Intracellular calcium accumulation and responsiveness to progesterone in capacitating human spermatozoa.

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Intracellular Calcium Accumulation and Responsiveness to Progesterone in Capacitating Human Spermatozoa

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ABSTRACT: Progesterone induced a rapid, long-lasting, dose-dependent increase of intracellular free calcium concentration ([Ca2+]i) in human sperm capacitated overnight. This effect was not counteracted by the cytosolic progesterone receptor antagonist RU486 (1 μmol/L) nor by the GABA-A receptor antagonists bicuculline (10 μmol/L) and picrotoxin (50 μmol/L). Also, the rank order of potency of several progestative steroids on [Ca2+]i differed from that previously reported for uterine intracellular progesterone receptor or for P-GABA interaction in the central nervous system, indicating a different pathway for progesterone stimulation of human sperm. Modifications of basal and progesterone-stimulated [Ca2+]i during sperm capacitation were also studied. A progressive, parallel increase of basal and progesterone-stimulated [Ca2+]i in capacitating spermatozoa was found. In particular, progesterone-stimulated [Ca2+]i increased from a basal concentration of 147% ± 17% at 10 minutes to 327% ± 65% after 120 minutes of incubation in capacitating medium. This increase was well correlated with basal [Ca2+]i ($r = 0.93$). In contrast, basal and progesterone-stimulated [Ca2+]i concentrations were constantly low in spermatozoa incubated in noncapacitating medium. In capacitated spermatozoa, initial responsiveness to progesterone and basal [Ca2+]i was higher than in capacitating and noncapacitated samples, and remained constant throughout the duration of the experiment. The progressive, parallel increase of [Ca2+]i and response to progesterone observed during in vitro capacitation of human spermatozoa might be physiologically relevant in vivo during capacitation of sperm in the female genital tract.

Key words: Spermatozoa, [Ca2+]i, progesterone, capacitation.

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Capacitation of mammalian spermatozoa can be achieved in several chemically defined media supplemented with albumin (Chang, 1984) after a period of time that varies depending on the species (Yanagimachi, 1988). Sperm capacitation is accompanied by biochemical and biophysical alterations of the plasma membrane, including changes in ion permeability, which prepare the cell to undergo the acrosome reaction (Yanagimachi, 1988). In particular, calcium (Ca^{2+}) influx into spermatozoa during capacitation has been demonstrated in several species using fluorescent intracellular Ca^{2+} indicators (White and Aitken, 1989; Zhou et al, 1990), or other methods (Singh et al, 1978; Coronel and Lardy, 1987; Ruknudin and Silver, 1990). In contrast, capacitation of rabbit spermatozoa in a hypertonic medium is not accompanied by an increase of intracellular free Ca^{2+} concentration ($[Ca^{2+}]_i$) (Mahanes et al, 1986). Data on $[Ca^{2+}]_i$ changes occurring during capacitation of human spermatozoa are fragmentary, and it is not known whether this phenomenon is associated with an increase of $[Ca^{2+}]_i$ (Yanagimachi, 1988).

Recently, progesterone (P), which is present in high concentrations in the cumulus matrix (Osman et al, 1989), has been proposed as a physiologic stimulus for initiation of the acrosome reaction in human spermatozoa (Osman et al, 1989). P induces an increase in the concentration of $[Ca^{2+}]_i$ in human spermatozoa (Thomas and Meizel, 1989; Blackmore et al, 1990), followed by Ca^{2+}-dependent activation of phospholipase C (Thomas and Meizel, 1989). Elevation of $[Ca^{2+}]_i$ depends on extracellular Ca^{2+} (Thomas and Meizel, 1989; Blackmore et al, 1990). However, the exact mechanism underlying P-stimulated calcium entry in human sperm has not been fully elucidated. Studies have been conducted to determine if the P-stimulated $[Ca^{2+}]_i$ increase in human sperm is mediated by the classic intracellular P receptors (Spelsberg et al, 1989), or by "atypical" interactions with GABA-A receptors, as recently reported in the central nervous system (Majewska et al, 1986; Turner et al, 1989).

We also designed experiments to evaluate basal and P-stimulated $[Ca^{2+}]_i$ during capacitation in human spermatozoa loaded with the intracellular calcium indicator, fura 2/AM. Changes in the percentage of acrosome-reacted sperm in response to P and the calcium ionophore A23187 were also studied.

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Materials and Methods

Chemicals

Fura 2/AM (F2/AM) and calcium ionophore A23187 were obtained from Calbiochem (San Diego, CA). Bicuculline, picrotoxin, GABA, homotaurine, steroids (progesterone, 11β-hydroxy-progesterone, 17α-hydroxy-progesterone, 5α-pregnan-3,20-dione, 5β-pregnan-3α-ol-20-one, 4-androstendione, 5α-pregnan-3α-ol-20-one, and 5α-pregnan-3α-21-diol-20-one), and all the other reagents were obtained from Sigma Chemical Company (St. Louis, MO). Steroids were dissolved in dimethylsulfoxide (DMSO) at an initial concentration of 2 mg/ml, and further diluted in FM buffer (see below) supplemented with 7 mg/ml bovine serum albumin (BSA), according to the method of Thomas and Meizel (1989). As variability in the capacitating ability of different albumin preparations has been reported (Aitken et al, 1984), BSA from the same lot was always used (Sigma catalogue number A-7888). Stock solutions of A23187 (100 mmol/L) were made in 100% DMSO.

Preparation and Incubation of Spermatozoa

Human semen was collected by masturbation, after 4 days of sexual abstinence, from 30 men undergoing semen analysis for infertility. Samples with less than 3.0×10^7 sperm/mL, a linear progressive motility of less than 25% after 60 minutes, and with leukocytes and/or immature germ cell concentrations greater than 10^6/ml were not included in the study. After complete liquefaction, two or three semen samples were pooled and divided into two aliquots; one was prepared for sample capacitation, and the other served as a noncapacitated control and was processed as indicated in Figure 1. Samples were carefully layered on a discontinuous Percoll gradient (40% and 80%) containing or not containing (in order to avoid initial capacitation of the noncapacitated samples) 0.3% BSA, and processed as described by Yates et al (1989). Cells were collected from the 40% and 80% Percoll layers, washed once in an equal volume of BWW medium (Biggers et al, 1971) containing or not containing 3 mg/ml BSA, and finally resuspended in the same medium at a concentration of 5×10^6 cells/ml. Some of the capacitated samples were incubated overnight at 37°C in a cell incubator saturated with 5% carbon dioxide. Spermatozoa capacitated overnight were used for experiments of P dose–response curves, for testing the effects of the different steroids, and for evaluating the effects of the various agonists/antagonists on the $[Ca^{2+}]_i$ response to P.

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

Sperm suspensions, prepared as described above, were incubated in the dark with 2 μmol/L F2/AM for 45 minutes at 37°C. Fol-

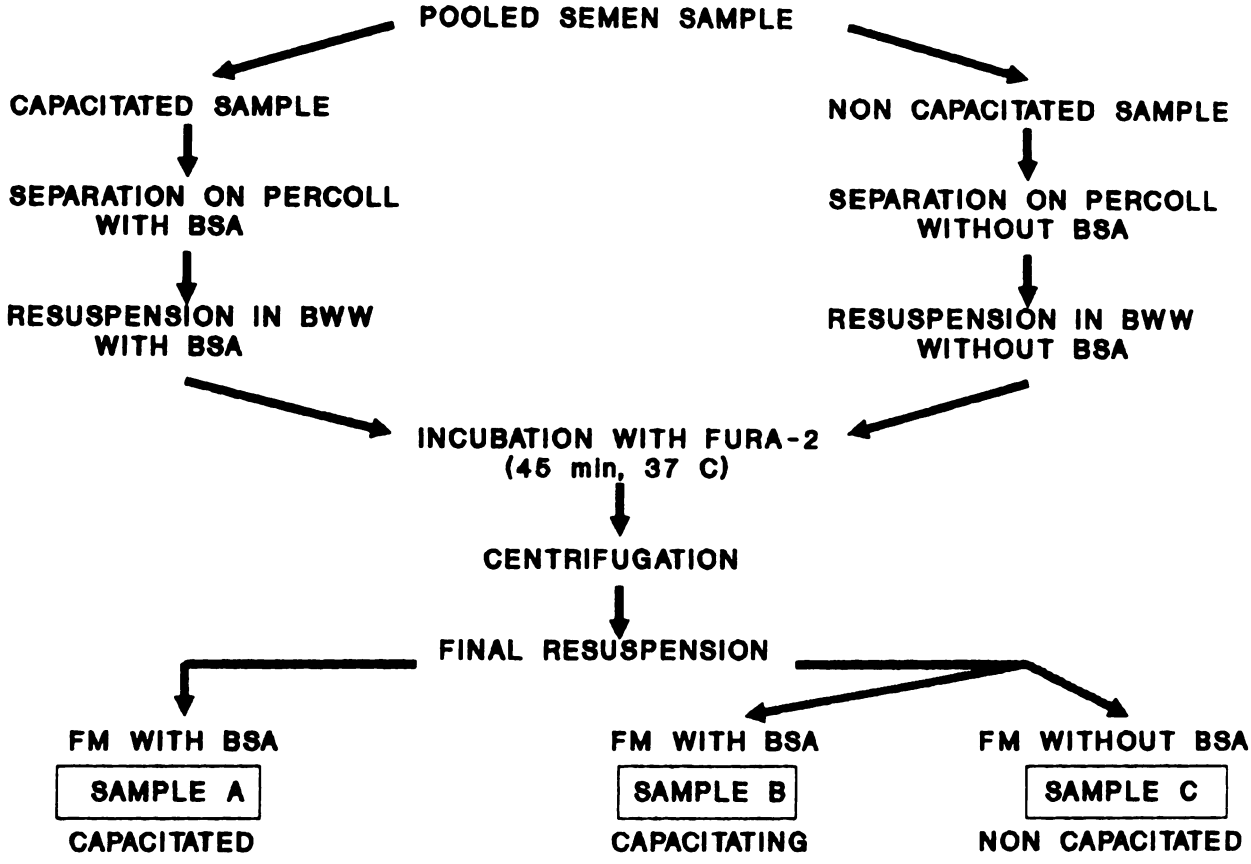

FIG. 1. The experimental design. Percoll separation was performed by centrifugation at 600*g* for 30 minutes; fura2/AM (F2/AM) was loaded by incubation at 37°C for 45 minutes with 2 μmol/L F2/AM, followed by 15 minutes of incubation at 37°C in FM medium without F2/AM to allow further deesterification of the dye.

lowing centrifugation, capacitated samples were resuspended in FM medium (125 mmol/L NaCl, 10 mmol/L KCl, 2.5 mmol/L $CaCl_2$, 0.25 mmol/L $MgCl_2$, 19 mmol/L Na lactate, 2.5 mmol/L Na pyruvate, 20 mmol/L Hepes/NaOH; Thomas and Meizel, 1988) containing 3 mg/ml BSA. Noncapacitated spermatozoa were resuspended in FM medium devoid of BSA. Cells were incubated for 15 more minutes at 37°C, centrifuged, and resuspended at a concentration of 5×10^6 cells /ml. As indicated in Figure 1, capacitated sperm were resuspended in FM medium with BSA (sample A); noncapacitated sperm were either resuspended in FM medium containing BSA (capacitating after fura 2 incubation: sample B), or in FM medium without BSA (noncapacitated: sample C).

The F2 loading protocols did not affect sperm motility, as determined by counting motile sperm under an optical microscope (35% ± 5% motile sperm before vs. 32% ± 4% after incubation, n = 12). F2/AM-loaded cells were kept in the dark at 37°C for the duration of the experiments. For fluorescence measurements, 2.5×10^6 cells were centrifuged for 1 minute at 6,000 rpm in a microfuge (ALC, Milan, Italy) in order to remove extracellular F2 that might have leaked from spermatozoa. Cells were reconstituted in the same volume and transferred to a quartz cuvette in a total volume of 2 ml. Fluorescence was measured using a spectrofluorometer (University of Pennsylvania Biomedical Group, Philadelphia, PA) set at 340 nm excitation with emission at 510 nm. Fluorescence measurements were converted to $[Ca^{2+}]_i$ after determining the maximal fluorescence (F_{max}) by lysing the cells with 0.01% digitonin followed by minimal fluorescence (F_{min}) with 10 mmol/L EGTA, pH 10. Intracellular calcium was then calculated according to Grynkiewicz et al (1985), assuming a dissociation constant of fura 2 for Ca^{2+} of 224 nmol/L.

Determination of Acrosome Reaction

Acrosome-reacted spermatozoa were evaluated by a double-stain technique as described by De Jonge et al (1988). Spermatozoa were prepared and incubated as described for determination of $[Ca^{2+}]_i$ (Fig 1), except that F2/AM was not added to the suspension. After 15 minutes of incubation at 37°C with calcium ionophore A23187 (final concentration 10 μmol/L), 2 μg/ml P, or control buffer (final concentration of DMSO 0.01%), 1.25×10^6 spermatozoa were fixed in 3% glutaraldehyde for 30 to 40 minutes, centrifuged, and washed twice by centrifugation at 600g for 10 minutes in distilled water. The pellet was then resuspended in 25 μl distilled water, layered on a glass slide, and dried under a stream of air. Slides were stained with 0.8% Bismark brown (at 37°C for 10 minutes), washed in distilled water, and stained in 0.8% Rose Bengal for 30 to 40 minutes at room temperature. After a second washing procedure, slides were dehydrated in alcohol series (50%, 95%, and 100%), cleared in xylene for 10 minutes, and examined by light microscopy in a blinded fashion. Results are expressed as the change in the proportion of acrosome-reacted spermatozoa (percent acrosome-reacted sperm in response to the stimulus minus control).

Statistical Analysis

Results are expressed as the mean ± SEM. Statistical comparisons were made using analysis of variance and student's t test. Dose–response curves were analyzed with the ALLFIT program (De Lean et al, 1978). Correlations were calculated using Pearson's correlation coefficient.

Results

Effect of Progesterone on $[Ca^{2+}1]_i$ in Human Sperm

The response of F2-loaded spermatozoa to progesterone was characterized by an immediate initial transient increase of intracellular Ca^{2+} followed by a long-lasting sustained phase whose length was dependent on P concentration (Fig 4). At concentrations of 0.1 μg/ml and higher, the sustained phase lasted at least 5 minutes, and in some cases basal

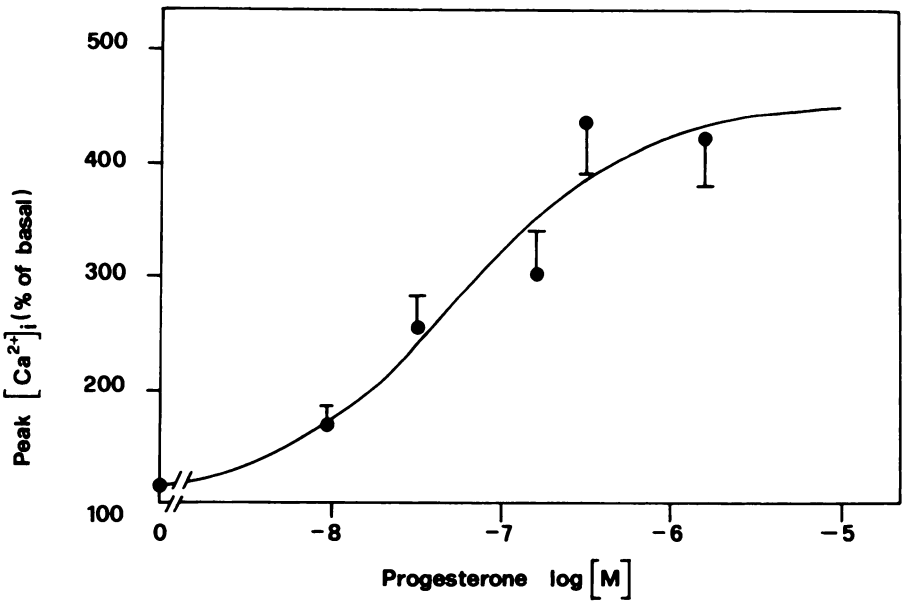

FIG. 2. Dose–response curve of P-stimulated $[Ca^{2+}]_i$ in capacitated human spermatozoa. Spermatozoa (5×10^6 cells/ml) were capacitated overnight in complete BWW medium and loaded with the intracellular Ca^{2+} indicator fura 2/AM. Fluorescence was measured at 340 nm excitation and 510 nm emission. Progesterone was added directly to the sperm suspension at the indicated final concentrations. Results are expressed as mean ± SEM percentage increase of $[Ca^{2+}]_i$ (n = 7 experiments).

values were not reached even after a longer time. In human sperm capacitated overnight, P dose-dependently increased $[Ca^{2+}]_i$, reaching a plateau at a concentration of 318 nmol/L (0.1 μg/ml) (Fig 2). The EC_{50} of the curve (defined as the concentration with expected half-maximal response) was 53 ± 45 nmol/L (7 ng/ml, n = 5 experiments), with a slope of 0.78 ± 0.4 and a maximal effect of 457% ± 93%.

Effect of Capacitation on Basal $[Ca^{2+}]_i$

Average basal $[Ca^{2+}]_i$, measured approximately 10 minutes after loading procedure, was significantly greater ($P < 0.05$) in capacitated sperm (137.5 ± 10.7 nmol/L, n = 9; sample A: sperm prepared in medium containing BSA) than in capacitating sperm (72.6 ± 16.5 nmol/L, n = 6; sample B: sperm prepared in a medium initially devoid of BSA, which was added after F2 loading procedure) and noncapacitated sperm (70.1 ± 28.7 nmol/L, n = 6; sample C: sperm incubated without BSA). There were no differences in basal $[Ca^{2+}]_i$ between samples B and C at this time (Fig 3). During the subsequent incubation in medium containing BSA, there was a progressive increase in basal $[Ca^{2+}]_i$ in capacitating sperm (sample B), which became significant at 60 minutes, and reached a plateau 120 minutes after loading with F2/AM (Fig 3). Free intracellular calcium did not change in sperm continuously kept in medium devoid of BSA (sample C), or in capacitated sperm (sample A), throughout the duration of the experiment.

After overnight incubation in capacitating medium, average basal $[Ca^{2+}]_i$ was 155.1 ± 30.4 nmol/L (n = 7). This value was stable after further 3-hour incubation in FM medium containing 3 mg/ml BSA (data not shown).

Changes in Responsiveness to Progesterone During Capacitation

Following the F2-loading procedure, initial responsiveness (transient increase of intracellular Ca^{2+}) to P (0.1 μg/ml) was greater in samples prepared in medium containing BSA (sample A; (Figs 4A, 5). By contrast, samples prepared in medium without BSA (sample C) had a much lower response. Responsiveness to P in samples A and C did not change significantly throughout the incubation period (Figs 4 A–C, 5). Responsiveness of capacitating sperm (sample B) to P, although initially comparable to sample C, progressively increased during incubation in medium containing 3 mg/ml BSA, reaching a plateau around 60 to 90 minutes (Figs 4B, 5). Average P-stimulated peak heights of $[Ca^{2+}]_i$ (expressed as percent of basal $[Ca^{2+}]_i$) in the three different samples at the various incubation times is shown in Figure 5. Statistical analysis indicated a significant increase of transient peak height in response to P in sample B at 30, 60, 90, 120, and 150 minutes following the beginning of incubation in medium containing BSA (Fig 5).

We examined next whether P-stimulated $[Ca^{2+}]_i$ increase in human sperm was related to the basal $[Ca^{2+}]_i$ in

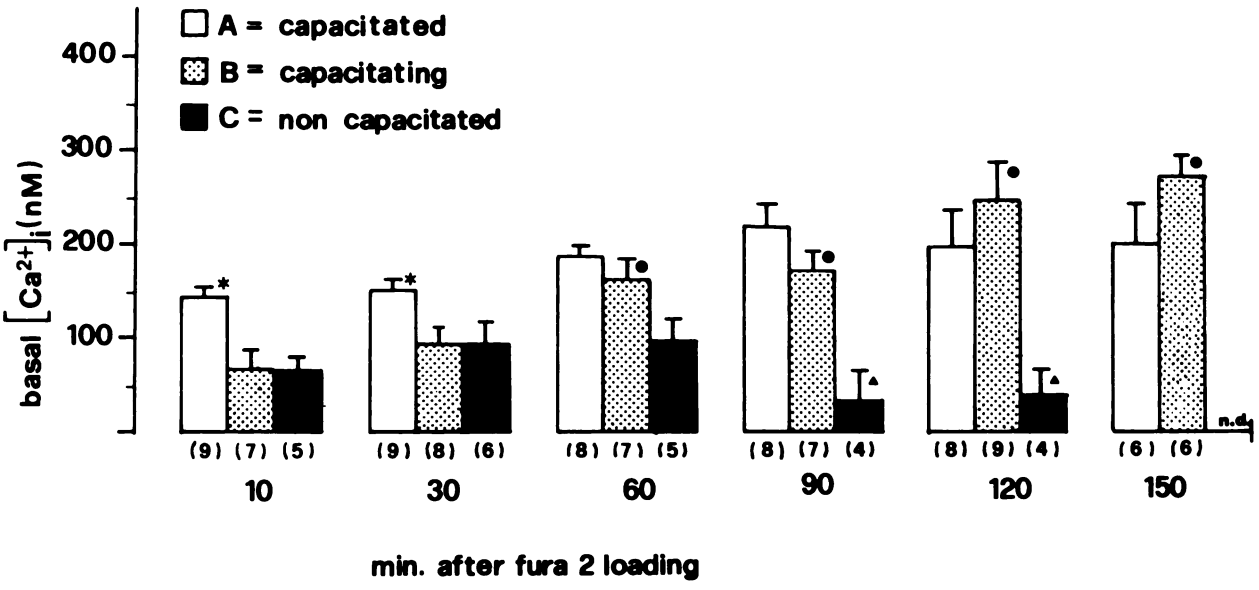

FIG. 3. Mean basal $[Ca^{2+}]_i$ in capacitated (column A), capacitating (column B), and noncapacitated (column C) human spermatozoa. Spermatozoa (5×10^6 cells/ml) obtained after migration on Percoll gradient in the presence (A) or absence (B,C) of BSA were loaded with the intracellular Ca^{2+} indicator fura 2/AM and resuspended in FM medium containing (A,B) or not containing (C) BSA. Fluorescence was measured at 340 nm excitation and 510 nm emission. Basal $[Ca^{2+}]_i$ was calculated at the indicated incubation times as described by Grynkiewicz et al (1985) after calibration with 0.01% digitonin (for determination of F_{max}) followed by 10 mmol/L EGTA at pH 10 (for determination of F_{min}). The number of experiments is listed in parentheses. *$P < 0.05$ vs B and C (10 and 30 minutes); •$P < 0.005$ vs B (10 minutes); ▲$P < 0.005$ vs A (90 and 120 minutes) and B (90 and 120 minutes). n.d. = not determined.

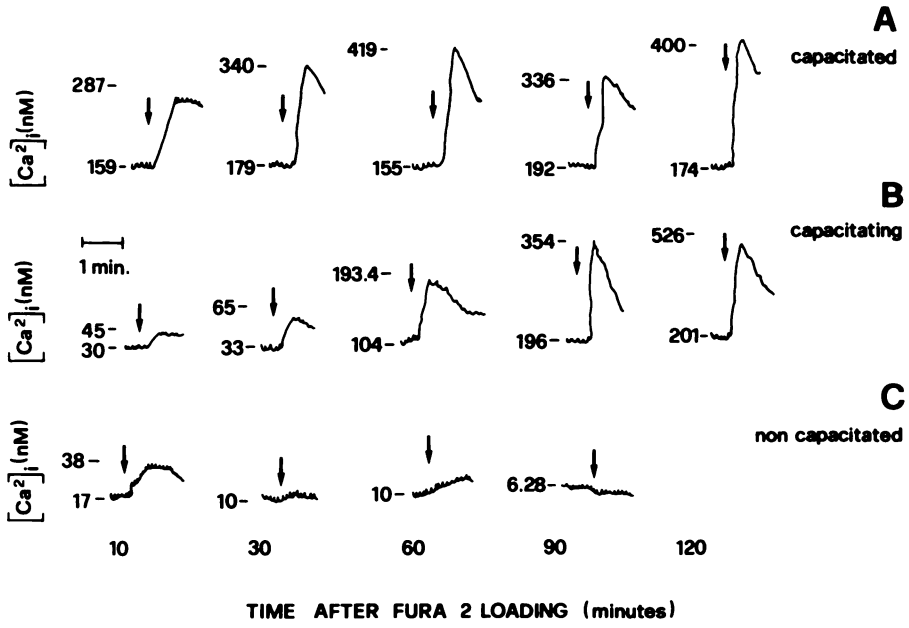

FIG. 4. $[Ca^{2+}]_i$ increase in response to P in capacitated (panel A), capacitating (panel B), and noncapacitated (panel C) human spermatozoa. Spermatozoa (5 × 10^6 cells/ml) obtained after migration on Percoll gradient containing (panel A) or not containing (panels B,C) BSA were loaded with the intracellular Ca^{2+} indicator fura 2/AM and resuspended in FM medium in the presence (panels A,B) or absence (panel C) of BSA. Fluorescence was measured at 340 nm excitation and 510 nm emission; arrows indicate the addition of P (0.1 μg/ml final concentration). Results of a typical experiment are shown.

the three different samples. A significant correlation between basal $[Ca^{2+}]_i$ and increase of transient peak ($[Ca^{2+}]_i$ in response to P minus basal $[Ca^{2+}]_i$) was present in both samples A and B. The correlation coefficient was greater in capacitating spermatozoa (sample A: $r = 0.47$, $P < 0.02$; sample B: $r = 0.93$, $P < 0.001$); no correlation was present in spermatozoa incubated in BSA-free medium (sample C: $r = 0.26$, $P = 0.25$).

P- and calcium ionophore A23187-stimulated acrosome reactions were measured at 10, 60, and 120 minutes of incubation. Results showed a similar pattern to P-stimulated $[Ca^{2+}]_i$ increase. The percentage of acrosome-reacted spermatozoa was maximal at any incubation time in sample A, both in response to P and A23187, whereas a significant increase in acrosome-reacted sperm was observed at 60 and 120 minutes of incubation in capacitating spermatozoa

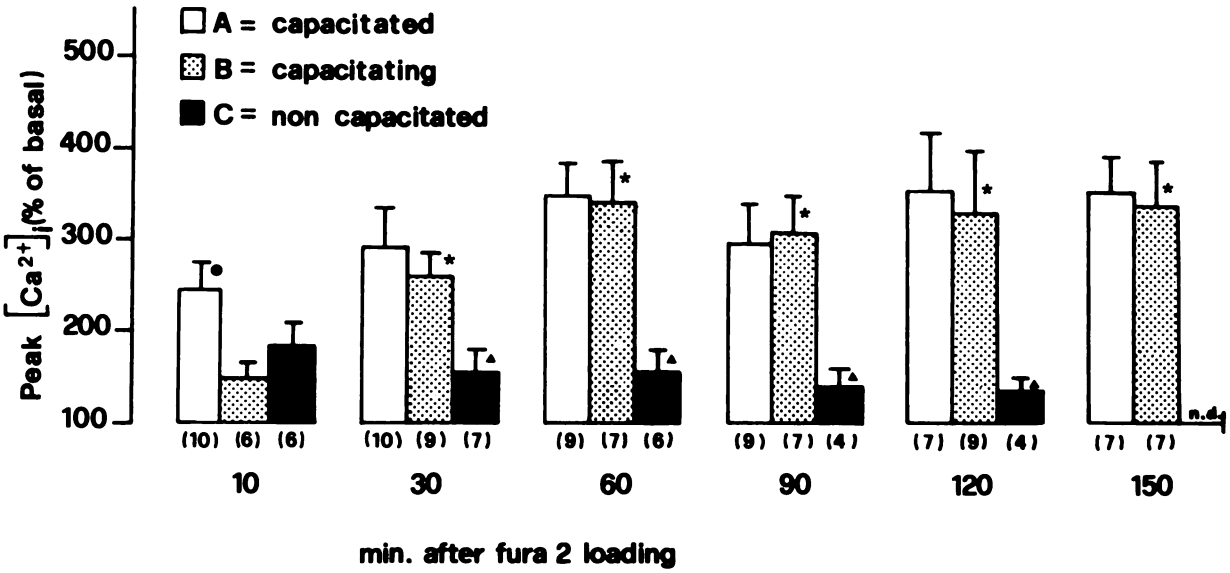

FIG. 5. Mean P-stimulated $[Ca^{2+}]_i$ increase in capacitated (column A), capacitating (column B), and noncapacitated (column C) human spermatozoa. Spermatozoa (5 × 10^6 cells/ml) obtained after migration on Percoll gradient in the presence (A) or absence (B,C) of BSA, were loaded with the intracellular Ca^{2+} indicator fura 2/AM and resuspended in FM medium containing (A,B) or not containing (C) BSA. Fluorescence was measured at 340-nm excitation and 510-nm emission. Peak $[Ca^{2+}]_i$ was calculated at the indicated incubation times according to Grynkiewicz et al (1985) after calibration with 0.01% digitonin (for determination of F_{max}) followed by 10 mmol/L EGTA at pH 10 (for determination of F_{min}). The number of experiments is listed in parentheses. *$P < 0.01$ vs B (10 minutes); •$P < 0.005$ vs B (10 minutes) and C (10 minutes); ▲$P < 0.005$ vs A and B (30, 60, 90, and 120 minutes). n.d. = not determined.

Table 1. *Acrosome reaction in response to P and calcium ionophore A23187 in capacitated and capacitating human sperm*

Minutes after final resuspension	Sample A		Sample B	
	A23187	P	A23187	P
10	14.7 ± 5.8 (4)	7.0 ± 1.6 (7)	4.7 ± 1.5 (4)	0.6 ± 0.4† (6)
60	16.1 ± 2.5 (7)	8.0 ± 1.8 (7)	7.6 ± 0.9 (5)	7.8 ± 1.5‡ (6)
120	13.1 ± 2.5 (6)	5.7 ± 1.8 (7)	14.6 ± 2.6* (6)	14.1 ± 2.2‡ (6)

Values are presented as the mean ± SEM percentage of acrosome-reacted spermatozoa in response to the stimuli minus control. The number of experiments is listed in parentheses.

* $P < 0.05$ vs A23187 10-minute sample B.

† $P < 0.005$ vs P 10-minute sample A.

‡ $P < 0.05$ vs P 10-minute sample B.

(sample B; Table 1). By contrast, there were no differences between stimulated, acrosome-reacted spermatozoa and control in noncapacitated sperm (sample C; data not shown).

Effect of Agonists/Antagonists Response to Progesterone

The effect of the P antagonist RU486 (Philibert et al, 1985; (final concentration: 1 μmol/L) on P-mediated Ca^{2+} increase in human sperm was tested in two different experiments by adding the antagonist 1 minute before increasing concentrations of P. The P dose–response curve was not affected by this treatment, indicating that classic intracellular P receptors are not involved in this effect (results not shown).

To test the hypothesis that P-stimulated intracellular Ca^{2+} rise in human sperm occurred through interaction with putative GABA-A receptors, we investigated the effects of two GABA-A receptor antagonists, bicuculline and picrotoxin, on P-stimulated Ca^{2+} increase. The two antagonists were added directly to the sperm suspension 1 minute before P. Bicuculline, at a final concentration of 10 μmol/L, and picrotoxin at 50 μmol/L, did not significantly affect the dose–response curve of P nor modify the EC_{50} for the steroid (n = 3 experiments for each antagonist, data not shown). We also tested the effect of GABA (10 to 500 μmol/L) and homotaurine (10 to 100 μmol/L), a GABA-A partial agonist, on $[Ca^{2+}]_i$ in human spermatozoa. Neither agonists affected intracellular Ca^{2+}, or modified the dose–response to P (n = 2 experiments for each agonist, data not shown).

Effect of Steroids on the Stimulation of $[Ca^{2+}]_i$ Increase

The effect of other steroids on $[Ca^{2+}]_i$ was tested in human spermatozoa capacitated overnight. Each steroid was added at a final concentration of 0.3 μmol/L. P, 11β-hydroxyP, and 17α-hydroxyP were the most potent stimulators of Ca^{2+} increase, followed by 5α-pregnane-3,20-dione (Table 2). Other steroids produced lesser effects, ranging from 0% to 30% of P response. A comparison of the binding activity of some of the tested steroids on intracellular P receptor in the uterus (Kontula et al, 1975; Gee et al, 1988) and their effect on the $[Ca^{2+}]_i$ concentration in human sperm (Table 2) revealed no correlation between the parameters. Moreover, 5α-pregnane-3α-ol-20-one, 5β-pregnane-3α-ol-20-one, and 5α-pregnane-3α-21-diol, 20-one, which are very potent modulators of GABA-A receptors in the central nervous system (Gee et al, 1988; Morrow et al, 1990), were ineffective in stimulating Ca^{2+} rise in spermatozoa, which argues against an interaction of P with putative GABA receptors in human spermatozoa.

Discussion

In this study, we confirm previous evidence (Blackmore et al, 1990; Thomas and Meizel, 1989) that P is a potent stimulator of calcium entry into capacitated human spermatozoa. The exact mechanism underlying P-stimulated Ca^{2+} increase in spermatozoa is still in question (Blackmore et al,

Table 2. *Effect of different steroids on $[Ca^{2+}]_i$ in capacitated human spermatozoa**

Steroid	% of P-stimulated $[Ca^{2+}]_i$ increase†	% of P binding to uterine intracellular receptor
Progesterone	100	100
11β-hydroxyprogesterone	79.8 ± 12.7	—
17α-hydroxyprogesterone	78.1 ± 10.5	0.7‡
5α-pregnane,3-20-dione	50.8 ± 11.2	30‡
5β-pregnane-3α-ol-20one	27.9 ± 11.4	—
4-androstendione	14.8 ± 3.5	0.1‡
5α-pregnane-3α-ol-20one	5.3 ± 3.5	0§
5α-pregnane3α-21diol-20one	0	0§

* Steroids were added directly to the sperm suspension at a final concentration of 0.3 μmol/L.

† The increase was measured as peak height of the response. Values are presented as the mean ± SEM; n = 3 experiments.

‡ Kontula et al, 1975.

§ Gee et al, 1988.

1990), although recent evidence suggests an action at the level of the sperm plasma membrane (Meizel and Turner, 1991). Binding of P and other steroids to rat and human spermatozoa has been shown (Galena et al, 1974; Chang et al, 1981), but the exact nature of P receptors on spermatozoa has not been elucidated. The dose–response relationship of P action on sperm intracellular Ca^{2+} concentrations suggests the presence of specific receptors; however, it is not known whether this receptor is the same or analogous to the intracellular P receptor. We demonstrate that treatment with the potent progestin and glucocorticoid antagonist RU486 (Philibert et al, 1985) did not affect P-stimulated Ca^{2+} accumulation in human spermatozoa. Moreover, there was no apparent correlation between the rank order of potency on uterine intracellular P receptors and the relative effect of some steroids on $[Ca^{2+}]_i$ in human sperm. These findings argue against similarities between the intracellular uterine P receptor and the human sperm P receptor.

Another possible mechanism of P interaction with the sperm surface is suggested by the recent finding that P and its reduced analogs induce sedation and anesthesia in experimental animals through modulation of GABA-A receptors in the central nervous system (Maiewska et al, 1986; Turner et al, 1989). High concentrations of GABA have been reported both in male and female reproductive tracts (Ritta and Calandra, 1986; Gimeno et al, 1986), and the hypothesis of a modulatory role for this amino acid on sperm function is attractive, although GABA receptors on spermatozoa have not yet been demonstrated. In our study, the two GABA-A receptor antagonists bicuculline and picrotoxin slightly (10% to 20%) inhibited P-stimulated Ca^{2+} accumulation in human sperm. By contrast, both antagonists completely abolished the central effects of progesterone analogs (Brann et al, 1990; Im et al, 1990). Moreover, GABA and the GABA-A partial agonist homotaurine did not affect sperm $[Ca^{2+}]_i$, nor modify the response to P. Furthermore, the P analogs 5α-pregnane-3α-ol,20-one and 5β-pregnane-3α-ol, 20-one, two potent modulators of GABA receptors in the central nervous system (Gee et al, 1988; Morrow et al, 1990), were less effective than other P analogs in stimulating Ca^{2+} increase. Cumulatively, these findings refute the interaction of P with putative GABA receptors on the sperm surface.

Mammalian spermatozoa acquire their ability to fertilize eggs following several biochemical alterations of plasma membrane composition, collectively known as capacitation (Yanagimachi, 1988). Physiologic capacitation occurs in the female genital tract, but sperm capacitation can also be achieved *in vitro* in a variety of capacitating media (Yanagimachi, 1988). Capacitation is characterized by profound changes in the protein and lipid composition of the sperm plasma membrane, including alterations of membrane permeability to ions, which prepare the spermatozoon to undergo the acrosome reaction (Yanagimachi, 1988).

An influx of Ca^{2+} during capacitation has been demonstrated in spermatozoa of several mammalian species (Singh et al, 1978; Coronel and Lardy, 1987; White and Aitken, 1989; Ruknudin and Silver, 1990; Zhou et al, 1990); however, Mahanes et al (1986) did not show any change of $[Ca^{2+}]_i$ in capacitating rabbit spermatozoa. We report here that basal $[Ca^{2+}]_i$ increased in capacitating human spermatozoa incubated in the presence of 3 mg/ml BSA. Furthermore, incubation in medium containing BSA resulted in a progressive increase in responsiveness to progesterone (increase of stimulated $[Ca^{2+}]_i$ transience and induction of acrosome reaction). Similarly, the percentage of acrosome-reacted sperm increased in response to calcium ionophore A23187 in capacitating spermatozoa, while remaining constant in capacitated and noncapacitated samples. These findings confirm previous results showing that albumin, present in oviductal and follicular fluids, stimulates capacitation of mammalian spermatozoa (Meizel, 1985), and that hamster spermatozoa do not undergo physiologic acrosome reactions in the absence of albumin (Andrews and Bavister, 1989). These results indicate a role for this protein in the induction of acrosome reaction in cooperation with physiologic stimuli. In our study, P-stimulated increase of $[Ca^{2+}]_i$ in capacitating human spermatozoa was highly correlated with basal $[Ca^{2+}]_i$. Such correlation was less evident in capacitated spermatozoa and absent in noncapacitated sperm. This finding suggests that the increase in responsiveness to P parallels the increase in $[Ca^{2+}]_i$ that occurs in capacitating spermatozoa, and that the ability of sperm to accumulate Ca^{2+} in their cytoplasm is important for preparing the cell to undergo the acrosome reaction. The presence of serum albumin in the medium increases the permeability of the cells, which then become able to undergo the acrosome reaction in response to P. The parallel increase of acrosome-reacted spermatozoa and Ca^{2+} accumulation in response to P suggests either an increase of responsiveness of the single cell and/or a progressive recruitment of responsive cells during the process of capacitation.

In conclusion, our results demonstrate an increase of $[Ca^{2+}]_i$ in human spermatozoa during capacitation in the presence of albumin. This increase is accompanied by an increase in responsiveness to P, suggesting either a progressive recruitment of responsive cells and/or increased responsiveness of the single cell during the process of capacitation. This mechanism could be physiologically relevant *in vivo* during capacitation of spermatozoa in the female genital tract.

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