Altered androstenedione to testosterone ratios and LH concentrations during musth in the captive male Asian elephant (Elephas maximus)

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Summary. Greater concentrations of androstenedione than testosterone were usually present during periods of non-musth in plasma collected weekly for various periods up to 2 years in 8 male Asian elephants (4–35 years of age). For the 6 males that exhibited musth the androstenedione/testosterone ratio shifted greatly in favour of testosterone. The severity of musth was assessed weekly using a scale of 1 to 5 for each of 8 behavioural traits including urine dribbling, temporal gland secretion and aggression. A significant correlation (P < 0.05) was noted between plasma testosterone concentrations and the musth score value in 5 of 6 musth episodes. Brief shifts in the ratio of the two androgens when testosterone predominated (n = 106) were seen during the non-musth period in 3 of the males studied continuously for 2 years. In 82% of these instances, stimuli of a sexual or aggressive nature had occurred in the preceding 48 h (x², P < 0.01).

A heterologous bovine assay was used to measure LH values in plasma collected every 15 min for 12 h. Increases in testosterone concentrations followed pulsatile increases in plasma LH concentrations during 7 non-musth periods in 4 animals. Apart from pulse frequency, increases in the variables describing pulsatile LH secretion were seen in 2 strong musth and 2 mild musth episodes compared to non-musth values. A strong musth, however, was characterized by a much greater increase in pulsatile testosterone secretion than was a mild musth and which may be a function of the duration of musth.

Keywords: elephant; musth; androgens; luteinizing hormone

Introduction

Musth is a temporary aggressive state which may occur annually or semi-annually in the mature male Asian elephant (Eisenberg et al., 1971; Jainudeen et al., 1972a, b; Rasmussen et al., 1984). It has also been documented in wild (Poole & Moss, 1981; Hall-Martin & van der Walt, 1984) and captive (Brannian et al., 1989) African bulls. A male elephant in musth has elevated testosterone levels (Jainudeen et al., 1972a; Hall-Martin & van der Walt, 1984; Poole et al., 1984; Rasmussen et al., 1984) and shows urine dribbling, swollen temporal glands which eventually drain and increased aggression toward other males (Jainudeen et al., 1972a; Rasmussen et al., 1984; Poole & Moss, 1981; Poole, 1987; Hall-Martin, 1987). Although a wild bull can breed in or out of musth, reproductive success is believed to be increased in the musth state (Eisenberg et al., 1971; Hall-Martin, 1987; Poole, 1987, 1989a, b).

In the wild state, despite the high levels of aggression, both Asian and African bulls in musth are usually more tolerant towards cows and have been observed mating (Eisenberg et al., 1971; Kurt, 1974; Poole, 1987, 1989a, b). However, in captivity, male African and Asian elephants in musth

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become decreasingly responsive to their handlers and may be extremely dangerous to keepers and often to other elephants. The length and severity of musth are variable, creating a problem in the management of this species. The following experiments were an attempt to gain an improved understanding of the hormonal basis of musth in conjunction with behavioural changes.

Materials and Methods

**Animals and sampling.** Eight male Asian elephants (*Elephas maximus*) aged 4–35 years were used. Blood was taken weekly from an ear vein between 08:00 and 10:00 h from all animals for various lengths of time up to 2 years, including periods of musth and non-musth. A catheter was placed in the saphenous vein of 4 of the elephants to facilitate more intensive sampling. Blood samples were centrifuged immediately after each collection, the plasma divided into appropriate aliquots and frozen at −20°C.

**Assessment of musth.** The severity of musth was assessed weekly by the same handlers using a scale of 1 to 5 for each of 8 behavioural traits: degree of swelling and drainage from the temporal gland; presence or absence of urine dribbling; changes in appetite or weight loss; increased signs of aggression towards unknown people, towards animals, towards known people and towards inanimate objects; and increased activity level. Thus a total possible score of 40 for a bull in musth could be achieved. These semi-objective behavioural scores were later examined in association with the fluctuations of the weekly plasmas androgen concentrations leading into, during and out of musth.

**Radioimmunoassay of LH.** A double-antibody, heterologous assay based on the method of Niswender et al. (1969) was used to estimate LH values. Bovine LH (UCB-Bioproducts S.A., Braine-L’Alleud, Belgium) was iodinated using chloramine T (Greenwood et al., 1963) and isolated by gel filtration through a Bio-gel P60 column. Anti-bovine LH (UCB-Bioproducts S.A., Belgium) was diluted to a final working volume of 1:14 000. The second antibody used was goat anti-rabbit globulin. To confirm the suitability of the assay, a crude extract of elephant pituitary powder at a concentration of approximately 100 mg pituitary extract/4 ml in 0-01 n-NaHCO₃ (pH 8-5) was treated according to McNeilly et al. (1983). Parallelism was seen when various aliquots of putative peak concentrations of LH in elephant plasma, as well as the pituitary extract were assayed at various dilutions in the bovine LH assay system. Results are reported in terms of ng bovine LH (UCB) which is ×2-7 the potency of the NIH bovine standard (LH-B5). The sensitivity of the LH assay was 9 pg based on 2 standard deviations from the zero value. The intra-assay and inter-assay coefficients of variation were 8·5% (*n* = 9) and 10·4% (*n* = 9) respectively.

**Steroid radioimmunoassays.** Plasma concentrations of testosterone and androstenedione were measured in duplicate by specific radioimmunoassay procedures, after extraction with diethyl ether. The method of Castro et al. (1974) was used to measure concentrations of testosterone, while the assay for androstenedione was based on that of Raeside & Xun (1986). The cross-reactivity of the testosterone antibody (UCB-Bioproducts S.A., Belgium) was 50% with dihydroxytestosterone and <0·5% for 17α-hydroxyprogesterone, androsterone, androstenedione and oestradiol. Antibody to androstenedione (UCB-Bioproducts S.A., Belgium) cross-reacted 4% with androsterone, 2% with testosterone and <1% with dihydroepiandrosterone, dihydroxytestosterone, progesterone and 17α-hydroxyprogesterone.

Mean recovery values for testosterone and androstenedione were >85% based on extraction/recovery procedures with radioactive labelled steroid in elephant plasma. The sensitivities of the assays were 165 pg/ml (*n* = 10) for testosterone, and 187 pg/ml (*n* = 10) for androstenedione. The intra-assay coefficient of variation for testosterone was 4·23% (*n* = 10) and for androstenedione was 5·6% (*n* = 10). The inter-assay coefficients of variation for testosterone and androstenedione were 7·4% (*n* = 10), and 9·1% (*n* = 10), respectively.

**Statistical analysis.** Pulse parameters were assessed by the cluster analysis method of Veldhuis & Johnson (1986). The variance model used was the median experimental within-sample standard deviation determined from the 49 samples in each animal sampling period. The test nadir and peak sizes were 1 and 2 samples respectively with a *t* statistic of 2·62 which allows for a maximal false-positive rate of 5% on random measurements. χ² analysis was performed to examine the significance of association between changes in the ratio of plasma androgens and the occurrence of behavioural events. Correlation coefficients were calculated for the weekly changes in testosterone concentrations and the assigned musth score values.

Results

**Weekly hormone concentrations**

The overall mean (±s.d.) weekly plasma concentrations of testosterone and androstenedione during non-musth for 8 male Asian elephants are given in Table 1. During musth the mean androgen ratio shifted to 0·41 from a mean value of 1·0 during non-musth. For the 6 males which
Table 1. Mean (± s.d.) plasma concentrations of testosterone (T), androstenedione (A) and ratios of A/T in blood samples collected at weekly intervals from 8 male Asian elephants (*Elephas maximus*) during the non-musth period and episodes of musth

<table>
<thead>
<tr>
<th>Elephant</th>
<th>Age (years)</th>
<th>Severity of musth*</th>
<th>n†</th>
<th>T (ng/ml)</th>
<th>A (ng/ml)</th>
<th>A/T ratio</th>
<th>Musth</th>
<th></th>
<th></th>
<th>Non-musth</th>
</tr>
</thead>
<tbody>
<tr>
<td>Buke</td>
<td>22</td>
<td>Strong</td>
<td>19</td>
<td>116·64</td>
<td>36·94</td>
<td>0·33</td>
<td>68</td>
<td>7·80</td>
<td>10·27</td>
<td>1·32</td>
</tr>
<tr>
<td>Tommy</td>
<td>35</td>
<td>Strong</td>
<td>1</td>
<td>152·53</td>
<td>35·27</td>
<td>0·23</td>
<td>2</td>
<td>2·36</td>
<td>2·57</td>
<td>1·08</td>
</tr>
<tr>
<td>Indy</td>
<td>17</td>
<td>Medium</td>
<td>20</td>
<td>38·21</td>
<td>8·41</td>
<td>0·22</td>
<td>18</td>
<td>6·71</td>
<td>4·19</td>
<td>0·62</td>
</tr>
<tr>
<td>Rex</td>
<td>18</td>
<td>Mild</td>
<td>1</td>
<td>14·5</td>
<td>15·97</td>
<td>1·10</td>
<td>86</td>
<td>6·44</td>
<td>10·00</td>
<td>1·55</td>
</tr>
<tr>
<td>Vance</td>
<td>17</td>
<td>Mild</td>
<td>4</td>
<td>15·35</td>
<td>5·07</td>
<td>0·33</td>
<td>12</td>
<td>2·45</td>
<td>3·37</td>
<td>1·38</td>
</tr>
<tr>
<td>Rajah</td>
<td>16</td>
<td>Mild</td>
<td>2</td>
<td>20·70</td>
<td>5·99</td>
<td>0·29</td>
<td>7</td>
<td>3·50</td>
<td>2·43</td>
<td>0·70</td>
</tr>
<tr>
<td>Tusko</td>
<td>17</td>
<td>—</td>
<td>2</td>
<td>6·10</td>
<td>1·76</td>
<td></td>
<td>87</td>
<td>3·88</td>
<td>3·28</td>
<td>0·85</td>
</tr>
<tr>
<td>Hans</td>
<td>4</td>
<td>—</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>10</td>
<td>1·92</td>
<td>2·46</td>
<td>1·28</td>
</tr>
</tbody>
</table>

*Strong = 20–40; medium = 10–20; mild = 5–10.
†No. of samples.

Fig. 1. Weekly plasma concentrations of androstenedione (●) and testosterone (○) throughout 1 year in a 22-year-old male Asian elephant (*Elephas maximus*). Behavioural signs of musth were observed from Week 4 to Week 16. Inset shows hormone values for Weeks 41–56 when specific behavioural events occurred: m, mating; ♀, presence of new female; e, presence of oestrous females; f, fighting.
exhibited musth, the severity of musth increased with age and only bulls > 20 years of age showed a strong musth.

Figure 1 illustrates the weekly fluctuations in plasma androgen concentrations during non-musth and an episode of musth through a 1-year period (September 1987–1988) in one elephant. During musth (Weeks 4–16), testosterone was the principal androgen being secreted, although androstenedione production was also greater than that produced during non-musth. Apart from the time of musth, androstenedione was generally the principal androgen present in plasma. There were, however, occasions during non-musth when brief shifts in the androgen ratio occurred, resulting in greater testosterone concentrations. Retrospective analysis of the daily observational records kept by the handlers revealed that specific events, e.g. mating, fighting amongst males, introduction of new females or the presence of oestrous females, occurred during the 48-h period preceding sample collection and appeared to be associated with these brief androgen ratio shifts (inset Fig. 1). A change in the ratio of androstenedione to testosterone <1 during non-musth occurred on 106 occasions in the 3 male elephants studied over a continuous 2-year period. These shifts occurred in association with a behavioural event 82% of the time ($\chi^2$, $P < 0.01$).

Figure 2 depicts the temporal relationships between behavioural scores and the corresponding androgen changes during 2 musth periods in each of 3 male elephants. The male on the left (Buke) exhibited an annual musth of extreme severity, lasting from October to January, and which was closely synchronized from year to year. The male Indy exhibited a more moderate musth biannually, with a spring musth and a summer musth. Rex exhibited a short spring musth in 1988 while the musth for 1989 appeared in August. The severity of the behavioural changes shown by these males during musth appeared to parallel the increasing plasma androgen concentrations and to reflect the different intensities of musth exhibited. A significant correlation coefficient ($P < 0.05$) between plasma testosterone and musth score value was present in 5 of 6 musth episodes. Buke had 4 times the androgen values compared to those of Indy and Rex, with peak values of 223.4 ng/ml and 81.4 ng/ml respectively for testosterone and androstenedione versus 62.8 ng/ml and 12.6 ng/ml for Indy and 17.4 ng/ml and 15.97 ng/ml for Rex.

**Intensive collection periods**

More detailed observations regarding the changes in pulsatile hormone concentrations were obtained with frequent blood sampling during 12-h periods. Figure 3 shows the pattern of secretion during musth and non-musth for one of the elephants. A summary of the variables describing the pulsatile increases in LH and testosterone in 4 of the male elephants during 7 non-musth episodes are given in Table 2. Only 2 of the elephants were available for sampling during musth and the mean values for 2 successive years are shown in Table 3. No appreciable differences between musth and non-musth were seen in pulse rates for LH and testosterone. The percentage increases during musth in pulse amplitude, area and mean value for LH were similar in both animals and varied from 150 to 220%. However, marked differences were evident between the 2 animals in the increase in testosterone values present during musth. Buke exhibited increases in pulse amplitude, pulse area and mean concentration that were 4, 2.3 and 8 times greater, respectively, than those present in Rex.

**Discussion**

Jainudeen *et al.* (1972a) and Rasmussen *et al.* (1984) have reported that increased concentrations of testosterone are present in the circulation of Asian elephants during musth. The present results extend these findings and suggest that the severity of musth is correlated with the degree of increase in testosterone values. The development of a musth behaviour score chart was an attempt to provide a semi-objective means of comparing the severity of musth from year to year and between
Musth in the Asian elephant

Fig. 2. Weekly musth behaviour score value and plasma concentrations of testosterone and androstenedione through 2 musth episodes in each of 3 male Asian elephants (*Elephas maximus*) (Buke, 22 years, strong musth; Indy, 17 years, medium musth; Rex, 18 years, mild musth). Note different scale for androgen concentrations for Buke.

Fig. 3. Plasma concentrations of LH and testosterone in samples collected every 15 min for 12 h from a 22-year-old male Asian elephant (*Elephas maximus*) during non-musth and musth.
Table 2. Mean (± s.d.) pulsatile plasma hormone variables for luteinizing hormone (LH) and testosterone during 7 non-musth episodes in adult male Asian elephants (Elephas maximus) based on sampling every 15 min for 12 h

<table>
<thead>
<tr>
<th></th>
<th>LH</th>
<th>Testosterone</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of pulses/12 h</td>
<td>4.14 ± 1.34</td>
<td>3.71 ± 1.88</td>
</tr>
<tr>
<td>Pulse amplitude (ng/ml)</td>
<td>0.53 ± 0.35</td>
<td>5.50 ± 1.97</td>
</tr>
<tr>
<td>Pulse area (ng/h)</td>
<td>26.79 ± 27.67</td>
<td>375.61 ± 135.82</td>
</tr>
<tr>
<td>Mean (ng/ml)</td>
<td>0.85 ± 0.71</td>
<td>5.50 ± 3.21</td>
</tr>
</tbody>
</table>

Table 3. Mean pulsatile plasma hormone variables for luteinizing hormone (LH) and testosterone for 2 non-musth (NM) periods and 2 musth (M) episodes (Buke, strong; Rex, mild) in each of 2 adult male Asian elephants (Elephas maximus) based on sampling every 15 min for 12 h

<table>
<thead>
<tr>
<th></th>
<th>LH</th>
<th>Testosterone</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Buke</td>
<td>Rex</td>
</tr>
<tr>
<td>No. of pulses/12 h</td>
<td>NM</td>
<td>3.5</td>
</tr>
<tr>
<td></td>
<td>M</td>
<td>4.0</td>
</tr>
<tr>
<td>Pulse amplitude (ng/ml)</td>
<td>NM</td>
<td>0.48</td>
</tr>
<tr>
<td></td>
<td>M</td>
<td>0.74</td>
</tr>
<tr>
<td>Pulse area (ng/h)</td>
<td>NM</td>
<td>21.93</td>
</tr>
<tr>
<td></td>
<td>M</td>
<td>48.28</td>
</tr>
<tr>
<td>Mean (ng/ml)</td>
<td>NM</td>
<td>0.41</td>
</tr>
<tr>
<td></td>
<td>M</td>
<td>0.81</td>
</tr>
</tbody>
</table>

animals. Mild, medium and strong musth episodes were clearly evident and maximal scores of 0–10, 10–20 and 20+, respectively, were noted. A behavioural score value has the potential of providing a practical, comparative method of assessing the severity of musth and the effectiveness of medication that might moderate musth behaviour in captive animals.

During periods of non-musth, androstenedione concentrations generally exceeded those of testosterone. Urinary concentrations of androstenedione have been reported to increase in an African bull (Brannian et al., 1989) during musth. Although an increase in plasma androstenedione values occurred during musth the shift in the androstenedione/testosterone ratio was clearly in favour of testosterone. In seasonal breeders such as sheep (Sanford et al., 1974), horses (Thompson et al., 1985) and deer (Mirarchi et al., 1978) increases in testosterone in the male are present during the mating season. While the preponderance of births in zoos seems to have resulted from non-musth matings when male to male competition is presumably not a factor, the resulting more aggressive behaviour when testosterone concentrations are elevated may enhance reproductive success for the wild male elephant. Eisenberg et al. (1971) and Kurt (1974) observed wild Asian bulls in musth mating cows while Poole & Moss (1981) and Hall-Martin (1987) have reported that an African elephant in musth is a more successful breeder compared with males not in musth. In addition to the greater testosterone increase relative to androstenedione during musth, sporadic shifts in the androstenedione/testosterone ratio in favour of testosterone were seen during the non-musth period. In the majority of these instances, stimuli of an aggressive or sexual nature had occurred shortly before the brief elevation in plasma testosterone concentrations. The elephant appears to react similarly to the domestic bull (Katongole et al., 1971) and boar (Liptrap & Raeside, 1978) in which increases in testosterone occur after copulation and aggression.
Luteinizing hormone was measured in plasma collected at more frequent intervals during non-musth and musth. The immunoreactive values measured in the Asian male elephant with a heterologous bovine assay system were considerably lower than the concentrations of LH determined in African bull elephants with an ovine assay (McNeilly et al., 1983). This may be due to the different assay sytems used, because parallelism was present in both assays with elephant plasma and elephant pituitary extract.

Lincoln et al. (1985) have reviewed much of the evidence in sheep indicating that LH is released in a pulsatile manner under the influence of gonadotrophin-releasing hormone secretion by a putative hypothalamic pulse generator. Pulses of testosterone followed those of LH in a pattern more reminiscent of the ram than that of man on which pulses of LH and testosterone are not as tightly coupled. Although only 2 of the males exhibiting musth were available for more intensive sampling, these were examined on 2 successive musth episodes. Based on these limited data the number of pulses of LH and testosterone appeared to change very little from non-musth to musth. In seasonally breeding animals the pulsatile pattern of LH may vary during the year. Rams have a shorter interpulse interval during the breeding season (Lincoln & Short, 1980). The increase in values of the variables describing the pulsatility of LH were similar (150–220%) for both the strong (Buke) and mild (Rex) musth episodes. More dramatic increases in testosterone, however, were present in the strong musth compared to those in the mild musth. The strong musth lasted approximately 3 months compared to a 3 or 4-week duration of mild musth. The length of time the testes are exposed to an increase in LH secretion may be an important factor in determining the extent of increase in testosterone concentrations and hence the severity of signs associated with musth.

The present study indicates that a consistent shift in the plasma androstenedione/testosterone ratio in favour of testosterone is present during musth in the captive Asian elephant. Brief shifts in the ratio may also occur in association with aggressive or reproductive behaviour. The results suggest that the immediate hormonal regulatory mechanism is similar to that reported for males of other mammalian species. Further work, however, is necessary to determine what triggers the chain of hormonal events resulting in musth.

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References


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