LETTER

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The CatSper channel mediates progesterone-induced Ca^{2+} influx in human sperm

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In the oviduct, cumulus cells that surround the oocyte release progesterone. In human sperm, progesterone stimulates a Ca^{2+} increase by a non-genomic mechanism[1–3]. The Ca^{2+} signal has been proposed to control chemotaxis, hyperactivation and acrosomal exocytosis of sperm[4–8]. However, the underlying signalling mechanism has remained mysterious. Here we show that progesterone activates the sperm-specific, pH-sensitive CatSper Ca^{2+} channel[9–11]. We found that both progesterone and alkaline pH stimulate a rapid Ca^{2+} influx with almost no latency, incompatible with a signalling pathway involving metabotropic receptors and second messengers. The Ca^{2+} signals evoked by alkaline pH and progesterone are inhibited by the Ca_v channel blockers NNC 55-0396 and mibefradil. Patch-clamp recordings from sperm reveal an alkaline-activated current carried by mono- and divalent ions that exhibits all the hallmarks of sperm-specific CatSper Ca^{2+} channels[10,11]. Progesterone substantially enhances the CatSper current. The alkaline- and progesterone-activated CatSper current is inhibited by both drugs. Our results resolve a long-standing controversy over the non-genomic progesterone signalling. In human sperm, either the CatSper channel itself or an associated protein serves as the non-genomic progesterone receptor. The identification of CatSper channel blockers will greatly facilitate the study of Ca^{2+} signalling in sperm and help to define further the physiological role of progesterone and CatSper.

Progesterone-evoked Ca^{2+} influx in human sperm does not involve classical regulation of transcription by nuclear receptors. Various candidate membrane receptors for progesterone have emerged, including a novel G-protein-coupled-type progestin receptor (mPR) and a single-pass receptor (progesterone-receptor membrane component, PGRMC)[12]. The pathway downstream of the non-genomic progesterone receptor has been proposed to involve cAMP and cGMP, protein kinase A and G, Ca^{2+} release from intracellular stores, store-operated Ca^{2+} channels and cGMP-activated channels[1,8,13].

Here we study progesterone action in human sperm using optical and electrophysiological techniques. In sperm loaded with the Ca^{2+} indicator Fluo-4, progesterone elicited a change in intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$). The biphasic signal[3,14] recorded in a plate reader consisted of a rapid Ca^{2+} transient followed by a slower, sustained Ca^{2+} elevation (Fig. 1a). The dose–response relation of the transient and the sustained components (Fig. 1b) yielded constants of half-maximal activation ($K_{1/2}$) of 42 ± 15 nM and 91 ± 31 nM (six experiments), respectively.

We tested whether the progesterone-induced Ca^{2+} signal is different in capacitated and non-capacitated sperm. Under the capacitating conditions used here, the amplitude and time course of Ca^{2+} signals were overall unaffected by incubation conditions that promote sperm capacitation (Supplementary Fig. 1a). However, under capacitating conditions, the $K_{1/2}$ values (10 ± 10 nM for the transient and 24 ± 35 nM for the sustained component, six experiments) were shifted to lower concentrations, indicating that capacitation renders sperm more sensitive to progesterone (Supplementary Fig. 1b).

Signalling involving activation of metabotropic receptors is usually characterized by a latency of the cellular response, for example chemosensory Ca^{2+} signalling in sea urchin sperm or G-protein-coupled receptor signalling in vertebrate olfactory neurons[15–17]. In contrast, insect olfactory neurons, which use ionotropic chemoreceptors, respond with almost no latency[17]. To test whether progesterone activates a G-protein-coupled receptor pathway, we recorded Ca^{2+} signals using the kinetic stopped-flow technique (Fig. 1c). The progesterone-induced Ca^{2+} signals recorded in a plate reader (Fig. 1a, b) and in the stopped-flow apparatus (Fig. 1c, d) were similar. The Ca^{2+} response at first saturated at about 1 μM and then continued to rise at concentrations greater than 2 μM (Fig. 1c, d), suggesting that progesterone binds to sites of high and low affinity. The $K_{1/2}$ of the high-affinity site was 42.3 ± 9.3 nM (three experiments) (Fig. 1d). Owing to the poor aqueous solubility of progesterone, we could not determine a $K_{1/2}$ value for the low-affinity site. At all progesterone concentrations, $[Ca^{2+}]_i$ rose within the time resolution of the stopped-flow apparatus (36 ms) (Fig. 1e). The instantaneous rise of $[Ca^{2+}]_i$ argues for an extracellular action of progesterone on a membrane protein, consistent with the observation that impermeable progesterone derivatives also stimulate a Ca^{2+} response[18].

We investigated which signal component rests upon Ca^{2+} entry. Sperm were mixed in the stopped-flow apparatus with a Ca^{2+}-free medium containing progesterone and different concentrations of the Ca^{2+} chelator BAPTA (Fig. 1f): thereby, within milliseconds, a well-defined external Ca^{2+} concentration ($[Ca^{2+}]_o$) is adjusted, which prevents depletion of intracellular Ca^{2+} stores before progesterone stimulation. The amplitude of the transient Ca^{2+} signal evoked by 1 μM progesterone strongly depended on $[Ca^{2+}]_o$ (Fig. 1f). At 28 nM $[Ca^{2+}]_o$, even high progesterone concentrations (10 μM) no longer produced a Ca^{2+} response (Supplementary Fig. 2a). Moreover, at such low $[Ca^{2+}]_o$, the slow sustained component was also abolished (Supplementary Fig. 2b). We conclude that both transient and sustained Ca^{2+} signals result from Ca^{2+} entry. Alternatively, the sustained component might reflect Ca^{2+} release from intracellular stores involving inositol trisphosphate (IP_3), a mechanism that is also triggered by Ca^{2+} influx ('Ca^{2+}-induced Ca^{2+} release')[1,2,19,20].

The instantaneous rise of $[Ca^{2+}]_i$ is difficult to reconcile with a mechanism involving metabotropic receptors or the synthesis of second messengers. However, progesterone has been reported to elevate cAMP levels[8,21] and cAMP to elevate $[Ca^{2+}]_i$ (refs 9, 22). We failed to reproduce these results. Progesterone did not elevate cAMP levels (Fig. 2a). Moreover, colforsin, which activates all membrane adenylyl cyclases[23], did not affect cAMP levels, arguing altogether against canonical G-protein-coupled receptor-operated cAMP signalling in human sperm. By contrast, HCO_3^-, which stimulates

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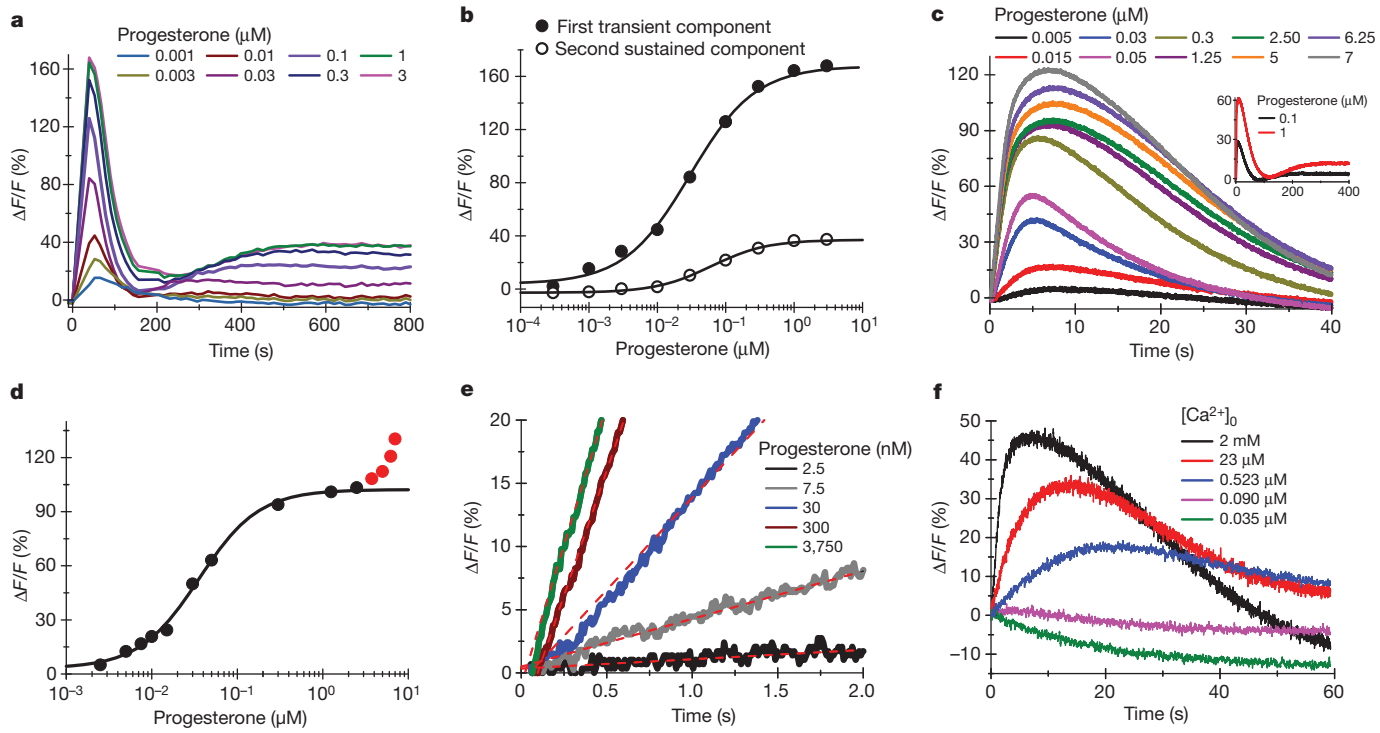

Figure 1 | Progesterone-induced Ca^{2+} signals in human sperm. **a**, Signals measured in a fluorescence plate reader. **b**, Dose–response relation of the two kinetic signal components from a ($K_{1/2}$: first component 33 nM; second component 68 nM). **c**, Signals measured in a stopped-flow apparatus. Inset: signals recorded for 400 s. **d**, Dose–response relation of signals from c ($K_{1/2}$: 35 nM). Red dots: continuous rise of $[Ca^{2+}]_i$ at progesterone concentrations greater than 2 μM. **e**, First 2 s of Ca^{2+} signals. Red lines: linear fit to their initial slope. **f**, Ca^{2+} signals evoked by 1 μM progesterone at different external Ca^{2+} concentrations $[Ca^{2+}]_o$ in the stopped-flow apparatus.

a soluble adenylyl cyclase[23], enhanced cAMP levels by fourfold. Isobutylmethylxanthine (IBMX), which inhibits phosphodiesterases, increased cAMP levels by 4.9-fold. A combination of HCO_3^- and IBMX augmented cAMP levels by 10.5-fold: that is, beyond values reached by each substance alone (Fig. 2a). Importantly, a rise of cAMP either by photolysis of caged cAMP or stimulation by HCO_3^- did not evoke Ca^{2+} increase (Fig. 2b). If anything, HCO_3^- caused a small decrease of $[Ca^{2+}]_i$. In mouse sperm also, HCO_3^- does not stimulate a Ca^{2+} response[24]. Therefore, cAMP signalling is not directly involved in the rapid Ca^{2+} response to progesterone.

The principal voltage-gated Ca^{2+} channel (Ca_v) in epididymal mouse and ejaculated human sperm is CatSper[10,11], which is activated at alkaline intracellular pH (refs 10, 11). We reasoned that CatSper mediates progesterone-induced Ca^{2+} entry. We tested several inhibitors of Ca_v channels for their ability to suppress progesterone- and alkaline-evoked Ca^{2+} responses. Only the T-type channel blockers NNC 55-0396 and mibefradil significantly impaired the progesterone-induced Ca^{2+} response (Fig. 3a, b). In addition, both compounds significantly impaired the alkaline-induced Ca^{2+} signals evoked by NH_4Cl (10 mM) (Fig. 3c–e). However, we emphasize that, in Ca^{2+} fluorimetry, the pharmacology of these drugs is complex: at concentrations greater than 10 μM NNC 55-0396 and greater than 40 μM mibefradil, the drugs evoked Ca^{2+} responses themselves (Supplementary Fig. 3a, b). This prevented us testing whether higher drug concentrations completely inhibited the progesterone- and alkaline-evoked Ca^{2+} signals. Progesterone does not stimulate Ca^{2+} entry by alkalization; in contrast to

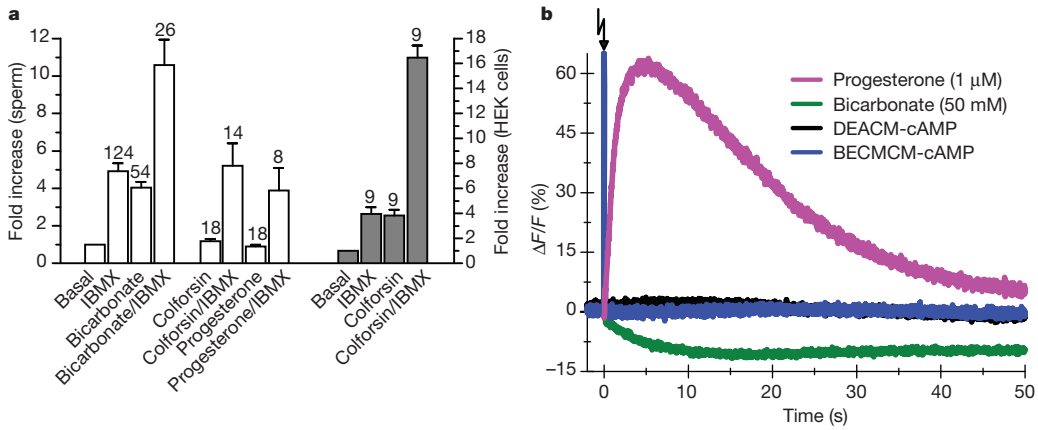

Figure 2 | Progesterone does not activate a cAMP-signalling pathway. **a**, Changes in total cAMP concentration in human sperm (white) and HEK 293 cells (grey) evoked by 0.5 mM IBMX, 50 mM bicarbonate, 50 μM colforsin, 1 μM progesterone (mean ± s.e.m.; number of measurements above the bars, from 3 to 26 experiments). At rest, 10^6 human sperm contain 0.087 ± 0.002 pmol cAMP (mean ± s.e.m., 290 measurements from 26 experiments), corresponding to approximately 2.5 μM cAMP (sperm volume ~ 35 fl). **b**, Ca^{2+} signals produced by photolysis of DEACM-cAMP and BECMCM-cAMP, and by bicarbonate and progesterone. Experiments were done in a stopped-flow apparatus.

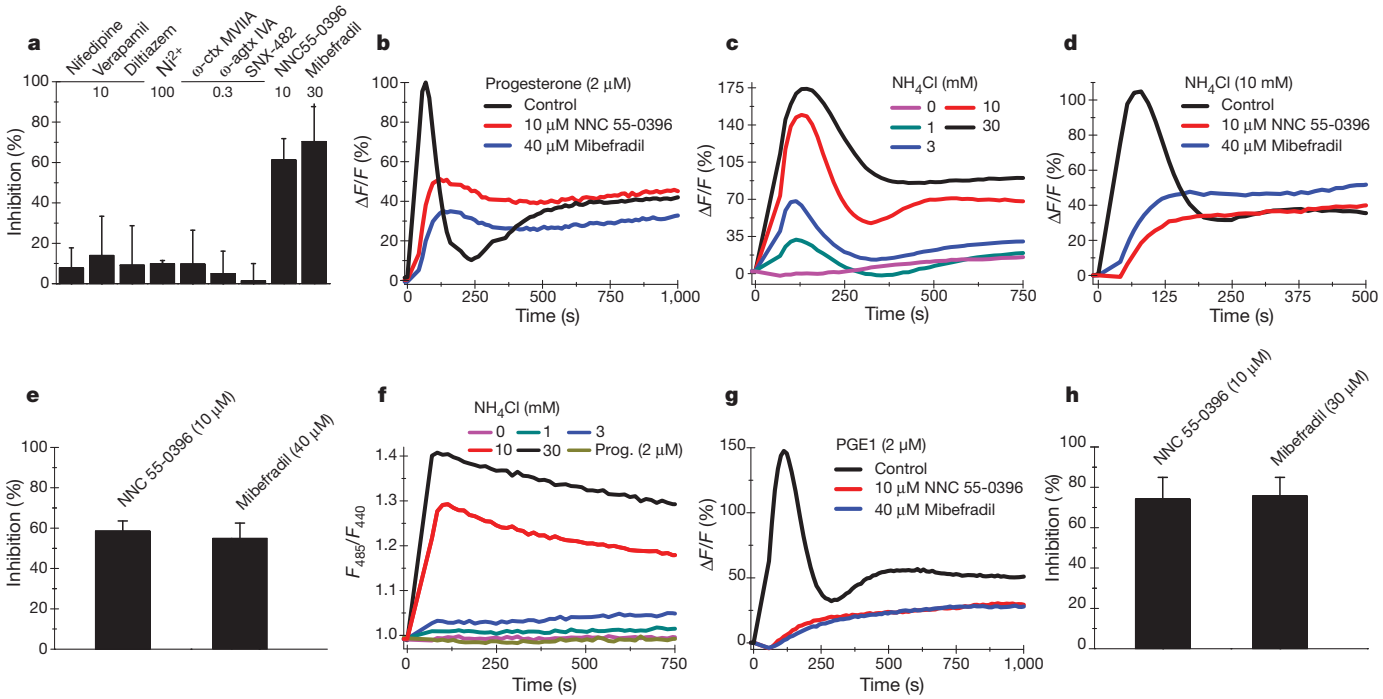

Figure 3 | Pharmacology of progesterone-induced Ca^{2+} signals. **a**, Relative inhibition of progesterone-evoked (2 μM) Ca^{2+} signals by Ca_v channel blockers (three to five experiments, inhibitor concentrations in μM). **b**, Progesterone-evoked Ca^{2+} signals are inhibited by NNC 55-0396 or mibefradil. **c**, NH_4Cl-evoked Ca^{2+} signals. **d**, NH_4Cl responses are inhibited by NNC 55-0396 or mibefradil. **e**, Relative inhibition of NH_4Cl-induced Ca^{2+} signals by NNC 55-0396 or mibefradil (four experiments). **f**, NH_4Cl-evoked changes in pH_i. Progesterone did not change pH_i. **g**, PGE1-evoked Ca^{2+} signals inhibited by NNC 55-0396 or mibefradil. **h**, Relative inhibition of PEG1-evoked Ca^{2+} signals by NNC 55-0396 and mibefradil (four experiments). Experiments were done in a fluorescence plate reader; data are given as mean ± s.d.

NH_4Cl, progesterone (2 μM) did not change pH_i (Fig. 3f). Taken together, these results suggest that progesterone activates CatSper.

Prostaglandin E1 (PGE1) and progesterone produce similar biphasic Ca^{2+} signals[25] (Figs 1a and 3g and Supplementary Fig. 4). The pharmacology of the PGE1 response is incompatible with known prostanoid receptors[25]. The PGE1-induced Ca^{2+} signal is also suppressed by NNC 55-0396 and mibefradil, indicating that PGE1 also activates CatSper (Fig. 3g, h). Sequential application of 17-OH-progesterone and progesterone, but not of PGE1 and progesterone[25], leads to cross-desensitization (Supplementary Fig. 4), suggesting that progesterone and PGE1 use distinct binding sites or signalling mechanisms.

We studied membrane currents in mature non-capacitated sperm by whole-cell patch-clamp recordings. In standard extracellular solution containing Ca^{2+} and Mg^{2+}, steps in membrane voltage V from 0 mV to ± 80 mV produced hardly any currents[11] (Fig. 4a). When switching to divalent-free solution (NaDVF + 0 Prog; Fig. 4a), monovalent currents carried by CatSper channels appeared[10,11]. Progesterone evoked a dose-dependent increase of monovalent current (Fig. 4a) with a $K_{1/2}$ of 66 ± 19 nM (five experiments) (Fig. 4b). Mean inward currents at −80 mV were −59.4 ± 26.8 pA, range −30.3 to −102.9 pA (0 progesterone) and −180.8 ± 73.5 pA, range −126.9 to −264.5 pA (10 μM progesterone). Remarkably, the reversal potential (V_{rev}) of the monovalent current (35 ± 5 mV; five experiments) did not change during progesterone stimulation (Fig. 4c), indicating that V_{rev} of progesterone-induced and CatSper currents is similar. RU486, an antagonist for nuclear progesterone receptors, did not inhibit the progesterone-induced currents (Supplementary Fig. 5). Stimulation with NH_4Cl (10 mM), or progesterone (1 μM), or both, potentiated monovalent currents, again with no change in V_{rev} (Fig. 4d). Moreover, PGE1 also activated a monovalent current with a V_{rev} similar to that of CatSper currents (Supplementary Fig. 6a). Most importantly, mibefradil (30 μM) completely blocked the monovalent current activated by NH_4Cl, progesterone (Fig. 4d) and PGE1 (Supplementary Fig. 6b). This suggests that progesterone- and PGE1-induced currents are carried by CatSper.

Under physiological conditions, CatSper primarily carries divalent currents. Therefore, we studied the voltage dependence, pH sensitivity and pharmacology of Ba^{2+} tail currents elicited after repolarization from various test voltages (Fig. 4e, g). At pH_i 6, almost all CatSper channels are closed. At pH_i 6, progesterone (1 μM) or NH_4Cl (10 mM), however, induced sizeable Ba^{2+} tail currents (Fig. 4e). Simultaneous application of NH_4Cl and progesterone enhanced tail currents in a non-additive manner (Fig. 4e): the current stimulated by progesterone/NH_4Cl was three times larger (2.96 ± 0.7; five experiments at +70 mV) than the superposition of currents produced by each substance alone. Alkalization and progesterone shifted the activation curve to less positive voltages (Fig. 4f). The voltage shift evoked by progesterone/NH_4Cl was larger than that caused by progesterone or NH_4Cl alone (Fig. 4f). At pH_i 8, a significant fraction of CatSper channels was activated (Fig. 4g, h); and progesterone (1 μM) further enhanced the Ba^{2+} tail currents (Fig. 4g, h). NNC 55-0396 (10 μM) inhibited these tail currents (Fig. 4h). In summary, the similar V_{rev}, the non-additive action of pH_i and progesterone, and the blockage by NNC 55-0396 and mibefradil demonstrate that alkaline pH_i, progesterone and PGE1 activate CatSper. We recorded progesterone-evoked CatSper currents using pipette solutions without ATP and GTP, arguing against an activation mechanism involving second messengers, phosphorylation or G proteins.

Here we show that, in human sperm, progesterone activates CatSper either by binding to the channel itself or to an associated protein. Based on electrophysiological analysis of progesterone-induced currents in sperm and isolated flagella, Lishko *et al.*[26] come to a similar conclusion. In mice, CatSper controls hyperactivation, a mode of sperm motility required for fertilization. Considering the function of progesterone as a chemoattractant[6,8], we suggest that CatSper serves in human sperm as a Ca^{2+} channel for both hyperactivation and chemotaxis. CatSper channels are also expressed in other mammalian species. Rabbit, human and bovine follicular factors display chemotactic activity across species, arguing that mammals use a common or similar chemoattractant[27]. Further studies are required to examine whether other mammals also use progesterone as a chemoattractant and whether in these

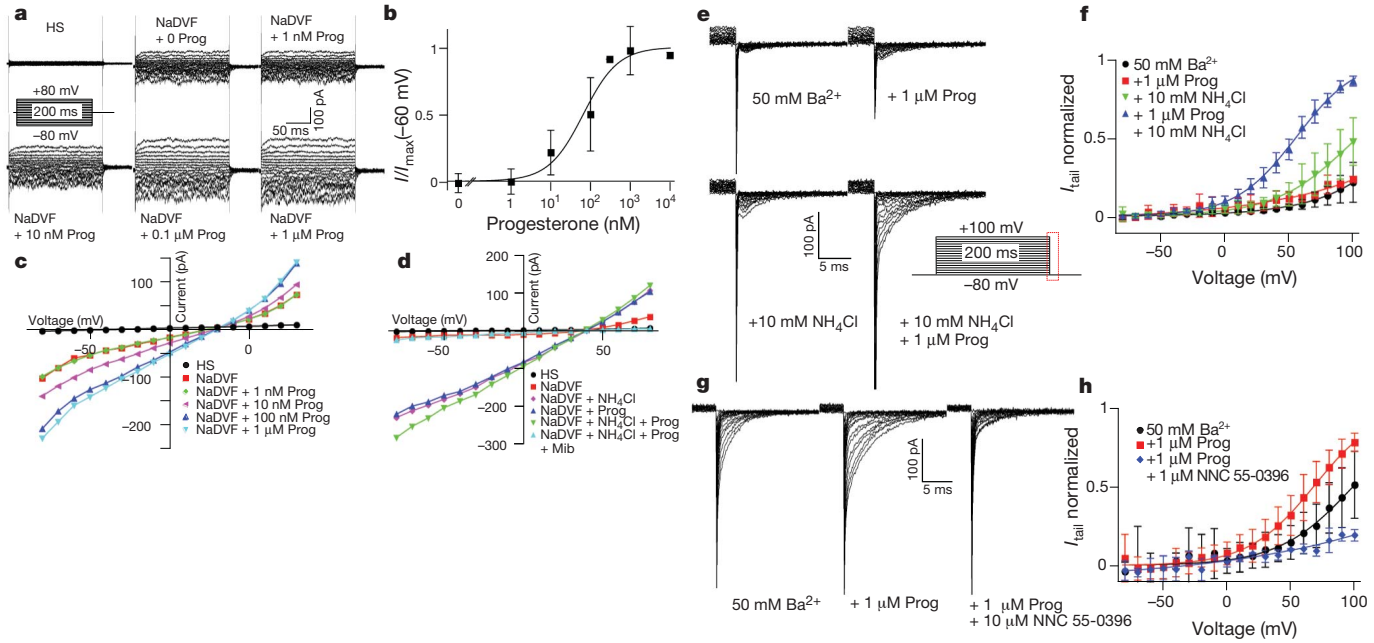

Figure 4 | Electrophysiological characterization of whole-cell CatSper currents from human sperm cells. **a**, Control currents in standard extracellular solution (HS) and monovalent currents in divalent-free Na^+-based bath solution (NaDVF), in the absence of intracellular divalent ions. Currents were recorded at pH_i 7.3 after extracellular addition of different progesterone concentrations. Voltage was stepped from −80 mV to +80 mV in increments of 10 mV. **b**, Dose–response relation of the mean current at −60 mV ($K_{1/2}$: 66 ± 19 nM, Hill coefficient 1 ± 0.2; mean ± s.d of five experiments). **c**, Current–voltage relation of monovalent currents at different extracellular progesterone concentrations. **d**, Activation of monovalent CatSper currents at pH_i 7.3 by 1 µM extracellular progesterone, or intracellular alkalization with 10 mM NH_4Cl, or both. Currents were completely blocked by 30 µM mibefradil. **e**, Ba^{2+} tail currents at −80 mV after voltage steps from −80 mV to +100 mV in increments of 10 mV at pH_i 6. The red box in the voltage protocol indicates the part of the current traces shown. **f**, Ba^{2+} tail currents at pH_i 6; mean ± s.d. of four or five experiments; currents were normalized to the interpolated maximum current evoked by simultaneous application of progesterone and NH_4Cl. **g**, Ba^{2+} tail currents at −80 mV after voltage steps from −80 mV to +100 mV in increments of 10 mV at pH_i 8. Same protocol used as in **e**. **h**, Ba^{2+} tail currents at pH_i 8; mean ± s.d. of two to six experiments; currents were normalized to the interpolated maximum current evoked by 1 µM progesterone.

species progesterone also activates CatSper. However, at least in mice, progesterone does not activate CatSper (Lishko *et al.*[26]). Moreover, many substances as diverse as odorants and prostaglandins evoke Ca^{2+} responses in human sperm[22,25,28], and several chemicals or factors have been proposed to attract sperm[4,28]. Chemotaxis in the female genital tract might be a multistep process that involves distinct chemoattractants and even thermotaxis[4]. Future work needs to show whether other substances that activate CatSper serve as chemoattractants, and whether substances that reportedly display chemotactic activity also open CatSper.

We propose that CatSper represents a ligand-gated Ca^{2+} channel that can be activated by lipophilic compounds. However, the binding site for progesterone has yet to be determined. We cannot exclude the possibility that progesterone binds to proteins such as the mPRs and/or PGRMCs that might associate with CatSper and convey the binding event to the channel. Such a mechanism, where distinct proteins form a receptor/channel complex, exists in insect olfactory neurons[17,29,30]. Alternatively, the accessory subunits CatSper-β or CatSper-G might host the binding site. The availability of CatSper blockers provides a powerful tool to establish unequivocally progesterone as a chemoattractant and CatSper as a chemotaxis transduction channel, but also to interfere pharmacologically with fertilization.

METHODS SUMMARY

Sperm preparation. Sperm were purified by a 'swim-up' procedure in human tubular fluid (HTF) medium. Sperm were washed and resuspended in HTF^{++} containing 4 mM $NaHCO_3$ and 3 mg ml^{-1} human serum albumin (Irvine Scientific). Before experiments, sperm were incubated for at least 1 h in HTF^{++} at 37 °C. Under these conditions, the sperm are non-capacitated. For capacitation, sperm were incubated in 25 mM $NaHCO_3$ and 3 mg ml^{-1} human serum albumin for at least 2 h. Unless otherwise specified, experiments were done with non-capacitated sperm.

Measurement of changes in intracellular Ca^{2+} and pH. Changes in $[Ca^{2+}]_i$ and pH_i were measured in sperm loaded with Fluo-4 and BCECF (Molecular Probes), respectively, in 384 multi-well plates in a fluorescence plate reader (Fluostar Omega, BMG Labtech) at 29 °C and in a rapid-mixing device in the stopped-flow mode (SFM400, Bio-Logic) at 37 °C. After loading, sperm were washed and resuspended in HTF^+ containing 4 mM $NaHCO_3$ but no serum albumin, unless otherwise specified. Ligands or inhibitors were dissolved in HTF^+ as well.

Caged compounds and photolysis. We used the caged cyclic nucleotides BECMCM-caged cAMP and DEACM-caged cAMP. The caged compound was photolysed by an ultraviolet flash from a xenon flash lamp (DP-10, Rapp OptoElectronic).

Determination of cAMP content. Sperm in HTF^{++} were mixed 1:1 (v/v) with substances dissolved in HTF^{++}. The cAMP content was determined by radioimmunoassays (^{125}I-labelled cAMP; IBL).

Patch-clamp recordings. We recorded from sperm in the whole-cell configuration. Seals between pipette and spermatozoa were formed either at the cytoplasmic droplet or in the neck region in standard extracellular solution (HS) containing divalent cations. Monovalent currents were recorded in a sodium-based divalent-free solution (NaDVF). Bath solutions for recording divalent CatSper currents contained 50 mM Ba^{2+}. Different pipette solutions were used depending on whether monovalent or divalent currents were recorded.

Data analysis. Data are given as mean ± s.d. (number of experiments) if not otherwise stated.

Full Methods and any associated references are available in the online version of the paper at www.nature.com/nature.

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Supplementary Information is linked to the online version of the paper at www.nature.com/nature.

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Author Contributions T.S., N.G., C.B., N.K. and I.W. designed and performed experiments. R.S. designed experiments. T.S. and U.B.K. conceived the project. T.S. and U.B.K. wrote the manuscript. All authors revised and edited the manuscript.

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METHODS

Sperm preparation. Human semen samples were obtained from healthy volunteers with their consent. Fresh ejaculates were allowed to liquefy at room temperature for 30–60 min. Sperm were purified by a 'swim-up' procedure. Liquefied semen (0.5–1 ml) was layered in a 50-ml falcon tube below 4 ml of HTF medium containing (in mM) 97.8 NaCl, 4.69 KCl, 0.2 $MgSO_4$, 0.37 KH_2PO_4, 2.04 $CaCl_2$, 0.33 sodium pyruvate, 21.4 sodium lactate, 2.78 glucose, and 21 HEPES, adjusted to pH 7.3–7.4 with NaOH. Motile sperm were allowed to swim-up into the HTF layer for 60–90 min at 37 °C and washed two times (700*g*, 20 min, room temperature (approximately 22 °C)). For cAMP determination, 'swim-up' sperm from several donors were pooled. For cAMP determination, samples were washed three times. The three washing steps are necessary to remove from seminal fluid an unknown factor that interferes with the cAMP assay and produces artificially high cAMP levels. Sperm number was determined in a Neubauer cell counter. Washed sperm were re-suspended in HTF^{++} containing 4 mM $NaHCO_3$ and 3 mg ml^{-1} human serum albumin (Irvine Scientific) at a density of 2×10^7 ml^{-1}, unless otherwise specified. Capacitation was assessed using the FITC-CD46 assay, which probes the complete acrosome reaction induced by a Ca^{2+} ionophore.

Measurement of changes in intracellular Ca^{2+} and pH. Changes in $[Ca^{2+}]_i$ and pH_i were measured in sperm loaded with Fluo-4 and BCECF (Molecular Probes), respectively, in 384 multi-well plates in a fluorescence plate reader (Fluostar Omega, BMG Labtech) at 29 °C and in a rapid-mixing device in the stopped-flow mode (SFM400, Bio-Logic) at 37 °C. Sperm were loaded with the fluorescent Ca^{2+} indicator Fluo-4 AM (10 μM) in the presence of Pluronic F-127 (0.05% v/v) at 37 °C for 45 min (ref. 31). To measure pH_i changes in the plate reader, sperm were loaded for 15–20 min with the fluorescent pH indicator BCECF (10 μM). After incubation, excess dye was removed by two centrifugation steps (700*g*, 10 min, room temperature (approximately 22 °C)). The pellet was re-suspended in the same volume of HTF containing 4 mM $NaHCO_3$ but no human serum albumin (HTF^+) and equilibrated for 5 min at 37 °C. Each well was filled with 27 μl (1×10^7 sperm ml^{-1}) or 54 μl (5×10^6 sperm ml^{-1}) of the sperm suspension; the fluorescence was excited at 480 nm (Fluo-4) or alternating at 440 nm and 480 nm (BCECF, dual excitation). Fluorescence emission was recorded at 520 nm with bottom optics. Fluorescence was recorded before and after injection of 27 μl (1:1 dilution) or 6 μl (1:10 dilution) of solutions containing ligands, inhibitors or HTF^+. The solutions were injected into the wells manually with an electronic multichannel pipette.

In the stopped-flow device, the sperm suspension was rapidly mixed (1:1 v/v; flow rate 1 ml s^{-1}) with HTF^+ solutions containing different concentrations of ligand or other substances. At this flow rate, no physical damage of sperm was detected and their swimming behaviour appeared normal. Fluorescence excitation and recording were as described[31].

Caged compounds and photolysis. The following caged cyclic nucleotides were used: BECMCM-caged cAMP and DEACM-caged cAMP[15,16]. Sperm were loaded with 30 μM caged cyclic nucleotides for 30–45 min in the presence of Fluo-4 AM and Pluronic F-127. The ultraviolet flash was delivered to the cuvette by a liquid light guide and passed through a 295–395 (1 mm) band-pass filter (Rapp OptoElectronic).

Determination of cAMP content. The sperm suspension (1.25×10^6 cells) was mixed 1:1 (v/v) with different substances in HTF^{++}. The final concentration of substances was as follows (in mM): 50 HCO_3^-, 0.5 IBMX; (in μM): 50 colforsin, 1 progesterone. The final concentration of solvent (0.5% DMSO) did not affect the resting cAMP level. After stimulation for 5 s to 25 min at 37 °C, the reaction was quenched with $HClO_4$ (1:3 (v/v); 0.5 M final concentration). Samples were neutralized by K_3PO_4 (0.24 M final concentration). The salt precipitate and cell debris were sedimented by centrifugation (15 min, 15,000*g*, 4 °C). The cAMP content in the supernatant (200 μl) was determined by radioimmunoassay that included an acetylation step for higher sensitivity (^{125}I-labelled cAMP; IBL). Calibration curves were obtained by serial dilutions of cAMP standards. The basal cAMP concentration was constant for up to 3 h. The cAMP increase produced by IBMX was largely identical for stimulation periods between 15 s and 300 s; pre-incubation of sperm with IBMX was for 2 min.

Patch-clamp recordings. We recorded from sperm in the whole-cell configuration. Seals between pipette and spermatozoa were formed either at the cytoplasmic droplet or in the neck region in standard extracellular solution (HS) containing (in mM) 135 NaCl, 5 KCl, 1 $MgSO_4$, 1 $CaCl_2$ 5 glucose, 1 sodium pyruvate, 10 lactic acid, 20 HEPES (pH 7.4 was adjusted with NaOH). Voltage pulses of 500–650 mV for 0.5 ms combined with light suction achieved transition into the whole-cell mode. Monovalent currents were recorded in divalent-free solution (NaDVF) (in mM): 140 NaCl, 40 HEPES and 1 EGTA (pH 7.4 was adjusted with NaOH); the pipette (10–15 MΩ) solution contained (in mM) 121 Cs-methanesulphonate, 65 HEPES, 4.5 EGTA, 4.5 CsCl (pH 7.3 was adjusted with CsOH); up to 20 mM glucose were added to the pipette solution to balance osmolarity. Pipette (15–25 MΩ) solution for recording of divalent currents contained (in mM) 165 *N*-methyl-D-glucamine (NMDG), 5 CsCl, 10 ethylene glycol tetraacetic acid (EGTA) and 10 MES or HEPES (pH 6.0 or pH 8.0 was adjusted with methanesulphonic acid (CH_3SO_3H)). Bath solution for recordings of divalent CatSper currents contained (in mM) 50 $Ba(OH)_2$, 90 NMDG, 20 HEPES (pH 7.4 was adjusted with methanesulphonic acid). NH_4Cl, progesterone, PGE1, NNC 55-0396 and mibefradil were added as indicated. Experiments were performed at 24 °C.

Data analysis. Data are given as mean ± s.d. (number of experiments) if not otherwise stated.

31. Kilic, F. *et al.* Caged progesterone: a new tool for studying rapid nongenomic actions of progesterone. *J. Am. Chem. Soc.* **131,** 4027–4030 (2009).