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A novel functional estrogen receptor on human sperm membrane interferes with progesterone effects

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Abstract

We have identified an estrogen receptor of approximately 29 kDa apparent molecular weight in human sperm membranes by ligand and Western blot analysis, respectively, using peroxidase-conjugated E_2 and an antibody directed against the ligand binding region of the genomic receptor (αH222). Such receptor is functional since $17\beta E_2$ induces a rapid and sustained increase of intracellular calcium concentrations ($[Ca^{2+}]_i$) which is completely eliminated by preincubation with αH222. $17\beta E_2$ effects on calcium are clearly mediated by a membrane receptor, as they are reproduced by the membrane-impermeable conjugate of the hormone BSA-E_2. Dose-response curve for this effect is biphasic with EC_{50}s in the nanomolar and micromolar range. In addition to calcium increase, $17\beta E_2$ stimulates tyrosine phosphorylation of several sperm proteins including the 29-kDa protein band. Preincubation of human sperm with $17\beta E_2$ inhibits calcium and acrosome reaction increases in response to progesterone. We conclude that estrogens may play a role in the modulation of non-genomic action of progesterone (P) in human sperm during the process of fertilization. © 2000 Elsevier Science Ireland Ltd. All rights reserved.

Keywords: Estrogen receptor; Human sperm membrane; Progesterone

1. Introduction

Increasing evidence indicates that specific cell membrane receptors are involved in rapid effects of steroids (Wehling, 1997). As far as estrogens concerns, non-genomic effects of $17\beta E_2$ have been demonstrated on intracellular calcium concentrations ($[Ca^{2+}]_i$), cyclic adenosine monophosphate levels (cAMP), mitogen activated protein kinase activity, phospholipase C and A_2, protein kinase C in different tissues and cell lines (Nemere and Farach-Carson, 1998). Estrogens are present at micromolar concentrations in follicular fluid (Frederick et al., 1991), a location that suggests a possible role of these molecules in the regulation of male and female gamete function. However, while several studies have demonstrated rapid effects of $17\beta E_2$ in human oocytes and documented its role in oocyte activation and development (for review see Revelli et al., 1998), little is known about the estrogen effects in spermatozoa (Revelli et al., 1998). The influence exerted by the lack of estrogen receptors on sperm function in estrogen receptor knock-out (ERKO) mice have been recently investigated (Couse and Korach, 1999), showing reduced motility and absence of fertilizing potential for α-estrogen receptor and no substantial modifications of male fertility for β-estrogen receptor.

2. Presence of membrane estrogen receptor in human sperm

The presence of specific binding sites for $17\beta E_2$ on human sperm surface has been suggested by several studies (Hernandez-Perez et al., 1979) although the nature of these receptors has not been investigated in these studies. We have recently identified and characterized a receptor for estrogen on human sperm membrane (Luconi et al., 1999) using functional and biochemical approaches similar to those applied by our group to characterize the non-genomic receptor for P on human sperm surface (Luconi et al., 1998a). In particular, we have employed ligand and Western blot analysis of human sperm lysates and membranes using, respec-

Abbreviations: BSA, Bovine serum albumin; CHO, Chinese hamster ovary; EGTA, Ethylene glycol-bis (β-aminoethyl ether)N,N,N′,N′-tetraacetic acid; POD, Peroxidase.

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tively, E_2-POD and the monoclonal antibody αH222, directed against the steroid-binding domain of the genomic receptor (Greene et al., 1984), as probes. αH222 antibody has been shown to recognize a membrane estrogen receptor in rat pituitary tumor cells (Pappas et al., 1995). Moreover, the approach of using an antibody produced against the steroid binding sequence of the genomic receptor was applied by our (Luconi et al., 1998a) and other groups (Sabeur et al., 1996; Cheng et al., 1998) to identify the putative membrane receptors for P in human spermatozoa. We detected a single band of approximately 29 kDa molecular weight by both techniques (Luconi et al., 1999; Fig. 1(A, B)), although longer exposures of the αH222-stained blots revealed the presence of two additional bands of about 42–45 and 54–58 kDa (not shown). The same protein band of 29 kDa was detected on purified sperm membranes stained with αH222 antibody (Luconi et al., 1999). An estrogen-binding protein of similar molecular weight has been also described in other cell types (Bukusoglu and Krieger, 1994, 1996; Monje and Boland, 1999). In particular, a protein with molecular mass of about 29 kDa, identified by photoaffinity labeling with progesterone-11α-hemisuccinate-(2-[^{125}I]iodohistamine) and specifically displaced by incubation with estradiol, has been detected in mouse brain membranes (Bukusoglu and Krieger, 1994, 1996), and has been suggested to be the putative membrane binding site for estrogen (Bukusoglu and Krieger, 1996). Moreover, Monje and Boland (1999) using monoclonal antibodies against different domains of the intracellular estrogen receptor, identified on uterine membranes a 28–32-kDa protein, besides the expected 65-kDa band representing one of the genomic receptors. Furthermore, by Western blot analysis of MCF-7 cell lysates with αH222 antibody we detected a band of 29 kDa in addition to the expected protein bands corresponding to the genomic estrogen receptor (Luconi et al., unpublished result). Whether such protein band may represent the non-genomic receptor mediating the rapid effects of estrogen observed in this mammary cancer cell line (Migliaccio et al., 1993) has to be demonstrated. On the other hand it is worth noting that transfection of CHO cells with both α and β estrogen receptor isoforms results in expression of both proteins in the cell membrane (although at much lower levels compared to the nucleus) as well as in activation of rapid signal transduction pathways by estrogens (Razandi et al., 1999), indicating that classical estrogen receptors may be located on cell membranes. In this scenario, it can be postulated that both classical estrogen receptors located in the cell membrane as well as other (cell-type specific?) surface proteins may be involved in transducing rapid effects of estrogens.

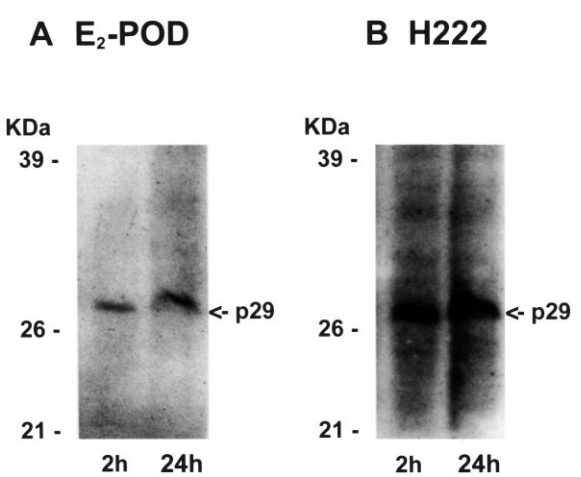

Fig. 1. Identification of sperm 17βE_2-binding proteins by ligand and Western analysis. Total extracts (30 μg/lane) or purified membranes (50 μg/lane) from 2 and 24 h capacitated spermatozoa were separated on reducing 10% SDS-PAGE. (A): Ligand blot analysis of the sperm lysates using peroxidase-conjugated estradiol (E_2-POD, 0.5 μM) reveals a single binding protein of 29 kDa molecular weight. (B) After stripping, the same blot as in (A) was re-probed for estrogen receptor with αH222 antibody (1:400) followed by BM detection system. An exact alignment of the blots indicates that the E_2-POD binding protein of 29 kDa (A) coincides with the band revealed by αH222 (B) antibody.

3. Biological effects of estrogen on human spermatozoa

3.1. Effects of intracellular calcium concentrations ($[Ca^{2+}]_i$)

Addition of 17βE_2 to fura-loaded spermatozoa induced a rapid and sustained rise in $[Ca^{2+}]_i$ in a dose-dependent manner. Fig. 2 shows the typical calcium waves observed in response to 17βE_2 (panel A) in comparison with the typical wave obtained in response to progesterone (P, panel B), a well known stimulator of calcium increase and acrosome reaction of human spermatozoa (Baldi et al., 1999). The shapes of calcium waves were quite different, indeed, while P induces a first rapid peak followed by a long sustained plateau (Fig. 2(B)), 17βE_2 induced a slow and sustained response (Fig. 2(A)). The increase in $[Ca^{2+}]_i$ following 17βE_2 addition was totally dependent on the presence of extracellular calcium, since the response was absent if spermatozoa were stimulated in calcium-depleted medium in the presence of 2 mM EGTA and was restored by subsequent replacement of external calcium to normal levels. The dose-response curve for the calcium effect of 17βE_2 was biphasic showing a first component with an EC_{50} of 0.60 ± 0.12 nM and a second component with an EC_{50} of 3.80 ± 0.26 μM. Since interactions between P and estrogen have been reported in brain tissues (Bukusoglu and Krieger, 1994, 1996) and in spermatozoa (Hyne and Boettcher, 1977), we tested the hypothesis of an eventual functional interference between the effects of the two steroids. We

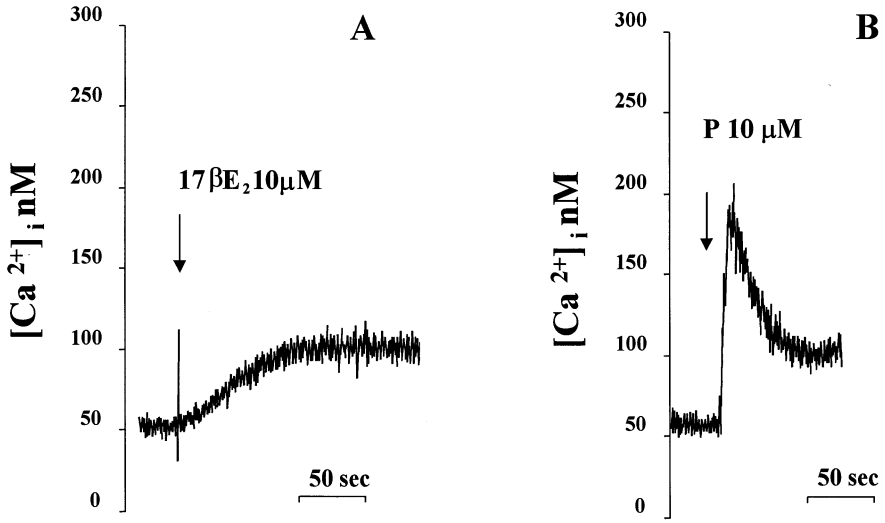

Fig. 2. Typical calcium waves observed in response to $17\beta E_2$ (panel A) and P (panel B) in fura-2-loaded capacitated human spermatozoa. The arrows indicate the moment of addition of stimuli.

found that the typical $[Ca^{2+}]_i$ transient in response to P (10 μM) was reduced in a dose-dependent manner by a previous administration of $17\beta E_2$ both in the peak and plateau components (not shown, Luconi et al., 1999). Both effects of $17\beta E_2$ on calcium levels and on calcium response to P were not antagonized by the cytosolic estrogen receptor antagonist tamoxifen (not shown), suggesting that the classical estrogen receptors are not involved.

The effect of $17\beta E_2$ on $[Ca^{2+}]_i$ appeared to be specific as comparable concentrations of 17α-estradiol ($17\alpha E_2$) even at 10 μM concentration neither stimulated $[Ca^{2+}]_i$ rise nor interfered with P-induced response. In addition, the membrane-impermeable estradiol conjugate BSA-E_2 induced an $[Ca^{2+}]_i$ increase similar to that of $17\beta E_2$ (not shown), further indicating a membrane location of estrogen receptor. In addition, since pre-incubation of sperm samples with αH222 antibody (1:20 dilution), but not with normal rat serum, reduced $17\beta E_2$ stimulation of calcium influx (not shown), we infer that the sperm membrane receptor recognized by this antibody (Fig. 1) is involved in the calcium rise induced by the steroid.

3.2. Effects of $17\beta E_2$ on sperm tyrosine phosphorylation

Tyrosine phosphorylation of proteins is an important regulating event in spermatozoa (Baldi et al., 1996). A large increase of protein phosphorylation occurs spontaneously during capacitation (Baldi et al., 1996), the maturational process that confers to the sperm the ability to undergo acrosome reaction (AR) and to fertilize an egg, as well as following stimulation with inducers of AR such as zona proteins and P (Baldi et al., 1996). We therefore investigated the effect of $17\beta E_2$ on this important sperm event. Brief (5–10 min) stimulation with this steroid produced an increase of phosphorylation in tyrosine residues of several protein bands, among which a band at 29 kDa (not shown, Luconi et al., 1999) which co-migrated with the putative estrogen receptor (Fig. 1), indicating that the sperm membrane receptor for $17\beta E_2$ can be regulated by tyrosine phosphorylation. On the other hand, tyrosine phosphorylation of estrogen genomic receptor has been described as one of the mechanisms of receptor transactivation (Weigel, 1996).

We also observed an increase of tyrosine phosphorylation in a sperm protein of 42–44 kDa molecular weight following addition of $17\beta E_2$. The presence of extracellular-signal regulated kinases-1 and 2 (ERK1 and ERK2), which have a molecular weight in the 42–44-kDa range, has recently been shown in human sperm (Luconi et al., 1998b). In addition, the activity of these two enzymes appears to be regulated by tyrosine phosphorylation during capacitation (Luconi et al., 1998b) and in response to P (Luconi et al., 1998c). Therefore, it is possible that stimulation of ERK1/ERK2 activation is part of the signal transduction cascade induced by $17\beta E_2$ in human sperm and such possibility is at present under investigation in our laboratory.

3.3. Effects of $17\beta E_2$ on AR

As $[Ca^{2+}]_i$ rise induced by P leads to an increase in AR of human spermatozoa (Baldi et al., 1999), we investigated whether $17\beta E_2$ effects on calcium were also involved in regulation of AR. Two hours incubation of capacitated spermatozoa with increasing concentrations of $17\beta E_2$ induced only a slight, not significant, stimulation of AR at 10 μM concentration (Table 1). However,

if spermatozoa were incubated with $17\beta E_2$ before addition of P, the AR-inducing effect of the latter was blunted (Table 1). Such effect was present with all the concentrations of $17\beta E_2$ tested and does not appear to be dose-dependent (Table 1). The precise mechanism involved in $17\beta E_2$ inhibition of calcium and AR response to P in human spermatozoa is still unclear. It is possible that the partial stimulation by $17\beta E_2$ of the same signal transduction pathways of P interferes with the biological response to the latter leading to inhibition of AR. However, the possibility that $17\beta E_2$ and P compete for the same receptors cannot be excluded.

4. Conclusions

In conclusion, the estrogen receptor detected in human sperm surface is apparently involved in the activation of two different signal transduction pathways, namely an increase of $[Ca^{2+}]_i$ and of tyrosine phosphorylation of proteins confirming the findings in other cell types for non-genomic/rapid actions of estrogens (Revelli et al., 1998). The effects of the estrogen result in inhibition of P-stimulated calcium influx and AR. Since both these steroids are present in the follicular fluid (Frederick et al., 1991) and in the female genital tract (Batra et al., 1980) at concentrations similar to those inducing the biological effects observed in vitro in human spermatozoa, it is conceivable that these effects may be physiologically relevant. In particular, the strict cross talk between sperm membrane receptors for $17\beta E_2$ and P may be important for an appropriate timing of capacitation and AR in the female genital tract.

Further studies are required to elucidate whether environmental chemicals with estrogen action might have similar effects on human sperm and to evaluate whether the absence of sperm response to P in several case of idiopathic male infertility (Baldi et al., 1999) may be due to alteration in the interactions between these two steroids.

Table 1
Effect of different doses of $17\beta E_2$ on AR in response to progesterone (10 μM) in capacitated human spermatozoa[a]

$17\beta E_2$ (M)	C	P	P+E
0	5 ± 0.3 ($n = 6$)	11.8 ± 1.23 ($n = 6$)	–
1E-9	5.6 ± 1.07 ($n = 6$)	–	6.9 ± 1.01 ($n = 6$)
1E-7	6.3 ± 1.02 ($n = 4$)	–	6.5 ± 0.6 ($n = 4$)
1E-5	7.75 ± 0.5 ($n = 6$)	–	8.8 ± 1.44 ($n = 6$)

[a] Data represents the mean ± S.E.M. percentage acrosome reacted spermatozoa for the number of experiments indicated in parenthesis. C = basal AR; P = AR in response to progesterone; P+E = AR in response to progesterone in the presence of the indicated concentration of $17\beta E_2$.

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