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BRIEF REPORT

Urinary Testosterone Levels of Wild Male Bonobos (*Pan paniscus*) in the Lomako Forest, Democratic Republic of Congo

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We collected urine samples from seven male bonobos (*Pan paniscus*) in the Eyengo community, Lomako Forest, Democratic Republic of Congo, and assayed them for testosterone (T). T levels averaged 525 pmol/mg Cr in adult males, and 309 pmol/mg Cr in subadult males. We collected hormonal and behavioral data during a period of relative social instability following the recent arrival of two immigrant males. In concordance with predictions derived from the challenge hypothesis [Wingfield et al., American Naturalist 136:829–846, 1990], which relates T to levels of reproductive aggression, the alpha male had the highest circulating levels of T. When we removed the two recent immigrant males from the analysis, there was a significant positive correlation between T levels and dominance rank for the long-term resident males (n=5, P=0.001, r^2=0.98). These are the first data on T levels in wild bonobos, and the results suggest that further study of the relationship between T levels and social context in this species could inform current models relating hormones and aggression in wild apes. Am. J. Primatol. 65:87–92, 2005.

Key words: bonobos; testosterone; aggression; urinary steroids

INTRODUCTION

Research on a wide variety of species has demonstrated that testosterone (T) is critical for facilitating male aggression in the context of reproductive competition. Wingfield et al. [1990] hypothesized that increases in T during the breeding season were direct responses to aggressive challenges from conspecific males. This ''challenge hypothesis'' has received considerable support from studies of birds [e.g., Beletsky et al., 1995; Wingfield et al., 2000]. Evidence from

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some primate species also supports the challenge hypothesis. In several seasonally breeding primate species (e.g., ring-tailed lemurs [Cavigelli & Pereira, 2000], rhesus macaques [Higley et al., 1996], and capuchin monkeys [Lynch et al., 2002]), T levels have been shown to increase dramatically during times when matings are most highly contested to substantially higher levels than would be required to maintain normal reproductive function alone [Dixon, 1998].

Muller and Wrangham [2004] found a strong correlation between dominance rank and urinary T in chimpanzees, and reported that male chimpanzees showed acute increases in T when parous females were maximally swollen. Both of these results show that the predictions of the challenge hypothesis are generally upheld in chimpanzees. While bonobos are closely related to chimpanzees and share many general aspects of their social organization, various measures of male aggression and status competition indicate that dominance relationships among bonobos are relatively egalitarian compared to male chimpanzees [de Waal, 1995; Muller & Wrangham, 2001]. Although dominance hierarchies are less intensely contested in bonobos than in chimpanzees, dominance ranks have been clearly observed in bonobo males [Furuichi, 1997; Furuichi & Ihobe, 1994; Kano, 1992] and high rank has demonstrable fitness benefits [Gerloff et al., 1999; Kano, 1996]. Male bonobos at Lomako compete for access to estrus females, and by doing so increase their mating opportunities [Hohmann & Fruth, 2003]. Following the logic of the challenge hypothesis, it is reasonable to predict that high-ranking male bonobos would have higher levels of T than low-ranking individuals. We therefore collected urine from wild male bonobos to test whether or not T levels were correlated with dominance rank.

MATERIALS AND METHODS

Study Site and Subjects

We collected data from habituated, individually recognized bonobos living in the Eyengo community, located in the Lomako Forest, Democratic Republic of Congo. In 1998, when these data were collected, this community consisted of up to 15 adult females, five adult males, and 20 subadults and infants [Hohmann & Fruth, 1994; Hohmann et al., 1999]. The immigration of two adult males during this period led to social instability between community members. The resulting high rates of agonistic interaction between all of the males allowed us to easily determine linear rank relationships using both agonistic interactions and the direction and outcome of non-agonistic displacements [Hohmann & Fruth, 2003].

Collection and Preservation of Urine Samples

We collected clean, uncontaminated urine samples in July and August 1998 from below nests in the early morning, and opportunistically during behavioral follows throughout the day, following established methods [Knott, 1997; Muller & Wrangham, 2004]. Since some urine samples passed through canopy leaves before they were collected, and other samples were collected directly from leaves, we conducted a test to address possible contamination effects (following Muller and Wrangham [2004]). We obtained clean collections of urine from two human males, and separated them into untreated control and test groups. We poured six replicates of each of the test samples over leaves of the five plant species from which urine samples were typically pipetted. After 5–10 min, we collected and preserved these samples following the procedures described below for bonobo urine.

We utilized methods for processing urine samples that were based on techniques developed for humans [Campbell, 1994], wild apes [Knott, 1997], and captive primates [Shideler et al., 1995]. We pipetted 200 μl of urine onto 2.5 × 2.5 cm squares of absorbent filter paper in the evening of the day on which they were collected. We dried and stored five replicates of these filter paper samples in airtight plastic containers with silica gel.

Laboratory Analysis

A.J.M. analyzed all urine samples in the Reproductive Ecology Laboratory at Harvard University within 4 months of collection. We measured steroid levels using standard radioimmunoassay (RIA) techniques [Ellison, 1988] that were modified for use with primate urine samples. We deconjugated the samples by hydrolysis using the enzyme β-glucuronidase-arylsulfatase (Boehringer Mannheim, La Jolla, CA), and assayed T in the extracted samples using commercially available radioactive T (a four-position tritium competitor provided by Amersham-Searle, Arlington Heights, IL) and androgen antibodies (an antiserum to tetosterone-11-BSA, provided by Gordon Niswender of Colorado State University, Colorado). After incubating the samples for 24 hr, we separated bound and unbound steroids by adsorbing the unbound steroids to dextran-coated charcoal. The amount of bound competitor T was measured in a RacBeta liquid scintillation counter (LKB/Wallac). We ran all samples in duplicate, and each of the two standard curves in triplicate. Assay sensitivity averaged 10 pmol/mL. The average intra-assay variability (coefficient of variation, CV) at the 50% binding point of the standard curve was 7.2% (range=6.3–8.2%). The interassay variability (CV) averaged 4–10% for the high, medium, and low pools. We verified the linearity of response by assaying serial dilutions of T standard (predicted vs. observed values: $r^{2=}0.999$, $P<0.0001$). The average recovery value of the tritiated T was 88.8%. To correct for variation between samples due to variable clearance rates, we indexed all T values to creatinine (following Erb [1970], Robbins and Czekala [1997], and van Schaik et al. [1991]). We calculated creatinine levels colorimetrically using the Jaffe reaction [Taussky, 1954]. See Knott [1997] and Muller and Wrangham [2004] for a detailed explanation and validation of the methods used.

RESULTS

We collected a total of 17 urine samples from the seven males present at Lomako during the collection period. We excluded one additional sample (from BLA, the alpha male) from the analysis because the creatinine levels were too low (<0.06 mg/ml) for the T level to be reliably indexed. The mean creatinine level in all other samples was 0.225 mg/ml (SD=0.101, range=0.098–0.416 mg/ml). Pairwise comparisons of untreated controls and test samples of human urine pipetted from the leaves of five plant taxa at Lomako revealed no significant differences in T or Cr levels for any of the plant species tested ($n_{test}=6$, $n_{control}=6$, U=9.0–12.5 , $P>0.48$ for all tests), which suggests that the collection of urine by the methods described above was unlikely to have introduced significant contaminants into our samples.

The mean level of T in all samples (n=17) was 419 pmol/mg Cr. The mean level of all samples taken for the five adult males (n=9) was 538 pmol/mg Cr, and was significantly higher than the mean level for the samples taken from the two subadult males (n=8) 285 pmol/mg Cr (U=12, $n_{adult}=8$, $n_{subadult}=9$, $P=0.02$). There was substantial variation in T levels between samples within an individual. Although the sample sizes precluded the use of a formal test, T values appeared

TABLE I. Summary of Testosterone Results*

ID	AGE	RANK	AVG T (n)	AM T	PM T
BLA	>20	1	634 (3)	750	576
PLA	>35	2	531 (1)	531	–
PIN	~20	3	400 (1)	400	–
LEO	10–11	4	357 (2)	357	–
MON	10–11	5	261 (6)	300	241
ER	>20	6	474 (3)	542	338
XE	>35	7	586(1)	586	–

*This table provides summary data for the seven individuals from whom samples were collected. ''AVG T'' is the average levels of T in all samples collected from that individual. The number of samples is noted in parentheses. AVG T levels were higher in the five adult males than the two subadult males (U=10, N_1=5, N_2=2, P=0.053), and excluding the two new immigrant males (ER and XE), there was a significant positive correlation between T level and rank (P=0.001, r^2=0.98). ''AM T'' records mean testosterone levels in all samples collected before 10:00 hr. ''PM T'' lists T levels for samples collected after 10:00 hr. All T values are reported in pmol T/mg creatinine. Blank cells indicate that no sample was collected during the specified period. Age estimates are based on size and overall condition.

higher in the morning samples than in the afternoon samples. The results of the T assays are summarized in Table I.

To control for the fact that different numbers of samples were collected from each individual, we calculated an overall mean T value for each individual using the average of all samples collected from that individual. Using the mean T value for each male (n=7), the average T level was 463 $\pm$ 50 SE pmol/mg Cr. Using the same corrected mean values, adult males averaged 525 $\pm$ 41 SE pmol/mg Cr (n=5), and the subadult male average was 309 $\pm$ 48 SE pmol/mg Cr (n=2). Relative and absolute T values are not substantially changed if analysis is confined to only samples collected in the morning.

In accordance with predictions derived from the challenge hypothesis, the alpha male (BLA) had the highest T levels of any male. There was no significant overall correlation between average T and dominance rank. However, when the two recent immigrant males (ER and XE) were removed, there was a significant correlation between T levels and dominance rank of the remaining five males (n=5, P=0.001, r^2=0.98). This effect is found regardless of whether the mean or the morning T value is used for each male.

DISCUSSION

This study presents the first data on T levels in wild male bonobos. Although the relatively small number of samples collected precludes us from drawing definitive conclusions, the results suggest that circulating T levels in male bonobos are responsive to social context. Our results are compatible with the possibility that T levels in bonobo males respond in ways similar to those observed in male chimpanzees [Muller & Wrangham, 2004].

The observation that T levels were highest in the alpha male and that there was a positive correlation between T and rank for the long-term resident males in the Eyengo community suggest that T in male bonobos is responsive to dominance rank. However, these results are equivocal since there was no correlation between T and dominance rank across all males. It is unlikely that the relationship between dominance and T is as direct in bonobos as it is in chimpanzees, since dominance rank in bonobos reflects not only a male's competitive ability but also the rank and status of his mother [Furuichi, 1997; Kano, 1992], and perhaps his relationships with other females in the community [Hohmann et al., 1999]. The low rank of two

adult males (ER and XE) probably reflects the fact that both had recently joined the Eyengo community [Hohmann, 2001] and lacked the support of female allies. Both were more often involved in aggressive interactions than other males, and were targets of coalitionary charges by residents. The high rates of aggression associated with these two individuals may explain why they both had higher T levels than would be predicted by their rank. As noted above, if these two individuals are excluded, the T levels of the long-term residents do mirror rank.

For three of the four males for which we had multiple samples, there was substantial variation in T levels between samples from the same individual. The stability of our control samples over time and the almost identical values we found in multiple samples from human males collected at the same time and under the same conditions (unpublished data) suggest that the variations in our bonobo samples are not the product of assay error or differences in storage conditions. Rather, they most likely reflect real differences in circulating T levels at the time the samples were collected. The different samples were collected from individuals at different times on different days, often in the presence of different individuals, and it is reasonable to postulate that these differences resulted in varying circulating T levels.

Clearly, the results reported here must be considered preliminary because of the small number of samples that were available for analysis. However, these are the first data on T levels in wild bonobos, and the results suggest that further study of the relationship between T and behavior in bonobos can be expected to provide important insights into the physiological mechanisms underlying bonobo behavior [Hohmann & Fruth, 2003]. Of particular interest would be a comparison of the T levels found in our sample with a comparably collected and analyzed set of samples from wild male chimpanzees. The reduced levels of aggression in bonobos relative to chimpanzees suggest that T levels would be found to be lower in bonobos [Muller & Wrangham, 2001]. In addition, the collection of a larger number of samples over a longer period of time (i.e., during times of both stability and instability, or varying food availability) will elucidate the role of T in mediating behavioral differences between these closely related species, and help to clarify the nature of the interactions between T, dominance, and aggression in wild apes.

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