LETTER

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Progesterone activates the principal Ca^{2+} channel of human sperm

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Steroid hormone progesterone released by cumulus cells surrounding the egg is a potent stimulator of human spermatozoa. It attracts spermatozoa towards the egg and helps them penetrate the egg's protective vestments[1]. Progesterone induces Ca^{2+} influx into spermatozoa[1–3] and triggers multiple Ca^{2+}-dependent physiological responses essential for successful fertilization, such as sperm hyperactivation, acrosome reaction and chemotaxis towards the egg[4–8]. As an ovarian hormone, progesterone acts by regulating gene expression through a well-characterized progesterone nuclear receptor[9]. However, the effect of progesterone upon transcriptionally silent spermatozoa remains unexplained and is believed to be mediated by a specialized, non-genomic membrane progesterone receptor[5,10]. The identity of this non-genomic progesterone receptor and the mechanism by which it causes Ca^{2+} entry remain fundamental unresolved questions in human reproduction. Here we elucidate the mechanism of the non-genomic action of progesterone on human spermatozoa by identifying the Ca^{2+} channel activated by progesterone. By applying the patch-clamp technique to mature human spermatozoa, we found that nanomolar concentrations of progesterone dramatically potentiate CatSper, a pH-dependent Ca^{2+} channel of the sperm flagellum. We demonstrate that human CatSper is synergistically activated by elevation of intracellular pH and extracellular progesterone. Interestingly, human CatSper can be further potentiated by prostaglandins, but apparently through a binding site other than that of progesterone. Because our experimental conditions did not support second messenger signalling, CatSper or a directly associated protein serves as the elusive non-genomic progesterone receptor of sperm. Given that the CatSper-associated progesterone receptor is sperm specific and structurally different from the genomic progesterone receptor, it represents a promising target for the development of a new class of non-hormonal contraceptives.

CatSper is a sperm-specific Ca^{2+} channel located in the principal piece of the flagellum[11,12]. Weakly voltage-dependent, pH-sensitive CatSper is the only constitutively active Ca^{2+} conductance present in mouse and human spermatozoa as recorded using the whole-cell patch-clamp technique[12,13]. Ca^{2+} influx through CatSper triggers sperm hyperactivation, a special high-amplitude asymmetrical flagellar beat required for penetration through viscous luminal fluids of the female reproductive tract and protective vestments of the egg[14–16]. CatSper is also ideally positioned to control sperm chemotaxis, because 'chemotactic turns' that guide spermatozoa towards the egg depend on asymmetrical flagellar motion triggered by Ca^{2+} influx into the flagellum[4,17]. Furthermore, CatSper-mediated Ca^{2+} influx into the flagellum leads to Ca^{2+} elevation even in the sperm head[18] (probably by causing Ca^{2+}-dependent Ca^{2+} release from the intracellular store located in the sperm neck[19]) and thus can contribute to Ca^{2+}-dependent acrosome reaction[20]. Because CatSper may cause all three Ca^{2+}-dependent responses triggered by progesterone (hyperactivation, chemotaxis and acrosome reaction), we tested the hypothesis that progesterone activates CatSper using the recently developed method for patch-clamping completely mature ejaculated human spermatozoa[13].

Under normal physiological conditions, mouse and human CatSper channels are Ca^{2+} selective, but pass monovalent ions (Cs^+ or Na^+) under divalent-free conditions[12,13]. Because monovalent CatSper currents are significantly larger, we studied CatSper currents under divalent-free conditions. The monovalent human CatSper current ($I_{CatSper}$) was overall smaller (Fig. 1a, blue) than mouse $I_{CatSper}$ (Fig. 1c, blue), especially at negative membrane potentials (inward current). The virtual absence of human $I_{CatSper}$ at the negative potentials normally found across the sperm plasma membrane was puzzling. Interestingly, addition of 500 nM progesterone to the bath solution dramatically increased the amplitude of human monovalent $I_{CatSper}$ (Fig. 1a, red). Mouse monovalent $I_{CatSper}$ did not increase after addition of 500 nM progesterone (Fig. 1c, red) or 10 μM progesterone (Supplementary Fig. 1).

Similar results were obtained with divalent $I_{CatSper}$. At intracellular pH 7.0, human Ba^{2+} $I_{CatSper}$ was very small but increased dramatically after addition of 500 nM progesterone (Supplementary Fig. 2a). In contrast, mouse Ba^{2+} $I_{CatSper}$ was easily detected at intracellular pH 7.0 and was not affected by progesterone (Supplementary Fig. 2b).

After potentiation with P, the amplitude of human $I_{CatSper}$ remained stable. The potentiation was reversible and could be reproduced again on the same cell (Supplementary Fig. 3). Another major ovarian steroid hormone, oestradiol (17-β-oestradiol)[9,10], did not affect human CatSper (Supplementary Fig. 4).

We next conducted a series of experiments to confirm that the progesterone-activated current is indeed mediated by CatSper. Triturating human spermatozoa with a micropipette leads to the separation of a few spermatozoa into the head and the flagellum (containing midpiece and principal piece) at the neck region. Patch-clamp recording from isolated flagella detected monovalent CatSper currents that were strongly potentiated by progesterone and were indistinguishable from those recorded from whole human spermatozoa (Fig. 1b, compare with Fig. 1a). Mean amplitudes of currents recorded from whole human spermatozoa before and after stimulation with progesterone also closely matched those recorded from flagella (Fig. 2a), suggesting that similar to CatSper, the progesterone-activated conductance originates from the sperm flagellum.

Next, we discovered that human $I_{CatSper}$ is potently blocked by an inhibitor of T-type voltage-gated Ca^{2+} channels, NNC55-0396 (ref. 21) (NNC, Fig. 2b, left panel), and tested whether NNC also inhibits the progesterone-activated current. Indeed, 2 μM NNC completely inhibited the whole-cell current in the presence of progesterone (Fig. 2b, right panel), confirming that the progesterone-activated current is carried by CatSper. Interestingly, because just 2 μM NNC completely suppressed human $I_{CatSper}$, we estimate that the affinity of NNC to CatSper is at least ten times greater than that to T-type voltage-gated Ca^{2+} channels (half-maximum inhibitory concentration (IC_{50}) ≈ 7 μM for T-type channels)[21]. NNC blocked mouse CatSper in a similar range of concentrations (Supplementary Fig. 5). Furthermore, the Cs^+ $I_{CatSper}$ was blocked by the same concentrations of Ca^{2+} as the progesterone-activated current, suggesting that the progesterone-activated channels

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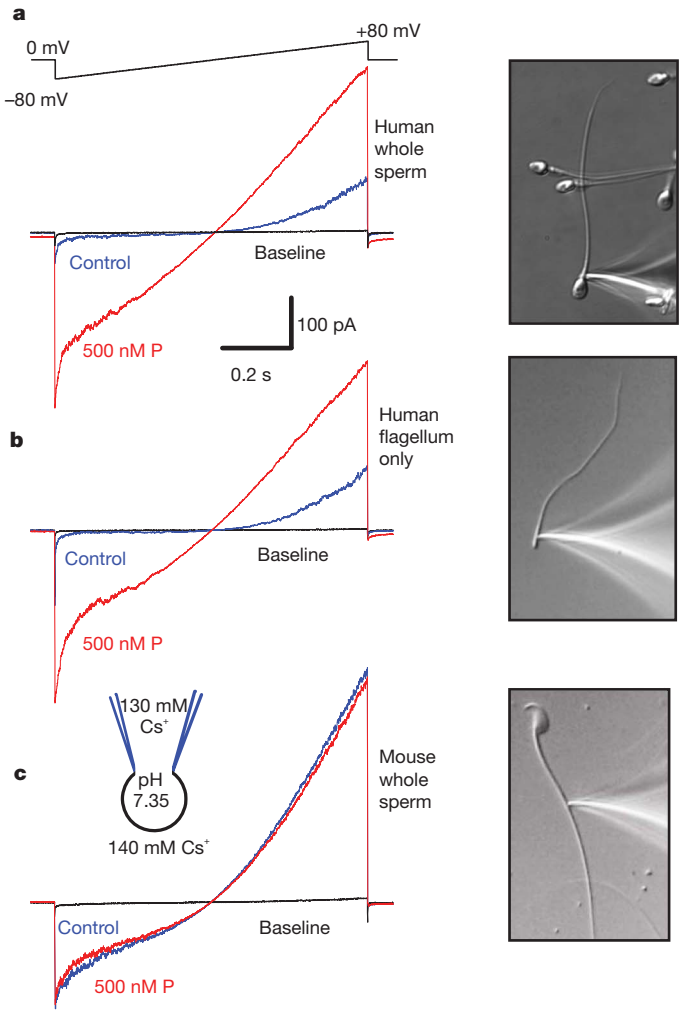

Figure 1 | Progesterone strongly potentiates human but not mouse CatSper. **a**, Representative monovalent whole-cell $I_{CatSper}$ recorded from a human spermatozoon in the absence (blue) and presence (red) of 500 nM progesterone (P). Right, a human spermatozoon attached to the recording pipette. **b**, Representative whole-flagellum human $I_{CatSper}$. Right, sperm flagellum attached to the recording pipette. **c**, Representative whole-cell $I_{CatSper}$ recorded from a mouse spermatozoon in the absence (blue) and presence (red) of 500 nM progesterone. Right, mouse spermatozoon attached to the recording pipette. Voltage protocol is shown in **a**. Baseline indicates currents recorded in HS solution.

contain a Ca^{2+}-binding site in the pore region that has the same affinity to Ca^{2+} as the binding site of CatSper (Fig. 2c and Supplementary Fig. 6).

Finally, we demonstrated that similar to CatSper, the progesterone-activated current is strongly potentiated by intracellular alkalinization (Fig. 2d and Supplementary Fig. 7). Because the CatSper and progesterone-activated currents have identical properties, we conclude that the progesterone-activated current is mediated by CatSper. Identical slopes of the voltage-activation curves (with the same maximal conductance) for sperm Ca^{2+} currents observed with or without progesterone (Fig. 3c) also support this conclusion.

Progesterone potentiated CatSper with a half-maximum effective concentration (EC_{50}) of 7.7 ± 1.8 nM (Fig. 3a). The progesterone-binding site associated with CatSper is external, because a membrane-impermeable conjugate of progesterone and bovine serum albumin (BSA), P-3-BSA, activated $I_{CatSper}$ similar to progesterone (Fig. 3b). Interestingly, progesterone modified with BSA at position C_{11} (P-11-BSA) did not potentiate $I_{CatSper}$ (Fig. 3b). A potent competitive antagonist of the nuclear progesterone receptor RU486 (mifepristone)[22] did not inhibit potentiation of CatSper by progesterone (Supplementary Fig. 8), suggesting that the ligand-binding site of the CatSper-associated progesterone receptor is structurally different from that of the classic nuclear progesterone receptor.

CatSper activation by intercellular alkalinization can be explained at least partially by shifting voltage-activation (G/V) curve to more negative, physiologically relevant potentials[12]. Therefore we tested whether progesterone activates human CatSper through a similar mechanism. Indeed, at intracellular pH 7.4, addition of 500 nM progesterone shifted CatSper half-activation voltage ($V_{1/2}$) from +85 mV to +52 mV (Fig. 3c, black curves, and Supplementary Fig. 9a). On the other hand, the maximal amplitude of the CatSper tail currents did not change significantly after addition of 500 nM progesterone (control maximal tail current was 97 ± 3% of that with progesterone ($n = 7$); see Supplementary Fig. 9a). These results suggest that progesterone activates human CatSper primarily by shifting its voltage dependence towards more physiological, negative membrane potentials, while the single-channel conductance is not significantly affected.

Because *in vitro* capacitation (incubation of spermatozoa under conditions similar to those found in the Fallopian tubes) was shown to increase progesterone-induced Ca^{2+} transients in human spermatozoa[23], we investigated the effect of capacitation on the progesterone-induced shift in the CatSper G/V curve. Capacitation caused a 15 mV negative shift of the CatSper G/V curve, and slightly increased the amplitude of the negative shift induced by progesterone: addition of 500 nM progesterone to capacitated spermatozoa shifted CatSper half-activation voltage ($V_{1/2}$) from +70 mV to +30 mV (Fig. 3c, red curves, and Supplementary Fig. 9b). Again, no change in the maximal amplitude of the CatSper tail currents was observed after stimulation of capacitated spermatozoa with 500 nM progesterone: control maximal tail current was 99 ± 4% of that with progesterone ($n = 6$, Supplementary Fig. 9b). Thus capacitation enhances the effect of progesterone upon CatSper channel by providing additional negative shift of the CatSper G/V curve.

The experiments with CatSper voltage activation also revealed why, in the absence of progesterone, human $I_{CatSper}$ is much smaller than mouse $I_{CatSper}$ at negative membrane potentials (compare Fig. 1a and c). At intracellular pH 7.4, $V_{1/2}$ of human CatSper is approximately +85 mV (Fig. 3c), whereas under similar conditions ($pH_i = 7.5$), $V_{1/2}$ of mouse CatSper is approximately +11 mV (ref. 12). Moreover, the G/V curve of human CatSper is steeper (slope factor $k \approx 20$; Fig. 3c) than that of mouse CatSper ($k \approx 30$)[12]. Slope factors for strongly voltage-sensitive ion channels are approximately 4. A more positive $V_{1/2}$ and a steeper slope of human CatSper G/V curve result in a smaller fraction of channels activated at negative membrane potentials compared with mouse CatSper. By inducing a negative shift in the G/V curve, progesterone helps human CatSper achieve a degree of activation at physiological potentials that is similar to mouse CatSper.

Prostaglandin E_1 (PGE_1) causes sperm intracellular Ca^{2+} transients similar in amplitude and waveform to those of progesterone-induced Ca^{2+} transients[24–26]. Therefore we tested whether PGE_1 also potentiates CatSper. Indeed, addition of 500 nM PGE_1 induced strong potentiation of human monovalent $I_{CatSper}$ (Fig. 4a), similar to that induced by progesterone. In contrast, mouse CatSper was not potentiated by PGE_1, even at 10 μM (Supplementary Fig. 10). In human sperm, the large current recorded in the presence of PGE_1 was fully inhibited by 2 μM NNC (Fig. 4a), a potent CatSper channel blocker (Fig. 2b). As with progesterone-induced current, the PGE_1-induced current originated from the flagellum (Fig. 4b, c) and was activated by intracellular alkalinization induced by addition of 10 mM NH_4Cl (Fig. 4d and Supplementary Fig. 11). These findings confirm that, similar to progesterone, nanomolar concentrations of PGE_1 potentiate CatSper.

Although progesterone and PGE_1 activate the same channel, addition of a saturating concentration of PGE_1 (2 μM) after potentiation of $I_{CatSper}$ with a saturating concentration of progesterone (2 μM) causes additional increase in the current amplitude and vice versa (Supplementary Fig. 12a, b). We used 2 μM as saturating concentration

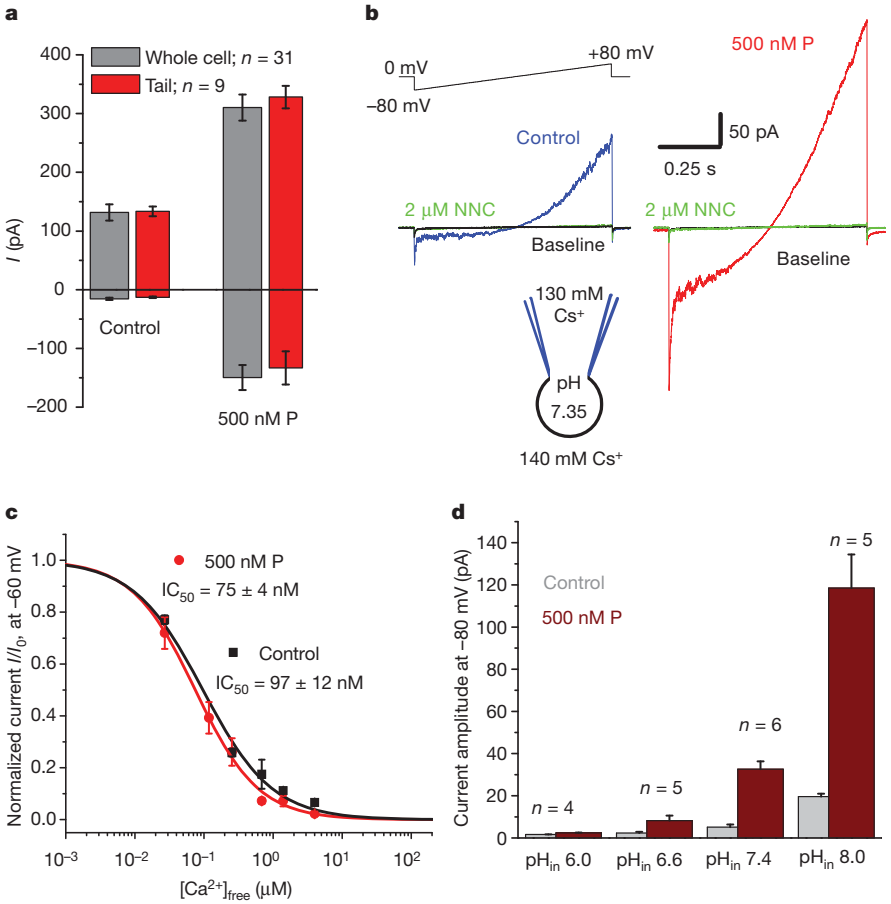

Figure 2 | Progesterone-activated current of human sperm is mediated by CatSper. **a**, Averaged $I_{CatSper}$ amplitudes recorded from a whole spermatozoon (grey) and a flagellum (red) in the absence and presence of progesterone (P). Current amplitudes at −80 mV (negative) and +80 mV (positive) are shown. **b**, Representative monovalent $I_{CatSper}$ (left, blue) and the current in the presence of progesterone (right, red) were inhibited by 2 μM NNC (green). **c**, Dose–responses of Ca^{2+} inhibition of inward monovalent $I_{CatSper}$ in the absence (black) and presence (red) of 500 nM progesterone. Amplitudes were recorded at −60 mV, $n = 10$. Representative current traces are shown in Supplementary Fig. 6. **d**, Amplitudes of inward monovalent $I_{CatSper}$ at varying intracellular pH in the absence (grey) and presence (maroon) of 500 nM progesterone. Current amplitudes were measured at −80 mV. For representative traces, see Supplementary Fig. 7. Data are mean ± s.e.m.

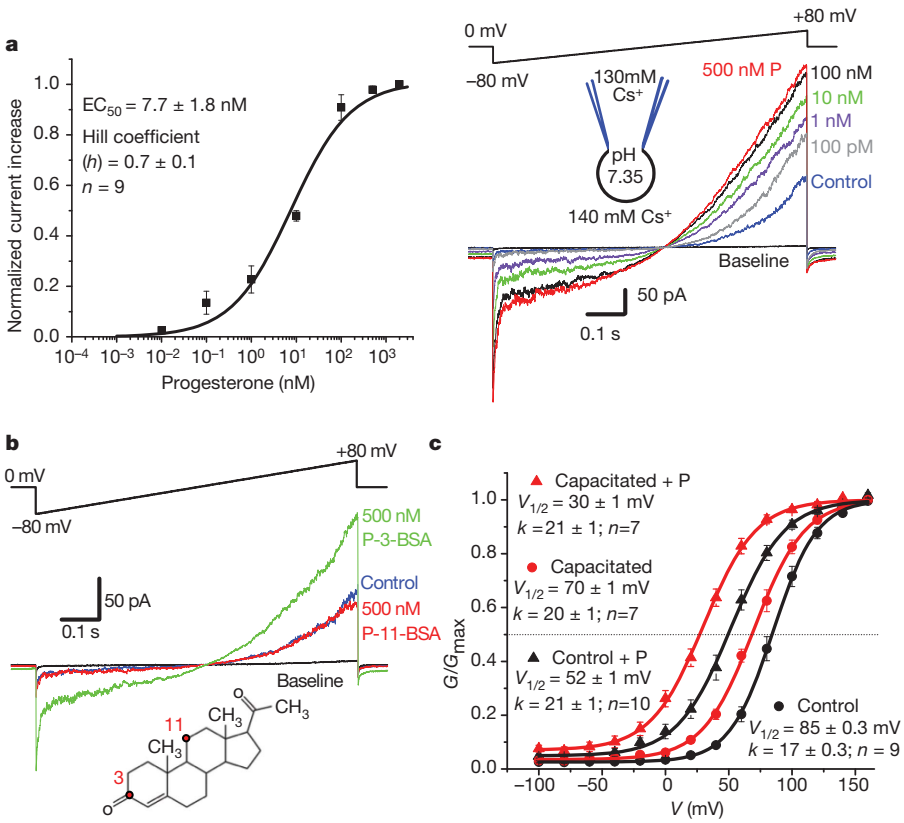

Figure 3 | Mechanism of CatSper potentiation by progesterone. **a**, Dose dependence of potentiation of monovalent inward $I_{CatSper}$ by progesterone (P; left panel) and representative monovalent whole-cell $I_{CatSper}$ recorded from a human spermatozoon in the presence of varying concentrations of progesterone (right panel). Baseline indicates current recorded in HS solution. Current amplitudes were measured at −60 mV by averaging five to ten original current traces. **b**, Monovalent $I_{CatSper}$ in control (blue) and in the presence of membrane-impermeable progesterone derivatives, P-3-BSA (green) and P-11-BSA (red). Below: chemical structure of progesterone with C_3 and C_{11} positions indicated. **c**, Voltage activation (G/V) curve of Ba^{2+} $I_{CatSper}$ in the absence (circles) and presence (triangles) of 500 nM progesterone recorded in 10 mM Ba^{2+} bath solution. Black curves represent non-capacitated spermatozoa, whereas red curves represent capacitated spermatozoa. For representative traces, see Supplementary Fig. 9. Data are mean ± s.e.m.

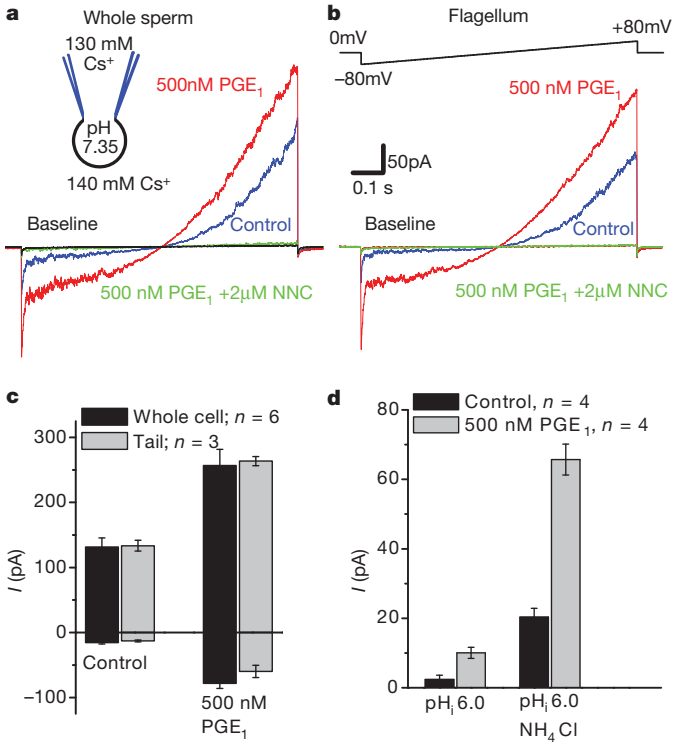

Figure 4 | Prostaglandin E_1 potentiates human CatSper. **a**, Representative whole-cell CatSper currents recorded from human spermatozoon in the absence (blue) and presence (red) of 500 nM PGE_1. Current with 500 nM PGE_1 was completely inhibited by 2 μM NNC (green). Baseline indicates current recorded in HS solution. **b**, Experiment from **a** reproduced with whole flagellum (principal piece and midpiece). **c**, Averaged $I_{CatSper}$ amplitudes from a whole human spermatozoon (black) and a flagellum (grey) in the absence and presence of 500 nM PGE_1. Amplitudes of CatSper currents at −80 mV (negative) and +80 mV (positive) are shown. **d**, Potentiation of $I_{CatSper}$ by 500 nM PGE_1 before and after induction of intracellular alkalinization with 10 mM NH_4Cl in the bath (initial intracellular pH = 6.0). Averaged $I_{CatSper}$ amplitudes (at −80 mV) before (black) and after (grey) addition of 500 nM PGE_1. Data are mean ± s.e.m.

of PGE_1, because 10 μM PGE_1 did not induce any additional potentiation of $I_{CatSper}$ (Supplementary Fig. 12c). These results suggest that the CatSper-associated binding sites for progesterone and PGE_1 are different and confirm a previous observation that progesterone and PGE_1 induce Ca^{2+} entry into human spermatozoa through different binding sites[25,26].

Prostaglandin $F_{1\alpha}$ ($PGF_{1\alpha}$) activated CatSper as strongly as PGE_1. Prostaglandins E_2 (PGE_2) and A_1 (PGA_1) had weaker but significant effects, whereas prostaglandin D_2 (PGD_2) had almost no effect (Supplementary Fig. 13). The relative effects of all activators of human CatSper identified in this work followed the sequence progesterone $>$ $PGF_{1\alpha}$ $\approx$ PGE_1 $>$ PGA_1 $>$ PGE_2 $\gg$ PGD_2 and are summarized in Supplementary Fig. 14.

The principal piece of the human sperm flagellum contains a voltage-gated proton channel H_v1 that may activate CatSper by causing intraflagellar alkalinization[13]. Because progesterone and PGE_1 had no effect on human sperm H_v1 current (Supplementary Fig. 15), we conclude that H_v1 is not involved in the sperm Ca^{2+} influx induced by progesterone or prostaglandins.

In summary, we demonstrate that human CatSper is strongly potentiated by progesterone and select prostaglandins, and that progesterone and PGE_1 apparently use different binding sites to activate CatSper. Because progesterone and prostaglandins potentiated CatSper in the absence of intracellular Ca^{2+}, ATP or GTP, their effects are not mediated by G-proteins, protein kinases or second messengers. The simplest explanation of these results is that receptors for progesterone and prostaglandins are located within the CatSper channel complex (consisting of CatSper1–4, CatSper-γ, CatSper-β[27]). However, membrane progestin receptors (mPR-α, -β, -γ, -δ and -ε) or progesterone receptor membrane component 1 (PGRMC1) that have been proposed to function as membrane progesterone receptors in different tissues[28,29] may also serve as CatSper-associated progesterone receptors. Precise identification of the progesterone- and prostaglandin-binding sites is complicated by an inability to express functional CatSper channel complex in heterologous expression systems.

CatSper is present in different species including invertebrates, but orthologues of CatSper subunits have low identity (50% or less)[27,30], which probably reflects the fact that regulation of the main sperm Ca^{2+} entry pathway in species with different mechanisms of fertilization also differs. Indeed, in contrast to human CatSper, mouse CatSper is not activated by progesterone or prostaglandins.

The kinetic rapid-mixing fluorimetry used by Strünker *et al.*[31] also demonstrates that CatSper is activated by progesterone and prostaglandins, without involvement of metabotropic receptors or second messengers.

METHODS SUMMARY

Gigaohm seals between the patch pipette and mouse spermatozoa were formed at the cytoplasmic droplet. Human spermatozoa were patched either at the cytoplasmic droplet or, if the cytoplasmic droplet was inconspicuous, at the neck region. Seals were formed in HS solution comprising (in mM): 130 NaCl, 5 KCl, 1 $MgSO_4$, 2 $CaCl_2$, 5 glucose, 1 sodium pyruvate, 10 lactic acid, 20 HEPES, pH 7.4 adjusted with NaOH. Pipettes for whole-cell patch-clamp recordings of monovalent CatSper currents were filled with (in mM): 130 Cs-methanesulphonate ($CsMeSO_3$), 70 HEPES/MES, 3 EGTA, 2 EDTA, 0.5 TrisHCl, pH 6.0–8.0 adjusted with CsOH. Bath divalent-free solution for recording of monovalent CatSper currents contained the following (in mM): 140 $CsMeSO_3$, 40 HEPES/MES, 1 EDTA, pH 7.4 adjusted with CsOH. HS solution was used to record baseline current while measuring monovalent CatSper currents. Bath solution for recording monovalent CatSper currents at different bath $[Ca^{2+}]_{free}$ contained the following (in mM): 140 $CsMeSO_3$, 40 HEPES/MES, 1 EDTA, 1 BAPTA, 1 HEDTA, pH 7.4 adjusted with CsOH. $CaCl_2$ was added to this solution in accordance with WinMAXC version 2.05 (C. Patton, Stanford University) to obtain required free $[Ca^{2+}]$. Pipettes for whole-cell patch-clamp recordings of Ba^{2+} CatSper currents were filled with the following (in mM): 145 NMDG, 100 HEPES, 10 BAPTA, 0.5 TrisHCl, pH 7.4 with $HMeSO_3$. Bath solution for recordings of Ba^{2+} currents contained the following (in mM): 0–50 Ba^{2+}, 150-90 NMDG, 100 HEPES, pH 7.4 adjusted with $HMeSO_3$. Mg^{2+} bath solution used as a control (baseline) solution contained the following (in mM): 2 $MgCl_2$, 150 NMDG, 100 HEPES, pH 7.4 adjusted with $HMeSO_3$. Proton currents were recorded in divalent-free solution as described in Lishko *et al.*[13]. All electrophysiology experiments were performed at 24 °C. Data were analysed with Origin 7.0 and Clampfit 9.2. Statistical data were calculated as the mean ± s.e.m., and *n* indicates number of experiments.

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

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1. Publicover, S., Harper, C. V. & Barratt, C. $[Ca^{2+}]_i$ signalling in sperm – making the most of what you've got. *Nature Cell Biol.* **9,** 235–242 (2007).
2. Blackmore, P. F., Beebe, S. J., Danforth, D. R. & Alexander, N. Progesterone and 17 alpha-hydroxyprogesterone. Novel stimulators of calcium influx in human sperm. *J. Biol. Chem.* **265,** 1376–1380 (1990).
3. Harper, C. V., Barratt, C. L. & Publicover, S. J. Stimulation of human spermatozoa with progesterone gradients to simulate approach to the oocyte. Induction of $[Ca^{2+}]_i$ oscillations and cyclical transitions in flagellar beating. *J. Biol. Chem.* **279,** 46315–46325 (2004).
4. Eisenbach, M. & Giojalas, L. C. Sperm guidance in mammals – an unpaved road to the egg. *Nature Rev. Mol. Cell Biol.* **7,** 276–285 (2006).
5. Revelli, A., Massobrio, M. & Tesarik, J. Nongenomic actions of steroid hormones in reproductive tissues. *Endocr. Rev.* **19,** 3–17 (1998).
6. Roldan, E. R., Murase, T. & Shi, Q. X. Exocytosis in spermatozoa in response to progesterone and zona pellucida. *Science* **266,** 1578–1581 (1994).
7. Teves, M. E. *et al.* Progesterone at the picomolar range is a chemoattractant for mammalian spermatozoa. *Fertil. Steril.* **86,** 745–749 (2006).
8. Uhler, M. L., Leung, A., Chan, S. Y. & Wang, C. Direct effects of progesterone and antiprogesterone on human sperm hyperactivated motility and acrosome reaction. *Fertil. Steril.* **58,** 1191–1198 (1992).
9. Evans, R. M. The steroid and thyroid hormone receptor superfamily. *Science* **240,** 889–895 (1988).

10. Losel, R. & Wehling, M. Nongenomic actions of steroid hormones. *Nature Rev. Mol. Cell Biol.* **4,** 46–56 (2003).
11. Ren, D. *et al.* A sperm ion channel required for sperm motility and male fertility. *Nature* **413,** 603–609 (2001).
12. Kirichok, Y., Navarro, B. & Clapham, D. E. Whole-cell patch-clamp measurements of spermatozoa reveal an alkaline-activated Ca^{2+} channel. *Nature* **439,** 737–740 (2006).
13. Lishko, P. V., Botchkina, I. L., Fedorenko, A. & Kirichok, Y. Acid extrusion from human spermatozoa is mediated by flagellar voltage-gated proton channel. *Cell* **140,** 327–337 (2010).
14. Carlson, A. E. *et al.* Identical phenotypes of CatSper1 and CatSper2 null sperm. *J. Biol. Chem.* **280,** 32238–32244 (2005).
15. Carlson, A. E. *et al.* CatSper1 required for evoked Ca^{2+} entry and control of flagellar function in sperm. *Proc. Natl Acad. Sci. USA* **100,** 14864–14868 (2003).
16. Qi, H. *et al.* All four CatSper ion channel proteins are required for male fertility and sperm cell hyperactivated motility. *Proc. Natl Acad. Sci. USA* **104,** 1219–1223 (2007).
17. Kaupp, U. B., Kashikar, N. D. & Weyand, I. Mechanisms of sperm chemotaxis. *Annu. Rev. Physiol.* **70,** 93–117 (2008).
18. Xia, J., Reigada, D., Mitchell, C. H. & Ren, D. CATSPER channel-mediated Ca^{2+} entry into mouse sperm triggers a tail-to-head propagation. *Biol. Reprod.* **77,** 551–559 (2007).
19. Olson, S. D., Suarez, S. S. & Fauci, L. J. A model of CatSper channel mediated calcium dynamics in mammalian spermatozoa. *Bull. Math. Biol.* **72,** 1925–1946 (2010).
20. Xia, J. & Ren, D. Egg coat proteins activate calcium entry into mouse sperm via CATSPER channels. *Biol. Reprod.* **80,** 1092–1098 (2009).
21. Huang, L. *et al.* NNC 55–0396 [(1*S*,2*S*)-2-(2-(*N*-[(3-benzimidazol-2-yl)propyl]-*N*-methylamino)ethyl)-6-fluoro-1,2,3,4-tetrahydro-1-isopropyl-2-naphtyl cyclopropanecarboxylate dihydrochloride]: a new selective inhibitor of T-type calcium channels. *J. Pharmacol. Exp. Ther.* **309,** 193–199 (2004).
22. Baulieu, E. E. Contragestion and other clinical applications of RU 486, an antiprogesterone at the receptor. *Science* **245,** 1351–1357 (1989).
23. Neri-Vidaurri Pdel, C., Torres-Flores, V. & Gonzalez-Martinez, M. T. A remarkable increase in the pH_i sensitivity of voltage-dependent calcium channels occurs in human sperm incubated in capacitating conditions. *Biochem. Biophys. Res. Commun.* **343,** 105–109 (2006).
24. Aitken, R. J., Irvine, S. & Kelly, R. W. Significance of intracellular calcium and cyclic adenosine 3′,5′-monophosphate in the mechanisms by which prostaglandins influence human sperm function. *J. Reprod. Fertil.* **77,** 451–462 (1986).
25. Schaefer, M., Hofmann, T., Schultz, G. & Gudermann, T. A new prostaglandin E receptor mediates calcium influx and acrosome reaction in human spermatozoa. *Proc. Natl Acad. Sci. USA* **95,** 3008–3013 (1998).
26. Shimizu, Y. *et al.* Prostaglandins induce calcium influx in human spermatozoa. *Mol. Hum. Reprod.* **4,** 555–561 (1998).
27. Ren, D. & Xia, J. Calcium signaling through CatSper channels in mammalian fertilization. *Physiology (Bethesda)* **25,** 165–175 (2010).
28. Baldi, E. *et al.* Nongenomic activation of spermatozoa by steroid hormones: facts and fictions. *Mol. Cell. Endocrinol.* **308,** 39–46 (2009).
29. Gellersen, B., Fernandes, M. S. & Brosens, J. J. Non-genomic progesterone actions in female reproduction. *Hum. Reprod. Update* **15,** 119–138 (2009).
30. Liu, J., Xia, J., Cho, K. H., Clapham, D. E. & Ren, D. CatSperβ, a novel transmembrane protein in the CatSper channel complex. *J. Biol. Chem.* **282,** 18945–18952 (2007).
31. Strünker, T. *et al.* The CatSper channel mediates progesterone-induced Ca^{2+} influx in human sperm. *Nature* doi:10.1038/nature09769 (this issue).

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

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Author Contributions P.V.L. and Y.K. conceived the project, designed the experiments and wrote the manuscript. P.V.L. performed most of the experiments. I.L.B. helped with pilot experiments for the project. All authors discussed the results and commented on the manuscript.

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METHODS

Materials and reagents. PGE_1, PGA_1 and $PGF_{1\alpha}$ were obtained from Avanti Polar Lipids. Oestradiol, PGD_2 and PGE_2 were purchased from Cayman Biochemicals. Progesterone was obtained from Sigma and Calbiochem. Progesterone conjugates were from aBiox. NNC 55-0396 was from Tocris. All other chemicals were purchased from Sigma.

Isolation of spermatozoa. Protocols for the human sperm studies were approved by the Committee on Human Research at the University of California, San Francisco. Freshly ejaculated sperm samples were obtained from four healthy young donors by masturbation and allowed to liquefy for 30–60 min at 22 °C before processing. Human spermatozoa were purified by the swim-up method in artificial human tubal fluid solution (in mM): 98 NaCl, 4.7 KCl, 0.3 KH_2PO_4, 2 $CaCl_2$, 0.2 $MgSO_4$, 21 HEPES, 3 glucose, 21 lactic acid, 0.3 sodium pyruvate, pH 7.4 (NaOH). Spermatozoa were stored in this medium at 22 °C for up to 6 h. For *in vitro* capacitation, isolated human spermatozoa were incubated in the capacitating medium as previously described[13]. Electrophysiological properties of non-capacitated and capacitated spermatozoa from the same donor were compared during the same day. Mouse spermatozoa were isolated from cauda epididymis as previously described[13].

Patch-clamp recordings. Gigaohm seals between the patch pipette and mouse spermatozoa were formed at the cytoplasmic droplet. Human spermatozoa were patched either at the cytoplasmic droplet or, if the cytoplasmic droplet was inconspicuous, at the neck region. Seals were formed in HS solution comprising the following (in mM): 130 NaCl, 5 KCl, 1 $MgSO_4$, 2 $CaCl_2$, 5 glucose, 1 sodium pyruvate, 10 lactic acid, 20 HEPES, pH 7.4 adjusted with NaOH. Transition into the whole-cell mode was performed by applying short (1 ms) 499–611 mV voltage pulses, sometimes combined with light suction. Access resistance was 25–70 MΩ, depending on the intracellular solution used (lower for Cs-based compared with NMDG-based solutions). Cells were stimulated every 5 s. Data were sampled at 2–5 kHz and filtered at 1 kHz.

Pipettes (11–17 MΩ) for whole-cell patch-clamp recordings of monovalent CatSper currents were filled with the following (in mM): 130 Cs-methanesulphonate, 70 HEPES/MES, 3 EGTA, 2 EDTA, 0.5 TrisHCl, pH 6.0–8.0 adjusted with CsOH. Bath divalent-free solution for recording of monovalent CatSper currents contained the following (in mM): 140 Cs-methanesulphonate, 40 HEPES/MES, 1 EDTA, pH 7.4 adjusted with CsOH. HS solution was used to record baseline current while measuring monovalent CatSper currents (Ca^{2+} contained in HS solution inhibits monovalent CatSper currents and causes Ca^{2+}-dependent inactivation of CatSper channels). Bath solution for recording monovalent CatSper currents at different bath $[Ca^{2+}]_{free}$ contained the following (in mM): 140 Cs-methanesulphonate, 40 HEPES/MES, 1 EDTA, 1 BAPTA, 1 HEDTA, pH 7.4 adjusted with CsOH. $CaCl_2$ was added to this solution in accordance with WinMAXC version 2.05 (C. Patton, Stanford University) to obtain required free $[Ca^{2+}]$.

Pipettes (20–30 MΩ) for whole-cell patch-clamp recordings of Ba^{2+} CatSper currents were filled withthe following (in mM): 145 NMDG, 100 HEPES, 10 BAPTA, 0.5 TrisHCl, pH 7.4 with methanesulphonic acid (Fig. 3c and Supplementary Fig. 9). Ba^{2+} currents in Supplementary Fig. 2 were obtained with pipette solution (in mM): 140 NMDG, 100 HEPES, 5 EDTA, 5 EGTA, 0.5 TrisHCl, pH 7.0 with methanesulphonic acid. Bath solution for recordings of Ba^{2+} currents contained the following (in mM): 0–50 Ba^{2+}, 150-90 NMDG, 100 HEPES, pH 7.4 adjusted with methanesulphonic acid. Mg^{2+} bath solution used as a control (baseline) solution while recording Ba^{2+} currents contained the following (in mM): 2 $MgCl_2$, 150 NMDG, 100 HEPES, pH 7.4 adjusted with methanesulphonic acid.

Proton currents were recorded in divalent-free solution as described in Lishko *et al.*[13]. Osmolarities of all electrophysiological solutions were approximately 321 and 335 mOsm l^{-1} for bath and pipette solutions, respectively. All electrophysiology experiments were performed at 24 °C. Data were analysed with Origin 7.0 and Clampfit 9.2. Statistical data were calculated as the mean ± s.e.m., and *n* indicates number of experiments.