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

(cation channels/fertilization/G proteins/seminal plasma Zn^{2+})

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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 propose 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 μM 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 [α-^{32}P]GTP azidoanilide into immunoprecipitated $G\alpha_{q/11}$ 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 (1). 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 (1). 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 ($0.5–2 \times 10^7$ 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).

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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 Axiovert 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 presence of digitonin (50 μM) 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 (1×10^6 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 μl 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°C until analysis.

Membranes prepared from mouse L cells (16) served as positive controls. Membrane proteins (50 μg 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 (α_s), AS 233 (α_{12}), AS 343 (α_{13}), and AS 368 ($\alpha_{q/11}$) (20, 21).

Photolabeling of Sperm Membrane G Proteins. The photolabeling of $G_{q/11}$ proteins with [α-^{32}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 immunoprecipitates, nitrocellulose membranes were immunostained with a biotinylated IgG fraction prepared from AS 370 ($\alpha_{q/11}$) and horseraddish peroxidase-conjugated streptavidin.

[^{3}H]PGE_1-Binding. Binding of [^{3}H]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 [^{3}H]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 μM unlabeled PGE_1.

RESULTS

Prostanoids Inducing Ca^{2+} Influx in Human Sperm. Prostaglandins produced by ovarian follicular cells and locally within the oviduct 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 induced a rapid and transient increase in $[Ca^{2+}]_i$ in capacitated human spermatozoa displaying a kinetic profile similar to that evoked by progesterone (Fig. 1 *A* 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. 1*A* and *B*). A costimulation of sperm with PGE_1 and progesterone had an additive effect with respect to the peak $[Ca^{2+}]_i$. The decline of elevated $[Ca^{2+}]_i$ to baseline levels, however, was substantially retarded (Fig. 1*C*). On the contrary, a second challenge of sperm with 17α-hydroxyprogesterone (1 μM) subsequent to a primary stimulation with progesterone (1 μM) did not give rise to a second Ca^{2+} transient, and the simultaneous addition of both steroids at maximally effective concentrations (1 μM) 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.

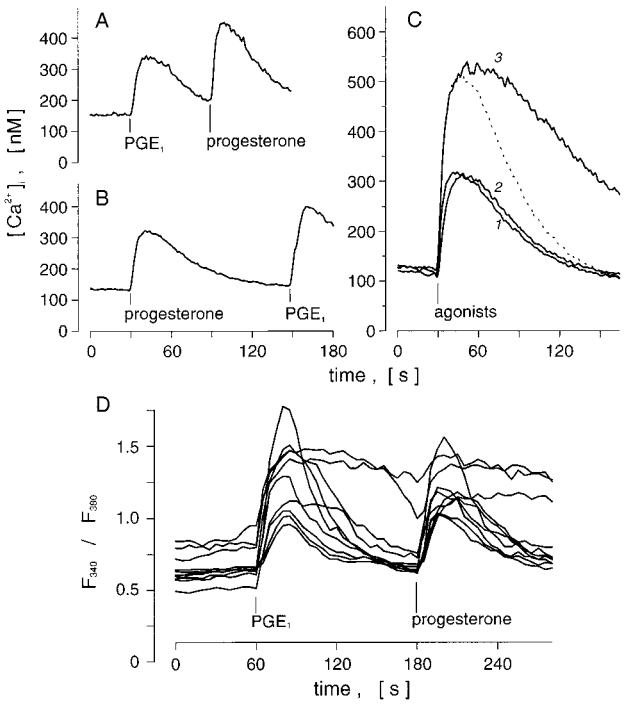

FIG. 1. Time courses of intracellular Ca^{2+} concentrations in human spermatozoa stimulated with different agonists. (*A* and *B*) Human sperm suspensions were loaded with fluo-3/AM and stimulated with 1 μM concentrations of PGE_1 and progesterone as indicated. (*C*) Recordings of intracellular Ca^{2+} concentrations after stimulation with 1 μM PGE_1 (1) or 1 μM progesterone (2) were superimposed. Trace 3 was obtained by simultaneous application of both agonists. The dotted lined represents a calculated addition of increases in $[Ca^{2+}]_i$ over basal values elicited by PGE_1 and progesterone in (1) and (2), respectively. One representative experiment of five is shown. (*D*) Capacitated human sperm loaded with fura-2/AM were immobilized on poly(L-lysine)-coated coverslips and stimulated with PGE_1 (1 μM) and progesterone (1 μM) as indicated. Fluorescence ratios (F_{340}/F_{380}) obtained from measurements of 10 single sperm were calculated after subtraction of autofluorescence. One representative experiment of three is depicted.

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. 1*D*). 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 μM) with a second increase in $[Ca^{2+}]_i$. The kinetics of Ca^{2+} transients, however, displayed some variability in that

sustained elevations of $[Ca^{2+}]_i$ were observed after addition of the first agonist (Fig. 1*D*) in a minor cell fraction.

In contrast to human sperm, bull and boar spermatozoa were unaffected by PGE_1 stimulation (data not shown). These findings are in accord with results from binding studies (Fig. 2). Human sperm membranes displayed specific and saturable $[^3H]PGE_1$ binding sites (Fig. 2*A*). Scatchard analysis of the data revealed a K_d value of 20.4 nM and a B_{max} value of 88 fmol/mg protein (Fig. 2 *B* and *C*). Specific binding was detected only in human but not in bull and boar sperm membranes (Fig. 2*C*).

To characterize prostaglandin-evoked Ca^{2+} transients, various prostanoids were tested for agonistic properties on human sperm (Fig. 3*A*). Apart from PGE_1, PGE_2 was the only additional prostaglandin found to raise $[Ca^{2+}]_i$ in a concentration-dependent manner (Fig. 3*A*), whereas $PGF_{2\alpha}$, PGD_2, PGA_1, PGI_2, and the synthetic prostanoids cicaprost, U46619 (data not shown), iloprost and sulprostone, an EP_3 and EP_1 receptor-selective agonist, were ineffective (Fig. 3*A*). Unexpectedly, PGE_1 was found to be more potent and effective than PGE_2 (Fig. 3*A*). One-half maximally effective concentrations (EC_{50}) of PGE_2 were ≈300 nM; the EC_{50} value for PGE_1 was below 10 nM. HEL cells express both EP_1 and EP_3 receptors (26, 27), which mediate phosphoinositide breakdown and Ca^{2+} mobilization. To control for the bioactivity of agonist preparations, prostanoid-induced Ca^{2+} transients were measured in HEL cells (Fig. 3*B*) by using the same agonist dilutions as in experiments with human sperm. In HEL cells, PGE_1, PGE_2, and sulprostone caused concentration-dependent rises of $[Ca^{2+}]_i$ characterized by EC_{50} values around 80 nM. Iloprost, a full agonist for IP and partial agonist for EP_1 receptors, also was effective at 10 μM (Fig. 3*B*). These findings indicate that human sperm express a EP receptor whose activation results in rapid Ca^{2+} transients.

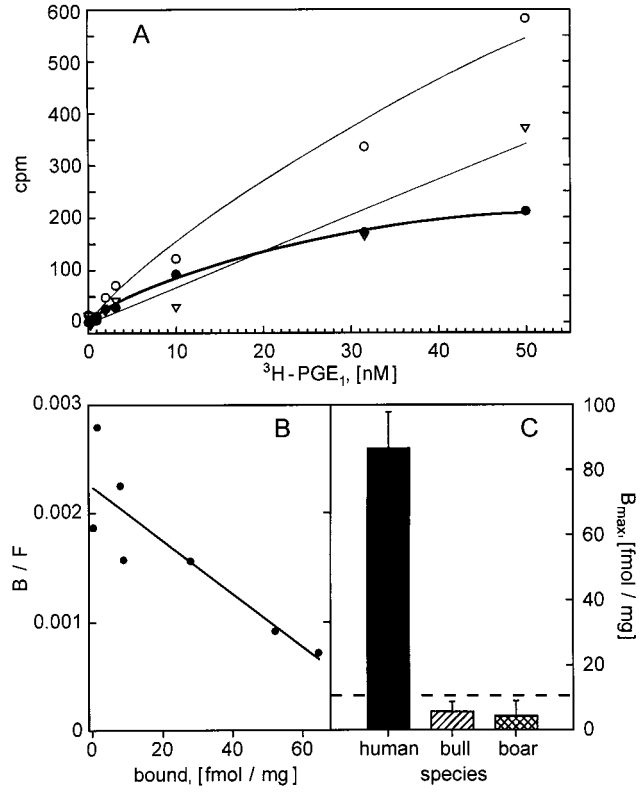

FIG. 2. Specific binding of $[^3H]PGE_1$ to human sperm membranes. (*A*) Sperm membranes (60 μg) were incubated with increasing concentrations of $[^3H]PGE_1$, and total (○), nonspecific (▽), and specific (●) binding was determined. (*B*) Scatchard transformation of binding data presented in *A*. (*C*) Human, bull, and boar sperm membranes were incubated with 50 nM of $[^3H]PGE_1$, and specific binding was determined. The dotted line indicates the upper limit of the 95% confidence interval of $[^3H]PGE_1$ binding to glass fiber membranes. Data represent means ± SD of three independent experiments each performed in duplicate.

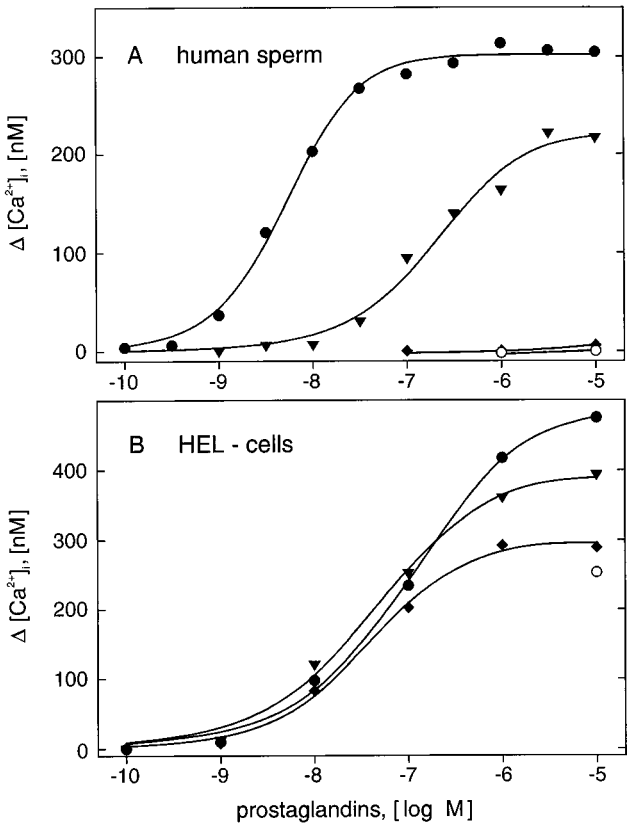

FIG. 3. Pharmacological characterization of prostaglandin effects on human spermatozoa. (*A*) Human sperm were loaded with the fluorescent dye fluo-3/AM and stimulated with different concentrations of PGE_1 (●), PGE_2 (▼). Sulprostone (◆) was tested at 0.1, 1, and 10 μM and iloprost (○) was tested at 1 and 10 μM. Agonist-induced increases in $[Ca^{2+}]_i$ are indicated. Data represent means of three experiments. (*B*) HEL cells were loaded with fluo-3/AM and stimulated with agonists used in *A*. Agonist-induced rises in $[Ca^{2+}]_i$ were recorded. Iloprost (○) was tested at a concentration of 10 μM only. Data represent means of three experiments.

Blockers of Ca^{2+} Transients. The Ca^{2+} signal in HEL cells appears to rely on agonist-dependent phospholipase C activation because the aminosteroid U73122 (10 μM) completely inhibited prostanoid-induced rises of $[Ca^{2+}]_i$ (data not shown). When applied to human sperm, U73122 and its inactive analog U73343 evoked substantial and irreversible rises of $[Ca^{2+}]_i$. However, U73122 (10 μM) reduced agonist-dependent Ca^{2+} transients in PGE_1-challenged spermatozoa by ≈50% (data not shown). Considering that known EP_3 receptors primarily couple to G_i proteins (28), human sperm were treated with 1 μg/ml of pertussis toxin (PTX) for 6 h during the capacitation period. Such a regimen has been reported previously to efficiently ADP ribosylate $G_{i/o}$ proteins in human sperm (29). We observed that PTX treatment did not affect progesterone- and PGE_1-induced Ca^{2+} transients, arguing against a participation of $G_{i/o}$ proteins (data not shown).

Progesterone- and PGE_1-evoked Ca^{2+} transients were carried by extracellular Ca^{2+} ions. Preincubation in Ca^{2+}-free medium containing 1 mM EGTA precluded any rise of $[Ca^{2+}]_i$. Mn^{2+}-quenching experiments with fura-2-loaded sperm incubated in Ca^{2+}-free medium demonstrated that progesterone- and PGE_1-induced increase in $[Ca^{2+}]_i$ mainly relied on influx of Ca^{2+} as opposed to mobilization from internal stores. In addition to Ca^{2+} and Mn^{2+}, other divalent cations like Sr^{2+} and Ba^{2+} also entered human spermatozoa upon progesterone and PGE_1 stimulation. Agonist-induced Ca^{2+} influx could be attenuated effectively by

lanthanids (La^{3+}, Gd^{3+}) and Zn^{2+} in a concentration-dependent fashion (data not shown), whereas we observed Ni^{2+}, Cd^{2+}, and Co^{2+} to be ineffective up to a concentration of 1 mM. The IC_{50} values for La^{3+}, Gd^{3+}, and Zn^{2+} were 2, 10, and 30 μM, respectively. Zn^{2+} concentrations physiologically occurring in seminal plasma (≈2 mM; 30) completely block progesterone- and PGE_1-mediated Ca^{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^{2+} concentrations did not affect sperm viability. Blockers of voltage-gated L-type Ca^{2+} channels like nicardipine (10 μM) and methoxyverapamil (10 μM) as well as pimozide (5 μM), a blocker of T-type voltage-gated Ca^{2+} channels, did not affect progesterone- or PGE_1-elicited Ca^{2+} transients. Incubation of sperm suspensions with 100 μM 8-bromo-cAMP (100 μM) or 8-bromo-cGMP (100 μM) did not raise $[Ca^{2+}]_i$, and agonist-induced Ca^{2+} peaks were not attenuated by L-*cis*-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 $[Ca^{2+}]_i$ was reflected in the comparably weak effect of this prostaglandin (1 μM) to stimulate AR (≈1.4-fold, Fig. 4). The $G_{i/o}$ protein-activating peptide mastoparan (50 μM) and the Ca^{2+} ionophor 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^{2+} transient in human sperm (data not shown) but markedly affected the ability of these agents to evoke AR (Fig. 5 *A–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. 5*C*). 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. 5*D*) demonstrating that bicarbonate is not required for progesterone- and PGE_1-induced AR in capacitated human spermatozoa. In the presence of 200 μM Zn^{2+}, however, progesterone- and PGE_1-induced AR was abolished completely (Fig. 5*E*).

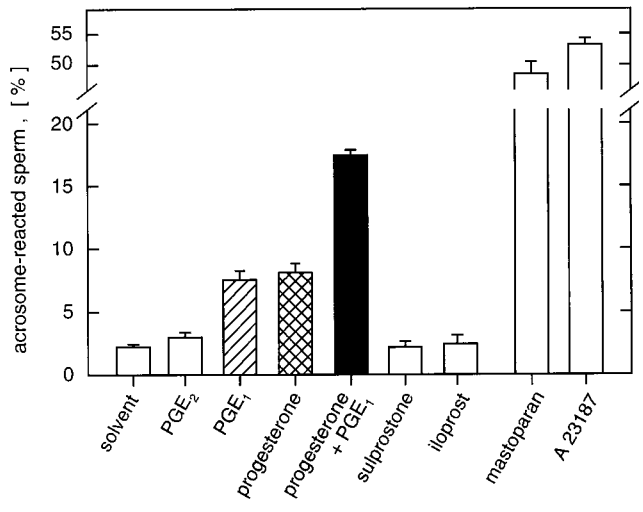

FIG. 4. Agonist-induced acrosomal exocytosis in capacitated human sperm. Capacitated human spermatozoa were incubated for 30 min in the presence of modulators as indicated. Mastoparan (50 μM) and the Ca^{2+} ionophore A23187 (10 μM) served as positive controls. All of the other agents were applied at 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.

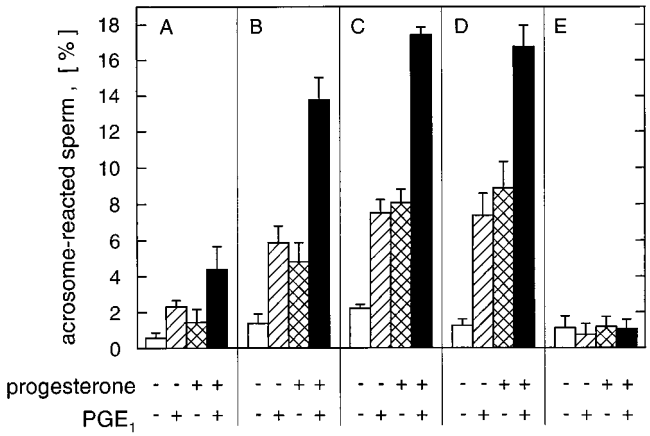

FIG. 5. Effect of capacitation and Zn^{2+} on agonist-induced AR. Swim-up human sperm were capacitated for 2 (*A*), 4 (*B*), or 6 (*C*) h in BWW-FCS medium, incubated for 30 min with progesterone (1 μM), PGE_1 (1 μM), or a combination of the two agonists (application of agonist indicated by +), and the 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^{2+}. Data represent means ± SEM of four independent experiments each performed in quadruplicate.

cAMP Levels in Human Sperm. Because the known EP_2 and EP_4 receptors couple to the G_s/adenylyl 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 ± 15 (after 5 min) and 117 ± 7 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/0^6 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^{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 antiserum 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. 6*A*). 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_{12} (AS 233) and α_{13} (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. 6*A*). 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 $G\alpha_s$ in human spermatozoa.

Photolabeling of Receptor-Activated G Proteins. To study coupling of activated EP receptors to G proteins of the $G_{q/11}$ family, sperm membranes were photolabeled with [α-^{32}P]GTP azidoanilide in the absence and presence of 1 μM PGE_1. Labeled G protein α subunits were immunoprecipitated with a specific antiserum and resolved by SDS/PAGE (Fig. 6*B Left*). In human

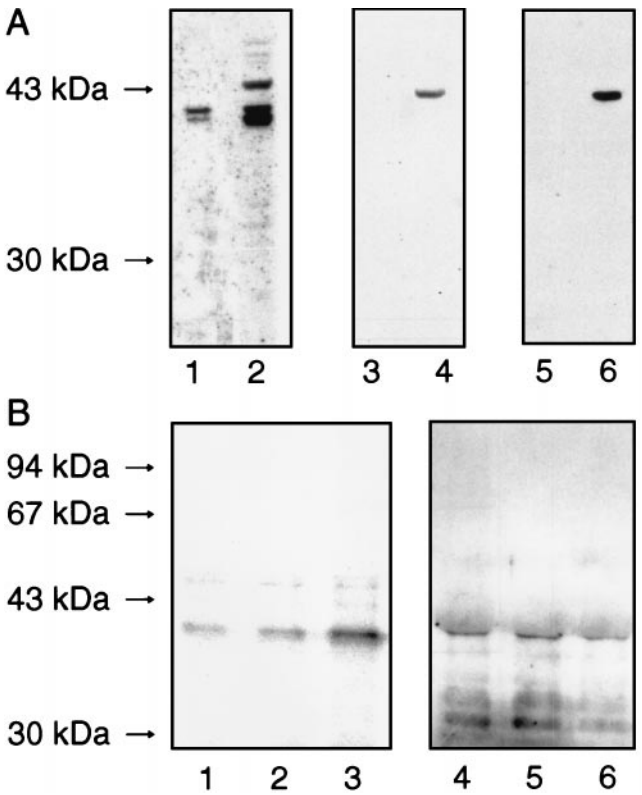

FIG. 6. Immunoblot analysis of PTX-insensitive G proteins and PGE_1-stimulated photolabeling of G protein α subunits in human sperm membranes. (*A*) Membrane proteins (50 μg per lane) of human sperm (lanes 1, 3, and 5) and of mouse L cells (lanes 2, 4, and 6) were resolved by 13% SDS/PAGE, blotted, and incubated with antisera recognizing $\alpha_{q/11}$ (lanes 1 and 2), α_{12} (lanes 3 and 4), and α_{13} (lanes 5 and 6). Molecular masses (kDa) are indicated on the left. (*B Left*) Membranes (150 μg per tube) were photolabeled with [α-^{32}P]GTP azidoanilide at 4°C (lane 1) or 30°C (lanes 2 and 3) in the absence (lanes 1 and 2) or presence (lane 3) of 1 μM prostaglandin E_1 and immunoprecipitated with antiserum AS 370 ($\alpha_{q/11}$). Immunoprecipitates were resolved by 10% SDS/PAGE, and labeled α subunits were visualized by autoradiography. One of seven independent experiments is shown. (*B Right*) The amount of immunoprecipitated $\alpha_{q/11}$ was determined by immunoblotting with a biotinylated IgG fraction of AS 370 and peroxydase-conjugated streptavidin. Lanes 4, 5, and 6 correspond to lanes 1, 2, and 3, respectively.

sperm membranes, PGE_1 led to increased incorporation of radioactivity into $G\alpha_{q/11}$ proteins (239 ± 43%, mean ± SEM, n = 7). Thus, we show that in human spermatozoa PGE_1 activates $G_{q/11}$ proteins. Probing the immunoprecipitates with a biotinylated $G\alpha_{q/11}$-specific antibody showed that different loads of immunoprecipitated protein were not responsible for the autoradiographic results (Fig. 6*B Right*). Incubation of membranes with 1 μM progesterone did not result in increased photolabeling of $G\alpha_{q/11}$ proteins (data not shown).

DISCUSSION

To date, ZP proteins and progesterone are regarded as physiological stimuli leading to AR in human sperm (5, 6). There is evidence that multiple binding proteins or receptors function to mediate the dual biological functions of ZP3, i.e., sperm binding and induction of the AR (5). Signal transduction cascades that are initiated following binding of sperm to ZP3 employ distinct sperm G_i proteins as critical transducing elements (29, 31, 32). On the contrary, discrete signaling steps set in motion by progesterone acting at a plasma membrane receptor are less understood, and a diverse array of molecular mechanisms has been proposed (33). 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 $PGF_{2\alpha}$ for the mammalian sperm AR (37, 38). Applying the Ca^{2+} indicator quin-2, Aitken *et al.* (39) observed a rise of $[Ca^{2+}]_i$ in human sperm subsequent to exposure to PGE_1 and PGE_2. Because these effects were detected with high prostaglandin concentrations (170 μM) 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 prostanoid 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 $PGF_{2\alpha}$, PGD_2, and U-46619 have 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 iloprost stimulation rules out the EP_1 receptor because this compound behaves as a partial EP_1 receptor agonist in somatic cells (40), and the ineffectiveness of sulprostone strongly argues against EP_3 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 dihydropyridines and benzothiazepines in excess of what is required to block classical Ca^{2+} channels had no significant effect on progesterone- and PGE_1-induced Ca^{2+} transients. Whereas pimozide, a selective blocker of T-type Ca^{2+} channels, effectively attenuated increases in $[Ca^{2+}]_i$ in ZP3-stimulated capacitated mouse sperm (41), this compound did not affect Ca^{2+} transients in human spermatozoa incubated with progesterone and PGE_1. In summary, these results do not favor the concept of L- or T-type Ca^{2+} channels in rapid Ca^{2+} transients induced by progesterone and PGE_1. Incubation of capacitated human sperm with membrane-permeable cyclic nucleotides did not evoke Ca^{2+} influx, and L-*cis*-diltiazem, a selective blocker of cyclic nucleotide-gated cation channels, was ineffective in decreasing agonist-elicited $[Ca^{2+}]_i$ rises. Thus, cyclic nucleotide-gated channels appear not to be involved in the generation of progesterone- and PGE_1-induced Ca^{2+} 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 lanthanids 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 $G\alpha_q$ and $G\alpha_{11}$ can be detected in sperm membranes whereas no appreciable amounts of G proteins of the $G_{12/13}$ family nor G_s were observed. Photolabeling experiments showed that in human sperm membranes the activated EP receptor stimulates G proteins of the $G_{q/11}$ family. Thus, in human sperm, classical receptor G protein-meditated signaling cascades are employed to achieve a biological effect.

In human semen, spermatozoa are exposed to high PGE_1 concentrations of $\approx$80 μM (30). The simultaneous presence of mM Zn^{2+} concentrations protects sperm from a massive Ca^{2+} influx which has been shown to profoundly impair the fertilizing potential of spermatozoa (36). It has been suggested that Zn^{2+} may stabilize sperm membranes during storage and ejaculation, and that Zn^{2+} removal may be required to prepare sperm for fertilization (42). The latter notion is supported by the fact that incubation of sperm with Zn^{2+} chelators capacitates hamster sperm (43). An inhibitory effect of Zn^{2+} on human sperm motility and AR has been observed (44). Our findings indicate a role of Zn^{2+} as an endogenous cation channel blocker to protect and maintain spermatozoa in a transitory quiescent state. During their ascent in the female reproductive tract, spermatozoa escape millimolar seminal fluid Zn^{2+} concentrations and are further on exposed to serum levels of Zn^{2+} ($\approx$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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