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Progesterone and RU486: Opposing effects on human sperm

(capacitation/hyperactivation/acrosome reaction/intracellular calcium concentration)

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ABSTRACT **Progesterone induced a rapid influx of calcium in capacitated human sperm, followed by a long-lasting, dose-dependent increase of intracellular free calcium. Thereafter, progesterone increased the fraction of hyperactivated sperm and the acrosome reaction. On the contrary, the progesterone antagonist RU486 (mifepristone) induced an immediate and transient, dose-dependent decrease of intracellular free calcium and a drop in the values of sperm movement parameters related to hyperactivation. Moreover, RU486 counteracted the effects of progesterone on calcium influx, lateral sperm head displacement, and the acrosome reaction. Therefore, RU486 effects were opposite to those of progesterone. The nature of the membrane receptor(s) involved is unknown. Several steroids bearing 11β-phenyl substitutions, with different pharmacological profiles, were also investigated. It was concluded that the steroid structure and chemical groups added to the 11β-phenyl influence effects on calcium influx.**

Capacitation leads to functional changes of the sperm that are characterized by modified movement parameters designated as hyperactivation and by exocytotic events (the acrosome reaction) (1–3). The acrosome reaction is an essential step in the complex series of processes implied in Eutherian fertilization, necessary for sperm penetration through the zona pellucida and fusion with the oocyte plasma membrane. It can be induced by follicular fluid, cumulus cells (4), or zona pellucida (5). Progesterone (PROG) was identified as a major component of the follicular fluid for inducing the acrosome reaction in human sperm (6), preceded by an immediate, transient calcium influx into spermatozoa (7, 8). These rapid biological effects suggest a nongenomic mechanism and a cell surface receptor for PROG like the one responsible for the meiotic maturation of *Xenopus laevis* oocyte (9).

The antiprogestin RU486 {RU38486, mifepristone, 17β-hydroxy-11β-[4-(dimethylamino)phenyl]-17α-propynylestra-4,9-dien-3-one} binds with high affinity to the intracellular PROG receptor in most vertebrate species (10). It has been reported that this potent antiprogestin has either a small or negligible inhibitory effect on PROG-mediated calcium influx into human sperm (11, 12). In this study, we evaluated the effect of RU486, alone or in combination with PROG, on intracellular free calcium concentration ($[Ca^{2+}]_i$) and the movement of human sperm and acrosome reaction, two calcium-related events (3, 13).

MATERIALS AND METHODS

Materials. PROG, human serum albumin (HSA) fraction 5, Fura2-AM, Fluo3-AM, Hoechst 33258, fluorescein-labeled *Pisum sativum* agglutinin, verapamil, EGTA, Triton X-100, sodium pyruvate, and Hepes were purchased from Sigma. RU486, 17β-hydroxy-11β-{4-[2-dimethylamino)ethoxy]phenyl}-17α-propynylestra-4,9-dien-3-one (RU39009), 11β-[4-(dimethylamino)phenyl]-17α-propynylestra-1,3,5(10)-triene-3,17β-diol (RU41291), 11β-{4-[2-(dimethylamino)ethoxy]-phenyl}estra-1,3,5(10)-triene-3,17β-diol (RU39411), corticosterone, testosterone, estradiol, and 17α-methylnorpregna-4,9-diene-3,20-dione (R5020) were kindly provided by Roussel-UCLAF. Ionomycin (Iono) was purchased from Calbiochem. All other chemicals (e.g., salts for buffers) were purchased from Merck.

Sperm Preparation. Human semen was obtained from healthy donors. Motile spermatozoa were selected by centrifugation through a two-step (47.5–95%) mini-Percoll gradient and resuspended in a hypertonic BWW medium (14) containing 166 mM NaCl, 5 mM KCl, 1.3 mM $CaCl_2$, 1.2 mM KH_2PO_4, 1.2 mM $MgSO_4$, 5.5 mM glucose, 0.25 mM sodium pyruvate, 21 mM sodium lactate, 25 mM $NaHCO_3$, 20 mM Hepes, and 0.8% HSA [BWW/HSA(+)] (410 mosmoles/liter, pH 7.4 at room temperature).

Measurement of Intracellular Calcium. Measurements were performed on selected sperm kept at least 2 hr in capacitating medium BWW/HSA(+), unless otherwise stated. Cells (5–10 × 10^6 per ml) were incubated with Fura2-AM (2 μM final concentration) at 37°C, for 45 min. After washing (600 × g, 10 min) in BWW without HSA (BWW), 4 × 10^6 cells per ml were resuspended in BWW. Fluorescence signal was recorded at 37°C using a spectrofluorometer at excitation wavelengths of 340 nm and 380 nm (PTI M-2001) (Kontron, Zurich) or at excitation wavelengths of 340 nm, 360 nm, and 380 nm (Hitachi F-2000) (Braun Science Tec, Les Ulis, France). Fluorescence emission was monitored at 505 nm. Either PROG or the steroid hormone corticosterone, testosterone, or estradiol, the progestin R5020, or the 11β-phenyl-substituted steroid RU486, RU39009, RU41291, or RU39411 dissolved in absolute ethanol was added to the incubation medium at 0.1% final ethanol concentration. At the end of each assay, 5 μM Iono was added to the sample to measure the maximum signal of fluorescence; then sperm was permeabilized with 0.05% Triton X-100 and depleted of calcium by the addition of 10 mM EGTA (pH 9.5) to measure the minimum signal of fluorescence. These values were used to calculate $[Ca^{2+}]_i$ according to Grynkiewicz *et al.* (15).

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Abbreviations: PROG, progesterone; R5020, 17α-methylnorpregna-4,9-diene-3,20-dione; RU38486 (RU486, mifepristone), 17β-hydroxy-11β-[4-(dimethylamino)phenyl]-17α-propynylestra-4,9-dien-3-one; RU39009, 17β-hydroxy-11β-{4-[2-(dimethylamino)ethoxy]phenyl}-17α-propynylestra-4,9-dien-3-one; RU39411, 11β-{4-[2-(dimethylamino)ethoxy]phenyl}estra-1,3,5(10)-triene-3,17β-diol; RU41291, 11β-[4-(dimethylamino)phenyl]-17α-propynylestra-1,3,5(10)-triene-3,17β-diol; $[Ca^{2+}]_i$, intracellular free calcium concentration; HSA, human serum albumin; Iono, ionomycin; EC_{50}, half-maximally effective concentration; VSL, straight line velocity; VCL, curvilinear velocity; LIN, linearity; ALH, amplitude of lateral head displacement; MOT, motile cells.

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Movement Analysis. After capacitation in BWW/HSA(+) under 5% CO_2 in air at 37°C for 2 hr and incubation at 37°C for 2 min with 31.4 μM PROG, 23.4 μM RU486, or 10 μM Iono, sperm samples were deposited into capillary tubes of 200 μm inner depth. Sperm movement was analyzed at 37°C with a computerized automatic system (HT M-2030) (Hamilton Thorn Research). The rate of frame acquisition was 25 Hz. The parameters, measured according to the international terminology (16), on at least 100 motile cells were (*i*) the straight line velocity (VSL) evaluated as the linear distance covered by the sperm during 1 s (μm/s); (*ii*) the curvilinear velocity (VCL) evaluated as the actual distance covered by the head of sperm during 1 s (μm/s); (*iii*) the linearity (LIN), which is the VSL/VCL ratio × 100; (*iv*) the amplitude of the lateral head displacement (ALH) expressed in μm; and (*v*) the percentage of motile cells (MOT). Finally, the sort fraction was calculated as the percentage of sperm with VCL > 100 μm/s, ALH > 6 μm, and LIN < 60 and considered as hyperactivated cells (modified from Robertson *et al.*) (17).

Acrosome Reaction. After capacitation in BWW/HSA(+) at 37°C, under 5% CO_2 in air for 22 hr, sperm (6×10^6 per ml) were incubated with steroids at 37°C, for 30 min. Iono (10 μM) dissolved in dimethyl sulfoxide (0.5% final concentration) was used as positive control. The acrosome status and viability of sperm were determined according to Cross *et al.* (18). The amount of live sperm that underwent acrosome reaction (acrosome reacted sperm) was expressed in percent of total sperm number. At least 200 sperm were evaluated in each experiment.

Statistical Analysis. Results were expressed as mean ± SEM. A paired *t* test was used for statistical evaluation.

RESULTS

Effects of Steroids on $[Ca^{2+}]_i$. In a first set of experiments, we observed that PROG induced a transient increase of $[Ca^{2+}]_i$, followed by a second phase when the level of $[Ca^{2+}]_i$ remained slightly elevated above the basal level within the usual observation time (10 min) (Fig. 1*A*) and even after 30 min. The effect of PROG on $[Ca^{2+}]_i$ was dose dependent (Fig. 1*B*). The maximum response was obtained with 1 μM PROG, and the half-maximally effective concentration (EC_{50}) was about 3 nM. The increase of $[Ca^{2+}]_i$ induced by PROG (10 μM) was inhibited when sperm were preincubated with 1 mM verapamil for 2 min (Fig. 1*B*). Under the same experimental conditions, the steroid hormones corticosterone, testosterone, and estradiol and the synthetic progestin R5020 added at 10 μM concentration also induced transient increases of $[Ca^{2+}]_i$ of 63.3% ± 5.3%, 46.3% ± 8.8%, 43.6% ± 6.3%, and 30.4% ± 4.1%, respectively, of the increase produced by PROG (mean ± SEM, $n = 5$). PROG (10 μM) added 2 min after these steroids produced a further transient increase of $[Ca^{2+}]_i$, although it was smaller than that produced by PROG alone. Conversely, there was no further increase in $[Ca^{2+}]_i$ when either PROG or the other steroids (10 μM) were added 2 min after PROG (10 μM) (data not shown). When RU486 was added to the sperm suspensions under the same experimental conditions, the fluorescence signal decreased at 340 nm, increased at 380 nm, and did not change at 360 nm (Fig. 2). According to Grynkiewicz *et al.* (15), such fluorescence changes characterize a decrease in $[Ca^{2+}]_i$. The fluorescence signal of 1 μM unesterified Fura-2 added to a control solution, mimicking internal medium and containing no calcium, was not modified by RU486 (data not shown). The decrease of the fluorescence signal was maximum 15 s after addition of RU486 (Fig. 3*A*) and was dose-dependent (Fig. 3*B*). The maximum decrease of $[Ca^{2+}]_i$ produced by RU486 (10 μM) was 57.5% ± 3.8% (mean ± SEM, $P < 0.001$, $n = 11$) of the basal level. With the 100 μM RU486 dose, a parasite effect, increasing the fluorescence signal at 340 nm and 380 nm, was superimposed on the signal provided by the decrease of $[Ca^{2+}]_i$. At later times, $[Ca^{2+}]_i$ returned to the basal level for dose ≤ 1 μM, whereas it remained under basal level at doses > 1 μM within the 10-min observation time (Fig. 3*A*). The decrease of $[Ca^{2+}]_i$ induced by RU486 was confirmed in human sperm loaded with another calcium indicator, 1 μM Fluo-3 AM, at 506 nm excitation wavelength and 526 nm emission wavelength (Hitachi, F-2000). Preliminary results were also obtained with three analogs of RU486. RU39009 differs from RU486 only by the tamoxifen-like side chain at position 4 of the 11β-phenyl. It is also an antiglucocorticosteroid and a potential antiprogestin (Table 1) (19). RU41291 has the same 11β and 17α side chains as RU486 but is a phenol steroid like estrogen. It is a potential antiglucocorticosteroid

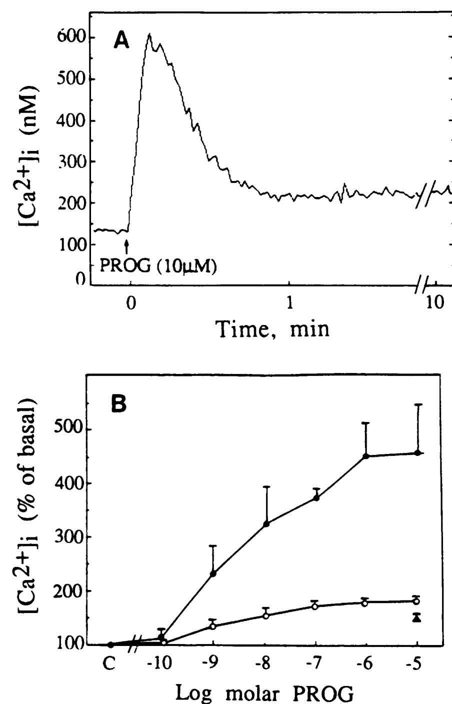

FIG. 1. PROG increases $[Ca^{2+}]_i$ in human sperm. The sperm were loaded with Fura-2 (see text). PROG was dissolved in absolute ethanol and added to the incubation medium (0.1% final ethanol concentration); 0.1% ethanol has no effect on $[Ca^{2+}]_i$. The fluorescence signal was measured with a PTI M-2001 spectrofluorometer. (*A*) Time course of the effect of 10 μM PROG on $[Ca^{2+}]_i$. (*B*) Dose–response curves of $[Ca^{2+}]_i$ to PROG [C, control level (100%) in absence of PROG]. The peak of $[Ca^{2+}]_i$ (●) and the calcium level after 10 min (○) were recorded (mean ± SEM, $n = 3$). After preincubation with verapamil (1 mM) for 2 min, the peak of $[Ca^{2+}]_i$ produced by 10 μM PROG was abolished (▲).

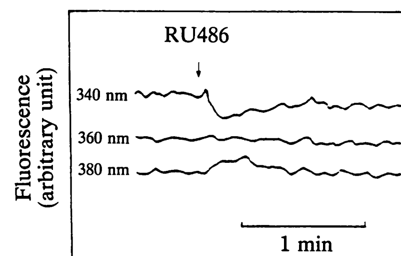

FIG. 2. Characterization of $[Ca^{2+}]_i$ changes induced by RU486 in human sperm. Spermatozoa (4×10^6 per ml) were capacitated in BWW/HSA(+) and then resuspended in BWW and loaded with Fura2-AM at 2 μM final concentration. The fluorescence signal was measured with excitation wavelengths at 340 nm, 360 nm, and 380 nm and emission wavelength at 505 nm (Hitachi F-2000). The final concentration of RU486 was 2.3 μM in 0.1% ethanol.

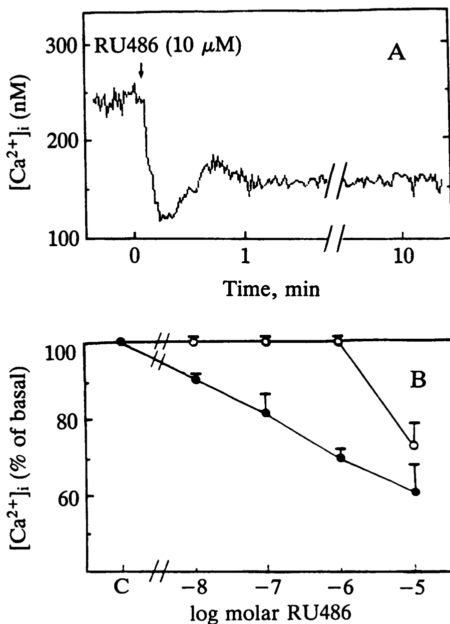

FIG. 3. RU486 decreases $[Ca^{2+}]_i$ in human sperm. RU486 was added to Fura-2-loaded spermatozoa. The fluorescence signal was measured with a PTI M-2001 spectrofluorometer. (*A*) Time course of the effect of RU486 (10 μM) on $[Ca^{2+}]_i$. (*B*) Dose–response curve of $[Ca^{2+}]_i$ to RU486 [C, control level (100%) in absence of RU486]. The peak of $[Ca^{2+}]_i$ (●) and the calcium level after 10 min (○) were recorded (mean ± SEM, $n = 4$).

and antiprogestin. RU39411 has the steroid structure of estradiol with the 11β side chain of RU39009. It is an antiestrogen with slight estrogen agonist activity (20–22). At 10 μM concentration, RU39009 and RU39411 decreased $[Ca^{2+}]_i$ to 47.3% ± 4.6% and 57.7% ± 5.7%, respectively, of basal level, whereas RU41291 increased $[Ca^{2+}]_i$ to 248.3% ± 36.1% of basal level (mean ± SEM, $n = 3$).

The effect of PROG was again tested after a 2-min preincubation interval with RU486. At the equimolar concentration of 10 μM, RU486 significantly inhibited the PROG response by 59.7% ± 11.5% (mean ± SEM, $P < 0.02$, $n = 7$) (Table 2). After a 10-min preincubation, RU486 at concentrations of 0.01, 0.1, and 1 μM did not counteract the stimulation produced by PROG when it was incubated at 0.01 μM concentration, which already increases $[Ca^{2+}]_i$ by 3-fold as shown in Fig. 1*B*. Only at 10 μM did RU486 significantly decrease the effect of 0.01 μM PROG by 69.0% ± 13.8% (mean ± SEM, $P < 0.05$, $n = 4$) (Fig. 4). We could not test the inhibitory effect of 100 μM RU486 because of its parasite effect. RU486 was also tested in combination with the other steroid hormones and progestin. Preincubation with 10 μM RU486 for 2 min also inhibited the increase of $[Ca^{2+}]_i$ produced by 10 μM corticosterone, testosterone, estradiol, or R5020 by 55.0% ± 11.9%, 26.3% ± 11.2%, 66.3% ± 25.0%, and 29.7% ± 20.6%, respectively (mean ± SEM, $n = 3$). At the same 10 μM concentration as RU486, RU39009, RU41291, and RU39411 inhibited the stimulation of $[Ca^{2+}]_i$ produced by PROG to about 75% of control level with PROG alone ($n = 3$).

Table 1. Relative binding affinities (%) of RU38486, RU39009, RU41291, and RU39411 for intracellular steroid hormone receptors

Compound	PR	GR	MR	AR	ER
RU38486	530	300	<0.1	25	<0.1
RU39009	11	170	1	1	<0.1
RU41291	130	120	<0.1	1.6	<0.1
RU39411	0.5	0.8	<0.1	6	180

The relative binding affinities of test compounds were determined for the following cytosol receptors: progesterone receptor (PR) of rabbit uterus, glucocorticosteroid receptor (GR) of rat thymus, mineralocorticosteroid receptor (MR) of rat kidney, androgen receptor (AR) of rat ventral prostate, and estrogen receptor (ER) of rat uterus. The radioactive reference steroids were PROG, dexamethasone, aldosterone, testosterone, and estradiol. Their affinity for the corresponding receptor was taken as 100%. Cytosol samples were incubated with the relevant radioligand in the presence of increasing concentrations of nonradioactive reference or test steroids, at 0°C for 24 hr (except in the case of ER, which was incubated at 25°C for 5 hr).

Table 2. Effects of PROG and RU486, alone or combined, on $[Ca^{2+}]_i$ in human sperm

Treatment	Peak $[Ca^{2+}]_i$, % of basal level
PROG	546.0 ± 28.3*
RU486	56.0 ± 1.8*
+ PROG	254.1 ± 14.3†‡

The steroids were added (10 μM concentration) to Fura-2-loaded spermatozoa. The peak response was measured and expressed as % of basal level. For competition experiments, the sperm suspensions were preincubated with RU486 for 2 min before the addition of PROG. The values are mean ± SEM of seven separate experiments. *, $P < 0.001$ versus basal; †, $P < 0.01$ versus basal; ‡, $P < 0.02$ versus PROG.

Effect of Steroids on Sperm Movement. Since the variations in $[Ca^{2+}]_i$ mediated by steroids occurred in a few seconds, the effects of steroids on sperm movement parameters were tested after a 2-min incubation interval (Table 3). After 2 hr of capacitation, MOT, VSL, and LIN were unchanged in the presence of 31.4 μM PROG, whereas VCL, ALH, and the sort fraction were significantly increased. In contrast, MOT, ALH, and the sort fraction were significantly decreased in the presence of 23.4 μM RU486. Moreover, in separate experiments, the increased values of sperm movement parameters induced by 31.4 μM PROG were partially inhibited by preincubation with 23.4 μM RU486 for 1 min, although the only statistically significant decrease was the one of ALH (6.0 ± 0.3 μm with PROG alone versus 5.5 ± 0.2 μm with RU486 plus PROG; mean ± SEM, $P < 0.02$, $n = 7$).

Induction of the Acrosome Reaction by Steroids. Human sperm were capacitated for 22 hr and then incubated at 37°C for 30 min with increasing concentrations of PROG. The percentages of live acrosome reacted sperm were significantly increased by PROG at 3.1 μM (7.9% ± 0.8%) and 31.4 μM (11.7% ± 2.5%) concentrations, compared to control (4.0% ± 1.1%) (mean ± SEM, $P < 0.05$, $n = 3$). The difference between both doses of PROG was not statistically

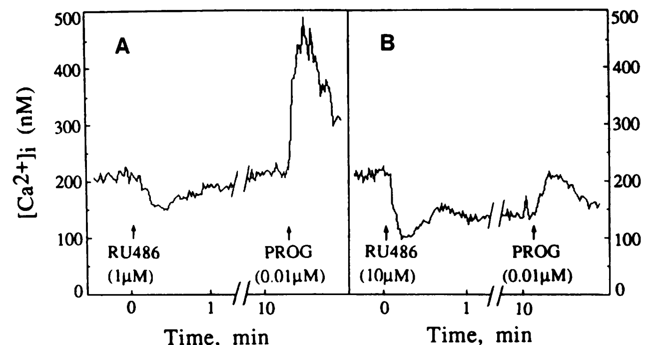

FIG. 4. Effects of PROG on $[Ca^{2+}]_i$ in human sperm preincubated with RU486. PROG (0.01 μM) was added to Fura-2-loaded sperm after preincubation with 1 μM RU486 (*A*) or 10 μM RU486 (*B*) for 10 min. The fluorescence signal was measured with a PTI M-2001 spectrofluorometer. An experiment representative of four is shown.

Table 3. Capacitated sperm movement parameters: Modulation by PROG or RU486

Parameter	Control	PROG§ (31.4 μM)	RU486§ (23.4 μM)	Iono¶ (10 μM)
MOT, %	73.6 ± 2.8	74.1 ± 2.4	67.4 ± 3.1‡	70.0 ± 3.2
VCL, μm/s	81.9 ± 3.0	88.7 ± 2.6†	77.2 ± 4.3	92.6 ± 4.0‡
VSL, μm/s	53.6 ± 3.1	54.9 ± 2.7	53.2 ± 4.0	54.6 ± 3.1
LIN, %	65.3 ± 2.2	63.0 ± 1.8	68.0 ± 2.3	57.8 ± 2.2
ALH, μm	5.6 ± 0.2	6.2 ± 0.2‡	5.1 ± 0.2*	6.7 ± 0.3‡
Sort fraction, %	9.3 ± 1.3	13.5 ± 2.0‡	6.6 ± 1.0‡	18.7 ± 3.4‡

Steroids with 1% ethanol final concentration were added to 1×10^6 spermatozoa per ml suspended in BWW/HSA(+) after 2 hr of capacitation; 1% ethanol was also added to the control incubation. After 2 min at 37°C, the sperm movements were recorded, and the different parameters were automatically computed. The values are means ± SEM. *, $P < 0.05$, †, $P < 0.02$, ‡, $P < 0.01$ versus control.
§n = 11 experiments.
¶n = 7 experiments.

significant and 62.8 μM PROG did not further increase the numbers of acrosome reacted sperm.

When PROG was replaced by 23.4 μM RU486, no increase of live acrosome reacted sperm occurred. However, the stimulatory effect of 31.4 μM PROG was significantly counteracted by preincubation with 23.4 μM RU486 for 2 min ($P < 0.02$, $n = 6$) (Fig. 5).

DISCUSSION

We report that PROG induces a rapid transient increase of $[Ca^{2+}]_i$ in human sperm, followed by a long-lasting phase when $[Ca^{2+}]_i$ stays above basal level (7, 8, 12). Under our experimental conditions, PROG was definitely more active than previously described (8, 12), since a half-maximal increase of $[Ca^{2+}]_i$ was attained at PROG concentrations in the nanomolar range. The peak and plateau of $[Ca^{2+}]_i$ are PROG dose dependent. We confirm that three other steroid hormones—corticosterone, testosterone, and estradiol—and the synthetic progestin R5020 also stimulate calcium uptake, although at lower levels than PROG (8). A second application of PROG 2 min after a first incubation with a maximally active concentration of PROG (10 μM) does not affect $[Ca^{2+}]_i$ any more. This refractoriness to a second application of steroid also occurs with the four other compounds tested, thus suggesting a common regulatory mechanism.

Several sperm movement parameters, VCL, ALH, and sort fraction, which define the hyperactivated state of human spermatozoa (17), are significantly increased by PROG, as previously reported (23). The percentage of sperm achieving

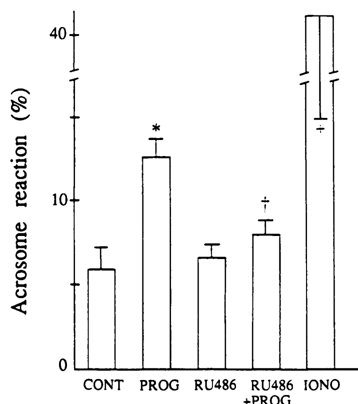

FIG. 5. RU486 inhibits the acrosome reaction of capacitated sperm produced by PROG. Sperm were incubated for 30 min either with 31.4 μM PROG, 23.4 μM RU486, or both steroids. RU486 was added 2 min before PROG. The acrosome status was evaluated as described in the text (mean ± SEM, $n = 6$). *, $P < 0.05$, and +, $P < 0.001$ versus control. †, $P < 0.02$ versus PROG. CONT, control; IONO, incubation with 10 μM Iono.

complete acrosome reaction is also significantly increased after 30 min of incubation with PROG.

The precise molecular mechanism of PROG action is not known, but its rapid effects on $[Ca^{2+}]_i$ (7, 8, 12), sperm movement, and onset of acrosome reaction (24) suggest an action at the membrane level. It has been proposed that voltage-dependent calcium channels (VDCCs) are responsible for the increase of $[Ca^{2+}]_i$ brought about by forskolin in porcine sperm (25) and for the acrosomal reaction produced by combined elevation of pH and membrane depolarization in ram and bull sperm (26). Similarly, we have found that the PROG effect on $[Ca^{2+}]_i$ was inhibited by the VDCC antagonist verapamil, at a concentration of 1 mM, suggesting that calcium channels may be involved in the effects of PROG in human sperm.

RU486 inhibits the uptake of calcium in sperm and counteracts the stimulation produced by PROG. The inhibitory action of RU486 *per se*, to our knowledge, has not been previously reported. Under our experimental conditions, this effect of RU486 on $[Ca^{2+}]_i$ was definite and was found in 11 of 12 experiments on different sperm samples. The PROG antagonist activity of RU486 on calcium uptake in human sperm is controversial and was not found in a previous report (12). RU486 has high affinity for the intracellular PROG receptor and displays strong competitive antagonism of the genomic effects of PROG (10). The situation is completely different in the case of sperm membrane: we have found that RU486 is a weak antagonist of PROG, as previously indicated (11). Indeed, the increase of $[Ca^{2+}]_i$ produced by 0.01 μM PROG was only partially inhibited by a 1000-fold excess of RU486, whereas an equimolar concentration of RU486 markedly counteracts the genomic effects of PROG (10). These results are consistent with the conclusion, previously proposed on the basis of different ligand affinities, that the sperm membrane receptor of PROG, as that of *Xenopus* oocyte membrane (27), is distinct from the classical intracellular PROG receptor. Although RU486 behaves like an inverse agonist of PROG, it cannot be determined whether the same membrane receptor is involved in the opposite effects of PROG and RU486 on calcium uptake in sperm and subsequent events. The present results are more in favor of distinct sites of action at the membrane level. This conclusion is based on the fact that the inhibitory effect of RU486 on $[Ca^{2+}]_i$ occurs with a threshold in the micromolar range, whatever the concentration of PROG. This is supported by the RU486-like activity of RU39009 and RU39411, the steroid structures of which are related to PROG and to estradiol, respectively, and which display completely different pharmacological activities (20, 21), although they have the same 11β side chain. However, RU41291, which has the same side chain as RU486, but has a 17α-propynyl estradiol steroid structure, behaves as a weak PROG agonist in sperm, as estradiol does. Therefore, the 11β phenyl side chain and the

steroid ring structure seem to be involved in the modulation of calcium uptake.

Binding sites for PROG–carboxymethyloxime–bovine serum albumin conjugates have been observed on a subpopulation of human sperm (28, 29), suggesting that only a small proportion of capacitated spermatozoa can respond to PROG. The percentage of live acrosome reacted sperm after PROG application in our experiments is in accordance with the proportion of cells binding the PROG–bovine serum albumin conjugates (29). Further work should determine whether the same cells do bind PROG, show an increased $[Ca^{2+}]_i$, are hyperactivated, and undergo exocytotic events. Although unlikely, it might be that different cells respond to PROG and to RU486.

Our results are consistent with the recently described (30) decrease of the fertilization rate observed after RU486 exposure of mouse sperm and oocytes, and not reverted by PROG.

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