3b), whereas testosterone secretion increased about 100-fold over baseline concentrations (Fig. 4b). Two days after Lupron Depot treatment, basal LH and testosterone were raised and the responses to GnRH were minimal (Fig. 4c). By 20 days after Lupron Depot treatment, LH and testosterone concentrations were reduced to pre-Lupron Depot values. After the GnRH challenge, serum LH increased only slightly, to a peak of 1.15 ng ml⁻¹, but subsequent testosterone secretion increased dramatically, exceeding 50 ng ml⁻¹ (Fig. 4d). On the basis of temporal gland secretions and hind leg staining, this male remained in musth during the 2 days after Lupron Depot treatment, but was out of musth by day 22.

Fig. 3. Mean ($\pm$ SEM) serum (a,b) LH and (d,e) testosterone concentrations in non-musth bull elephants administered ($\circ$) mannitol carrier ($n = 4$), ($\bullet$) GnRH antagonist (103-201-40; $n = 6$) or ($\blacktriangle$) GnRH agonist (Lupron Depot; $n = 4$), i.m. on day 0 (a,d) and given GnRH (300 μ g i.v.) on day 2 (b,e). (c) and (f) show LH and testosterone concentrations, respectively, after a GnRH challenge on day 20 for the Lupron Depot-treated group only. Blood samples were collected at intervals of 5 min for 90 min with all treatments administered after the third sample (arrow).

Discussion

Both GnRH agonists and antagonists suppress the pituitary–gonadal axis, albeit via different mechanisms. Superactive GnRH agonists inhibit reproductive function by initially causing pituitary hyperstimulation followed by a decline in LH secretion as the gonadotrophs become desensitized (Karten and Rivier, 1986). The low dose requirements and long duration of action of the depot forms of GnRH agonists have made them popular therapeutic agents. However, when an immediate reduction in testosterone secretion is required, the initial transitory increase in gonadotrophin and subsequent testosterone secretion is an undesirable side-effect. Conversely, antagonist analogues of GnRH compete with endogenous GnRH for pituitary binding sites, resulting in an immediate suppression of gonadotrophin and gonadal steroid production (Karten and Rivier, 1986). Although higher and more frequent doses of antagonists are generally required, transient increases in hormone secretion are not observed, suggesting they may be more appropriate for acute therapeutic needs or when agonist treatment fails (Vickery, 1986).

Administering the GnRH antagonist Detirelix induces a rapid and sustained suppression of LH and testosterone secretion in many species (Weinbauer *et al.*, 1984; see review, Vickery, 1985; Bremner *et al.*, 1991). Typically, Detirelix is administered daily (1–1700 μ g kg⁻¹ body weight), although its suppressive effects

can last for up to 96 h after a single injection (Weinbauer *et al.*, 1984; Vickery, 1985). We speculated that bull elephants might be highly sensitive to the suppressive effects of GnRH analogues because only a small amount of native GnRH (<0.05 μ g kg⁻¹) is generally needed to maximally stimulate endogenous LH release (J. L. Brown, unpublished observations). However, acute administration of Detirelix (0.8–2.4 μ g kg⁻¹) resulted in only partial inhibition of LH and testosterone secretion two days after treatment. Unfortunately, Detirelix was unavailable for re-testing in Study 2; therefore, a structurally similar antagonist (103-201-40) was used. However, despite an increase in dose, this compound failed to inhibit pituitary LH secretion when examined 48 h after treatment. Although higher or more frequent doses might completely block pituitary activity, the data raise doubts about the practicality of these antagonists for long-term suppression of gonadal activity in elephants. The results also suggest that even minor differences in analogue structure can greatly influence physiological effectiveness, although we cannot rule out the possibility that differences in season, environment or vehicle may also have been a factor.

The GnRH agonist Lupron Depot is currently marketed for treating human prostate cancer because it inhibits testosterone secretion. In men, castrate concentrations of serum testosterone are achieved within two to four weeks and remain suppressed for at least 60 days after Lupron Depot injection (equivalent to 7.5 mg leuprolide acetate) (J. Seeley, Takeda-Abbott, personal

Fig. 4. Serum (○) LH and (●) testosterone concentrations in a musth bull elephant administered (a) mannitol carrier i.m. on day 0 and (b) given GnRH (300 µg i.v.) on day 2. This animal was then given Lupron Depot i.m. after the last blood sample on day 2 (double arrow) and GnRH challenges (300 µg, i.v.) on days 2 (c) and 20 (d) after Lupron Depot treatment. Blood samples were collected at intervals of 5 min for 90 min with mannitol and GnRH treatments administered after the third sample (arrow).

communication). In adult male elephants, Lupron Depot administration initially stimulated and then suppressed basal LH and testosterone secretion in a similar way to that described for primates and sheep (Resko *et al.*, 1982; Mann *et al.*, 1985; Lincoln *et al.*, 1986). However, the pituitary was still responsive to GnRH 20 days after treatment, albeit weakly, as evidenced by a modest increase in LH release. The surprising observation was the magnitude of the resulting testosterone response. These data contrast with previous findings in rats which showed that GnRH agonist treatment inhibited testicular responsiveness by decreasing gonadal LH receptor concentrations and blocking the steroidogenic pathway at the 17-hydroxylase and 17,20-desmolase steps (Belanger *et al.*, 1979, 1980; Labrie *et al.*, 1980; Seguin *et al.*, 1981). However, in male rhesus monkeys and sheep, chronic GnRH agonist treatment decreased pituitary responsiveness without abolishing the testicular response to exogenous LH or hCG, although testosterone secretion never exceeded that measured in controls (Resko *et al.*, 1982; Mann *et al.*, 1985; Lincoln *et al.*, 1986). In this regard, our data more closely resemble those reported for beef bulls in which chronic administration of the GnRH agonists nafarelin acetate (0.15 mg day⁻¹; Melson *et al.*, 1986) or leuprolide (3.3 or 10 µg kg⁻¹ body weight day⁻¹; Ronayne *et*

al., 1990) blocked pulsatile LH secretion, but induced significant increases in basal testosterone secretion. In the former study, the increase in testosterone secretion coincided with a more than threefold increase in testicular LH receptor concentrations (Melson *et al.*, 1986). However, one major difference between elephants and bulls was that basal testosterone secretion was not increased in elephants. Instead, testicular hyper-responsiveness only became evident after GnRH treatment. However, it is possible that increased GnRH-induced testosterone secretion in agonist-treated elephants is related to increased testicular LH receptor binding.

It is clear that the mechanism of action of GnRH agonists is quite complex. While a disruption of normal hypothalamo-pituitary function is typically observed, testicular responsiveness can be inhibited, unaffected or even stimulated. These wide variations in response among the present and cited studies may be related to the use of different agonists, doses and treatment periods. Alternatively, there may be species differences in the pituitary-testicular response to these compounds. For example, in rats, direct agonist effects on the testis may be mediated through specific testicular GnRH receptors that are present in that species (Lefebvre *et al.*, 1980), but lacking in others (mice, Hunter *et al.*, 1982; humans and monkeys, Clayton and

Huhtaniemi, 1982; bulls and sheep, J. L. Brown and B. E. Melson, unpublished observations). Unfortunately, the use of different agonists and experimental protocols precludes direct species comparisons at this time.

We would have preferred testing the GnRH analogues in more musth bulls. However, during the time we were in the field only one musth bull was found for each study. High concentrations of testosterone were measured in serum of both of these bulls, confirming our visual observations of musth and the existence of a relationship between high circulating androgens and these physical cues. In Study 1, administering Detirelix significantly reduced basal testosterone concentrations, suggesting that this compound might inhibit testicular function during musth. However, the pituitary response to GnRH was only partially blocked and it was clear that the testes were still capable of responding with substantial testosterone production even to a very modest increase in endogenous LH. Thus, as with non-musth bulls, higher doses or more frequent injection schedules may be needed to inhibit reproductive function completely. In Study 2, the musth bull was initially treated as a control. Surprisingly, basal testosterone concentrations in this animal were also reduced on day 2, suggesting that anaesthesia or the stress of re-capture affected testicular function. This raised the question as to whether the observed inhibitory effects of GnRH analogues in non-musth bulls were due to treatment or the associated manipulation. However, pituitary–testicular function apparently was not significantly altered by capture or anaesthesia stress when data were compared between elephants evaluated after only a single anaesthetic episode (day 0 GnRH controls) and those subjected to repeated capture (Study 1 and 2 controls). Instead, it appears that perhaps only the testes of musth bulls are highly sensitive to these manipulatory procedures. Whether this effect is due to direct inhibitory effects of anaesthesia or stress hormones on the testis itself, to altered pulsatile gonadotrophin secretion, or to some other mechanism remains to be determined. In Asian elephants, increases in LH pulse area and amplitude, but not frequency, are observed during musth (Niemuller and Liptap, 1991). It is also known that many anaesthetics, including etorphine as was used in this study, can block gonadotrophin pulsatility or reduce the frequency or amplitude of pulses (Peet and Lincoln, 1977; Clark and Doughton, 1983).

Another interesting observation in both musth bulls was the hyper-responsiveness of the testes to increases in GnRH-induced LH concentration. Although basal testosterone concentrations were reduced to less than 4 ng ml^{-1} on day 2, each animal responded to exogenous GnRH with a striking increase in testosterone secretion that greatly exceeded the responses of non-musth bulls. The cause of this increased testicular sensitivity to gonadotrophic stimulation is not immediately apparent, but again may be related to increased testicular LH-receptor binding. In other ungulate species, increased testosterone secretion during the breeding season or rut is known to be related to increased testicular LH receptor concentrations (Barenton and Pelletier, 1983; Brown *et al.*, 1991). The fact that elephants in musth share many of the traits displayed by other mammalian species in rut (Poole, 1987) lends further support to this hypothesis.

In summary, because of the many management problems associated with musth, few male elephants are kept in captivity.

However, with numbers continuing to decline in the wild, a treatment that acutely suppresses cyclic musth behaviour would be a valuable regulatory tool for captive elephant breeding that would help perpetuate the species and maintain genetic diversity. The ability to prevent bulls from exhibiting aggressive (and dangerous) behaviour would allow more zoos to maintain male elephants and participate in propagation programmes. The results of this study suggest that GnRH analogues can disrupt normal pituitary function and may be useful to this end. It is clear, however, that controlling testosterone secretion in musth bulls will require further testing of other treatment regimens to block LH and subsequent testosterone secretion completely. Perhaps the greatest challenge will be dealing with the finding of testosterone hyper-responsiveness to even minute increases in pituitary LH. In a preliminary report, Miller *et al.* (1990) reported that a single injection of 45 mg Lupron Depot (twice the dose used in this study) to a musth bull inhibited basal testosterone concentrations to castrate concentrations from day 10 to day 60. However, although the visual signs of musth were attenuated, they were not completely alleviated. Perhaps the total elimination of musth behaviour will require the administration of an anti-androgen, such as cyproterone acetate or Danazol, to block the action of testosterone on behaviour as well as an inhibitor of pituitary–testicular function to reduce total testosterone output.

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