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Seasonal Changes in Urinary Prostate-Specific Antigenic Activity in Male Japanese Macaques (*Macaca fuscaa fuscata*)

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Abstract

Prostate-specific antigen (PSA) is usually detected in male adult urine and semen according to the Tanner stage development of males from birth to adolescence. To further study the pituitary-testicular axis in males, we determined urinary PSA levels in primates. Urinary PSA was detected with the use of anti-human PSA monoclonal antibody in male adult Japanese macaques (*Macaca fuscaa fuscata*) of seasonal breeding status. PSA activity in aseasonal animals (crab-eating macaques, *Macaca fascisularis*) did not change throughout the year; however, alterations in PSA activity were observed in Japanese macaques during breeding season, with the highest levels observed between October and January, the lowest levels between January and June, and a gradual increase in PSA activity observed from August until October. Although primate urinary PSA produces 2 polypeptide bands of approximately 55 and 33 kd, in addition to a band corresponding to human urinary PSA, the 33-kd polypeptide band was less pronounced during nonbreeding season in Japanese macaques. Urinary testosterone (T) levels in seasonally breeding animals (Japanese macaques) changed in parallel with urinary PSA levels. When urinary PSA and T levels were compared among animals during the breeding season (from October to February) and the nonbreeding season (from March to September), significantly increased PSA and T levels were observed during the

breeding season. Furthermore, PSA and T levels in a monkey housed in a cage placed between 2 female cages were elevated compared with other monkeys. Increased PSA activity was observed concurrent with menstrual blood loss in females. These results suggest a link between PSA activity and testosterone levels, which could be influenced by changes in the female menstrual cycle.

Key words: Prostate-specific antigen, testosterone, urine, seasonal breeding

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Human semen spontaneously coagulates into a semisolid gelatinous mass after ejaculation into the female genital tract by the action of semenogelin secreted from the seminal vesicles. After that, semen liquefaction occurs within 30 minutes by prostate-specific antigen (PSA) as a major component of prostatic fluid (McCormack et al, 1995; Michael et al, 2003). PSA secreted from the prostate belongs to the kallikrein family of serine proteases with chymotryptic-like activity (Robert and Gagnon, 1999; Michael et al, 2003). Sato et al (2002) have demonstrated that PSA can usually be detected in the semen and urine of males over 14 years of age and that urinary PSA activity appears in male infants during a brief period of 1 to 4 months after birth (Sato et al, 2007b). PSA activity is undetectable after this period until puberty. This finding indicates that testicular androgen enhances PSA synthesis by proliferation of acinar epithelial cells of the prostate in infants and that testicular function might depend on activation of the pituitary-testicular axis during the neonatal period (Sato et al, 2007b). In addition, elevated levels of testosterone (T) at the onset of puberty might induce PSA synthesis within the prostate (Kim et al, 1999).

The role of T in the development of social and sexual behavior, bone metabolism, as well as penile development and adequate number of sertoli cells from the neonatal period through adolescence has been demonstrated (Mann and Fraser, 1996). Moreover, a few studies have reported a correlation between T and seasonal variations in the testicular function of adult male nonhuman primates (Matsubayashi et al, 1991; Muehlenbein et al, 2002; Bansode et al, 2003; Itoh et al, 2003). However, few studies have examined seminal plasma proteins among adult male nonhuman primates. In addition, seasonal alterations in prostate and seminal vesicle function have not been examined. Monitoring urinary PSA levels over time might provide insight into variations in prostate function, as well as changes resulting in the development of secondary sexual characteristics because *PSA* gene similarities have been detected among several nonhuman primate species and humans, including macaques (Karr et al, 1995). To gain a better understanding of changes with breeding season in male monkeys, we therefore investigated whether seasonal variations in urinary PSA activity might correspond to breeding in male Japanese macaques. In addition, the role of T on PSA production during the breeding season was investigated.

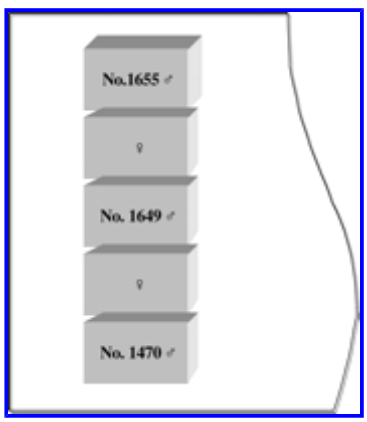

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Figure 1. Cage positions of the experimental animals used in this study.

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Animals and Housing

Three captive sexually mature male Japanese monkeys (*Macaca fuscata fuscata*) were used (1655, 1649, and 1470: 6, 7, and 11 years old, weighing 13.1, 11.7, and 14.3 kg, respectively). They were born between 1993 and 1996 and were maintained at the Primate Research Institute, Kyoto University (Inuyama, Japan). They were housed in individual cages (1.5 m wide, 2 m high, and 2 m in length) under a 10-hour light and 14-hour dark cycle and controlled temperature conditions (25.0 ± 5.0 °C) throughout the year. In the cage room, 2 captive multiparous female Japanese monkeys were placed adjacent to the male cages. Thus, the males had visual, auditory, and olfactory contact with adult females of the same species. External signs of estrus were monitored in females by daily visual inspection (ie, assessment of menstrual blood flow and general health). They were fed standard monkey pellets supplemented with fresh fruit daily and were given free access to water. The cage locations are shown in Figure 1. Our treatment of the monkeys adhered to *The Guide for the Care and Use of Laboratory Primates (2002) of the Primate Research Institute, Kyoto University.*

Urine Samples

Urine samples were collected from trays under the cages at 2-week intervals, as described in a previous report (Fujita et al, 2001). The study ran from October 2003 until September 2004. The breeding season of male Japanese macaques extends from October until January of the following year (Matsubayashi et al, 1991; Itoh et al, 2003). Each urine specimen was stored at −80 °C until the time of assay. Urine samples were also collected from 5 aseasonal male crab-eating macaques (*Macaca fascisularis*) as a control.

Assay Conditions

We measured PSA activity in urine with Seratec PSA Semiquant (Seratec Diagnostica, Gottingen, Germany) by an immunochromatographic membrane method (Sato et al, 2002, 2007b). Although the PSA membrane test provides only semiquantitative data regarding PSA levels up to 4 ng/mL, faint immunoreactions still occur in the range of 1 to 3 ng/mL, as stated by the manufacturer. Therefore, PSA concentrations were determined by judging the intensity of each red-purple immunoreactive line in the probe zone produced by the PSA gold-labeled anti-PSA antibody complex (Figure 2). All samples were compared with the reference line (4 ng PSA/mL) and ranked as follows: 3, strongly PSA positive (>10 ng/mL); 2, PSA positive (≒10 ng/mL); 1, weakly PSA positive (≒3 ng/mL); or 0, PSA negative (<1 ng/mL).

Figure 2. Urinary prostate-specific antigen (PSA) activity in crab-eating and Japanese monkeys determined with "Seratec PSA Semiquant" by the immunochromatographic membrane method. Positive PSA activity appears within the probe zone as a colored immunoreactive line (arrowhead). The intensity of the line was strongly positive (3) in all crab-eating monkeys examined and range from not positive to strongly positive (0–2) among the Japanese monkeys.

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Urinary T levels were measured with a commercially available solid-phase radioimmunoassay kit (Diagnostic Products Corp, Tokyo, Japan). Quantitative measurements of T were expressed in terms of micrograms per millimole of urinary creatinine (Irani et al, 1996; Sato et al, 2007b).

Immunoprecipitation was performed with a Seize X protein A immunoprecipitation kit (Pierce, Rockford, Ill) and a monoclonal anti-PSA mouse antibody (Santa Cruz Biotechnology Inc, Santa Cruz, Calif) according to the manufacturer's recommendations. The kit was used to bind mouse immunoglobulin G1. The adsorbed antibody was then separated on a12% (wt/vol) sodium dodecyl sulfate (SDS) polyacrylamide gel and stained with Coomasie Brilliant Blue (CBB).

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Detectable PSA in the Urine of Macaques

Figure 2 shows representative lines, ranked from weakly to strongly positive (1 to 3), on the PSA card. The membrane test card was used for semiquantitative detection of urinary PSA activity in male Japanese macaques and crab-eating macaques. Notably, differences in PSA activity among Japanese macaques during the breeding season were clearly identified by different line intensities.

Urinary Levels of PSA and Testosterone

Figure 3 provides semiquantitative information regarding PSA activity and T concentrations within urine samples from 3 animals over the course of a year. A gradual increase in urinary PSA activity was observed between August and January, after which, activity decreased and remained low until May. However, urinary T levels remained constant throughout the year, apart from 2 peaks in autumn and early summer (arrowheads in Figure 3). Because of these results, we examined urinary PSA and T levels in 3 animals during the breeding and nonbreeding seasons (Table). Urinary PSA activity was significantly greater between October and January compared with the period from March through June. Although concentrations of urinary T during breeding season were 1.2 times greater than during the nonbreeding season, a significant difference was not observed.

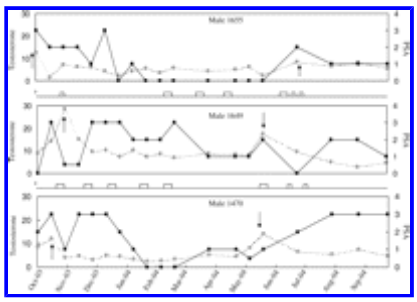

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Figure 3. Seasonal variations in urinary testosterone (T) and prostate-specific antigen (PSA) activity among 3 monkeys. Values are expressed as micrograms per millimole of urinary creatinine. Circles, squares, and arrows indicate testosterone (○), PSA (■), and T surges, respectively. R indicates female Japanese macaques housed in the same room (Figure 1); squares, daily menstrual blood loss, with the length of each bar indicating the number of days.

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Comparison of reproductive parameters between breeding and nonbreeding seasons in the Japanese macaque*

Detection of PSA in Urine

To examine the specificity of the monoclonal antibody for human PSA, urine samples obtained from human females, Japanese macaques during breeding season, and crab-eating macaques were immunoprecipitated with this antibody and analyzed by SDS polyacrylamide gel electrophoresis (SDS-PAGE). As shown in Figure 4A, human female urine did not produce any products of immunoprecipitation. In contrast, urine from the Japanese macaques during breeding season and from the crab-eating macaques yielded 2 polypeptide bands of approximately 55 and 33 kd (arrowheads) on CBB-stained gel, suggesting similarities among the 2 species. Interestingly, a decrease in intensity of the 33-kd polypeptide band was observed, after which it remained low during the nonbreeding season (Figure 4B).

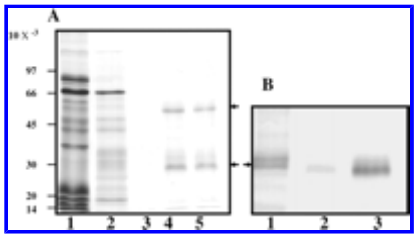

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Figure 4. Diagnostic pattern analysis of sodium dodecyl sulfate (SDS)–soluble biological samples. Proteins separated on a 12% (wt/vol) SDS polyacrylamide gel were stained with Coomasie Brilliant Blue. **(A)** Urinary samples collected from human females, Japanese macaques, and crab-eating macaques were concentrated with 10-fold volumes of Microcon YM-10 (Millipore Corp, Tokyo, Japan) and immunoprecipitated with anti-PSA antibody (lanes 3, 4, and 5). In the absence of immunoprecipitation, human seminal plasma and urine were applied to lanes 1 and 2, respectively. **(B)** Urinary samples collected from Japanese macaques during the breeding season (lane 1) and the nonbreeding season (lane 2) and from crab-eating macaques (lane 3) were immunoprecipitated with anti-prostate-specific antigen antibody.

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This study is the first to investigate urinary PSA activity according to breeding season in Japanese macaques. PSA was identified in the urine of male macaques by immunochromatography (Figure 2), and similarities with human urinary PSA (molecular mass 32 900; Shibata et al, 1997) were observed while looking at our immunoprecipitation data (Figure 4A). These results lend support to prior Southern blot and immunohistochemical studies demonstrating *PSA* gene expression and PSA activity in epithelial cells of the prostate (Karr et al, 1995). Although SDS-PAGE analysis indicated a molecular mass of 33 000 for PSA within human seminal plasma (Schaller et al, 1987), immunoprecipitation of urinary PSA from macaque monkeys demonstrated 2 bands of approximately 55 and 33 kd. Serum PSA binds to α1-antichymotrypsin, producing a molecular complex of 90 kd (Irani et al, 1996); however, the 55-kd band we identified by immunoprecipitation in this study is unlikely to represent this complex given a large difference in molecular mass. Furthermore, the band was not

identified after immunoprecipitation of human female urine (Figure 4A, lane 3). In addition, PSA cannot be detected in normal serum from healthy men (Pannek et al, 1997). These results, as well as those of previous reports, indicate that individual monomers, in addition to free PSA secreted from the prostate, can form dimmers within the urine of humans and macaques or combine with another member of the kallikrein family, but further experimentation is required to characterize this polypeptide bearing a PSA antigenic epitope.

We therefore demonstrated differences in PSA activity during breeding and nonbreeding seasons in male Japanese macaques (Figure 3). The results from 3 monkeys indicated a significantly greater PSA level during the breeding season compared with the nonbreeding season (Table), as can be seen from our immunoprecipitation results (Figure 4B). It is well known that androgen is required for masculinization and sexual and social behavior, as well as for development of reproductive organs in human males. Urinary T levels closely correlate with plasma T levels (Barrett et al, 2002). Matsubayashi et al (1991) have reported that plasma T levels in male Japanese macaques housed under laboratory conditions increase during the autumn and winter months, coinciding with increased urinary T levels, as observed in this study (Table). Remarkably, 2 peaks in urinary T were observed in Japanese macaques: 1 during the nonbreeding season (May to June) and 1 at the height of the breeding season (October to November) (Figure 3). Two of 3 individuals (1649 and 1470) exhibited increased urinary PSA activity at least 1 to 2 months after each T surge. This finding is in agreement with the relationship observed between T and PSA in humans in early infancy (Sato et al, 2007b).

Although this study was performed on a few animals, an interesting finding was obtained from monkey 1649. The animal had a mean urinary T concentration of 10.13 ± 5.66 (n = 19) μg/mmol of urinary creatinine (mean ± SD), approximately 1.8 times greater than the mean urinary T concentration of monkey 1655 or 1470 (5.61 ± 2.54 and 5.89 ± 3.14 μg/mmol of urinary creatinine, respectively). We speculate that monkey 1649 received more cues or exposure to pheromones secondary to the estrous call, exposure to smells from urine or menstrual blood (Figure 3), or both compared with the other monkeys because he was housed between 2 females (Figure 1). Therefore, we think that urinary T concentration remained elevated in monkey 1649 even during the nonbreeding season. However, this theory should be well demonstrated in more male monkey by rearranging cage locations.

Urinary levels of PSA and T in aseasonal animals (crab-eating macaques) were 1.8 ± 1.2 (n = 8) and 8.3 ± 1.2 (n = 15) μg/mmol of urinary creatinine, respectively (data not shown). Both levels were similar to those of Japanese macaques during the breeding season (Table). These data support previous reports that seasonal breeding in male Japanese macaques regulates not only testicular parameters (ie, T concentration, testicular size, total number of sperm; Matsubayashi et al, 1991) but also the synthesis of seminal plasma proteins, including PSA.

It is interesting to consider the possible role of seminal proteins in the propagation of a species. Several investigators demonstrated a link between the role of semenogelin in forming the semen coagulum and sperm competition in different mating systems in nonhuman primates (Irani et al, 1997; Robert and Gagnon, 1999; Kingan et al, 2003; Michael et al, 2003; Dorus et al, 2004). However, few studies have examined the role of PSA in semen liquefaction in nonhuman primates (Karr et al, 1995; Valtonen-Andrè et al, 2005).

Although both the prostate and the seminal vesicles are involved in semen production, we did not examine variation in semenogelin in this study because it cannot be detected in urine (Sato et al, 2001, 2002, 2007a; Michael et al, 2003).

Further research is required to investigate the possibility of seasonal variations in semenogelin activity within semen and urine using the immunochromatographic membrane test for semenogelin (Sato et al, 2004, 2007a) with an aim to improve our understanding of the relationship between seasonal breeding and semen composition in Japanese macaques.

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Footnotes

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