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A plasma-membrane progesterone receptor in human sperm is switched on by increasing intracellular free calcium

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Recent studies have revealed the presence of an atypical progesterone receptor on the plasma membrane of a relatively small subpopulation of human sperm cells. In this study, we show that the receptor is present in many cells in a latent form and that the receptor function in these cells is switched on asynchronously by increasing intracellular free Ca^{2+} levels. Under physiological conditions, this process occurs during sperm capacitation, but it can be mimicked by a short sperm exposure to the Ca^{2+}-mobilizing agent thapsigargin. These observations suggest a role of Ca^{2+} as a modulator of nongenomic steroid effects on the cell surface.

Progesterone receptor; Plasma membrane; Nongenomic steroid effects, Calcium mobilization; Human sperm

1. INTRODUCTION

The presence of external Ca^{2+} has been shown in many species to be an absolute requirement for the exocytosis of the acrosomal granule (the acrosome reaction) induced by physiological stimuli [1]. The acrosome reaction is thus a suitable model for the study of stimulus-induced, Ca^{2+}-dependent exocytosis. Recent data have indicated that a rapid Ca^{2+} influx, followed by the acrosome reaction, can be induced in human spermatozoa by the addition of progesterone [2,3] This progesterone effect is mediated by an atypical steroid receptor [4,5] that is localized on the plasma membrane covering the sperm acrosome [6]. The triggering of the Ca^{2+} influx by the receptor implies ligand-induced [7] or antibody-induced [8] receptor aggregation. As in many other systems in which ligand–receptor binding results in receptor aggregation, the ligand binding to the sperm-surface progesterone receptor stimulates tyrosine phosphorylation of a specific phosphoprotein [9].

Interestingly, the expression of the sperm-surface progesterone receptor is a selective phenomenon involving only about 10% of the sperm cell population [6,8]. The reason of this selectivity is not known. In general, two possibilities are to be envisaged: either the majority of sperm cells is inherently incapable of expressing the receptor, or the receptor function is under the control of some yet unknown factor(s) switching on the receptor in different cells at different times so that distinct progesterone-responsive cell subpopulation may succeed each other in time.

In this study, we tested a hypothesis that the expression of the active progesterone receptor on the sperm cell surface is a dynamic process controlled by the basal level of intracellular free Ca^{2+} ($[Ca^{2+}]_i$). We compared basal $[Ca^{2+}]_i$ levels and peak progesterone-induced $[Ca^{2+}]_i$ levels at different times of sperm incubation for in vitro capacitation, evaluated the relationship between the progesterone-induced Ca^{2+} influx and the number of progesterone-binding cells, examined the expression of the receptor after previous depletion of progesterone-binding cells from the sperm population, and studied the effect of the thapsigargin-induced Ca^{2+} mobilization on the receptor function.

2. MATERIALS AND METHODS

2.1. *Materials*

Progesterone, thapsigargin, FITC-labelled bovine serum albumin (BSA-FITC) and its conjugate with progesterone (P-BSA-FITC), acetoxymethyl ester of indo 1 (indo 1-AM), and EGTA were purchased from Sigma (La Verpillière, France).

2.2. *Sperm source and preparation*

Ejaculated spermatozoa were obtained from 24 healthy donors with normal sperm parameters. Spermatozoa were washed from seminal plasma and incubated for in vitro capacitation as described [10]. The same incubation media and conditions were also employed for sperm incubation with thapsigargin, P-BSA-FITC, and indo 1-AM

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Abbreviations BSA-FITC, fluorescein isothiocyanate-labeled bovine serum albumin; $[Ca^{2+}]_i$, intracellular free Ca^{2+}; FACS, fluorescence-activated cell sorting; indo 1-AM, acetoxymethyl ester of indo 1; progesterone-BSA-FITC, progesterone conjugated with BSA-FITC.

2.3. *Measurement of $[Ca^{2+}]i$*

Basal and progesterone-induced $[Ca^{2+}]_i$ levels were determined in sperm suspensions loaded with indo 1 exactly as decribed [8,11].

2.4. *Evaluation of progesterone-receptor ligand binding and biological activities*

The ability of sperm to bind progesterone to the cell surface and to generate biological response upon the hormone binding was evaluated with the use of a fluorochrome-labelled, cell-impermeant agonist (P-BSA-FITC) as described previously [6]. Briefly, this evaluation was based on the distinction of two typical agonist-binding patterns corresponding, respectively, to acrosome-intact sperm cells possessing progesterone binding sites (pattern A) and to those cells having undergone the acrosome reaction in response to the bound agonist (pattern B). After sperm incubation with P-BSA-FITC and washing from unbound agonist, the cells were examined by fluorescence microscopy or fluorescence-activated cell sorting (FACS) using the previously described parameter setup [6]. All steps subsequent to the incubation with P-BSA-FITC were carried out in the dark to prevent bleaching of the fluorescent signal. In some experiments, spermatozoa were exposed to 10 μM thapsigargin for 10 min preceding the addition of P-BSA-FITC. All subsequent steps were carried out as described above.

In other experiments, agonist-incubated and subsequently washed spermatozoa were incubated further in the absence of the agonist. The incubation in agonist-free medium was followed by a new exposure of spermatozoa to P-BSA-FITC under the same conditions and further incubation in agonist free medium; this cycle was repeated three times. Aliquots of sperm suspensions were taken at different times for fluorescence microscopy detection of sperm-bound agonist. The minimum numbers of spermatozoa examined in each treatment group in each replicate were 200 for fluorescence microscopy and 2000 for FACS

2.5. *Statistics*

Statistical analyses were performed with StatView II (Abacus Concepts, Berkeley, CA) statistical package Means were compared by Student's *t*-test. Percentual data were transformed by arc-sine prior to analysis Correlation between quantitative variables was evaluated using linear regression analysis.

3. RESULTS

When spermatozoa were incubated in capacitating conditions, the basal level of $[Ca^{2+}]_i$ after 1 h of incubation was not significantly different from that at the beginning of incubation ($P > 0.05$), but there was a significant increase ($P < 0.01$) between the 1-h and 2-h, and between the 2-h and 3-h time points, followed by a plateau between the 3-h and 6-h time points (Fig. 1A).

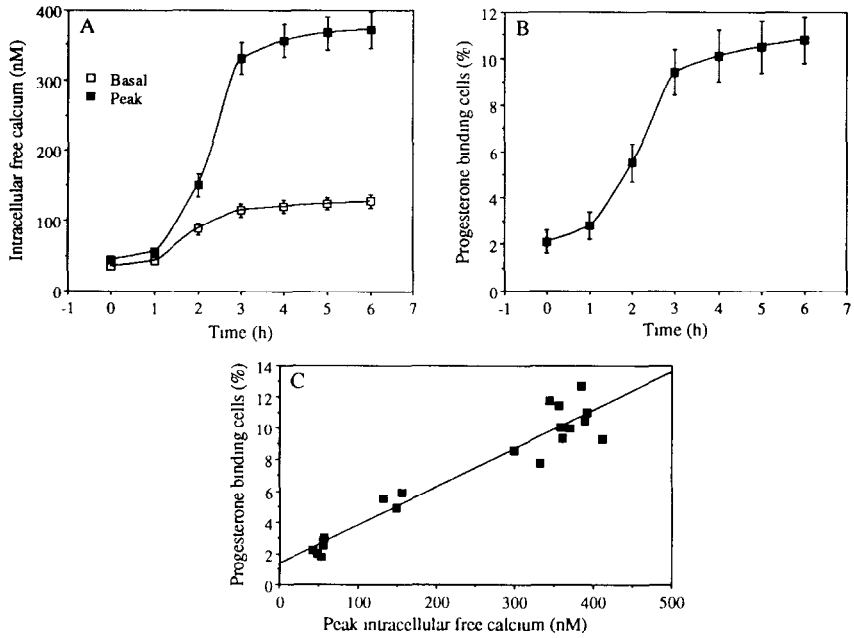

Fig. 1. The relationship between sperm capacitation, the concentration of intracellular free calcium ($[Ca^{2+}]_i$) in sperm cells and its changes after progesterone challenge, and the percentage of progesterone binding cells. (A) Sperm cells were washed from seminal plasma and incubated in capacitating conditions. At the times indicated, aliquots (2×10^6 cells/ml) of sperm suspensions were taken apart, 4 μM indo 1-AM was added to them, and the incubation was carried on in the dark for an additional 30 min. After washing (15 min), the samples were subjected immediately to spectrofluorimetric analysis using excitation and emission wavelengths of 331 nm and 410 nm, respectively. During the measurement, progesterone (3 μM) was added to the sperm suspensions. Basal values of $[Ca^{2+}]_i$ (just before hormone addition) and peak values achieved after hormone treatment were determined. Data are mean ± S.E.M. of 5 replicates. (B) Washed sperm cells were incubated in capacitating conditions as in (A). At the times indicated, aliquots of sperm suspensions were taken apart, 20 μg/ml of the fluorochrome-labelled agonist P-BSA-FITC (corresponding to 2 μM progesterone) was added to them, and the incubation was carried on in the dark for an additional 30 min. After washing (15 min), living cells were analyzed immediately by fluorescence-activated cell sorting. After the correction for nonspecific fluorescence (using parallel incubations with the same concentration of BSA-FITC instead of P-BSA-FITC), the percentages of specifically labelled cells were determined. Data are mean ± S.E.M of 5 replicates. (C) Correlation between the peak $[Ca^{2+}]_i$ levels achieved after the addition of 3 μM progesterone and the percentages of progesterone binding cells determined in aliquots of the same sperm samples (at different times of in vitro capacitation) as described in panels (A) and (B).

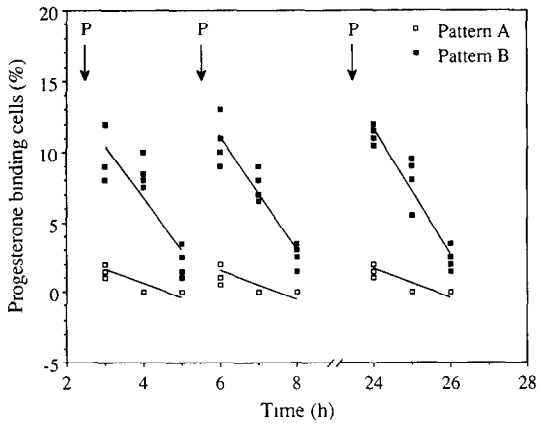

Fig. 2. Fluorescence microscopy analysis of the ability of sperm cells to bind, and respond to, progesterone during repeated cycles of sperm exposure to fluorochrome-labelled agonist (P-BSA-FITC) followed by incubation in agonist-free medium. Spermatozoa were washed from seminal plasma and incubated in capacitating conditions. At the times indicated, P-BSA-FITC (P) was added to sperm suspensions at a concentration of 50 μg/ml followed by 30 min of incubation and washing. Aliquots were taken for fluorescence microscopy, and the remaining cells were incubated further in agonist-free capacitating medium until the subsequent pulse with the agonist. Percentages of cells showing pattern A (agonist binding acrosome-intact spermatozoa) and pattern B (spermatozoa having undergone the acrosome reaction in response to bound agonist) were determined. Data of 4 replicates and corresponding regression lines are shown.

A similar increase, restricted to the period between the 1st and the 3rd hour of incubation, was observed for peak $[Ca^{2+}]_i$ achieved after the subsequent addition of progesterone; moreover, the difference between the peak and the basal levels also augmented during this period (Fig. 1A). The time course of this increase in the progesterone-induced Ca^{2+} influx matched with the increase in the relative number of sperm cells binding the progesterone receptor agonist P-BSA-FITC (Fig. 1B) and undergoing the acrosome reaction in response to P-BSA-FITC binding (data not shown). When peak $[Ca^{2+}]_i$ levels following progesterone addition were related to the actual number of progesterone-binding cells in individual sperm samples (after different times of in vitro capacitation) a strong correlation ($r = 0.962$) was obtained (Fig. 1C).

To determine whether new progesterone-reactive cells appear during in vitro capacitation, the progesterone-binding cell subpopulation was depleted by incubation with P-BSA-FITC leading to the induction of the acrosome reaction with the accompanying loss of binding sites in the responsive cells. Spermatozoa were then washed and incubated in agonist-free medium followed by a new exposure to P-BSA-FITC. After this new exposure, the whole cycle was repeated once more. The percentage of progesterone-binding cells was determined at the end of each exposure to P-BSA-FITC and during the periods of incubation without the agonist (Fig. 2). These experiments showed that the relative numbers of progesterone binding cells decreased with the time of incubation in agonist-free medium, but each new exposure to the agonist resulted in a new sharp increase in these numbers (Fig. 2). When spermatozoa previously incubated with P-BSA-FITC were newly exposed to this agonist immediately after washing following the first incubation, leaving out the incubation in agonist-free medium between the two pulses with P-BSA-FITC, no augmentation of the number of progesterone binding cells was detected (data not shown). The reappearance of progesterone-binding cells, observed after 2 h of incubation in agonist-free medium between the two pulses with P-BSA-FITC, was not due to agonist binding to those cells that already bound the agonist during the first exposure because the binding induced the acrosome reaction in most of those cells; acrosome-reacted spermatozoa have been shown previously to lose the ability to bind progesterone [6].

When thapsigargin was added to sperm suspensions at the same time as P-BSA-FITC (Fig. 3), the maximum number of progesterone-responsive cells was attained as early as time 0 of incubation in capacitating conditions, and this number decreased progressively between the 2-h and 6-h time points. In the presence of 5 mM EGTA, there was only a slight increase in the number of progesterone-responsive cells immediately after thapsigargin addition, and this early increase was followed by a continuous decrease during further incubation (Fig. 3).

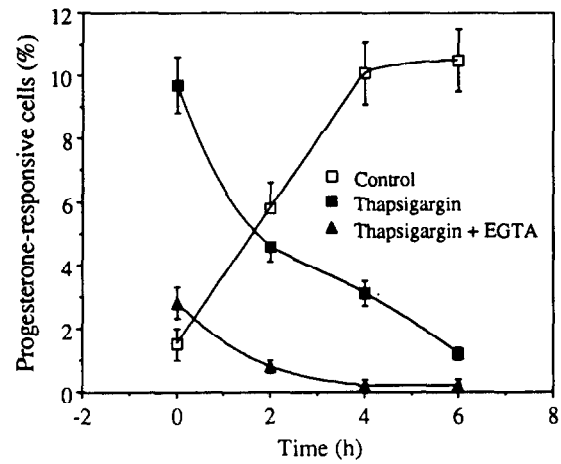

Fig. 3. The effect of thapsigargin on the time course of sperm responsiveness to progesterone. Spermatozoa were washed from seminal plasma and incubated in capacitating conditions. At the times indicated, three aliquots were taken from the sperm suspensions; they received, respectively, 10 μM thapsigargin, 10 μM thapsigargin together with 5 mM EGTA, and 0.5% dimethyl sulfoxide (control). After 10 min of further incubation, this addition was followed by 50 μg/ml of the agonist P-BSA-FITC, and the agonist binding was evaluated by fluorescence microscopy as described in Fig. 2. The representation of progesterone-responsive cells was determined as the percentage of cells showing the an agonist-induced acrosome reaction (pattern B of P-BSA-FITC binding). Data are mean ± S.E.M. of 3 replicates.

4. DISCUSSION

The restriction of the ability to bind, and respond to, progesterone to a relatively small subpopulation of human sperm cells has been described previously [6]. That observation opened the question whether the absence of hormone binding and response in the majority of cells is a sequela of an inability of those cells to express the receptor in its complete, functionally active form, or whether the receptor function is controlled by another, yet unknown mechanism. Since mature spermatozoa are terminally differentiated cells with arrested transcription, such a regulatory mechanism could only act at the posttranscriptional level.

In this study, we confirmed the previous observation that the progesterone-induced Ca^{2+} transient is more pronounced in those cell suspensions showing higher basal $[Ca^{2+}]_i$ levels [12]. Moreover, our data have shown that this increase in the global response detected in suspensions of various hundreds of thousands of cells can be explained by an increase in the number of those cells that actually respond to the hormone in these suspensions.

The data presented in this study also show that the number of cells potentially expressing the progesterone receptor activity is higher than the number of cells that actually bind, and respond to, the hormone. In other words, there are many cells which possess the receptor in a latent, functionally inactive form. The receptor function appears to be switched on asynchronously in different sperm subpopulations in the course of sperm capacitation. Since spermatozoa that are able to bind progesterone trigger a rapid acrosome reaction in response to the bound hormone [3,6,7], and prematurely acrosome-reacted spermatozoa lose the ability to penetrate the egg [13], the asynchronous activation of receptor function may serve to prevent a rapid depletion of potentially fertilizing spermatozoa by a premature acrosome reaction upon exposure to progesterone in the female genital tract.

In addition to the demonstration of the asynchronous activation of the sperm progesterone receptor function during sperm capaciation, our data point out the role of Ca^{2+} as a key-element switching on the receptor function. In fact, the need for sperm capacitation was circumvented when basal $[Ca^{2+}]_i$ was increased artificially by thapsigargin, a drug that probably acts by preventing Ca^{2+} ions that enter spermatozoa from the extracellular compartment from getting sequestered within the perinuclear cisterna [14]. The loss of the thapsigargin effect in the presence of excess EGTA is in agreement with the view that the source of Ca^{2+} accumulating in thapsigargin-treated spermatozoa is essentially extracellular [14]. The data from our thapsigargin experiments have also shown that only about 10% of spermatozoa are able to bind progesterone immediately after thapsigargin addition even though apparently much more spermatozoa possess a latent receptor that can be activated during sperm capacitation. Thus, the action of Ca^{2+} on the receptor may be both direct and indirect; the latter may require more time to bring into play other intracellular regulatory elements. Studies are in progress in our laboratories to elucidate the mechanism by which Ca^{2+} switches on the receptor and to determine the eventual participation of other intracellular signalling systems in this process.

REFERENCES

[1] Shapiro, B.M., Schackmann, R.W. and Gabel, C.A (1981) Annu. Rev. Biochem. 50, 815–843.
[2] Osman, R.A., Andria, M.L., Jones, D.A. and Meizel, S. (1989) Biochem. Biophys. Res Commun. 160, 828–833.
[3] Meizel, S., Pillai, M.C., Diaz-Pérez, E. and Thomas, P. (1990) in: Fertilization in Mammals (Bavister, B.D., Cummins, J. and Roldan, E.R.S. eds.) pp. 205–222, Serono Symposia USA, Norwell, MA.
[4] Blackmore, P.F. and Lattanzio, F.A. (1991) Biochem. Biophys. Res. Commun. 181, 331–336.
[5] Blackmore, P.F., Neulen, J., Lattanzio, F. and Beebe, S.J. (1991) J. Biol. Chem. 266, 18655–18659.
[6] Tesarik, J., Mendoza, C., Moos, J. and Carreras, A. (1992) Fertil. Steril. 58, 784–792
[7] Tesarik, J. and Mendoza, C. (1993) Exp. Cell Res. 205, 111–117.
[8] Tesarik, J., Mendoza, C., Moos, J., Fénichel, P. and Fehlmann, M. (1992) FEBS Lett. 308, 116–120.
[9] Tesarik, J., Moos, J. and Mendoza, C. (1993) Endocrinology, in press.
[10] Tesarik, J., Mendoza Oltras, C. and Testart, J. (1990) J. Reprod. Fertil. 88, 665–675.
[11] Tesarik, J., Carreras, A. and Mendoza, C. (1993) Mol. Reprod. Dev. 34, 183–189.
[12] Baldi, E., Casano, R., Falsetti, C., Krausz, C., Maggi, M. and Forti, G. (1991) J. Androl. 12, 323–330.
[13] Tesarik, J. (1992) J. Assist. Reprod. Genet. 9, 313–317.
[14] Blackmore, P.F. (1993) Cell Calcium 14, 53–60.