A new prostaglandin E receptor mediates calcium influx and acrosome reaction in human spermatozoa

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ABSTRACT Zona pellucida protein 3, a protein of the egg’s extracellular matrix, and progesterone secreted by granulosa cells surrounding the oocyte are regarded as physiological stimuli of sperm acrosome reaction. Signal transduction steps initiated by both stimuli result in influx of $Ca^{2+}$ from the extracellular space. Herein, we present a role for prostaglandin (PG) E as a physiological inducer of $Ca^{2+}$ influx and acrosome reaction in human spermatozoa. PGE$_1$ specifically binds to human sperm membranes ($K_d = 20.4$ nM; $B_{max} = 88$ fmol/mg protein) and induces a pertussis-toxin-insensitive, transient increase in intracellular $Ca^{2+}$ concentrations, which can be blocked by $\mu$M concentrations of La$^{3+}$, Gd$^{3+}$, and Zn$^{2+}$. The kinetic profile was similar to that observed after progesterone challenge. Sequential application of both agonists did not lead to cross-desensitization. E prostaglandins were found to be the only prostanoids with agonistic properties ($EC_{50}$ values for PGE$_1$ and PGE$_2$: <10 nM and 300 nM, respectively). Pharmacological characteristics were not compatible with those of cloned prostanoid receptors indicating the expression of a distinct membrane receptor. Activation of the sperm $E$ prostanoid receptor stimulates incorporation of $[^{3}P]GTP$ azidoanilide into immunoprecipitated G$_{q/11}$ subunits. Thus, in human sperm, PG induces $Ca^{2+}$ influx and acrosome reaction via a G$_{q/11}$-coupled E prostanoid receptor. The block of PGE$_1$-induced $Ca^{2+}$ transients and acrosome reaction by physiological Zn$^{2+}$ concentrations highlights a role of Zn$^{2+}$ as an endogenous $Ca^{2+}$ channel blocker present in seminal plasma protecting sperm from premature PGE$_1$-evoked increases in intracellular $Ca^{2+}$ concentrations.

In vivo, ejaculated and epididymal mammalian spermatozoa are not able to fertilize an egg immediately but have to undergo a process of maturation in the female reproductive tract. This time-dependent acquisition of fertilizing capacity called “capacitation” is correlated with changes in sperm motility, metabolism, plasma membrane fluidity, and intracellular ion concentrations. In capacitated spermatozoa, local stimuli acting in vicinity of the oocyte induce the acrosome reaction (AR), an exocytotic event leading to release of hydrolytic enzymes and substantial reorganization of the sperm plasma membrane. To date, two physiological inducers of the AR are known: Subsequent to species-specific binding of sperm to the zona pellucida (ZP), the oocyte’s extracellular matrix (2–4), one of the three major proteins forming the mouse ZP, ZP3, elicits the AR (5). Progesterone secreted by ovarian follicular cells surrounding the ovulated egg also initiates the AR (6), and a priming role of the steroid for the induction of the AR by the ZP has been suggested (7).

Signal transduction steps in sperm resulting in AR are understood poorly. One of the essential features of the AR is an influx of $Ca^{2+}$ from the extracellular space required to promote the fusion between the outer acrosomal membrane and the overlying sperm plasma membrane (1). The lack of knowledge on signaling steps leading to $Ca^{2+}$ influx and AR contrasts with the detailed information about the expression and subcellular localization of classical signal transduction components like G protein-coupled receptors (8), receptor kinases (9), G proteins (10, 11), and effectors such as enzymes (11) and ion channels (12). Thus, the delineation of signaling cascades in sperm is a crucial first step to understand the physiology of fertilization at the molecular level. In the present study, we identified a new role for E prostaglandins as physiological inducers of the AR in humans and provide evidence for the expression of a G protein-coupled E prostanoid (EP) receptor in human sperm.

MATERIALS AND METHODS

Sperm Sample Preparation. Human semen samples were obtained from couples undergoing in vitro fertilization because of female infertility. Sperm samples with normal parameters of sperm count, motility, and morphology were pooled and included in this study. Motile sperm fractions were isolated by a swim-up procedure in hypertonic Biggers Whitten and Whittingham (BWW) medium (for composition, see refs. 13 and 14) supplemented with 10% fetal calf serum (BWW-FCS). Capacitation was promoted by incubating sperm suspensions (0.5–2 × 10$^7$ per ml) for 6–8 h at 37°C in a humidified atmosphere containing 5% CO$_2$.

Assessment of Intracellular $Ca^{2+}$ Concentrations in Cell Suspensions and Immobilized Single Cells. For determination of intracellular $Ca^{2+}$ concentrations in sperm suspensions, the fluorescent indicator fluo-3/AM (2 µM; Molecular Probes) was added during the final 30 min of capacitation followed by a 15-min incubation at room temperature. To prevent precipitation of insoluble salts, bicarbonate, phosphate, and sulfate ions present in the original BWW medium were replaced by chloride ions when lanthanum, gadolinium, or zinc ions were added. Fluorescence (excitation wavelength 506 nm; emission wavelength 526 nm) was monitored at 37°C with an LS50B dual wavelength fluorescence spectrophotometer (Perkin–Elmer). To obtain $F_{max}$ and $F_{min}$, Triton X-100 (reduced form; final concentration, 0.1%) and EGTA (pH 8.4, 20 mM) were added to the incubation buffer, respectively. Intracellular $Ca^{2+}$ concentrations were calculated by assuming a $K_d$ of 400 nM (37°C) for fluo-3 (15). Stock solutions (10 mM) of lipophilic agonists were prepared in ethanol. Human erythroleukemia (HEL) 92.1.7 cells resuspended in culture medium were loaded with fluo-3/AM (2 µM) as outlined for fura-2/AM (16).

Abbreviations: AR, acrosome reaction; PG, prostaglandin; PTX, pertussis toxin; ZP, zona pellucida; ZP3, zona pellucida protein 3; EP, E prostanoid; HEL, human erythroleukemia; BWW, Biggers Whitten and Whittingham.

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For single-cell Ca\(^{2+}\) determinations, capacitated, fura-2/AM-loaded sperm suspensions (Molecular Probes) were washed in incubation buffer (IC) containing 138 mM NaCl, 6 mM KCl, 1.25 mM CaCl\(_2\), 1.25 mM MgCl\(_2\), 5.5 mM glucose, and 10 mM Hepes (pH 7.4) and layered onto poly(l-lysine)-coated coverslips, which were subsequently washed with incubation buffer twice. Intracellular Ca\(^{2+}\) concentrations in single sperm were monitored with a digital imaging system (TILL Photonics, Planegg, Germany) using an inverted microscope (Zeiss Axiosvert 100). Fura-2 fluorescence was excited alternately at 340 and 380 nm. Regions of interest were defined manually over single sperm. Background fluorescence was assessed by monitoring fluorescence signals in the absence of digitonin (50 \(\mu\)M) and MnCl\(_2\) (10 mM) and subtracted from fluorescence readings.

**Assessment of Sperm Acrosome Reaction and Determination of CAMP Levels.** Acrosome reaction assay was performed as described in ref. 17. Aliquots of capacitated sperm suspensions (1 \(\times\) 10\(^6\) cells/ml) were resuspended in BWW supplemented with 1 mM 3-isobutyl-1-methylxanthine and incubated with or without agonists for the times indicated. Cells were pelleted and supernatants were removed. CAMP was extracted from sperm pellets by resuspending cells in 100 \(\mu\)l of ice-cold water containing 0.1% Triton X-100. Proteins were denatured by boiling samples for 10 min. Cellular debris was removed by centrifugation, and the CAMP content of the supernatant was determined by radioimmunoassay (16).

**Membrane Preparation, SDS/PAGE, Immunoblotting, and G Protein Antibodies.** Membranes were prepared from washed and capacitated sperm according to Althouse et al. (18). Harvested membrane pellets were resuspended in Hepes-phosphate-buffered saline (pH 7.4) and stored at \(-70^\circ\)C until analysis.

Membranes prepared from mouse L cells (16) served as positive controls. Membrane proteins (50 \(\mu\)g per lane) were resolved by SDS/PAGE performed on 13% (wt/vol) acrylamide gels and blotted onto nitrocellulose filters. Immunoreactive bands were visualized as described (19). The following antisera were used: AS 348 (\(\alpha_\text{g}\)), AS 233 (\(\alpha_\text{q}\)), AS 343 (\(\alpha_\text{q}\)), and AS 368 (\(\alpha_9^{11}\)) (20, 21).

**Photolabeling of Sperm Membrane G Proteins.** The photolabeling of \(\alpha_\text{g}\) with \([\alpha^{32}\text{P}]\)GTP azidoanilide and immunoprecipitation was performed as described (22). Immunoprecipitated proteins (AS 368) were resolved by 10% SDS/PAGE and were visualized by blotting onto nitrocellulose membranes and autoradiography followed by analysis with a phosphorimager (Fuji BAS 1500). To test for comparable loading of immunoprecipitated nitrocellulose membranes were immunostained with a biotinylated IgG fraction prepared from AS 370 (\(\alpha_9^{11}\)) and horseradish peroxidase-conjugated streptavidin.

**\([\text{H}]\text{PGE}_1\) Binding.** Binding of \([\text{H}]\text{PGE}_1\) to human, bovine, and porcine sperm membranes was assessed as described (23). Sixty micrograms of sperm membranes were incubated for 1 h at room temperature with \([\text{H}]\text{PGE}_1\) at the concentrations indicated. Bound and free ligand were separated by filtration through GF/B glass fiber filters (Whatman). Nonspecific binding was determined in the presence of 10 \(\mu\)M unlabeled PGE\(_1\).

**RESULTS**

**Prostanoids Inducing Ca\(^{2+}\) Influx in Human Sperm.** Prostaglandins produced by ovarian follicular cells and locally within the ovum are assumed to be involved in the process of ovulation and in the regulation of tubal function, respectively (24, 25). Therefore, we set out to study the effect of prostanoids on human sperm. PGE\(_1\), a rapid and transient increase in [Ca\(^{2+}\)], was capacitated human spermatozoa displaying a kinetic profile similar to that evoked by progesterone (Fig. 1A and B). The peak response was reached within 10–20 sec. Sequential application of maximally effective concentrations of PGE\(_1\) and progesterone did not lead to cross-desensitization but resulted in two independent Ca\(^{2+}\) transients unaffected by the order in which agonists were applied (Fig. 1A and B). A costimulation of sperm with PGE\(_1\) and progesterone had an additive effect with respect to the peak [Ca\(^{2+}\)]. The decline of elevated [Ca\(^{2+}\)], to baseline levels, however, was substantially retarded (Fig. 1C). On the contrary, a second challenge of sperm with 17\(\alpha\)-hydroxyprogesterone (1 \(\mu\)M) subsequent to a primary stimulation with progesterone (1 \(\mu\)M) did not give rise to a second Ca\(^{2+}\) transient, and the simultaneous addition of both steroids at maximally effective concentrations (1 \(\mu\)M) did not yield a synergistic response (data not shown). Similar results were obtained on sequential or simultaneous application of PGE\(_1\) and PGE\(_2\). These data indicate that distinct signaling mechanisms are responsible for progesterone- and PGE\(_1\)-mediated Ca\(^{2+}\) influx in human sperm.

To exclude a potential contribution of contaminating round cells in sperm preparations to prostaglandin-induced Ca\(^{2+}\) transients and to determine the proportion of sperm responsive to prostanoids, single fura-2-loaded sperm were examined by fluorescence microscopy. Sperm were immobilized on poly(l-lysine)-coated glass coverslips and sequentially were stimulated with PGE\(_1\) and progesterone (Fig. 1D). Image analysis of Ca\(^{2+}\) transients in single sperm heads revealed that the cellular response to PGE\(_1\) was ubiquitous, and all of the fluorescent sperm reacted to agonist application with Ca\(^{2+}\) transients localized over the acrosomal region (n = 64 in three experiments). More than 90% of immobilized sperm responded to a subsequent progesterone challenge (1 \(\mu\)M) with a second increase in [Ca\(^{2+}\)]. The kinetics of Ca\(^{2+}\) transients, however, displayed some variability in that...
sustained elevations of \([\text{Ca}^{2+}]\) were observed after addition of the first agonist (Fig. 1D) in a minor cell fraction.

In contrast to human sperm, bull and boar spermatozoa were unaffected by PGE1 stimulation (data not shown). These findings are in accord with results from binding studies (Fig. 2). Human sperm membranes displayed specific and saturable \([^3\text{H}]\text{PGE}_1\) binding sites (Fig. 2A). Scatchard analysis of the data revealed a \(K_d\) value of 20.4 nM and a \(B_{\text{max}}\) value of 88 fmol/mg protein (Fig. 2B and C). Specific binding was detected only in human but not in bull and boar sperm membranes (Fig. 2C).

To characterize prostaglandin-evoked \(\text{Ca}^{2+}\) transients, various prostanoids were tested for agonistic properties on human sperm (Fig. 3A). Apart from PGE1, PGE2 was the only additional prostaglandin found to raise \([\text{Ca}^{2+}]\), in a concentration-dependent manner (Fig. 3A), whereas PGF2\(_{\alpha}\), PGD2, PGA1, PGI2, and the synthetic prostanoids cicaprost, U46619 (data not shown), iloprost and sulprostone, an EP1 and EP3 receptor-selective agonist, were ineffective (Fig. 3A). Unexpectedly, PGE1 was found to be more potent and effective than PGE2 (Fig. 3A).

One-half maximally effective concentrations (EC\(_{50}\)) of PGE2 were \(\approx 300 \text{nM}\); the EC\(_{50}\) value for PGE1 was below 10 nM. HEL cells express both EP1 and EP3 receptors (26, 27), which mediate phosphoinositide breakdown and \(\text{Ca}^{2+}\) mobilization. To control for the bioactivity of agonist preparations, prostanoid-induced \(\text{Ca}^{2+}\) transients were measured in HEL cells (Fig. 3B) by using the same agonist dilutions as in experiments with human sperm. In HEL cells, PGE1, PGE2, and sulprostone caused concentration-dependent rises of \([\text{Ca}^{2+}]\) characterized by EC\(_{50}\) values around 80 nM. Iloprost, a full agonist for IP and partial agonist for EP1 receptors, also was effective at 10 \(\mu\)M (Fig. 3B). These findings indicate that human sperm express a EP receptor whose activation results in rapid \(\text{Ca}^{2+}\) transients.

**Blockers of \(\text{Ca}^{2+}\) Transients.** The \(\text{Ca}^{2+}\) signal in HEL cells appears to rely on agonist-dependent phospholipase C activation because the aminosteroid U73122 (10 \(\mu\)M) completely inhibited prostanoid-induced rises of \([\text{Ca}^{2+}]\) (data not shown). When applied to human sperm, U73122 and its inactive analog U73343 evoked substantial and irreversible rises of \([\text{Ca}^{2+}]\). However, U73122 (10 \(\mu\)M) reduced agonist-dependent \(\text{Ca}^{2+}\) transients in PGE1-challenged spermatozoa by \(\approx 50\%\) (data not shown). Considering that known EP3 receptors primarily couple to Gi proteins (28), human sperm were treated with 1 \(\mu\)g/ml of pertussis toxin (PTX) for 6 h during the capacitation period. Such a regimen has been reported previously to efficiently ADP ribosylate Gi/o proteins in human sperm (29). We observed that PTX treatment did not affect progesterone- and PGE1-induced \(\text{Ca}^{2+}\) transients, arguing against a participation of Gi/o proteins (data not shown).

Progestosterone- and PGE1-evoked \(\text{Ca}^{2+}\) transients were carried by extracellular \(\text{Ca}^{2+}\) ions. Preincubation in \(\text{Ca}^{2+}\)-free medium containing 1 mM EGTA precluded any rise of \([\text{Ca}^{2+}]\). Mn\(^{2+}\)-quenching experiments with fura-2-loaded sperm incubated in \(\text{Ca}^{2+}\)-free medium demonstrated that progesterone- and PGE1-induced increase in \([\text{Ca}^{2+}]\) mainly relied on influx of \(\text{Ca}^{2+}\) as opposed to mobilization from internal stores. In addition to \(\text{Ca}^{2+}\) and Mn\(^{2+}\), other divalent cations like Sr\(^{2+}\) and Ba\(^{2+}\) also entered human spermatozoa upon progesterone and PGE1 stimulation. Agonist-induced \(\text{Ca}^{2+}\) influx could be attenuated effectively by
lanthanids (La$\text{^{3+}}$, Gd$\text{^{3+}}$) and Zn$\text{^{2+}}$ in a concentration-dependent fashion (data not shown), whereas we observed Ni$\text{^{2+}}$, Cd$\text{^{2+}}$, and Co$\text{^{2+}}$ to be ineffective up to a concentration of 1 mM. The IC$_{50}$ values for La$\text{^{3+}}$, Gd$\text{^{3+}}$, and Zn$\text{^{2+}}$ were 2, 10, and 30 μM, respectively. Zn$\text{^{2+}}$ concentrations physiologically occurring in seminal plasma (~2 mM; 30) completely block progesterone- and PGE$_1$-mediated Ca$\text{^{2+}}$ influx (data not shown). In these cell preparations, ~90% of sperm were viable as tested with the dye Hoechst 33258 and even high Zn$\text{^{2+}}$ concentrations did not affect sperm viability. Blockers of voltage-gated L-type Ca$\text{^{2+}}$ channels, such as pimozide (5 μM) and methoxyverapamil (10 μM) as well as pimozone (5 μM), a blocker of T-type voltage-gated Ca$\text{^{2+}}$ channels, did not affect progesterone- or PGE$_1$-elicited Ca$\text{^{2+}}$ transients. Incubation of sperm suspensions with 100 μM 8-bromo-cAMP (100 μM) or 8-bromo-cGMP (100 μM) did not raise [Ca$\text{^{2+}}$]$_i$, and agonist-induced Ca$\text{^{2+}}$ peaks were not attenuated by L-is-diltiazem (data not shown), proven to be an effective blocker of cGMP-gated cation channels.

**Stimulation of Acrosome Reaction.** The percentage of spontaneously acrosome-reacted sperm after a capacitation period of 5–8 h was 2.2 ± 0.2 (mean ± SEM, n = 24, four independent experiments) (Fig. 4). Treatment of sperm with PGE$_1$ (1 μM) or progesterone (1 μM) increased the fraction of acrosome-reacted sperm by a factor of 3.4–3.7 (Fig. 4). The simultaneous application of both agonists had an additive effect, and the proportion of acrosome-reacted sperm increased to nearly 20%. The reduced potency and efficacy of PGE$_2$ to increase [Ca$\text{^{2+}}$]$_i$ was reflected in the comparably weak effect of this prostanoid (1 μM) to stimulate AR (~1.4-fold, Fig. 4). The G$_{i/o}$ protein-activating peptide mastoparan (50 μM) and the Ca$\text{^{2+}}$ ionophore A 23187 (10 μM) served as positive controls and induced AR in 49 and 53% of spermatozoa examined (Fig. 4).

Capacitation had no effect on the ability of progesterone and PGE$_1$ to elicit a rapid Ca$\text{^{2+}}$ transient in human sperm (data not shown) but markedly affected the ability of these agents to evoke AR (Fig. 5A–C). We observed a time-dependent increase in the proportion of sperm having undergone AR in response to progesterone and PGE$_1$. Optimal effects were reached after capacitating spermatozoa for 6 h (Fig. 5C). At all of the time points tested, however, costimulation of sperm with both agonists yielded additive results. When sperm were stimulated in bicarbonate, phosphate, and sulfate ion-free BWW medium substituted with chloride ions, results were similar to those obtained in original BWW medium (Fig. 5D) demonstrating that bicarbonate is not required for progesterone- and PGE$_1$-induced AR in capacitated human spermatozoa. In the presence of 200 μM Zn$\text{^{2+}}$, however, progesterone- and PGE$_1$-induced AR was abolished completely (Fig. 5E).

**cAMP Levels in Human Sperm.** Because the known EP$_2$, and EP$_3$ receptors couple to the G$_{i/o}$/adenyl cyclase system in somatic cells, we examined the effect of PGE$_1$ and progesterone on intracellular cAMP formation in human sperm. The cAMP content of unstimulated cells was 101 ± 6 fmol per 10$^6$ sperm (after 15 min; mean ± SEM, n = 3). Intracellular cAMP concentrations were not altered significantly 5 or 15 min subsequent to stimulation of cells with either 1 μM PGE$_1$ (5 min: 102 ± 7; 15 min: 101 ± 7 fmol/10$^6$ sperm) or 1 μM progesterone (5 min: 102 ± 14; 15 min: 113 ± 16 fmol/10$^6$ sperm).

**PTX-Insensitive G proteins in Human Sperm Membranes.** Because PGE$_1$-dependent Ca$\text{^{2+}}$ transients were unaffected by PTX pretreatment, we hypothesized that PTX-insensitive G proteins may be involved in PGE$_1$-evoked cellular effects. Immunoblotting with an antisera specific for the G protein α subunits α$_{q}$ and α$_{11}$ (AS 368) revealed the expression of corresponding 42-kDa proteins in sperm and in L cell membranes, the latter serving as a positive control (Fig. 6A). Under the separation conditions chosen, the upper band represents α$_{11}$ and the lower band represents α$_{q}$. Immunoblotting of membranes with two antisera raised against the C-terminal sequence of α$_{11}$ (AS 233) and α$_{q}$ (AS 343) revealed one distinct protein for each antibody in L cell membranes, whereas no specific signal was recognized in equivalent amounts (50 μg) of sperm membranes (Fig. 6A). Thus, human sperm do not express appreciable amounts of G proteins belonging to the G$_{12}$/13 family. In addition, we did not obtain any indication for the expression of Go$_{q}$ in human spermatozoa.

**Photolabeling of Receptor-Activated G Proteins.** To study coupling of activated EP receptors to G proteins of the G$_{8/11}$ family, sperm membranes were photolabeled with [α-32P]GTP azidoanilide in the absence and presence of 1 μM PGE$_1$. Labeled G protein α subunits were immunoprecipitated with a specific antisera and resolved by SDS/PAGE (Fig. 6B). In human

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**Fig. 4.** Agonist-induced acrosomal exocytosis in capacitated human sperm. Capacitated human spermatozoa were incubated for 30 min in the presence of modulators as indicated. Mastoparan (50 μM) and the Ca$\text{^{2+}}$ ionophore A 23187 (10 μM) served as positive controls. All of the other agents were applied at 1-μM concentrations. The percentage of acrosome-reacted sperm is indicated. Two hundred sperm were evaluated per single assay. Data represent means ± SEM (n = 16–32) of four independent experiments.

**Fig. 5.** Effect of capacitation and Zn$\text{^{2+}}$ on agonist-induced AR. Swim-up human sperm were capacitated for 2 (A), 4 (B), or 6 (C) h in BWW-FCS medium, incubated for 30 min with progesterone (1 μM), PGE$_1$ (1 μM), or a combination of the two agonists (application of agonist indicated by +), and the percentage of acrosome-reacted sperm was determined as outlined in the legend of Fig. 4. (D) After a 6-h capacitation period in BWW-FCS, sperm were washed and resuspended in BWW-chloride ions. (E) Sperm were treated as in D but were stimulated in the presence of 200 μM Zn$\text{^{2+}}$. Data represent means ± SEM of four independent experiments each performed in quadruplicate.
In contrast to stimulation of sperm by ZP, there is currently no evidence for the involvement of G proteins in the progesterone-induced Ca\(^{2+}\) influx in human sperm (34, 35). The present study proposes the concept of E prostaglandins as physiological inducers of the AR in human sperm. We demonstrate that both progesterone and PGE\(_1\) evoke rapid Ca\(^{2+}\) transients with similar pharmacological properties, thus indicating that progesterone- and PGE\(_1\)-elicited signaling events converge on a common distal signaling molecule, for instance a Ca\(^{2+}\)-permeable cation channel. In accord with recent observations on the percentage of human sperm responding to progesterone (36), we detected that the cellular reaction to PGE\(_1\) challenge also was ubiquitous. The observation that the two agonists do not cross-desensitize indicates that progesterone and PGE\(_1\) exert their rapid action via different receptors.

Early reports on hamster and guinea pig sperm suggested a role for E prostaglandins and PGE\(_2\), for the mammalian sperm AR (37, 38). Applying the Ca\(^{2+}\) indicator quin-2, Aitken et al. (39) observed a rise of [Ca\(^{2+}\)], in human sperm subsequent to exposure to PGE\(_1\) and PGE\(_2\). Because these effects were detected with high prostaglandin concentrations (170 \(\mu\)M) only, an ionophore-like action of E prostaglandins was proposed. On the contrary, we present evidence for the expression of a G protein-coupled EP prostaglandin receptor in human spermatozoa. Among the cloned prostaglandin receptors, only the IP receptor shows a predilection for PGE\(_1\) over PGE\(_2\) (28, 40). The ineffectiveness of the IP receptor-selective agonist cicaprost, however, excludes this receptor species as a candidate for the PGE\(_1\) action on human sperm. Because PGE\(_{2\alpha}\), PGD\(_2\), and U-4619 show no agonistic properties on human sperm, F prostaglandin, D prostaglandin, and thromboxane receptors do not have to be considered either.

All of the known EP receptors, however, display a similar or even lower affinity for PGE\(_1\) as compared with PGE\(_2\) (28, 40). In addition, the complete lack of a functional response upon ioprost stimulation rules out the EP1 receptor because this compound behaves as a partial EP1 receptor agonist in somatic cells (40), and the ineffectiveness of sulprostone strongly argues against EP3 receptors. Thus, the biological effect of E prostaglandins on human sperm most likely is mediated by an unknown, PGE\(_1\)-preferring EP receptor. PGE\(_1\) not only evokes rapid Ca\(^{2+}\) transients in human sperm, but also is capable of inducing AR. The simultaneous application of PGE\(_1\) and progesterone had an additive effect on the induction of AR in capacitated spermatozoa. Whereas capacitation was not required for progesterone (34) and PGE\(_1\) to elicit rapid Ca\(^{2+}\) transients, agonist-induced AR strictly was capacitation-dependent.

In accord with early findings by Blackmore et al. (34), we found that concentrations of blockers of L-type voltage-gated Ca\(^{2+}\) channels like diltiazem, a selective blocker of cyclic nucleotide-gated channels, was ineffective in decreasing agonist-induced transients. Our own results described here as well as observations by Foresta et al. (35) that progesterone causes Na\(^+\) influx accompanied by depolarization and Ca\(^{2+}\) influx through the same channel, indicate that progesterone and PGE\(_1\) both open nonselective cation channels that can be blocked by lanthanides and Zn\(^{2+}\).

Mammalian sperm express PTX-sensitive G proteins of the G\(_i\),\(_o\)-family as well as G\(_z\) (10). In the present study, we analyzed...
the expression of PTX-insensitive G proteins in membranes from human sperm and showed that Goα and Gαi1 can be detected in sperm membranes whereas no appreciable amounts of G proteins of the G12/13 family nor Gβ were observed. Photolabeling experiments showed that in human sperm membranes the activated EP receptor stimulates G proteins of the Gβγ family. Thus, in human sperm, classical receptor G protein-mediated signaling cascades are employed to achieve a biological effect.

In human semen, spermatozoa are exposed to high PGE1 concentrations of ~80 μM (30). The simultaneous presence of mM Zn2+ concentrations protects sperm from a massive Ca2+ influx which has been shown to profoundly impair the fertilizing potential of spermatozoa (36). It has been suggested that Zn2+ may stabilize sperm membranes during storage and ejaculation, and that Zn2+ removal may be required to prepare sperm for fertilization (42). The latter notion is supported by the fact that incubation of sperm with Zn2+ chelators capacitates hamster sperm (43). An inhibitory effect of Zn2+ on human sperm motility and AR has been observed (44). Our findings indicate a role of Zn2+ as an endogenous cation channel blocker to protect and maintain spermatozoa in a transitory quiescent state. During their ascent in the female reproductive tract, spermatozoa escape millimolar seminal fluid Zn2+ concentrations and are further on exposed to serum levels of Zn2+ (~20 μM), nearly all of which is bound to albumin. When approaching the ovulated egg, sperm are exposed again to rising concentrations of E prostaglandins produced locally within the fallopian tube and by granulosa cells surrounding the oocyte (24). Thus, in the vicinity of the oocyte, a simultaneous stimulation of spermatozoa by E prostaglandins and by micromolar concentrations of progesterone within the cumulus oophorus may serve to prime spermatozoa to undergo effectively AR after binding to the ZP.

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