Journal of Andrology, Vol. 21, No. 4, July/August 2000
Copyright © American Society of Andrology

Progesterone-Induced Calcium Influx in Cynomolgus Monkey (*Macaca fascicularis*) Spermatozoa

T. GWATHMEY,*† P. F. BLACKMORE,‡ AND M. C. MAHONY*

*From the *Jones Institute for Reproductive Medicine, Department of Obstetrics and Gynecology, Eastern Virginia Medical School, Norfolk, Virginia; the †Department of Biological Sciences, Hampton University, Hampton, Virginia; and the ‡ Department of Physiological Sciences, Eastern Virginia Medical School, Norfolk, Virginia.*

ABSTRACT: For in vitro capacitation to occur in cynomolgus monkey (*Macaca fascicularis*) spermatozoa, there is an absolute requirement for exogenous stimulation with the sperm activators, caffeine (1 mM) and db–cyclic adenosine monophosphate (dbcAMP) (1 mM), which are known to induce capacitation-related hyperactivated motility. Tyrosine phosphorylation of sperm tail proteins is an integral component of this caffeine- and dbcAMP-stimulated hyperactivated motility. In both capacitated and noncapacitated human spermatozoa, progesterone (P_4) has been reported to elicit an immediate, potent increase in intracellular calcium ion concentrations $[Ca^{2+}]_i$. The objective of this study was to examine the effects of progesterone on requisite events in macaque fertilization, including $[Ca^{2+}]_i$, hyperactivated motility, and the concomitant tyrosine phosphorylation of sperm tail (STTP) proteins after treatment with caffeine and dbcAMP. The effect of 1 μM of progesterone on $[Ca^{2+}]_i$ was determined by spectrofluorometry with the fluorescent indicator, fura-2/AM, on hyperactivated motility using computer analysis (HTM-IVOS) with the sorting criteria lateral head amplitude (≥ 8.0 μm), curvilinear velocity (≥ 150 μm/s), linearity (≤ 60%), and on STTP by immunocytochemistry. The results showed that progesterone elicited a significant increase in $[Ca^{2+}]_i$ in caffeine- and dbcAMP-activated macaque sperm with maximal stimulation at 30 minutes after activation. The response in nonactivated sperm was dramatically reduced compared with the response in activated sperm. Basal $[Ca^{2+}]_i$ increased as a function of time in both activated and nonactivated control sperm although basal levels were significantly increased in activated sperm. Progesterone stimulation resulted in a small but significant increase in both hyperactivation and STTP when sperm were first pretreated with caffeine and dbcAMP. Our results provide evidence that macaque sperm activation with caffeine and dbcAMP is required for a progesterone-elicited response, which results in calcium influx, hyperactivated motility, and sperm tail tyrosine phosphorylation.

Key Words: Macaque, hyperactivation, tyrosine phosphorylation.

J Androl 2000;21:534–540

Mammalian sperm capacitation is a complex event that leads to the development of fertilizing capabilities by male gametes (Chang, 1951, 1955; Austin, 1952). In vivo capacitation normally occurs within the female reproductive tract in response to cellular and humoral components. In vitro capacitation may occur under appropriate capacitating conditions. Mammalian sperm also exhibit characteristic motility patterns associated with capacitation. This whiplash or hyperactivated motility, which is seen as wide amplitude flagellar beats (Yanagimachi 1969), could facilitate oviductal passage and zona penetration (Overstreet and Cooper, 1979; Stauss et al, 1995; Mahony et al, 1996). Attainment of in vitro capacitation is often evidenced by functional changes in sperm, including hyperactivated motility and completion of the acrosome reaction (Yanagimachi, 1994); however, the regulatory relationship between capacitation and hyperactivated motility is still in question.

Cynomolgus monkey (*Macaca fascicularis*) spermatozoa exhibit unique requirements for fertilization in vitro. Hyperactivated motility does not occur spontaneously; instead, this physiological event is dependent upon exogenous stimulation with the sperm activators, caffeine and db–cyclic adenosine monophosphate (dbcAMP; Boatman and Bavister, 1984). We recently demonstrated that tyrosine phosphorylation of sperm tail proteins is part of a signaling pathway involved in dbcAMP- and caffeine-stimulated hyperactivated motility in macaque sperm (Mahony and Gwathmey, 1999). In addition, in macaque sperm, hyperactivation occurs under different stimulating conditions than does induction of the acrosome reaction (VandeVoort et al, 1992; 1994).

Progesterone, a major component of the female reproductive tract milieu, induces the capacitation-related events of hyperactivation and acrosome reaction in human spermatozoa (Osman et al, 1989; Uhler et al, 1992; Oehninger et al, 1994). This steroid hormone exerts its effects on human sperm via a nongenomic surface receptor localized to the plasma membrane (Blackmore and Lattanzio, 1991; Meizel and Turner, 1991). Progesterone acti-

Supported by the Andrew W. Mellon Foundation.

Correspondence to: Mary C. Mahony, PhD, The Jones Institute for Reproductive Medicine, Department of Obstetrics and Gynecology, Eastern Virginia Medical School, 601 Colley Ave, Norfolk, VA 23507 (e-mail: mahonymc@evms.edu).

Received for publication May 3, 1999; accepted for publication January 12, 2000.

vates human sperm by inducing a rapid influx of extracellular calcium into the cell, which results in increased levels of free intracellular calcium, $[Ca^{2+}]_i$ (Thomas and Meizel, 1988; Blackmore et al, 1990).

This study was undertaken to examine the effects of progesterone (P_4) on macaque sperm. We analyzed whether macaque sperm would respond to stimulation by progesterone and if preincubation under capacitating conditions (ie, activation with caffeine and dbcAMP) was required to elicit an influx of calcium. In addition to assessing $[Ca^{2+}]_i$, we also examined the potential of progesterone to induce hyperactivated motility. Finally, we examined the influence of progesterone on tyrosine phosphorylation of sperm tail proteins as a biochemical marker and an integral signaling component of hyperactivated motility in macaque sperm (Mahony and Gwathmey, 1999).

Materials and Methods

Animals

Adult proven-breeder male cynomolgus monkeys (*Macaca fascicularis*) (n = 10) each weighing 5 to 8 kg were used in this study. Monkeys were housed in individual cages with a room temperature of 22°C, 12 hours of light per day, and fed a diet of commercially available monkey chow and water ad libitum. Serum testosterone levels were determined for each monkey before it was included in the study. Before the studies were initiated, protocols were approved by the Animal Care and Use Committee of Eastern Virginia Medical School and were in accordance with the *Guiding Principles for the Care and Use of Research Animals.*

Sperm Preparation

Semen samples were collected from 7 monkeys via electroejaculation directly into Talp-HEPES medium with a pH of 7.4 (Bavister et al, 1983) that had been pre-equilibrated to room temperature (approximately 25°C). Monkey spermatozoa were prepared according to previously reported techniques (Mahony et al, 1993, 1996). Samples were washed twice with Talp-HEPES for 8 minutes at 270 × *g*. Talp-HEPES media (1 mL) was layered over the sperm pellet and the pellet was dislodged. After a 1-hour incubation at room temperature in which the dislodged pellet was allowed to settle, the motile fraction in the supernatant was retrieved, adjusted to a sperm concentration of 20 million/mL and allowed to incubate at room temperature for an additional 1 hour (Lanzendorf et al, 1990a, 1990b; Mahony and Gwathmey, 1999).

Sperm Treatment

Sperm were transferred to Talp medium with a pH of 7.4 (Bavister et al, 1983) that had been re-equilibrated for at least 18 hours by incubation at 37°C in 5% CO_2 in water-saturated air. The motile sperm fractions were centrifuged for 8 minutes at 270 × *g* and the supernatant was discarded. The pellet was resuspended in Talp media and divided into 2 aliquots for a control sample (nonactivated) and for the addition of activators (1 mM of caffeine, 1 mM of dbcAMP) and incubated for a half hour at 37°C in 5% CO_2 in water-saturated air.

To examine the effect of progesterone, the control and caffeine-treated and dbcAMP-treated sperm were stimulated with either progesterone (Sigma Chemical Company, St Louis, Mo), 1 μM in dimethylsulfoxide (DMSO), or a vehicle (DMSO) control.

Measurement of Intracellular Calcium $[Ca^{2+}]_i$

The motile sperm fractions were incubated with 5 μM of the fluorescence indicator, fura-2/AM, for 1 hour at room temperature as described by Blackmore et al (1990). The motile fraction of sperm was loaded with the indicator, fura-2, by incubating the sperm with 4 μM of fura-2/AM for 1 hour at room temperature. Preliminary experiments demonstrated that macaque sperm treated with the fura-2/AM indicator had motion characteristics similar to those specimens that had not been treated with the indicator under the same experimental conditions. After centrifugation at 500 × *g* for 5 minutes, the sperm were resuspended in TALP medium and incubated at 37°C in 5% CO_2 in water-saturated air. Control (nonactivated) sperm and caffeine- and dbcAMP-treated (activated) sperm from the same monkey specimen were prepared as described earlier. The addition of each treatment was staggered to allow calcium to be measured at similar time points. Sperm suspensions of 0.5-mL aliquots were added to a 6-mm by 50-mm glass cuvette (Chrono-Log, Havertown, Penn) containing a magnetic stirring bar in a SPEX ARCM spectrofluorometer (SPEX Industries, Edison, NJ). Aliquots (500 μL) of the sperm suspension were treated with progesterone at a final concentration of 10^{-6}M for 30 seconds after data collection was started.

The sperm were excited at 340 nanometers and 380 nanometers, respectively, and emission was measured at 505 nanometers. To obtain the Ca^{2+}-saturated fluorescence values of fura-2 at each wavelength, 0.01% (wt/vol) of digitonin was added to the fura-2–loaded sperm suspension in the presence of 1 mM of Ca^{2+}. To obtain the Ca^{2+}-depleted fura-2 fluorescence values at each wavelength, 10 mM of ethylene-bis (oxyethylenenitrilo) tetra-acetic acid [EGTA] was added to the digitonin-treated sperm suspension. Fluorescence values at each wavelength were corrected for cellular autofluorescence by adding 2 mM of $MnCl_2$ in the presence of 20 μM of ionomycin to another aliquot of fura-2–loaded sperm (the Mn^{2+} quenches the fura-2 fluorescence). The autofluorescence values were then subtracted from the fura-2–loaded sperm fluorescence values obtained at both wavelengths. The level of $[Ca^{2+}]_i$ was calculated with dm3000 SPEX software using a K_D of 224 nM for the fura-2–Ca^{2+} complex.

Sperm Motion Assessment

Sperm motion was evaluated with the HTM-IVOS Motion Analyzer (Hamilton-Thorn Research, Danvers, Mass) as described previously for assessing cynomolgus monkey sperm (Mahony et al, 1993, 1996). The changes in the settings take into account the increased velocity of monkey sperm compared to human sperm. All sperm samples were equilibrated to and assessed at

37°C to allow for standardization between treatments. The pertinent settings used during the HTM assessment were as follows: frames acquired = 30, frame rate = 60 Hz, minimum contrast = 80, minimum cell size = 5 pixels, "slow cells" were accepted as motile. At the outset of each experiment, we verified that the settings permitted accurate differentiation of motile sperm vs. nonmotile sperm or debris by using the "playback" option, during which the motions of sperm in the previous field were replayed. For each frame, a green dot was positioned over the head of all motile spermatozoa and a red dot was positioned over the head of nonmotile spermatozoa. When errors were detected, we adjusted the settings until the problem was corrected.

To optimize the motion assessments, the sperm concentration of each sperm group was adjusted to 20 million/mL and analyzed using a MicroCell counting chamber (Conception Technologies, San Diego, Calif) with a chamber depth of 20 μm. Using these experimental conditions prevented the crossing of sperm tracks that would result in errors in data collection. Approximately 100 sperm were evaluated per baseline (untreated) and caffeine- and dbcAMP-treated groups.

Sperm samples were analyzed for motility with the HTM-IVOS Motion Analyzer to determine the percentage of hyperactivation by sorted criteria that were established by our laboratory (Mahony and Gwathmey, 1999). Percent hyperactivation was defined by the following parameters: curvilinear velocity (VCL), ≥ 150 μm/s; lateral head amplitude (ALH), ≥ 8 μm; and linearity (LIN), ≤ 60%.

Protein Tyrosine Phosphorylation

Sperm in the control and treated groups that had been evaluated for motion characteristics and calcium influx were treated with a sperm fixative containing 0.5% sodium azide and 0.1 μM of phenylmethylsulfonyl fluoride [PMSF] in phosphate-buffered saline (PBS). Each control and treatment group was loaded onto Teflon-coated spot slides, methanol fixed, and stored at −70°C until assayed. Slides were equilibrated to room temperature for evaluation. Nonspecific binding sites on sperm were blocked with PBS with 1% BSA. Next, control and treated sperm were incubated for 1 hour with a fluorescein-isothyocyanate (FITC)–conjugated monoclonal antibody against phosphorylated tyrosine residues on proteins (1 μg/mL; PY20, Zymed, San Francisco, Calif). Sperm were rinsed with PBS and an antiquenching mounting medium was added. Visualization of phosphotyrosine proteins was completed at a magnification of 600× by epifluorescence microscopy. In a blinded fashion, two investigators analyzed a minimum of 100 sperm per control group or treated group. Data were presented as the proportion of sperm that exhibited immunoreactivity in the principle piece region of the macaque sperm tail as previously described (Mahony and Gwathmey, 1999) as well as in human (Carrera et al, 1996) and mouse (Carrera et al, 1994). Specificity of the antibody for phosphotyrosine residues was demonstrated by competitive inhibition experiments with unlabeled phosphotyrosine (*O*-Phospho-L-Tyrosine, 20 mM; Zymed).

Statistical Evaluation

Data were analyzed using a student's paired *t* test or 2-factor split-plot analysis of variance (ANOVA); $P < .05$ was considered significant. All results are expressed as the mean ± standard error of the mean. The number of replicates is presented for each experiment, which represented ejaculates obtained from different monkeys.

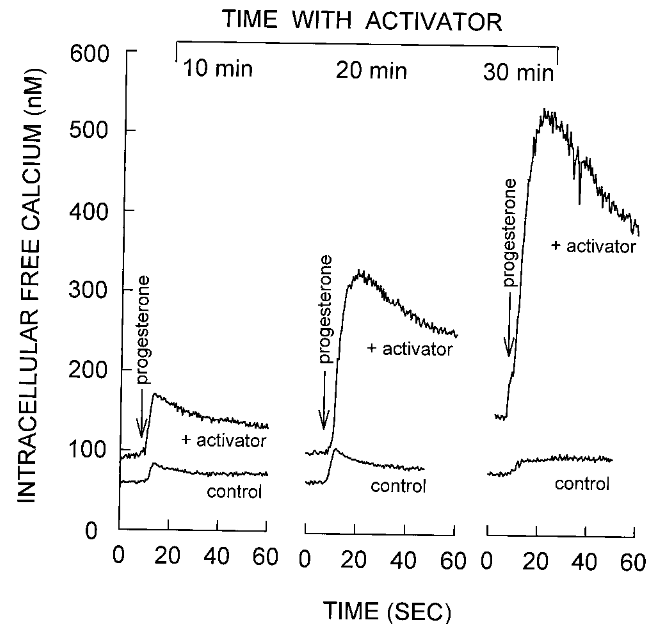

Figure 1. Effect of 1 mM of caffeine and 1 mM of dbcAMP on intracellular free calcium concentrations ($[Ca^{2+}]_i$) in sperm incubated in Talp-HEPES buffer. After incubating sperm for 1 hour with 5 μM of fura-2/AM they were sedimented by centrifugation at 500 × *g* for 5 minutes. Sperm were resusupended in Talp buffer at a concentration of approximately 5 million/mL. Progesterone (1 μM) stimulated $[Ca^{2+}]_i$ and was measured in the sperm at the times indicated after the addition of caffeine and dbcAMP (activator in buffer, 10% dilution) or control (buffer, 10% dilution). The figure is representative of data obtained from 7 cynomolgus monkey ejaculates.

Results

Progesterone-Induced Calcium Influx in Macaque Spermatozoa

The effects of progesterone on $[Ca^{2+}]_i$ after 10, 20, and 30 minutes of incubation with caffeine and dbcAMP treatment are presented in Figure 1. Progesterone was found to produce a rapid increase in $[Ca^{2+}]_i$ during each preincubation time with caffeine and dbcAMP, with the effect increasing substantially as a function of increasing incubation time with the 2 activators. The maximal increase in $[Ca^{2+}]_i$ was observed at approximately 10 seconds after progesterone was added. Although progesterone elicited a rise in $[Ca^{2+}]_i$ in nonstimulated sperm, its level was dramatically reduced compared to its effects on sperm that had been stimulated with caffeine and dbcAMP.

The addition of 10^{-6} M of progesterone to the caffeine-treated and dbcAMP-treated and nontreated populations

of sperm resulted in a rapid increase in $[Ca^{2+}]_i$; however, the levels of $[Ca^{2+}]_i$ reached were dependent upon pretreatment with caffeine and dbcAMP. The increasing effects of progesterone on $[Ca^{2+}]_i$ in the caffeine- and dbcAMP-stimulated groups were significantly greater at all time points observed, reaching at least a sixfold increase (450 nm) by 30 minutes after administration of the sperm stimulators (Figure 2A). For the control group without caffeine and dbcAMP treatment, no effect of progesterone on $[Ca^{2+}]_i$ was observed during the initial 15 minutes of incubation. (Figure 2B); however, there was a small but significant increase (50 nM) in $[Ca^{2+}]_i$ during the final 15 minutes of incubation for this group ($P < .05$).

Basal $[Ca^{2+}]_i$ was observed to be significantly greater in sperm that had been activated with caffeine and dbcAMP compared to that observed in nonactivated populations at each time point except at 30 minutes ($P < .05$; Figures 2A and B). Basal $[Ca^{2+}]_i$ in the activated group increased approximately fourfold over a 30-minute incubation period compared to the control group in which basal $[Ca^{2+}]_i$ increased only twofold over that same period of time.

Hyperactivated Motility

In order to determine if the effect of progesterone on macaque sperm led to hyperactivated motility, the motion parameters of each sample were compared using the previously described sorting criteria. As previously reported, treatment with caffeine and dbcAMP resulted in a significant increase in the proportion of sperm exhibiting hyperactivated motility ($P < .001$; Figure 3A). The addition of progesterone to the caffeine- and dbcAMP-treated group resulted in a significant increase in the proportion of macaque spermatozoa exhibiting hyperactivated motility ($P < .001$).

Tyrosine Phosphorylation of Macaque Sperm Tail Proteins

As previously reported, tyrosine phosphorylation of sperm proteins was localized to the principal piece of the tail region and a small region of the neck in methanol-fixed macaque spermatozoa. Labeling of these tyrosine-phosphorylated sperm tail proteins was very intense; however, in approximately 5% of labeled sperm, the labeling intensity was light compared with that observed in the majority of labeled sperm. Pretreatment of the PY-20 antiserum with unlabeled phosphotyrosine resulted in an absence of immunoreactivity, which indicates the specificity of the reaction.

As we have previously reported, there was a significant ($P < .001$) increase in phosphotyrosine immunoreactivity in macaque sperm with caffeine and dbcAMP treatment. As with hyperactivated motility, treatment with proges-

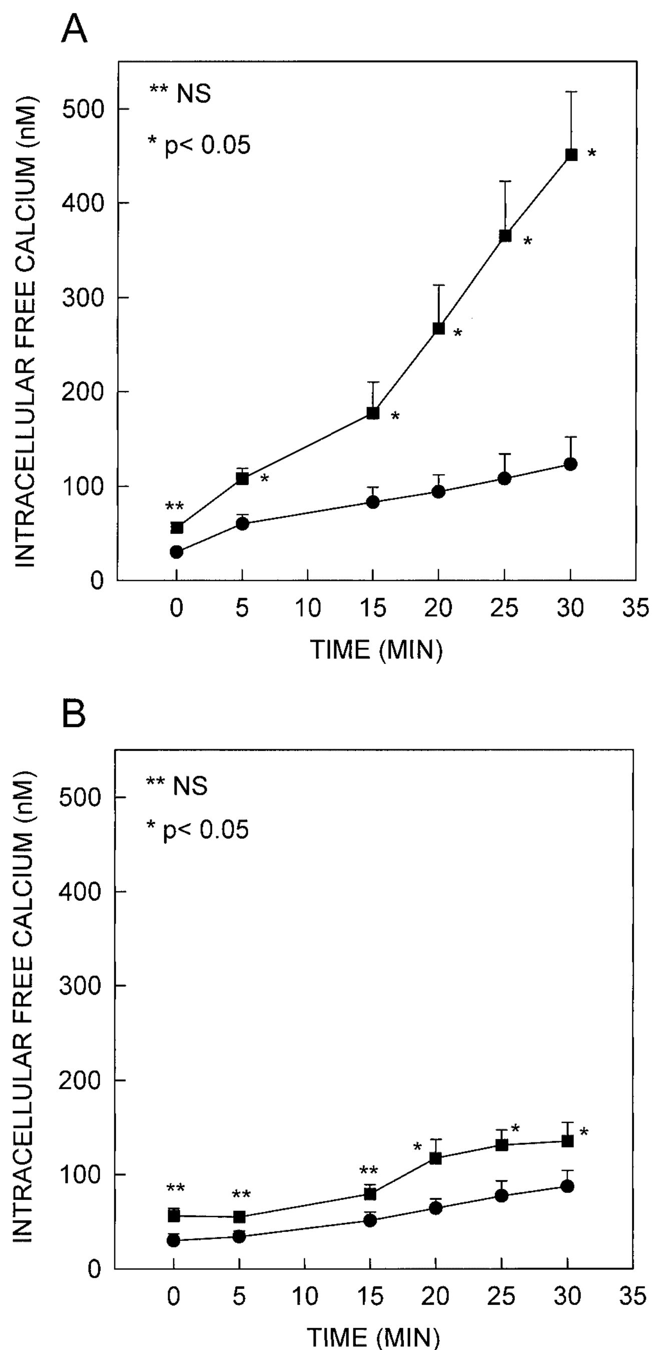

Figure 2. Effect of progesterone on increased concentrations of intracellular free calcium ($[Ca^{2+}]_i$) at various times of incubation **(A)** and on basal $[Ca^{2+}]_i$ **(B)** in control and caffeine- and dbcAMP-treated macaque sperm. After incubation for 1 hour with 5 μM of fura-2/AM, sperm were sedimented by centrifugation at 500 × *g* for 5 minutes. Sperm were resuspended in Talp buffer at a concentration of approximately 5 million/mL. $[Ca^{2+}]_i$ was measured in the sperm at the times indicated after the addition of caffeine and dbcAMP (activator in buffer, 10% dilution; ■) or control (buffer, 10% dilution; ●). Progesterone (1 μM) was added at various time points after the addition of caffeine and dbcAMP. Results presented are the mean ± standard error of the mean for data from 7 monkey ejaculates. Asterisks denote values that were significantly different from those of appropriate untreated or caffeine/dbcAMP-stimulated controls ($P < .05$.)

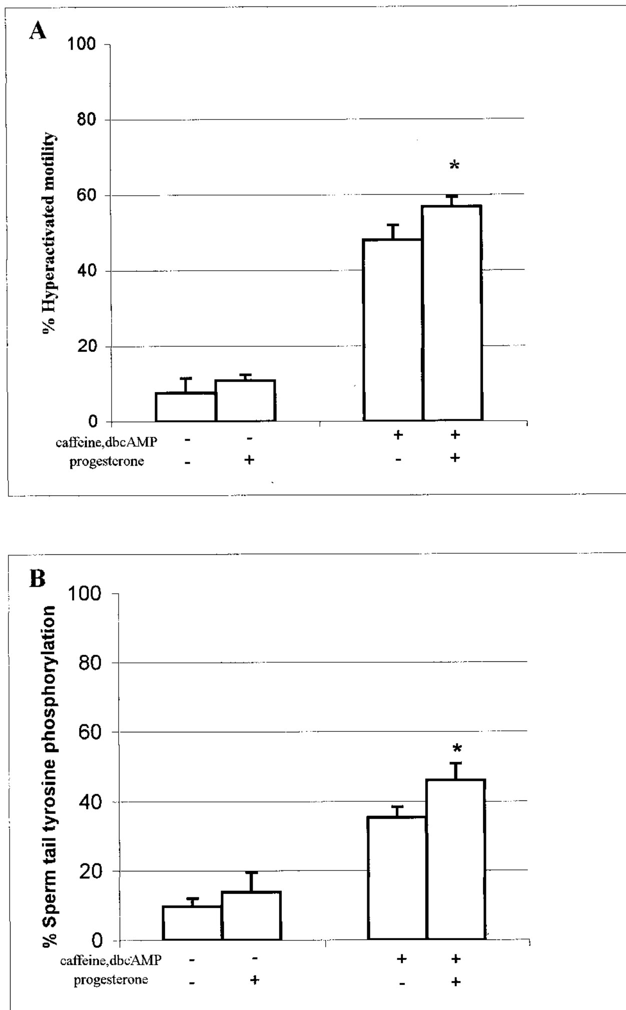

Figure 3. Effect of progesterone on hyperactivated motility (**A**) or phosphotyrosine immunoreactivity of sperm tail proteins (**B**) measured 30 minutes after either the addition of 1 mM of caffeine and 1 mM of dbcAMP or buffer. The results are the mean ± the standard error of the mean of determinations from 7 monkey ejaculates. Asterisk denotes a significant difference in progesterone-stimulated sperm compared to untreated matched controls $P < .001$.

terone resulted in a small but significant increase in the proportion of sperm exhibiting tyrosine phosphorylation of tail proteins when sperm were pretreated with the activators caffeine and dbcAMP ($P < .001$; Figure 3B).

Discussion

Cynomolgus monkey sperm provide a unique model for studying capacitation-related events. They exhibit an absolute requirement for exogenous stimulation with the cyclic nucleotide, dbcAMP, and the phosphodiesterase inhibitor, caffeine, to complete the steps that lead to successful in vitro fertilization, including hyperactivated motility, zona binding, and penetration (Boatman and Bavister, 1984; VandeVoort et al, 1992; 1994). This allows evaluation of these cellular processes to be made in a controlled system. We have previously demonstrated that tyrosine phosphorylation of sperm tail proteins is an integral signaling pathway that modulates caffeine- and dbcAMP-stimulated hyperactivated motility (Mahony and Gwathmey, 1999). This study was undertaken to examine the effects of progesterone on caffeine- and dbcAMP-activated sperm by evaluating a number of requisite events associated with successful fertilization in vitro.

We have shown that progesterone stimulates a significant increase in $[Ca^{2+}]_i$ in caffeine- and dbcAMP-activated sperm and, to a lesser degree, in nonstimulated sperm. The time required for maximal stimulation with progesterone was 30 minutes after activation with caffeine and dbcAMP, the condition known to induce capacitation in macaque sperm (Boatman and Bavister, 1984; VandeVoort et al, 1992, 1994). It is interesting to note that, in our experience, the usual time required for macaque sperm to fully achieve hyperactivated motility with caffeine and dbcAMP treatment is also approximately 30 minutes after treatment. Whereas $[Ca^{2+}]_i$ of nonactivated sperm increased by 50 nM during the final 15 minutes of incubation, $[Ca^{2+}]_i$ levels in caffeine- and dbcAMP-treated sperm increased more than sixfold during this same time period, which demonstrates a requirement that sperm must be more fully stimulated in order to optimally respond to stimulation with progesterone. The effect of progesterone on increasing $[Ca^{2+}]_i$ was observed approximately 10 seconds after the steroid was administered, which illustrates its nongenomic action at the sperm plasma membrane (Blackmore and Lattanzio, 1991; Meizel and Turner, 1991). Progesterone's effects on monkey sperm by increasing concentrations of intracellular calcium were also observed to be dose-dependent. A small effect (an approximately 5% increase) was observed with 1 nM; 10 nM produced a 40% to 50% increase, whereas a 100-nM dose increased $[Ca^{2+}]_i$ by 90% to 95%, and 1000 nM elicited the maximal response (data not shown).

An increase in $[Ca^{2+}]_i$ is believed to be a requirement for initiation and maintenance of hyperactivated motility; therefore, a progesterone-induced increase in $[Ca^{2+}]_i$ would also produce an increase in hyperactivated motility. Such an increase in hyperactivated motility with progesterone has been reported for human sperm (Uhler et al, 1992; Oehninger et al, 1994). Our findings show a consistent increase in the proportion of macaque sperm exhibiting hyperactivated motility with administration of progesterone. However, although the increase was significant, it was small. The degree of response to progesterone stimulation is similar to that often reported for human sperm (Uhler et al, 1992; Oehninger et al, 1994).

This increase in hyperactivated motility with progesterone treatment was observed only when sperm were

pretreated with caffeine and dbcAMP. These data are in contrast to those reported for human sperm in which progesterone significantly induced an increase in cAMP, which also correlated with a rise in hyperactivated motility (Parinaud and Milhet, 1996). In macaques, we observed a fourfold greater increase in hyperactivation with caffeine and dbcAMP compared to treatment with progesterone. These data would suggest, at least in macaque sperm, that the role of cAMP is upstream of the calcium influx, not downstream, as was suggested by LeClerc et al (1998), and reemphasizes the requirement for macaque sperm to be stimulated under these conditions for a full response to progesterone.

From these studies, it is likely that the major stimulator of hyperactivation is some component other than progesterone. Because even a full response to progesterone was small compared to that with caffeine and dbcAMP, it may be possible that the progesterone that the sperm will encounter in transit through the female reproductive tract will increase $[Ca^{2+}]_i$. This increase may facilitate the acrosome reaction when sperm bind to the zona pellucida, as was previously proposed (Roldan et al, 1994).

Phosphorylation of tyrosine residues on sperm tail proteins has been proposed as a consistent indicator of intracellular changes associated with hyperactivated motility in mouse and human (Carrera et al, 1994, 1996) and in macaque sperm (Mahony and Gwathmey, 1999). The response to progesterone resulting in an increase in tyrosine phosphorylation of sperm tail proteins mimicked that observed for hyperactivated motility and provides further evidence of the association of tyrosine phosphorylation of sperm tail proteins and hyperactivated motility in macaque sperm.

In our experiments, sperm responded to progesterone stimulation by a rapid influx of calcium and increased hyperactivation and tyrosine phosphorylation. However, all 3 processes required that sperm be pretreated with caffeine and dbcAMP for the maximal response. In humans, detectable progesterone receptor(s) have been reported only in the sperm head domain (Blackmore and Lattanzio, 1991; Miezel and Turner, 1991; Meizel et al, 1997). Whereas the acrosome reaction is a head-related phenomenon, hyperactivation and tyrosine phosphorylation of principal piece proteins are primarily tail-associated events. Suarez et al (1993) reported that intracellular calcium levels are significantly greater in the flagellar region of hyperactivated hamster sperm compared to nonhyperactivated sperm. Although it may be entirely plausible that progesterone receptors also exist on the sperm flagellum in levels that are beneath detection; theoretically, the relative number of head-to-tail receptors is considerably larger. This would allow a more profound effect of progesterone on sperm head–associated events unless intracellular calcium mobility from the head to tail compartments occurs.

In summary, we have demonstrated in macaque sperm that progesterone stimulates an increase in $[Ca^{2+}]_i$ with a concomitant increase in hyperactivated motility and tyrosine phosphorylation of tail proteins after pretreatment with caffeine and dbcAMP, conditions that are known to induce capacitation in this species. However, the response to caffeine and dbcAMP far surpassed that of progesterone, both for hyperactivated motility and tyrosine phosphorylation, which suggests a minor role for this steroid in these processes. Our results provide further evidence that macaque sperm must be in a specific physiological state for sperm to fully respond to progesterone stimulation. Finally, macaque sperm with their defined criteria for achieving capacitation continue to provide a unique model to delineate the cellular events defined in the process of capacitation. Such a system has potential application as a means to study methods of fertility enhancement and intervention in humans, as well as conservation of endangered nonhuman primates.

References

Austin CR. The ''capacitation'' of the mammalian sperm. *Nature.* 1952;170:326.

Bavister BD, Boatman DE, Leibfried L, Loose M, Vernon MW. Fertilization and cleavage of rhesus monkeys oocytes in vitro. *Biol Reprod.* 1983;28:983–999.

Blackmore PF, Beebe SJ, Danforth DR, Alexander NJ. Progesterone and 17α-hydroxyprogesterone: novel stimulators of calcium influx in human sperm. *J Biol Chem.* 1990;265:1376–1380.

Blackmore PF, Lattanzio FA. Cell surface localization of a novel nongenomic progesterone receptor on the head of human sperm. *Biochem Biophys Res Comm.* 1991;181:331–336.

Boatman DE, Bavister BD. Stimulation of rhesus monkey sperm capacitation by cyclic nucleotide mediators. *J Reprod Fertil.* 1984;71:357–366.

Carrera A, Gerton GL, Moss SD. The major fibrous sheath polypeptide of mouse sperm: structural and functional similarities to the A-kinase anchoring proteins. *Dev Biol.* 1994;165:272–284.

Carrera A, Moos J, Ning XP, Gerton GL, Tesarik J, Kopf GS, Moss SB. Regulation of tyrosine phosphorylation in human sperm by a calcium/calmodulin-dependent mechanism: identification of A kinase anchor proteins as major substrates for tyrosine phosphorylation. *Dev Biol.* 1996;180:284–296.

Chang MC. Fertilizing capacity of spermatozoa deposited into the fallopian tubes. *Nature.* 1951;168:697–698.

Chang MC. Development of fertilizing capacity of rabbit spermatozoa in the uterus. *Nature.* 1955;175:1036–1037.

Lanzendorf SE, Zelinski-Wooten MB, Stouffer RL, Wolf DP. Maturity at collection and the developmental potential of rhesus monkey oocytes. *Biol Reprod.* 1990a;42:703–711.

Lanzendorf SE, Gliessman PM, Archibong AE, Alexander M, Wolf DP. Collection and quality of rhesus monkey semen. *Mol Reprod Dev.* 1990b;25:61–66.

Leclerc P, de Lamirande E, Gagnon C. Interaction between Ca^{2+}, cyclic 3′5′ adenosine monophosphate, the superoxide anion, and tyrosine

phosphorylation pathways in the regulation of human sperm capacitation. *J Androl.* 1998;19:434–443.

Mahony MC, Gwathmey T. Protein tyrosine phosphorylation during hyperactivated motility of cynomolgus monkey (*Macaca fascicularis*) spermatozoa. *Biol Reprod.* 1999;60:1239–1243.

Mahony MC, Oehninger S, Doncel G, Morshedi M, Acosta A, Hodgen GD. Functional and morphological features of spermatozoa microaspirated from the epididymal regions of cynomolgus monkeys (*Macaca fascicularis*). *Biol Reprod.* 1993;48:613–620.

Mahony MC, Lanzendorf S, Gordon K, Hodgen GD. Effect of caffeine and dbcAMP on zona pellucida interactions by epididymal spermatozoa of cynomolgus monkeys (*Macaca fascicularis*). *Mol Reprod Dev.* 1996;43:530–535.

Meizel S, Turner KO. Progesterone acts at the plasma membrane of human sperm. *Mol Cell Endocrinol.* 1991;11:R1–R5.

Meizel S, Turner KO, Nuccitelli R. Progesterone triggers a wave of increased free calcium during the human sperm acrosome reaction. *Dev Biol.* 1997;182:67–75.

Oehninger S, Sueldo C, Lanzendorf S, Mahony MC, Burkman LJ, Alexander NJ, Hodgen GD. A sequential analysis of the effect of progesterone on specific sperm functions crucial to fertilization in vitro in infertile patients. *Hum Reprod.* 1994;9:1322–1327.

Osman RA, Andria ML, Jones AD, Meizel S. Steroid induced exocytosis: the human sperm acrosome reaction. *Biochem Biophys Res Commuun.* 1989;160:828–833.

Overstreet JW, Cooper GW. Effect of ovulation and sperm motility on the migration of rabbit spermatozoa to the site of fertilization. *J Reprod Fertil.* 1979;55:53–59.

Parinaud J, Milhet P. Progesterone induces Ca^{++}-dependent 3′,5′-cyclic adenosine monophosphate increase in human sperm. *J Clin Endocrinol Metab.* 1996;81:1357–1360.

Roldan ER, Murase T, Shi QX. Exocytosis in spermatozoa in response to progesterone and zona pellucida. *Science.* 1994;266:1578–1581.

Stauss CR, Votta TJ, Suarez SS. Sperm motility hyperactivation facilitates penetration of the hamster zona pellucida. *Biol Reprod.* 1995;53: 1280–1285.

Suarez SS, Varosi SM, Dai XB. Intracellular calcium increases with hyperactivation in intact, moving hamster sperm and oscillates with the flagellar beat cycle. *Proc Natl Acad Sci USA.* 1993;90:4660–4664.

Thomas P, Meizel S. An influx of extracellular calcium is required for the initiation of the human sperm acrosome reaction induced by human follicular fluid. *Gamete Res.* 1988;20:397–411.

Uhler ML, Leung A, Chan SYW, Wang C. Direct effects of progesterone and antiprogesterone on human sperm hyperactivated motility and acrosome reaction. *Fertil Steril.* 1992;58:1191–1198.

VandeVoort CA, Tollner TL, Overstreet JW. Separate effects of caffeine and dbcAMP on macaque sperm motility and interaction with the zona pellucida. *Mol Reprod Dev.* 1994;37:299–304.

VandeVoort CA, Tollner TL, Overstreet JW. Sperm-zona pellucida interaction in cynomolgus and rhesus macaques. *J Androl.* 1992;13:428–432.

Yanagimachi R. Mammalian Fertilization. In: Knobil E, Neill JD, eds. *The Physiology of Reproduction.* New York: Raven Press; 1994:189–317.

Yanagimachi R. In vitro capacitation of hamster spermatozoa by follicular fluid. *J Reprod Fertil.* 1969;18:275–286.