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CELL SURFACE LOCALIZATION OF A NOVEL NON-GENOMIC PROGESTERONE RECEPTOR ON THE HEAD OF HUMAN SPERM

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SUMMARY: Cell surface receptors for progesterone were visualized in human sperm using fluorescein isothiocyanate-progesterone 3-(0-carboxymethyl) oxime-bovine serum albumin (FITC prog CMO BSA). The receptors were confined to the head and not the midpiece or tail. FITC prog CMO BSA was also an effective stimulus to elevate intracellular free calcium in human sperm as detected by fura-2 fluorescence. The elevation of intracellular free calcium is a stimulus for the acrosome reaction, a process which is necessary to occur for sperm to fertilize the egg. It is proposed that progesterone, which is present in the female reproductive tract, can bind to progesterone receptors located in the plasma membrane of the sperm head and elicit an influx of Ca^{2+} into the underlying cytoplasm and or acrosome and induce the acrosome reaction and facilitate fertilization.

It was recently shown that progesterone, which is a component of follicular fluid, can induce the acrosome reaction in human sperm (1). It has also been demonstrated that progesterone and 17 α-hydroxyprogesterone induce a rapid influx of Ca^{2+} into human sperm (2,3) with a resultant increase in free intracellular calcium ($[Ca^{2+}]_i$). It was proposed from these experiments that a cell surface receptor for progesterone existed in human sperm (2). Studies using progesterone immobilized on albumin, a protein which is excluded from intact sperm (4), have confirmed this idea (4,5,6). In these studies progesterone 3-(0-carboxymethyl)oxime-bovine serum albumin (prog CMO BSA) as well as progesterone 3-(0-carboxymethyl)oxime (prog CMO) were full agonists at elevating $[Ca^{2+}]_i$, although they were less potent than progesterone at elevating $[Ca^{2+}]_i$ by approx 1.5 orders of magnitude (4). Both prog CMO BSA and prog CMO increased $[Ca^{2+}]_i$ by promoting Ca^{2+} influx as did progesterone (4). This unique cell surface receptor for progesterone exhibited unusual steroid specificity as the potent antiprogestins RU38486 and ZK98.299 were very ineffective at inhibiting progesterone effects (4). A related observation was that the potent synthetic genomic

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progestins megestrol, medroxyprogesterone acetate, norgestrel, norethynodrel, norethindrone, R5020 and cyproterone acetate were very weak stimulators at increasing $[Ca^{2+}]_i$ (4). These experiments using genomic antiprogestins and progestins showed that this sperm progesterone receptor was novel as far as its steroid specificity was concerned.

The aim of the present study was to identify where on the sperm surface the progesterone receptors were located. We utilized prog CMO BSA that was used previously to confirm that the progesterone receptor was on the cell surface (4), but to which fluorescein isothiocyanate (FITC) was conjugated. The FITC prog CMO BSA was added to live swim up sperm and the location of the progesterone receptors on the sperm visualized by fluorescence video microscopy. The receptors were confined to the head and not the midpiece or tail.

MATERIALS AND METHODS

Materials FITC prog CMO BSA progesterone, digitonin, and progesterone were from Sigma Chemical Co. Ionomycin was from Calbiochem. Fura-2/AM was from Molecular Probes, Eugene, OR.

Measurements of $[Ca^{2+}]_i$ The level of $[Ca^{2+}]_i$ in swim up sperm was measured as previously described (2). The Mn^{2+} quench technique (7) was also used to measure Ca^{2+} influx (2).

Visualization of cell surface progesterone receptors To a suspension of sperm (appro. 5×10^6 cells/ml) was added 10μM FITC prog CMO BSA. A 10 μl aliquot was placed onto a microscope slide, then a number 1 microscope cover glass was placed on top of the cell suspension. Slight pressure was applied to the cover glass to expel the excess liquid. This procedure immobilized most of the sperm so that clear video images could be obtained.

Light and fluorescence microscopy was performed using a Nikon microphot-FX microscope. Cells were observed using a Fluor 100x objective, excitation wavelength was 450-490 nm, barrier filter was 520-560 nm and the dichroic mirror was 510 nm. Images were recorded on VHS videotape using a MTI CCD72 camera and a Videoscope image intensifier. Images were printed on a Sony video printer UP-5000 W after 8 video frames were averaged with a Universal Imaging Image-1/AT system.

RESULTS AND DISCUSSION

The visualization of progesterone receptors on sperm can theoretically be observed if FITC-prog CMO BSA can actually bind to the cell surface progesterone receptors that stimulate Ca^{2+} influx. To confirm that FITC-prog CMO BSA binds to the progesterone receptor, we measured the ability of FITC-prog CMO BSA to elevate $[Ca^{2+}]_i$. The data in Fig 1 show the effect of two different concentrations of progesterone (10 and 100 nM) and one concentration (130 nM) of FITC prog CMO BSA to elevate $[Ca^{2+}]_i$. The effect of 130 nM FITC prog CMO BSA was equivalent to that seen with 10 nM progesterone. When higher concentrations of FITC prog CMO

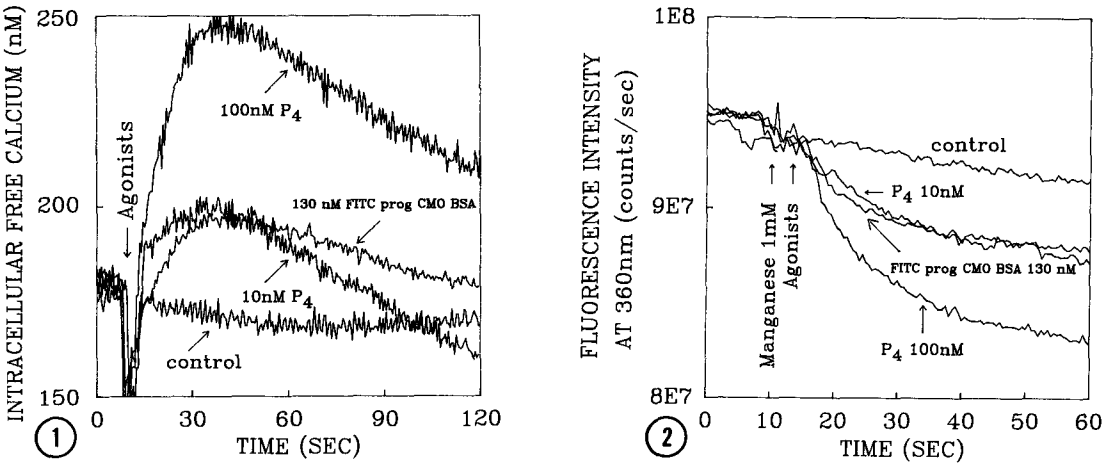

Figure 1. Effect of progesterone and FITC prog CMO BSA on $[Ca^{2+}]_i$ human sperm. Swim up sperm were loaded with fura-2 as previously described (2). Various concentrations of progesterone (P_4) were added at 10 sec and fluorescence changes at 340 and 380 nm measured. Also 130 nM FITC prog CMO BSA was added after 10 sec. The increase in $[Ca^{2+}]_i$ induced by 130 nM FITC prog CMO BSA was similar to that induced by 10 nM P_4. A representative experiment is shown.

Figure 2. Effect of FITC prog CMO BSA (130 nM) and progesterone (10 nM and 100 nM) to induce Ca^{2+} influx measured by observing the ability of Mn^{2+} added extracellularly to quench intracellular fura-2 fluorescence.

To fura-2 loaded sperm was added 1 mM Mn^{2+} at 10 sec. then at 15 sec. the various agonists were added. The excitation wavelength was 360 nm, which is the isobestic point for fura-2. A representative experiment is shown.

BSA were used such (e.g., 10 μM) the fluorescein produced such large changes in fluorescence emission wavelengths that no meaningful measurements of $[Ca^{2+}]_i$ could be made. Our earlier studies using 1 μM prog CMO BSA produced effects on $[Ca^{2+}]_i$ equivalent to that observed with approx 50 nM progesterone (4). One possible explanation for this lack of sensitivity of FITC prog CMO BSA is the fact that FITC prog CMO BSA only contained 8 moles of progesterone per mole of BSA, whereas the prog CMO BSA used earlier (4) contained 31 moles of progesterone per mole of BSA. Another approach used to measure Ca^{2+} influx is to observe the quenching of intracellular fura-2 by Mn^{2+}, which enters the cell via Ca^{2+} channels (2,7). The data in Fig 2 show that 130 nM FITC prog CMO BSA produced a rate of fura-2 quenching the same as 10 nM progesterone. The two experimental approaches (fura-2 fluorescence and Mn^{2+} quenching of fura-2) gave the same result in that 10 nM progesterone was approximately equivalent to 130 nM FITC prog CMO BSA. Thus, FITC prog CMO BSA did rapidly elevate $[Ca^{2+}]_i$ consistent with it binding to and stimulating the cell surface progesterone receptors. Having validated that FITC prog CMO BSA was able to stimulate the progesterone receptors, we then visualized the location of progesterone receptors by observing the location of the fluorescein fluorescence.

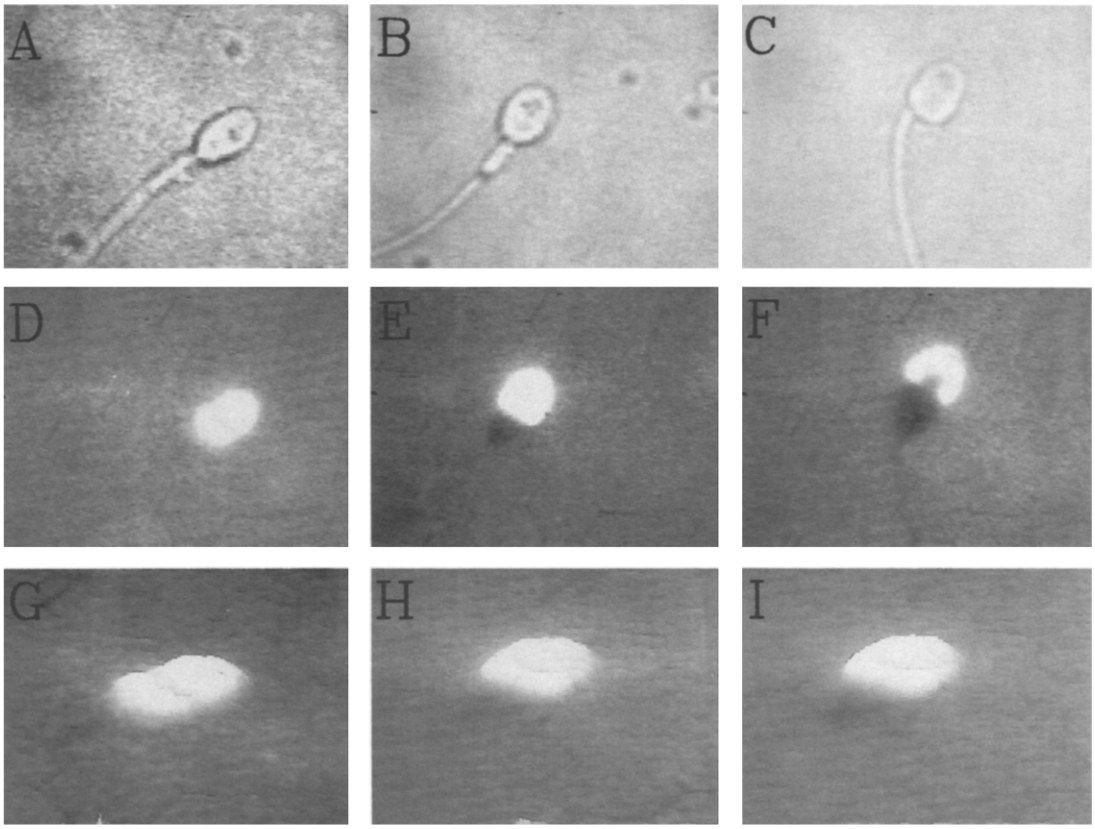

Figure 3. Localization of progesterone receptors on human sperm using FITC prog CMO BSA. In panels A, B, and C sperm are visualized by light microscopy. The same three sperm, in the presence of FITC prog CMO BSA, were visualized by epifluorescence (panels D, E, and F) as described in Methods section. Fluorescence intensity profiles of the same sperm are shown in panels G, H, and I. The fluorescence was associated with the head in each case.

The data in Fig 3 show three representative sperm under light and fluorescence microscopy in the presence of FITC prog CMO BSA and that appear to have progesterone receptors. In panels A,B, and C light microscopy of sperm clearly show the presence of the tail and head. When these same sperm are viewed under fluorescence microscopy, intense fluorescence was observed only on the head and not the midpiece and tail. In fact, there was a shadow in the midpiece region indicating exclusion of FITC prog CMO BSA from this area. There is some degree of heterogeneity of fluorescence patterns on the head, which can also be visualized by observing fluorescence intensity profiles in panels G, H, and I. The sperm in panels D and E show a band of fluorescence adjacent to the midpiece and a less intense area of fluorescence overlaying the cytoplasm and acrosomal region. The sperm in panel F shows fluorescence in a crescent shaped pattern in the cytoplasm and acrosomal area.

In our previous control studies we showed that FITC BSA was not associated with sperm in a nonspecific manner (4), thus the association of FITC prog CMO BSA with sperm was due to the presence of progesterone on the BSA and the presence of receptors for progesterone on the sperm head. Not all the sperm bound FITC-prog CMO BSA since only about 30% had fluorescence associated with the head. The data in Fig 4 show two representative sperm which do not appear to have cell surface progesterone receptors. In Fig 4 panels A and B two sperm are clearly visible by light microscopy, however, when they were observed by epifluorescence no FITC prog CMO BSA was associated with any structure, and in fact, a shadow was observed over the head and midpiece regions. The two populations of sperm (Figs 3 and 4) one showing the presence of cell surface progesterone receptors and the other apparently devoid of receptors may reflect sperm at various stages of maturity. In a few instances when sperm were observed swimming, fluorescence in a crescent shape was observed over

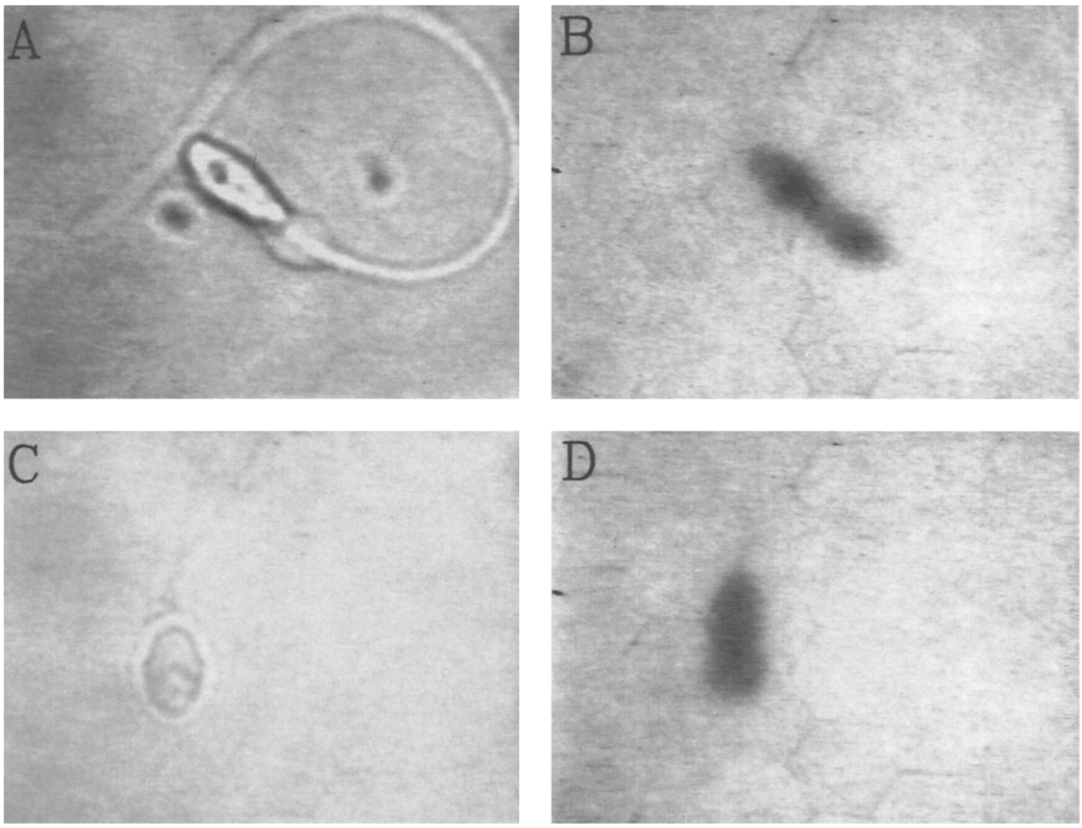

Figure 4. Lack of progesterone receptors on human sperm.
In panels A and B two individual sperm are visualized by light microscopy. The same two sperm in the presence of FITC prog CMO BSA were visualized by epifluorescence in panels C and D. In both instances no fluorescence was observed on any area of the sperm.

the anterior aspect of the head when the sperm were incubated with FITC prog CMO BSA (data not shown). This pattern of fluorescence indicates an uneven distribution of progesterone receptors on the head.

Several observations made in this present study need further investigation since they are not readily explainable. The first is the fact that progesterone receptors were only observed on approx 30% of the sperm. If only some of the sperm entering the female reproductive tract contain progesterone receptors then presumably they will respond to progesterone and undergo the acrosome reaction. Thus, the number of sperm that could conceivably bind to and fertilize the egg will be reduced since fully acrosome reacted sperm are unable to penetrate the zona pellucida, due to the loss of lytic enzymes, but they can bind to it (e.g., 8,9).

The implication from these experiments is that the receptors for progesterone are confined to the head, therefore, the receptors overlay the cytoplasm and acrosomal region of the sperm. It is generally agreed that induction of the acrosome reaction follows an influx of Ca^{2+} in human sperm (e.g., 10,11). Therefore, the progesterone receptors are in the appropriate region of the sperm head when they are stimulated by progesterone in the female reproductive tract. Stimulation by progesterone would cause an influx of Ca^{2+} into the cytoplasm and induce the acrosome reaction.

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