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NOVEL STIMULATORS OF CALCIUM INFLUX IN HUMAN SPERM*

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Progesterone and 17α-hydroxyprogesterone (but not other steroids such as testosterone, corticosterone, β-estradiol, estrone, dehydroepiandrosterone, 20α-hydroxyprogren-3-one, androstendione, and pregnenolone) were shown to cause an immediate increase, in free cytosolic calcium ([Ca2+]i) in both capacitated and noncapacitated human sperm, using the fluorescent indicator fura 2. Significant increases in [Ca2+]i were observed with 10 ng/ml progesterone, while maximum effects were seen with 1 μg/ml progesterone. Two other steroids 11β-hydroxyprogesterone and 5α-pregnane-3,20-dione exhibited significant activity to increase [Ca2+]i. This increase in [Ca2+]i elicited by progesterone was entirely due to Ca2+ influx from the extracellular medium since the increase in [Ca2+]i was blocked by the Ca2+ chelator EGTA (2.5 mM) and the Ca2+ channel antagonist La3+ (0.25 mM) when added to the medium containing 2.5 mM Ca2+. Progesterone also stimulated the uptake of Mn2+ into sperm as measured by the quenching of fura 2 fluorescence. Progesterone has been found in human follicular fluid at levels capable of stimulating increases in [Ca2+]i. The similarities in responses induced by human follicular fluid and progesterone suggest that the factor responsible for inducing an increase in [Ca2+]i, and hence the acrosome reaction, is progesterone and/or 17α-hydroxyprogesterone. Progesterone (1 μg/ml) did not increase [Ca2+]i in somatic cells such as adipocytes, hepatocytes, Balb/c 3T3 cells, normal rat kidney, or DDT1 MF-2 cells. The effects of these progestins to increase [Ca2+]i, by activating a receptor-operated calcium channel, is the first report of such an activity in sperm. This phenomenon possibly opens up a new field of steroid action in the area of sterility, fertility, and contraception at the level of the sperm.

Several studies have shown that fluid aspirated from preovulatory human ovarian follicles can stimulate the acrosome reaction (AR) in human (e.g. see reviews, Yanagimachi, 1988; Kopf and Gerton, 1990). The AR involves fusion of the acrosomal membrane with the plasma membrane at several sites, thus releasing the contents of the acrosome to the extracellular space (Yanagimachi, 1988; Kopf and Gerton, 1990). This AR can only occur after the sperm undergo a process of capacitation, following ejaculation. The AR normally occurs in the female reproductive tract; however, this process can also occur in vitro (Yanagimachi, 1988; Kopf and Gerton, 1990).

Controversy surrounds the precise time at which the AR occurs during the fertilization process; however, it is agreed that it has to occur before the sperm can penetrate the zona pellucida of the oocyte (Yanagimachi, 1988; Kopf and Gerton, 1990). We have initiated a study to identify and characterize the agent(s) responsible in human follicular fluid (hFF) for initiating the AR in human sperm (e.g. Thomas and Meizel, 1988; Suarez et al., 1986; Tesarik, 1985). An early event involved in the AR is an obligatory increase in free cytosolic calcium ([Ca2+]i) (Yanagimachi, 1988; Kopf and Gerton, 1990). We have measured this parameter as a possible index of AR in capacitated human sperm. The method utilized to measure [Ca2+]i involves the calcium indicator fura 2 (Grynkiewicz et al., 1985).

Initial characterization studies revealed that the factor in hFF that initiates the AR is in the 50-kDa fraction (Thomas and Meizel, 1988; Suarez et al., 1986; Siiteri et al., 1988). A recent study suggested that progesterone and 17α-hydroxyprogesterone may stimulate the AR in human sperm within 10 min, both of these steroids are present in hFF (Osman et al., 1989). Here we show that progesterone and 17α-hydroxyprogesterone can stimulate an immediate (within several seconds) calcium influx into capacitated or noncapacitated human sperm. This report thus shows that certain steroids can initiate a very rapid biological effect in human sperm and suggest a unique mode of action of these steroids involving cell surface receptors for the steroid and not the classical intracellular receptor involved in gene transcription (Duval et al., 1986), a relatively slow event.

EXPERIMENTAL PROCEDURES

Methods

Measurements of [Ca2+]i in Sperm—Human sperm, following overnight capacitation or noncapacitated, were loaded with fura 2 essentially as previously described (Thomas and Meizel, 1988). Cells (5–10 × 10⁶ cells/ml) were incubated with 4 μM fura 2-AM for 45 min at 37°C. Following centrifugation (2000 × g for 5 min) cells were resuspended in FM3B buffer (Thomas and Meizel, 1988) then kept in the dark at room temperature to prevent photobleaching. Aliquots

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¶ The abbreviations used are: AR, acrosome reaction; hFF, human follicular fluid; [Ca2+]i, free cytosolic calcium; EGTA, (ethylene(oxythelylenenitrilo)tetraacetic acid.

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(0.5 ml) of cells were incubated at 37°C in 6 x 50-mm glass test tubes, containing a small magnetic stirring bar, in a SPEX ARMC spectrofluorometer. Aliquots of agents (2-5 μl) were then added to the sperm suspension 30 s after data collection was started. Cells were excited at 340 and 380 nm, respectively, and emission measured at 505 nm. Data was collected between 2 and 5 min depending on the protocol used. The integration time was usually 0.1 s with a time increment of 0.5 s. On completion of experiments, cells were lysed with 0.01% (w/v) digitonin, then 10 mM EGTA was added to obtain fluorescence values of fura 2 at both wavelengths when it was either saturated or depleted of calcium. Autofluorescence of the cells was determined at both wavelengths by adding 2 mM MnCl₂, in the presence of 20 μM ionomycin, to fura 2-loaded cells. The autofluorescence values were then subtracted from the values obtained in the fura 2-loaded cells and the levels of [Ca⁡⁺]i, calculated according to Grynkiewicz et al. (1985). The glass cuvette was washed with 96% ethanol after each experiment so as to remove any traces of steroids which adhere to glass.

Preparation and Incubation of Cells—Human semen was collected by masturbation from healthy donors. Approximately 0.5 ml of semen was placed under 2 ml of BWW buffer (Biggers et al., 1971) and incubated at 37°C for 90 min. The swim-up sperm was collected and the concentration adjusted to 20 million/ml. The sperm were then capacitated by incubating overnight in a CO₂ incubator or were used immediately.

Isolation of Preovulatory Human Follicular Fluid—Hff was isolated as previously described (Jones et al., 1982). Some of the hff was treated as follows to remove low molecular weight substances including steroids. To 1.0 ml of hff was added 2.5 mg of dextran plus 25 mg of Norit A, this mixture was incubated at 4°C for 24 h, then centrifuged at 60,000 x g for 30 min. Also, 1.0 ml of hff was diluted to 10 ml with phosphate-buffered saline and extracted with a Cs Sep-Pak (Waters Associates). The absorbed material was then eluted with 10 ml of methanol and evaporated to dryness under a stream of nitrogen. The dry material was then resuspended in 1.0 ml of 0.9% (w/v) NaCl containing 0.1% (w/v) bovine serum albumin.

Measurement of Motility—Motility analysis of capacitated sperm samples was performed using a Hamilton Thorn motility analyzer/ HFT M2000. Motility from random samples was 96.9 ± 1.9%, the mean path velocity was 57.5 ± 2.7 microns/s with 78.0 ± 3.2% having a velocity >25 microns/s, 9.5 ± 1.9% having a velocity <25 but >10 microns/s, 3.1 ± 1.7% having a velocity <10 microns/s, and 11.5 ± 1.6% being static. The mean lateral head displacement in microns was 3.47 ± 0.3 for sperm >70 microns long.

Materials—The following were purchased from Sigma: bovine serum albumin fraction V, Na pyruvate, digitonin, Na lactate, EGTA, penicillin G, streptomycin, progesterone, 17α-hydroxyprogesterone, corticosterone, estrone, 11β-hydroxyprogesterone, 5α-pregnane-3,20-dione, androsterone, pregnenolone, 20α-hydroxyprogenin-3-one, dehydroepiandrosterone, β-estradiol, and testosterone. Fura 2-AM, progesterone, and ionomycin were purchased from Behring Diagnostics. All other chemicals (e.g. salts for buffers) were purchased from Fisher. Steroids were either dissolved in dimethyl sulfoxide or 95% ethanol. The different solvents used did not influence the results, and the solvents had no effects on [Ca²⁺], when added alone.

RESULTS—The data in Fig. 1A shows the potency of hff to increase [Ca²⁺], in capacitated human sperm. The same results were observed using noncapacitated sperm (data not shown), adding higher concentrations of hff (e.g. 2-10%) caused very large changes in autofluorescence and hence are not shown here. However, the effect of hff was very potent at stimulating an increase in [Ca²⁺]. The effect of all concentrations of hff to increase [Ca²⁺], was transient; however, it was still slightly elevated above resting [Ca²⁺] by 5 min. Addition of more hff 5 min after the first addition did not stimulate a further increase in [Ca²⁺], (data not shown). The concentrations of hff used previously to stimulate the AR were between 10 and 50% (w/v) (Thomas and Meizel, 1988; Suarez et al., 1996). Also shown in Fig. 1A was the effect of the 1% buffer (v/v) (control) used to aspirate the hff since it contained heparin.

FIG. 1. Effect of hff (panel A) and progesterone (panel B) on [Ca²⁺], in capacitated human sperm measured using fura 2. In panel A the effect of three dilutions (2, 1, and 0.1%, v/v) of hff to increase [Ca²⁺], in sperm are shown. The hff was added at 30 s. These concentrations of hff when added to non-fura 2-loaded sperm did not significantly alter the fluorescence signals when the cells were excised at 340 and 380 nm and emission measured at 510 nm; however, higher concentrations did. The effect of heparin containing buffer used to flush out the hff is also shown, which had no effect. In panel B, the effect of various concentrations (1.0, 0.1, 0.01, 0.001, and 0.0001 μg/ml) of progesterone (Sigma) on [Ca²⁺], are shown; progesterone from Behring Diagnostics produced the same results. The progesterone was dissolved in dimethyl sulfoxide and at a dilution which resulted in a final concentration of 0.5% (v/v) of dimethyl sulfoxide. This concentration of dimethyl sulfoxide had no effect on [Ca²⁺], over the duration of the experiment; higher concentrations such as 1.0% (v/v) caused a slow increase in [Ca²⁺], which was not transient. After the low concentrations (0.001 and 0.0001 μg/ml) of progesterone were added at 30 s, a higher concentration of progesterone (1 μg/ml) was added at 60 s. This elicited an increase in [Ca²⁺], comparable to that observed when 0.1-1.0 μg/ml progesterone was added alone at 30 s.

(20 units/ml), an agent known to stimulate the AR (Stock et al., 1989), as can be seen it did not have any effect on [Ca²⁺], at this concentration. Several samples of human plasma (from both males and females) at a concentration of 1% (v/v) elicited variable but small responses on [Ca²⁺], (range of 8-20% and a mean of 15% of that observed with 1% hff for 14 plasma samples). A 40% ammonium sulfate precipitation of hff followed by resuspension of the precipitated protein resulted in a very little loss of the activity to increase [Ca²⁺]. Likewise, overnight dialysis of hff removed very little activity. This data suggests that the activity was either a large molecular weight protein, such as the 50-kDa glycoprotein described by Meizel and co-worker (Thomas and Meizel, 1988; Sitteri et al., 1988a), or that the active agent was very tightly bound to protein.

The data in Fig. 1B shows the dose-response for progesterone to increase [Ca²⁺], a maximum response was observed with 1.0 μg/ml, whereas a threshold response on [Ca²⁺], was seen with 0.001 μg/ml. Adding a higher concentration (1 μg/ ml) of progesterone to the cells previously stimulated with lower (0.001 and 0.0001 μg/ml) concentrations produced a further increase in [Ca²⁺]. The final concentration of progesterone (measured using a radioimmunoassay kit; Diagnostics Products Corp.) in hff added to the sperm was 130, 65, and
The concentration of Ca" in the buffer was 2.5 mM, this concentration being slightly less than the ED_{50} for progesterone to increase [Ca^2+]. Measurements of [Ca^2+] were made just before steroid addition and at the peak effect which was observed approximately 15 s later. The concentration of each steroid is shown as nanomolar, which were very similar to one another ranging from 28.9 to 37.0, since they all have very similar molecular weights. For progesterone the basal resting level of [Ca"^2+] was 53 nM, and the maximum effect was 149 nm. Results for each steroid are expressed as a percent of this effect. The results shown are representative of three such experiments.

Other steroids were examined to see if they would increase [Ca"^2+] in sperm; the effects of several are shown in Fig. 2, each being added at a final concentration of 1.0 μg/ml. The steroid 17α-hydroxyprogesterone was almost as effective as progesterone at elevating [Ca"^2+], whereas testosterone, corticosterone, and pregnenolone only produced minimal effects. When progesterone was added after corticosterone, progesterone, and pregnenolone, it produced a further increase in [Ca"^2+] almost equal to progesterone added alone except for cells previously treated with pregnenolone. Thus pregnenolone may be a partial progesterone antagonist, by binding to the putative progesterone receptor; it may prevent binding and activation by progesterone.

The data in Table I show a more extensive list of steroids that were examined to increase [Ca"^2+] in one being tested at a concentration of 0.01 pg/ml, which was close to the half-maximally effective dose of progesterone to increase [Ca"^2+]. Consistent with the data shown in Fig. 2, progesterone and 17α-hydroxyprogesterone were the most effective steroids at increasing [Ca"^2+], also 11β-hydroxyprogesterone and 5α-pregnan-3,20-diene produced significant increases in [Ca"^2+], whereas androstenedione, pregnenolone, corticosterone, 20α-hydroxyprogren-3-one, β-estradiol, testosterone, estrone, and dehydroepiandrosterone only produced small effects ranging between 7.3 and 15.6% of that seen with progesterone. Thus the response of sperm to steroids is relatively specific for the two progestins, progesterone and 17α-hydroxyprogesterone.

It was previously implied that the effect of hff to increase [Ca"^2+] was predominantly mediated by Ca"^2+ influx (Thomas and Meizel, 1988). The data in Fig. 3A supports this contention. Addition of EGTA at a concentration of 2.5 mM (the concentration of Ca"^2+ in the buffer was 2.5 mM) prevented hff from increasing [Ca"^2+]. When Ca"^2+ was added back at a concentration of 2 mM, there was an immediate rise in [Ca"^2+]. This result suggests that the predominant effect of hff was to induce Ca"^2+ influx and that the brief 30-s treatment with EGTA does not have any effect on either the sperm or the Ca"^2+ elevating activity in the hff. When Ca"^2+ was added back to cells treated with EGTA, but not exposed to hff, there was an apparent very small rise in [Ca"^2+]. However, this small increase is most likely due to a small amount of extracellular furfule 2 which had leaked out of the cells. Also shown in Fig. 3A is the effect of 0.25 mM La"^3+ on the effect of hff to increase [Ca"^2+]. La"^3+ was able to attenuate the early effects of hff, but not completely abrogate the effect of hff at later times.

![Fig. 2. Effect of various steroids on [Ca"^2+] in capacitated human sperm measured using fura 2. The steroids progesterone (P), 17α-hydroxyprogesterone (HP), corticosterone (C), testosterone (T), and pregnenolone (Pgn) were added at 30 s to a final concentration of 1.0 μg/ml (dimethyl sulfoxide concentration 0.5% (v/v)). Progesterone (1.0 μg/ml) was added at 65 s to all samples; only those cells treated with corticosterone, testosterone, and pregnenolone produced any further effect on [Ca"^2+].](http://www.jbc.org/)

![Fig. 3. Effect ofEGTA and La"^3+ on the ability of hff (1%) and progesterone (1 μg/ml) to increase [Ca"^2+] in sperm using fura 2. In panel A, 2.5 mM EGTA (pH 8.0) was added at zero time. When hff was added at 30 s, hff did not increase [Ca"^2+]. At 60 s, 2.0 mM Ca"^2+ was added, which produced an immediate increase in [Ca"^2+]. When 2.0 mM Ca"^2+ was added to cells treated with EGTA at 60 s, but not with hff, there was a very small increase in [Ca"^2+], compared to cells pretreated with hff. For comparison, the effect of hff on [Ca"^2+] is shown when cells were incubated in Ca"^2+ containing medium. In another batch of cells, La"^3+ was added at zero time; the effect of hff added at 30 s was greatly attenuated. In panel B, the same protocol as that shown in panel A was used except that 1.0 μg/ml progesterone was used.](http://www.jbc.org/)
times. This finding is consistent with the competitive nature of La⁺ to bind to the putative Ca²⁺ channel in the plasma membrane. Although not shown, relatively high concentrations (e.g. 20 μM) of the potential dependent Ca²⁺ channel blockers (e.g. verapamil and diltiazem) were able to attenuate the rise in [Ca²⁺], induced by 1% (v/v) hff by approximately 30%. Thus the Ca²⁺ channel is most likely not the classical voltage-gated Ca²⁺ channel, but more likely to be the receptor operated (ROC) type (Blackmore et al., 1989; Hosey and Lazdunski, 1988). Shown in Fig. 3B is the effect of progesterone on [Ca²⁺], in the absence and presence of extracellular Ca²⁺ and La³⁺. The data is qualitatively very similar to that obtained with hff (Fig. 3A). This data shows that progesterone increases [Ca²⁺], by stimulating Ca²⁺ influx. There is no evidence for intracellular Ca²⁺ mobilization since removal of extracellular Ca²⁺ completely attenuates the response, whereas Ca²⁺ readdition restores the full progesterone effect seen in the presence of extracellular Ca²⁺.

Another approach used to measure Ca²⁺ influx was to measure the ability of Mn²⁺ to quench intracellular fura 2 (Hallam and Rink, 1985). The data in Fig. 4 shows that 2 mM Mn²⁺ added to fura 2-loaded sperm produces a gradual decrease in fluorescence, and that this decrease in fura 2 fluorescence was greatly accentuated when either progesterone or hff was added to sperm. This is again consistent with progesterone activating a Ca²⁺ influx mechanism since Mn²⁺ enters the cell via the Ca²⁺ pathway (Hallam and Rink, 1985; Merritt et al., 1989). The data in Fig. 5 shows a dose-response relationship for [Ca²⁺], increases induced by progesterone and the rate of Mn²⁺ induced quenching of intracellular fura 2. It is evident that both processes have very similar concentration relationships. This result suggests that the increase in [Ca²⁺], induced by progesterone is due entirely to Ca²⁺ influx, since Mn²⁺ induced fura 2 quenching is due to influx through the Ca²⁺ channel (Merritt et al., 1989).

Other experiments to support the existence of progesterone in hff, which was causing the increase in [Ca²⁺], are as follows. Treatment of hff with charcoal, completely prevented hff from increasing [Ca²⁺], (data not shown). Aliquots of hff were also passed over a C₁₈ column. The column was then eluted with methanol. The eluate was able to stimulate an increase in [Ca²⁺], similar to, but slightly less than that seen with hff. When maximally effective concentrations of progesterone (1 μg/ml) and hff (1% dilution) were added simultaneously or 60 s apart, the effect to increase [Ca²⁺] was no longer than that observed when each agent was added alone. This result implies that the effect on [Ca²⁺], was mediated by the same agent, which was most likely progesterone or that both hff and progesterone can each maximally stimulate the Ca²⁺ influx process. Another more specific way to show that progesterone was the active agent in hff at increasing [Ca²⁺], was to utilize antibodies directed against progesterone. We utilized polypropylene test tubes coated with progesterone antibodies (Diagnostics Products Corp.) to which diluted hff was added and incubated for 3 h at room temperature. This treatment attenuated the effect of hff to increase [Ca²⁺], by approximately 30%. Since this antibody was selective for progesterone, the 50-kDa glycoprotein (Thomas and Meizel, 1986; Suarez et al., 1986). The effect of progesterone to increase [Ca²⁺], in several other cell types was examined. Progesterone added at a final concentration of 1.0 μg/ml to several somatic cells such as freshly isolated adipocytes (Blackmore and Auge, 1988), hepatocytes (Blackmore and Exton, 1985), cultured Balb/BTC3 cells, DDT, MF-2, and cultured normal rat kidney cells was without effect on [Ca²⁺]. These negative findings tend to rule out a general membrane permeabilizing effect of progesterone at this concentration.

**DISCUSSION**

The results presented in this paper are unique from several standpoints. First, the increase in [Ca²⁺], induced by progesterone is one of the most rapidly induced steroid effects so far reported. Progesterone was, however, shown to increase [Ca²⁺], in Xenopus laevis oocytes utilizing aequorin and microelectrodes (Wasserman et al., 1980; Moreau et al., 1980). The effect was seen as early as 1 min. Second, a steroid effect on [Ca²⁺], utilizing fura 2 has hitherto never been observed, and third, sperm ~[Ca²⁺], has been shown to be responsive to progesterone, again a completely unexpected and novel finding. Binding of progesterone to human sperm, however, has been shown (Hyne et al., 1978; Cheng et al., 1981) and an effect of several steroids on membrane potential have been demonstrated (Calzada et al., 1983).

The concentration of progesterone within the cumulus matrix has been estimated to be in excess of 1000 ng/ml (Osman et al., 1989). Thus the concentration of progesterone is in the appropriate dose range to elicit the AR in sperm. If progesterone does stimulate the AR in sperm, then there are two possible sources for this, either the cumulus cells or the hff.
The progesterone in hff will most likely be absorbed or diluted following ovulation, this will likely depend on the time after ovulation, whereas the progesterone produced by the cumulus cells is the most likely source for the increase in \([\text{Ca}^{2+}]_i\), which probably leads to the AR. Even if the sperm have undergone the AR before reaching the egg this does not appear to prevent them from binding to the zona pellucida (Morales et al., 1988; Myles et al., 1987; Yanagimachi, 1981); this has also been shown for human sperm (Mories et al., 1989). Perhaps the time after AR was initiated may influence these results; however, this point still remains controversial.

There is some debate as to when the acrosome reaction occurs during the fertilization process. Many studies have shown that binding of acrosome intact sperm to a component of the zona pellucida, known as ZP3, will initiate the AR (e.g. Vazquez et al., 1989). Perhaps there is a coordinated or synergistic interaction between ZP3 and progesterone to initiate the AR; this idea awaits further experimentation. An alternative role for progesterone may be to elicit the AR in most of the sperm before reaching the egg. If these sperm have complete AR, they would lose their acrosomal contents and be unable to penetrate the zona pellucida.

The mechanism of progesterone action is most probably mediated by a progesterone receptor resident in the plasma membrane of sperm. Whether or not this receptor is the same or similar to the previously characterized cytosolic progesterone receptor is presently not known and is the subject of our present investigations. Binding of progesterone to this receptor then activates a \([\text{Ca}^{2+}]_i\) channel in the plasma membrane or inhibits the plasma membrane \([\text{Ca}^{2+}]_i\) ATPase pump. Alternatively the progesterone receptor may have inherent \([\text{Ca}^{2+}]_i\) activity itself.

Since the present studies utilized populations of cells as suspensions to examine intracellular \([\text{Ca}^{2+}]_i\), we cannot say where the calcium transient(s) are occurring within the sperm. This is because the dye loading of mouse and bovine spermatozoa has been shown to be either localized in one compartment or uniformly distributed (Lee and Storey, 1988; Florman and Babcock, 1988). It has been shown that the first stage of the zona-induced acrosome reaction in mouse sperm is \([\text{Ca}^{2+}]_i\) influx into the acrosomal region of the sperm head (Lee and Storey, 1988). Further studies using image analysis will be required to determine where the intracellular \([\text{Ca}^{2+}]_i\) transient(s) are occurring in human sperm following progesterone stimulation. Also, a careful correlation study is required for the effect of progesterone on \([\text{Ca}^{2+}]_i\) and the AR. A tight coupling of \([\text{Ca}^{2+}]_i\) increases and the AR has been shown in bovine sperm using ZP3 as a stimulus (Florman et al., 1988).

The involvement of phosphoinositide turnover and guanine nucleotide regulatory-binding proteins (G protein) do not appear to be involved in the \([\text{Ca}^{2+}]_i\) influx response. Treatment of sperm with AlF₄⁻, an activator of G, in many systems (e.g. Blackmore et al., 1985), does not induce an increase in \([\text{Ca}^{2+}]_i\). Also, treatment of sperm with 3 mM neomycin, an agent (not specific, however) known to attenuate phosphoinositide turnover in many systems (e.g. Bosch et al., 1986), slightly potentiates the effect on \([\text{Ca}^{2+}]_i\). Recent studies, however, show that polyphosphoinositide breakdown occurs after \([\text{Ca}^{2+}]_i\) entry in mammalian spermatozoa (Roldan and Harrison, 1989). Another alternative may be that the progesterone receptor activates phospholipase D, and that the increase in phosphatidic acid promotes \([\text{Ca}^{2+}]_i\) influx since this compound has been shown in some studies to have \([\text{Ca}^{2+}]_i\) stimulatory activity (e.g. Bocckino et al., 1987). A phospholipase D activity has recently been described in sea urchin sperm (Dominio et al., 1989).

Finally, this study opens up entirely new areas of investigation in the fields of fertility, sterility, and contraception. It is anticipated that the area of male contraception may advance from these observations and possible defects in the sperm progesterone system will account for some cases of male infertility (Blumenfeld and Nahhas, 1989) and that this can be treated with steroids.

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