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Testosterone response to a gonadotrophin-releasing hormone agonist in Hawaiian monk seals (*Monachus schauinslandi*)

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Adult male Hawaiian monk seals were administered a gonadotrophin-releasing hormone (GnRH) agonist to determine its effectiveness in reducing the testicular production of testosterone. Blood samples were collected from four treated seals and two control seals at weekly intervals for 10 weeks and again at the beginning of the following breeding season. The GnRH-agonist had an initial, brief, stimulating effect on circulating testosterone, but this was followed by an inhibitory effect that lasted for 7 to 8 weeks. The plasma concentrations of testosterone were within normal ranges by the following spring. These results demonstrate a reversible form of long-term androgen suppression, which may have applicability in a variety of wildlife management programmes.

Introduction

The Hawaiian monk seal (*Monachus schauinslandi*) was listed as 'endangered' in 1976 after a 50% reduction in the number of seals observed in beach counts over 20 years (Johnson *et al.*, 1982). In addition to the reduction in the number of animals, three of five major breeding populations also showed an imbalance in the adult sex ratio. Although the cause and chronological development of this condition remain uncertain, by the early 1980s, when extensive monitoring of the population began, male Hawaiian monk seals outnumbered females by at least 2:1 at Laysan Island (Johnson and Johnson, 1981), Lisianski Island (Stone, 1984) and Kure Atoll (Gilmartin, *et al.*, 1986). The disparate sex ratio at Kure Atoll was resolved by management actions which included introducing females and enhancing survival of young females (Gerrodette and Gilmartin, 1990; Van Toorenburg *et al.*, in press).

The skewed adult sex ratio still exists at Laysan and Lisianski Islands and it is associated with anomalous breeding behaviour termed 'mobbing'. Mobbings are mass matings wherein groups of males (4–14 or more) attempt copulation with a female or with an immature seal of either sex. Mobbing events have been observed to last several hours and usually result in severe injury and often death of the adult female or immature seal, either directly from the injury or indirectly by shark attack (Johnson and Johnson, 1981; Johanos and Kam, 1986; Alcorn and Buelna, 1989). The differential mortality between the adult sexes owing to mobbing attacks appears to be a major factor in maintaining these skewed sex ratios in adults and perpetuating mobbing incidents.

The Hawaiian monk seal is a seasonal breeder (Kenyon and Rice, 1959; Atkinson and Gilmartin, 1992) and mobbing attacks by adult males occur during the height of the breeding season, from May to July (L. M. Hiruki and W. G. Gilmartin, unpublished), when most females have weaned their pups and are thought to be resuming ovarian activity. This period correlates with the annual peak in circulating testosterone in males (Atkinson and Gilmartin, 1992).

Gonadotrophin-releasing hormone (GnRH) agonists have been used in humans to suppress secretion of testicular androgen to reduce the probability of metastasis of androgen-dependent prostate carcinomas (Parmar *et al.*, 1985). Similarly, GnRH agonists have been shown to suppress androgen production in dogs (Sandow *et al.*, 1980), monkeys (Sundaram *et al.*, 1982) and rodents (Redding *et al.*, 1984), with an attendant decrease of libido in dogs (Sandow *et al.*, 1980). However, reduction of testosterone production alone, via castration, does not always reduce androgen-mediated behaviour in dogs (Le Boeuf, 1970). In the harbour seal (*Phoca vitulina*) testosterone reduction by GnRH agonist has been shown to correlate with a reduction in sociosexual behaviour (Yochem *et al.*, 1991). Thus, we hypothesized that treatment of Hawaiian monk seals with a GnRH agonist would suppress testicular production of testosterone. The research reported herein was designed to test this hypothesis in captive adult male monk seals and, if an effect was detected, to measure the duration of testosterone suppression.

Materials and Methods

Animals and treatments

Five captive adult male Hawaiian monk seals, housed at Sea Life Park and Waikiki Aquarium, Oahu, Hawaii, were used for this trial. All of the male seals were at least 10 years old and annual veterinary physical examinations suggested that they

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were in good health. No adult females were present in the enclosures at either site. In June 1990, during the natural breeding season and when secretion of testosterone was at its highest (Atkinson and Gilmartin, 1992), three of the five seals (A, N and P) were given 11.25 mg of a GnRH-agonist, D-Trp6-luteinizing-hormone-releasing hormone (LHRH) (Decapeptyl-CR: Ferring, NY). The dose was determined by multiplying the human dose (3.75 mg) by 3, to approximate an equivalent dose for the seals who weighed about 200 kg. Seals S and Z were not given the drug at this time, but were handled and sampled at the same frequency as the test animals. The GnRH agonist was injected i.m. at three sites in the pelvic area of each of the treated seals. Blood samples were collected before treatment with the agonist and then weekly from all five seals for 10 weeks after the GnRH agonist was administered. The blood sampling procedure has been described (Atkinson and Gilmartin, 1992). Plasma from these samples was stored frozen at $-20°C$ until radioimmunoassay for testosterone could be performed. Four weeks after the first three seals were injected, one of the control seals, animal Z, was given 5.6 mg of the GnRH agonist. Blood sampling for this animal was continued for 8 weeks. Early in the following breeding season, 9 months after the GnRH was given, an additional blood sample was collected from each seal.

Hormone measurement

Testosterone was measured in the plasma with a single antibody radioimmunoassay (Atkinson *et al.*, 1986; Atkinson and Gilmartin, 1992). All oestrogen and progesterone compounds had $< 1\%$ crossreactivity (Atkinson *et al.*, 1986). Crossreactivity with dihydrotestosterone, 4-androstene-3β,17β-diol and androstenedione was 98, 47 and 4.7%, respectively, thus measuring the majority of circulating androgens. Nonspecific binding of the antibody was $< 5\%$, and the sensitivity of the assay was 0.03 ng per tube. Extraction recovery was 88.1%. A pool of monk seal plasma containing 2.06 ± 0.18 ng testosterone ml^{-1} plasma was included in each assay to determine interassay variation, which was $< 15\%$.

Statistical analysis

Log–logit transformations were applied to the standard curve of the assay (Rodbard, 1974). Student's *t* test was used to compare the differences in hormone concentrations before and after injection.

Results

Two of the three seals, P and N, injected with 11.25 mg GnRH agonist, and seal Z, injected with 5.6 mg, showed transient increases in circulating testosterone 1 week after the injection (Fig. 1). Within 2 weeks of the injections, the plasma concentration of testosterone in all seals had fallen significantly ($P < 0.001$) and remained at similar values to that in castrated animals for 7 to 8 weeks. Between 9 and 10 weeks after the injection, testosterone concentrations in seals A, P and N began to rise (Fig. 1a–c). Testosterone concentrations in seal Z also increased, although the sampling frequency was reduced. The control seal (S) maintained high and relatively constant plasma testosterone concentrations throughout the study (Fig. 1e), while being exposed to the same sampling stress as the experimental protocol. Early in the following breeding season, the mean testosterone concentrations of the treated seals (0.52 ± 0.08 ng ml^{-1}) were similar to that of the control seal (S: 0.60 ng ml^{-1}).

Discussion

This study demonstrated that an injection of a GnRH agonist can be used to reduce circulating testosterone concentrations of Hawaiian monk seals for about 2 months. The GnRH agonist used in this study (D-Trp6-LHRH) is a potent synthetic LHRH analogue which differs from the naturally occurring LHRH in that the glycine in amino acid position 6 has been replaced by D-tryptophan. This replacement leads to enhancement of the affinity for the LHRH receptors in the pituitary and a longer than normal half-life of the analogue. GnRH agonists initially stimulate the anterior pituitary to produce increased amounts of luteinizing hormone (LH). This was apparent in three of the four seals injected in the present study. While the increase was variable in the animals, we suspect that this was due to the sampling interval and recognize the need to characterize this transient increase with more frequent sampling.

Large doses (Sundaram *et al.*, 1982) or chronic administration of GnRH agonists (Bergquist *et al.*, 1979) have the paradoxical effect of decreasing LH, which leads to a decrease in the production and secretion of gonadal steroids. This decrease in the pituitary response is thought to reflect a desensitization of the pituitary or a refractory phase, accompanied by the down-regulation of local LH receptors (Sundaram *et al.*, 1982). The decreased testosterone is probably due to a decrease in the concentrations of 17,20-desmolase and 17α-hydroxylase in the Leydig cells, leading to a block in the steroidogenic pathway to testosterone synthesis (Chasalow *et al.*, 1979).

All but one dose used in the present study were scaled on a body weight basis from the dose recommended for treatment of male humans with androgen-dependent prostate carcinomas. One seal in the study received 5.6 mg of the GnRH agonist, which is half the dose of the other three treated seals. This half dose resulted in the same reduction in plasma testosterone observed in A, N and P, suggesting that the dose administered to these seals was well above the threshold required to achieve the desired response.

The concentrations of testosterone at the start of the study were similar for all animals except for seal A. Although the testosterone concentration appeared high in seal A, it was within the normal range of captive and wild seals sampled to date (Atkinson and Gilmartin, 1992).

This study was undertaken as a preliminary assessment leading to use of a GnRH agonist as an agent to control aggressive sociosexual behaviour, mobbing, in Hawaiian monk seals in the wild. While the animals in this study were housed in naturalistic enclosures, they were not exposed to adult female monk seals. Thus, it was not possible to assess the effects of the treatment on libido or aggressive behaviour, which exist in the wild during the breeding season when oestrous female seals are available.

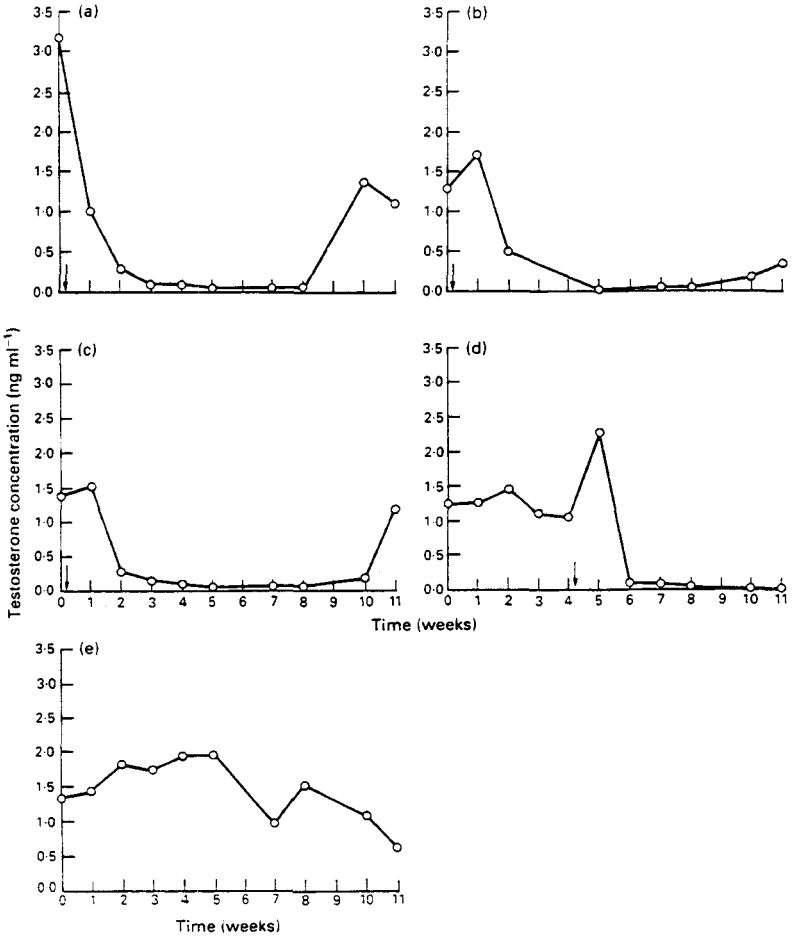

Fig. 1. Testosterone concentrations in the plasma of male Hawaiian monk seals injected with D-Trp6-luteinizing-hormone-releasing hormone (LHRH): 11.25 mg for seals (a) A, (b) P and (c) N and (d) 5.6 mg for seal Z. The arrow marks the time of injection. (d) Seal Z served as a control prior to administration of D-Trp6-LHRH at week 4. (e) Seal S served as a control throughout the experiment.

GnRH agonists have various effects on fertility, depending on the species. Dogs (Sandow *et al.*, 1980) appear to be more sensitive to the antigonadal effects of GnRH agonists than are primates (Sundaram *et al.*, 1982) or rats (Chasalow *et al.*, 1979). Sperm production and quality were not measured in the present study; however, the plasma testosterone concentrations of the treated seals did return to the normal breeding season concentrations (Atkinson and Gilmartin, 1992) by the following season, indicating that any effects on fertility were probably reversible. The effects on fertility are an obvious consideration that should be assessed, if restoration of breeding ability is deemed critical, before such treatments are used for behaviour modification in wildlife management programmes.

The GnRH agonist tested, D-Trp6-LHRH, demonstrated the ability to control production of testicular androgen in monk seals for about 2 months. Use of a repeated dose of this agent at 2 month intervals should allow suppression of testosterone for longer periods, increasing the probability that aggressive sexual behaviour in adult male Hawaiian monk seals may be controlled and the number of mobbing attacks reduced.

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