Disruption of the principal, progesterone-activated sperm Ca^{2+} channel in a CatSper2-deficient infertile patient

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Edited by Dejian Ren, University of Pennsylvania, Philadelphia, PA, and accepted by the Editorial Board March 4, 2013 (received for review September 25, 2012)

The female steroid hormone progesterone regulates ovulation and supports pregnancy, but also controls human sperm function within the female reproductive tract. Progesterone causes elevation of sperm intracellular Ca^{2+} leading to sperm hyperactivation, acrosome reaction, and perhaps chemotaxis toward the egg. Although it has been suggested that progesterone-dependent Ca^{2+} influx into human spermatozoa is primarily mediated by cationic channel of sperm (CatSper), the principal flagellar Ca^{2+} channel of sperm, conclusive loss-of-function genetic evidence for activation of CatSper by progesterone has yet to be provided. Moreover, it is not clear whether the responsiveness of CatSper to progesterone is an innate property of human spermatozoa or is acquired as the result of exposure to the seminal plasma. Here, by recording ionic currents from spermatozoa of an infertile CatSper-deficient patient, we demonstrate that CatSper is indeed the principal Ca^{2+} channel of human spermatozoa, and that it is strongly potentiated by progesterone. In addition, by recording CatSper currents from human epididymal and testicular spermatozoa, we show that CatSper sensitivity to progesterone arises early in sperm development and increases gradually to a peak when spermatozoa are ejaculated. These results unambiguously establish an important role of CatSper channel in human sperm nongenomic progesterone signaling and demonstrate that the molecular mechanism responsible for activation of CatSper by progesterone arises early in sperm development concurrently with the CatSper channel itself.

CatSper ion channel | male fertility | nongenomic steroid action | sperm physiology

Sperm ion channels play an important role in the process of fertilization and control sperm motility, chemotaxis toward the egg, and the acrosome reaction. Mutation or absence of some ion channels directly affects male fertility of mice and humans (1–6). Sperm Ca^{2+} signaling is especially important for male fertility (7), and defects in the primary calcium channel of sperm, the pH-sensitive cationic channel of sperm (CatSper), result in complete male infertility without other detected defects in phenotype (3–6, 8–15). The absence of CatSper channels was shown to cause male infertility in mice (6, 10, 14), primarily because of an inability of sperm to hyperactivate (4, 5). Reported cases of male infertility in patients, linked to the mutation or deletion in CatSper genes, were assumed to share the same etiology; however, no physiological data have been provided (3, 8, 12, 13, 16). Recently, we and others reported that the female steroid hormone progesterone, an important physiological activator of spermatozoa within the female reproductive tract, activates the principal Ca^{2+} channel of human sperm (17, 18), presumably CatSper.

It has long been known that human spermatozoa have an unusual nongenomic progesterone receptor, which upon activation, causes elevation of human sperm intracellular Ca^{2+} to trigger hyperactivated motility and the acrosome reaction (19, 20). Hyperactivated motility is characterized by whip-like, high amplitude asymmetrical beats of the sperm flagellum (Movies S1, S2, S3, and S4) and, together with the acrosome reaction, is crucial for sperm ability to penetrate through the egg protective vestments. Although it has been proposed that flagellar calcium channel CatSper mediates calcium influx into the spermatozoa upon progesterone stimulation, this hypothesis is based solely on electrophysiological and pharmacological evidence (17, 18). An essential step in the identification of CatSper as the Ca^{2+} channel associated with sperm nongenomic progesterone receptor, loss-of-function genetic evidence, has been missing partly because progesterone does not activate CatSper in mouse spermatozoa (18). Therefore, mouse CatSper knockout models cannot be used. Another important unresolved issue is whether exposure to seminal plasma is an essential step for spermatozoa to acquire the ability to respond to progesterone (21) and whether the obvious lack of sensitivity of mouse CatSper to progesterone is because experiments were performed with epididymal mouse spermatozoa (18) that have never been exposed to seminal plasma. Here we apply the patch-clamp technique to spermatozoa isolated from a CatSper2-deficient infertile patient to provide genetic evidence that the CatSper channel constitutes a dominant Ca^{2+} conductance of human spermatozoa that is potently activated by nanomolar concentrations of progesterone. We also show the successful recording from human testicular and epididymal spermatozoa to demonstrate that sensitivity of CatSper to progesterone arises early in sperm development and does not require exposure to seminal plasma.

Results

Disruption of CatSper Abolishes the Principal, Progesterone-Sensitive Ca^{2+} Channel of Human Spermatozoa. To date, *CatSper* mutations linked to male infertility have been found only in a few men with severe asthenozoospermia (13), but the mechanism by which these *CatSper* mutations leads to male infertility has not been determined. To test whether spermatozoa from such patients retain the principal pH-sensitive Ca^{2+} current presumably mediated by the CatSper channel (22) and whether this current is activated by nanomolar concentrations of progesterone, we

Author contributions: Y.K. and P.V.L. designed research; J.F.S., O.S., N.M., and P.V.L. performed research; J.F.S., M.F., C.S., Y.K., and P.V.L. contributed new reagents/analytic tools; J.F.S., O.S., C.S., N.M., Y.K., and P.V.L. analyzed data; and J.F.S., M.F., Y.K., and P.V.L. wrote the paper.

The authors declare no conflict of interest.

This article is a PNAS Direct Submission. D.R. is a guest editor invited by the Editorial Board.

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This article contains supporting information online at www.pnas.org/lookup/suppl/doi:10.1073/pnas.1216588110/-/DCSupplemental.

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obtained freshly ejaculated spermatozoa from a patient with a homozygous *CatSper2* deletion [see *Materials and Methods*; genetic background previously reported in Avidan et al. (8)]. As shown in Fig. 1*C*, semen samples obtained from this patient contained very few morphologically normal spermatozoa. Semen analysis revealed severe astheno-teratozoospermia with abnormal motility patterns (Movies S5 and S6), also mentioned previously in Avidan et al. (8). However, we were able to identify a few viable spermatozoa per ejaculate with clearly visible cytoplasmic droplets (Fig. 1*C*). Next, we recorded putative CatSper currents using a recently developed method for whole-cell patch-clamping ejaculated human spermatozoa (22, 23) by forming a gigaohm seal between the patch pipette and sperm cell at the cytoplasmic droplet (Fig. S1). Under normal physiological conditions, CatSper channels are Ca^{2+}-selective, but they can pass monovalent ions (Cs^+ or Na^+) when recorded under bath divalent free (DVF) conditions. Because cesium currents through CatSper are significantly larger than calcium currents, we used Cs^+-based divalent free (CsDVF) medium for recording under the conditions previously reported (18). The putative monovalent human CatSper current ($I_{CatSper}$) recorded from ejaculated spermatozoa of a fertile donor is shown in Fig. 1*A*, *Left* (blue trace); the red trace shows potentiation of presumably the same CatSper current upon addition of 500 nM progesterone to the bath solution (Fig. 1*A*, *Left*). In contrast, spermatozoa from the patient with the *CatSper2* deletion not only lacked an initial CatSper current (Fig. 1*A*, *Right*), but also failed to respond to concentrations of progesterone up to 1.7 μM (Fig. 1 *A*, *Right*, red trace, and *D*). Data from the murine CatSper knockout models revealed that absence of any of four pore-forming subunits of the CatSper channel (i.e., CatSper1–4) resulted in disappearance of the whole complex (10). Immunostaining of *CatSper2*-deficient human sperm for the CatSper beta subunit, which is a part of the CatSper complex, also revealed the absence of this subunit from *CatSper2*-deficient spermatozoa (Fig. 1*B*). As reported previously by Avidan et al. (8), this patient's genetic background contains homozygous deletions of the last two exons of *CatSper2* gene, with removal of 225 bp of the corresponding mRNA. This fragment corresponds to the C-terminal cytoplasmic region that may be required for protein–protein interactions that form the actual channel pore complex with other subunits, such as CatSper1, CatSper3, and CatSper4 (12). Combining these data with the absence of CatSper beta immunostaining, progesterone's inability to elicit any conductance across the patient's sperm plasma membrane, and the absence of CatSper residual currents, we concluded that the CatSper channel is the principal Ca^{2+} conductance of the human sperm plasma membrane and that human CatSper is activated by progesterone.

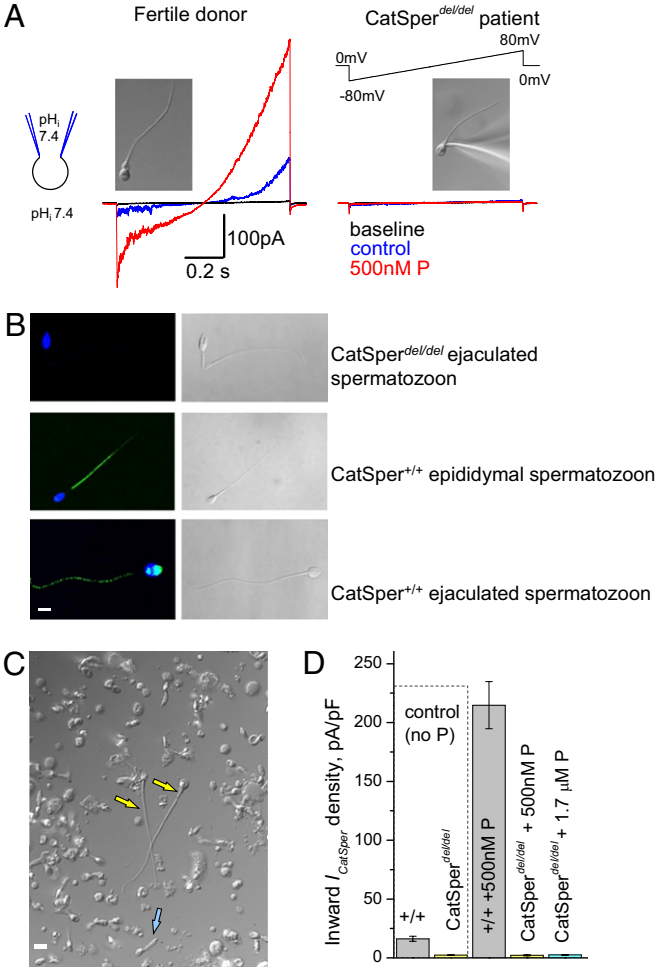

Fig. 1. Absence of CatSper abolishes the progesterone-activated current in human spermatozoa. (*A*, *Left*) Recording from ejaculated spermatozoon from a fertile donor; (*Right*) recording from an ejaculated spermatozoon from a *CatSper2*-deficient patient (CatSper$^{del/del}$). Representative monovalent whole-cell $I_{CatSper}$ in the absence (blue) and presence (red) of 500 nM progesterone (P). Currents were elicited by voltage ramps from a holding potential of 0 mV (*Right*). Ramps were applied from −80 mV to 80 mV. *Insets* show spermatozoa from a fertile donor and the *CatSper2*-deficient, patient (with a patch pipette attached).The bath solution contained 140 mM $CsMeSO_3$, 1 mM EDTA, 40 mM Hepes, pH 7.4. Pipettes were filled with 130 mM $CsMeSO_3$, 60 mM Hepes, 1 mM EDTA, 5 mM EGTA, pH 7.4. (*B*) Immunostaining of human spermatozoa. (*Right*) Differential interference contrast images of spermatozoa. (*Left*) The same cells probed with anti-CatSper beta antibody (green staining of the principal piece). Nuclei are stained blue by DAPI. (Scale bar, 5 μm.) (*C*) Ejaculate from CatSper$^{del/del}$ patient. Normal appearing spermatozoa are indicated by yellow arrows and comprise only 10% of all other spermatozoa in the ejaculate. The ejaculates were teratozoospermic, and most spermatozoa were immotile, with abnormally short flagella (blue arrows). (Scale bar, 10 μm.) (*D*) Densities of inward $I_{CatSper}$ recorded at −80 mV from normal human spermatozoa (n = 7) and spermatozoa from CatSper$^{del/del}$ patient (n = 11) in the absence (control) and presence of progesterone. Uncapacitated spermatozoa were used.

To confirm that CatSper2-deficient spermatozoa preserved functional integrity of their plasma membrane and retained other ion currents, we tested these cells for the presence of voltage-gated proton channel 1 (Hv1) (22), as well as for sperm potassium conductance KSper (1, 2, 24). The voltage gated proton channel Hv1 resides together with CatSper in the principal part of the human sperm flagellum (22), and may exert its action upon pH-sensitive CatSper by regulating sperm intracellular pH. Interestingly, spermatozoa from the *CatSper2*-deficient patient not only retained fully functional proton channel Hv1, but its current density was up-regulated (Fig. 2 *A* and *C*). This may indicate a possible linked developmental regulation between Hv1 and CatSper channels in human spermatozoa.

The predominant potassium conductance of mouse spermatozoa is represented by the pH-sensitive potassium channel of sperm (KSper), encoded by the potassium channel, subfamily U, member 1 (*KCNU1*) gene. KSper is essential for regulation of sperm membrane potential (1, 2, 24, 25). To determine whether similar conductance is present in human sperm cells, we recorded currents from ejaculated spermatozoa under conditions where potassium ion was the major permeant cation (*Materials and Methods*). To prevent conductance of potassium ions via CatSper channels, we added 1 mM Ca^{2+} to the bath solution. High micromolar concentrations of extracellular Ca^{2+} inhibit outward monovalent (Cs^+) current via the CatSper channel in human spermatozoa (Fig. S3). Both at negative and positive membrane potentials [(18) and Fig. S3], and in the presence of 1 mM Ca^{2+} in the bath solution, there was no measurable K^+

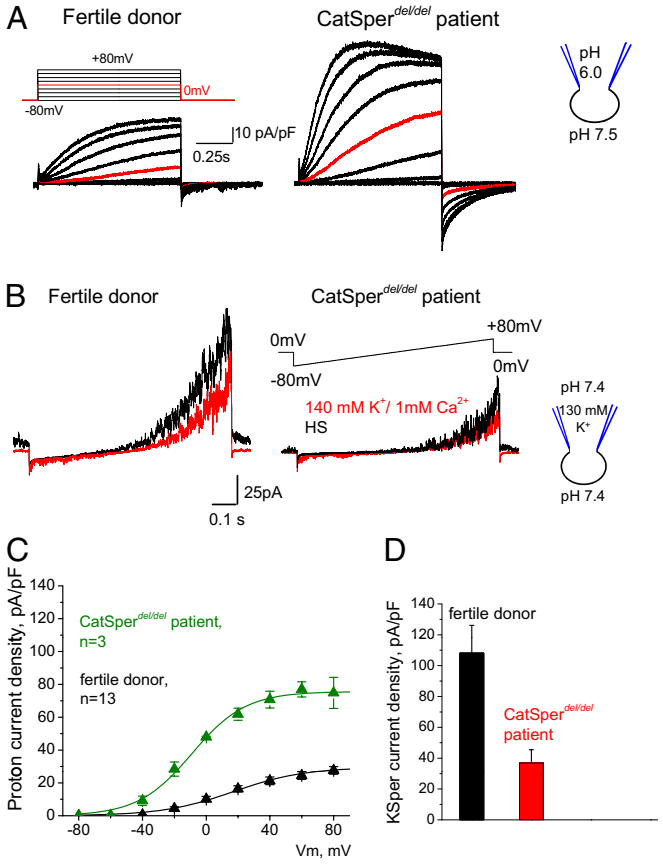

Fig. 2. Proton current Hv1 and potassium conductance KSper are retained in CatSper-deficient spermatozoa. (*A*) Representative Hv1 currents recorded from normal (*Left*) and CatSper$^{del/del}$ (*Right*) ejaculated spermatozoa in response to the voltage steps as shown. Note up-regulation of the Hv1 current in the CatSper$^{del/del}$ spermatozoon. (*B*) Representative KSper currents recorded from normal (*Left*) and CatSper$^{del/del}$ (*Right*) ejaculated spermatozoa in response to the voltage ramp protocol as shown. KSper currents were recorded in a solution containing 5 mM K^+ (HS solution, black traces) and a solution containing 140 mM K^+ (nominal free solution plus 1 mM Ca^{2+}, red traces). Note that KSper is reduced in CatSper $^{del/del}$ spermatozoa. (*C*) Current-voltage relationship of Hv1 recorded from ejaculated spermatozoa of fertile donor (black squares) and from CatSper$^{del/del}$ patient (green squares). Currents were recorded with a voltage step protocol as in *A* with current amplitudes measured at the end of each depolarizing pulse. (*D*) Averaged densities of the outward whole-cell KSper current from normal spermatozoa (n = 7, cells from three different fertile donors) and from CatSper$^{del/del}$ spermatozoa (n = 3) at pH_i = 7.4. Currents were recorded in the NMF bath solution with 1 mM Ca^{2+} as shown in *B*. Whole-cell KSper current amplitudes were measured at +80 mV and normalized to cell capacitances. Averaged data are presented as mean ± SEM, n indicates number of experiments. Uncapacitated spermatozoa were used. Vm, membrane voltage.

current through CatSper channels. Under these conditions, we recorded an outwardly rectifying K^+ current that resembled the KSper current (I_{Ksper}) previously reported in mouse epididymal sperm cells (1, 2, 24). The spermatozoa from the *CatSper2*-deficient patient retained this K^+ current, although its density was reduced (Fig. 2 *B* and *D*).

To probe whether the reduction in motility (asthenozoospermia) seen in spermatozoa from our patient is linked to the absence of the CatSper channel, we calculated sperm beat frequency of uncapacitated (freshly ejaculated) spermatozoa from fertile donors and compared this with the beat frequency of ejaculated fertile spermatozoa incubated with 1 μM of NNC 55-0396, a CatSper inhibitor (17, 18). No difference in the beat frequency was observed (Fig. S4 and Movies S5 and S7), which agrees with the previous observation that deletion of the CatSper channel in mice does not influence normal motility and only affects sperm hyperactivation (5, 6). It should be noted that the CatSper2-deficient patient also bears a microdeletion in a tandem repeat of chromosome 15 that removes another gene expressed in spermatozoa, diphosphoinositol pentakisphosphate kinase 1 (IP6K) (8). Although the function of IP6K in sperm physiology is not known, its absence may contribute to reduced motility.

CatSper Sensitivity to Progesterone Arises Early in Sperm Development. We have previously demonstrated that in contrast to the CatSper current recorded from human ejaculated spermatozoa, mouse CatSper from caudal epididymal spermatozoa is completely insensitive to progesterone (18). Although mouse caudal epididymal spermatozoa are considered mature and are able to fertilize the oocyte if subjected to capacitation in vitro, they never encounter seminal plasma and may possess different biochemical components compared with their ejaculated counterparts. Therefore, the dramatic difference in progesterone sensitivity between human and mouse CatSper may suggest either the lack of a CatSper-associated nongenomic progesterone receptor in mouse sperm, or the lack of responsiveness of CatSper to progesterone that is acquired as a result of exposure to seminal plasma. To differentiate between these two possibilities, we recorded CatSper channel activity from epididymal and testicular human spermatozoa.

Both epididymal and testicular spermatozoa had typical normal sperm morphology and slightly enlarged cytoplasmic droplets (Fig. 3*A* and Fig. S1 *B* and *C*). The monovalent human CatSper current ($I_{CatSper}$) recorded from both testicular (TS) and epididymal spermatozoa (ES) was similar to the CatSper current recorded from ejaculated spermatozoa (Fig. 3 *B* and *C*, blue traces, and Fig. S2*C*, control). Progesterone also was able to potentiate CatSper currents in TS and ES. However, the potentiation of the CatSper channel by progesterone, recorded from TS and ES, overall was twice as small (Fig. 3*B*, red traces; and Fig. S2*C*, +500 nM progesterone) as the activation of CatSper by progesterone recorded from ejaculated spermatozoa.

We next asked how early during spermatogenesis male germ cells acquire sensitivity to progesterone. As shown in Fig. S2, only weak progesterone response was recorded from elongated spermatids. Overall, the sensitivity to progesterone increased progressively throughout sperm development and the maximum sensitivity to progesterone was detected in ejaculated sperm cells (Fig. S2). This CatSper sensitivity to progesterone coincided with development of the flagellum and with establishment of the flagellar fibrous sheath (26), a dense support structure that lies beneath the flagellum plasma membrane and is believed to be a docking structure for many membrane proteins, including ion channels. This raises the possibility that CatSper is only fully functional after its subunits are properly positioned on the flagellum and are docked to underlying axonemal structures, therefore explaining unsuccessful earlier attempts to express this channel heterologously (15).

Our results indicated that the behavior of CatSper channels in ejaculated and epididymal spermatozoa was very similar, which further demonstrated that the progesterone regulation of human CatSper is clearly different from mouse CatSper. Also, our results showed that responsiveness of the human CatSper channel to progesterone occurs early during sperm development and is not imparted by exposure to the seminal plasma.

Repetitive Application of Progesterone to CatSper Channels Causes Its Desensitization in Ejaculated but Not in Epididymal or Testicular Spermatozoa. Interestingly, the amplitude of inward monovalent $I_{CatSper}$ in ejaculated spermatozoa decreased dramatically during repetitive applications of 500 nM progesterone interspersed with prolonged washout (Figs. 3*C* and 4), revealing the desensitization pattern known as tachyphylaxis. It has to be noted that $I_{CatSper}$ does not desensitize under continuing application of progesterone

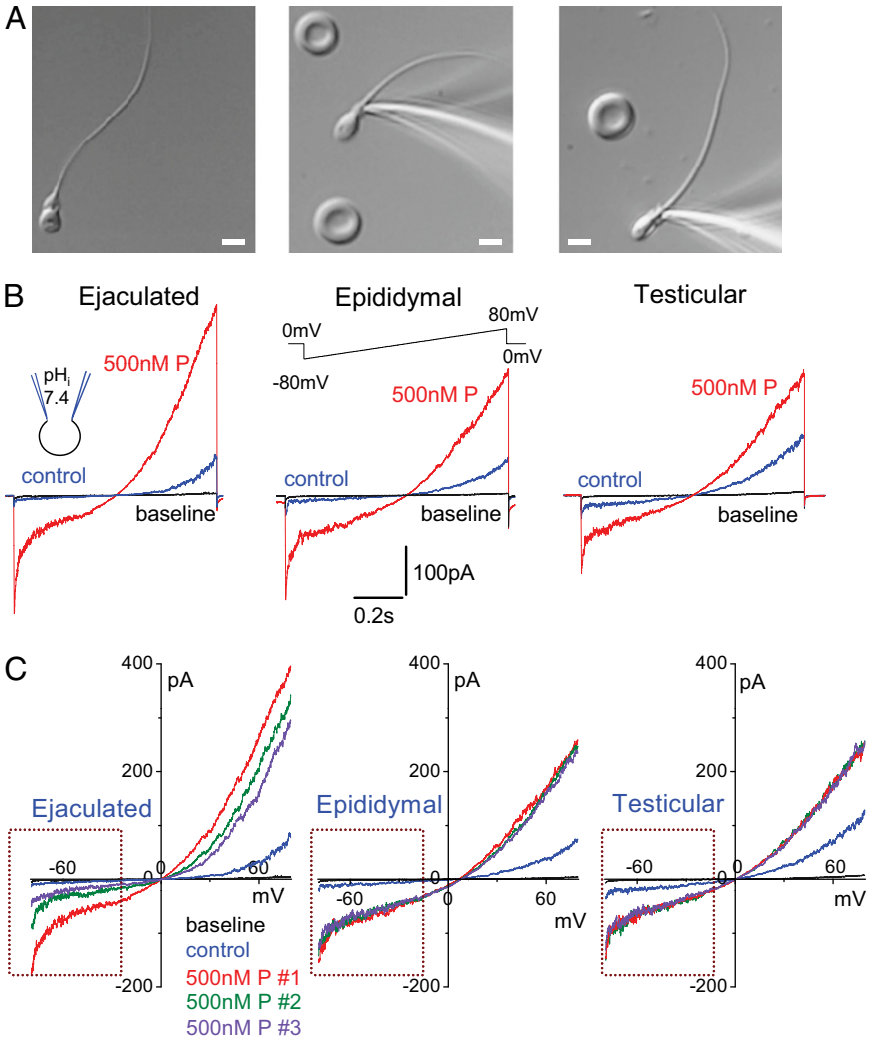

Fig. 3. Progesterone potentiates CatSper currents in TS, ES, and ejaculated human spermatozoa. (*A*) Human spermatozoa at different developmental stages: an ejaculated spermatozoon (*Left*); a spermatozoon isolated from corpus epididymis (*Center*, with the recording pipette); a testicular spermatozoon (right, with the recording pipette). Erythrocytes are seen in ES and TS preparations. (*B*) Representative monovalent whole-cell $I_{CatSper}$ recorded from human ejaculated (*Left*), epididymal (*Center*), and testicular (*Right*) spermatozoa in the absence (blue) and presence (red) of 500 nM progesterone (P). CatSper currents were obtained as in Fig. 1. (*C*) Whole-cell $I_{CatSper}$ recorded from human spermatozoa after repetitive application of 500 nM progesterone as indicated. The frames show diminishing response of inward $I_{CatSper}$ after second and third applications of progesterone in ejaculated spermatozoa, but not in ES or TS. Voltage protocol is shown in *B*. Baseline indicates currents recorded in HS solution (*Materials and Methods*). (Scale bar, 5 μm.) Uncapacitated spermatozoa were used.

in DVF solution [Fig. 4*A* and ref. (18)], but does so only after subsequent incubation in progesterone-free Ca^{2+}- containing high-saline (HS) solution (Fig. 4). This indicates that the observed tachyphylaxis of CatSper channel may be Ca^{2+}-dependent.

In contrast, monovalent $I_{CatSper}$ in TS and ES did not show desensitization (Figs. 3*C* and 4*A*, *Center* and *Right*). Fig. 4*B* shows $I_{CatSper}$ mean densities recorded from TS, ES, and ejaculated human spermatozoa before and after repetitive stimulation with progesterone. However, we found that exposure to the seminal plasma could promote tachyphylaxis of CatSper in ES. We compared CatSper amplitudes of sperm obtained from cauda epididymis of vas-obstructed patients before and after incubation with their sperm-free ejaculate (*Materials and Methods*). As shown in Fig. 4*B*, such incubation did not enhance CatSper sensitivity to progesterone, although we did observe desensitization of $I_{CatSper}$ in response to repetitive applications of progesterone. Therefore, although seminal plasma is not essential for responsiveness of CatSper to progesterone, it can modify CatSper-dependent nongenomic progesterone signaling.

Discussion

In summary, by applying the patch-clamp technique to spermatozoa of an infertile patient with a CatSper2 deletion, we confirmed that CatSper is the principal Ca^{2+} channel of human spermatozoa, similar to what has been observed for mouse spermatozoa (27). We also provided the missing genetic loss-of-function evidence that human CatSper is activated by the female steroid hormone progesterone.

In addition, we demonstrated that CatSper currents recorded from testicular and epididymal spermatozoa are sensitive to progesterone. Therefore, responsiveness of CatSper channel to progesterone is not acquired upon ejaculation as the result of exposure to the seminal plasma. Because mouse CatSper current recorded from epididymal spermatozoa is not sensitive to progesterone (18), we concluded that the sperm nongenomic progesterone receptor is either missing in mice or the mouse CatSper does not respond upon engagement of this receptor. CatSper is expressed in spermatozoa of many species, but orthologs of some CatSper subunits

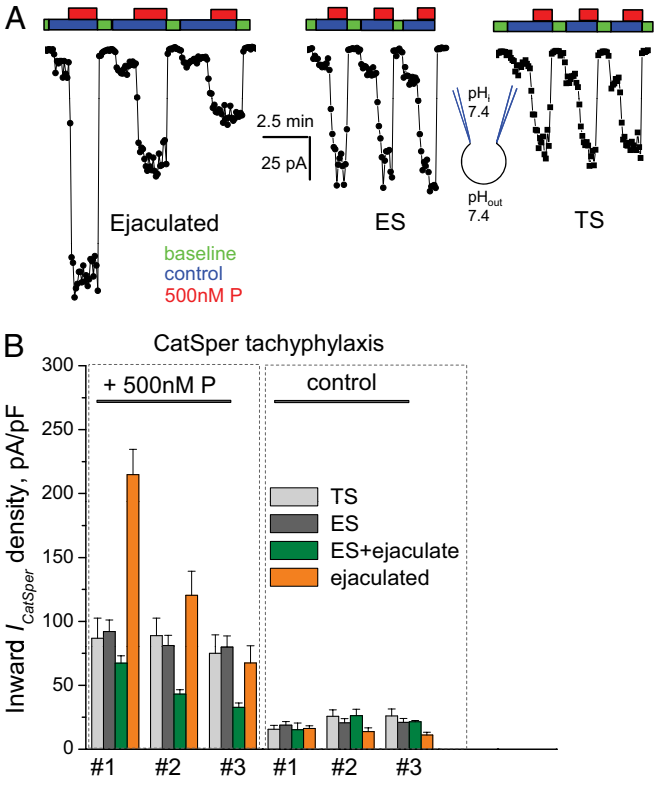

Fig. 4. CatSper tachyphylaxis. (*A*) Diminishing repetitive response of CatSper to progesterone (tachyphylaxis) in ejaculated spermatozoa (*Left*) but not in epididymal (ES, *Center*) or testicular (TS, *Right*) spermatozoa. $I_{CatSper}$ was recorded as in Fig. 3*C*, and amplitudes were measured at −80 mV and plotted against time. (*B*) CatSper tachyphylaxis in ejaculated spermatozoa, TS, ES, and epididymal spermatozoa treated with the seminal plasma (ES+ejaculate), as measured in *A*. The bar diagram shows mean densities of monovalent CatSper current upon three repetitive applications of divalent free Cs^+ bath solution (control, *Right*) and after subsequent stimulation with P (+500 nM P, *Left*). $I_{CatSper}$ amplitudes were measured at −80 mV from the following number of spermatozoa: ejaculated ($n = 7$), TS ($n = 4$), ES ($n = 10$), and ES treated with seminal plasma ($n = 3$). To obtain epididymal spermatozoa treated with seminal plasma (ES+ejaculate), ES obtained from the cauda epididymis of two vas-obstructed patients were treated with azoospermic ejaculate from the same patients (*Materials and Methods*). Data are mean ± SEM. Uncapacitated spermatozoa were used.

share low identity (50% or less) (28, 29), which can explain why regulation of the CatSper channel can differ between species.

Although responsiveness of human CatSper to progesterone arises early in sperm development concurrently with the pore of the CatSper channel, this observation does not necessarily mean that CatSper itself serves as the sperm nongenomic progesterone receptor. A model in which CatSper is activated by progesterone indirectly is supported by the observation that the CatSper channel of testicular and epididymal spermatozoa is potentiated by progesterone to a lesser degree than the CatSper channel of ejaculated spermatozoa.

The observation that progesterone activates the CatSper channel, not only in ejaculated but also in testicular and epididymal spermatozoa, argues strongly against the scenario where essential progesterone binding subunit(s) are acquired by spermatozoa from the seminal plasma, such as by transferring of the progesterone sensitive module from prostasomes. Although this possibility has been suggested by Park et al. (21), our results do not support it, at least with regard to the sperm nongenomic progesterone signaling associated with the CatSper channel. However, we do not exclude the possibility that the seminal plasma can have modulatory effects on CatSper channel response to progesterone. For example, our results suggest that exposure to seminal plasma can impart tachyphylaxis on the response of CatSper to progesterone. Such effects of the seminal plasma require additional investigation.

Asthenozoospermia seen commonly in infertile men may be mediated by impairment in ion channel function. This could have wide-ranging diagnostic implications for many men with infertility. The patch-clamp technique and molecular understanding of ion channel function may relate observations in single sperm with those at the population level. Currently, very few diagnostic tests are available to infertile men. For individuals seeking to understand why their sperm do not move well or for men with normal semen parameters unable to fertilize eggs well, using the patch-clamp technique could elucidate mechanistic reasons for these deficits. To ascertain this possibility, more work needs to be done at the population level.

Although male patients with defects in CatSper channel are likely sterile because of the inability of their spermatozoa to fertilize the oocyte on their own, such male infertility could be successfully treated by using in vitro fertilization with intracytoplasmic sperm injection. In addition, the progesterone-binding site on CatSper channel complex presents an attractive target for developing unisex contraceptives, which will have the potential to reduce sperm motility and successfully prevent fertilization.

Materials and Methods

Materials and Reagents. Progesterone was obtained from Sigma and Calbiochem. All other chemicals were purchased from Sigma.

Healthy Donors and Patients. A total of 10 healthy fertile volunteers aged 21–38 y old were recruited for this study. Freshly ejaculated semen samples were obtained by masturbation. Spermatozoa were purified by the swim-up technique as described (18).

Men with proven fertility who were undergoing sperm retrieval procedures or a vasectomy reversal in the University of California San Francisco Center for Reproductive Health were included in this study. As part of the ongoing Institutional Review Board–approved Lifestyle, Fertility, and Evaluation (LIFE) study, men who agreed to participate donated unused ejaculated semen samples and portions of surgical specimens. All men enrolled in the present study had a documented history of prior paternity and had undergone a vasectomy in the past. As part of routine clinical care, these men elected to undergo either a sperm retrieval procedure (microscopic epididymal sperm aspiration, percutaneous epididymal sperm aspiration, or testicular sperm extraction) combined with in vitro fertilization or a vasectomy reversal. An aliquot of testicular and epididymal fluid was used for the present study after patient consent. These men also provided a semen sample before undergoing sperm retrieval, allowing us to study the composition and effect of seminal fluid on sperm ion channel function.

Genetics and sperm analysis of the patient with homozygous *CatSper2* deletion (i.e., patient II-4) was reported previously (8). In brief, semen analysis revealed normal viscosity, pH 7.4, and ejaculate volume of 4 mL. Spermatozoa analysis revealed 16 $\times 10^6$ cells/mL with 10% normal morphology, of which 40% were motile. The patient's genetic background contained homozygous deletions of the last two exons of the *CatSper2* gene, with removal of 225 bp of the corresponding mRNA (8). This fragment corresponds to the C-terminal cytoplasmic region that may be required for protein–protein interactions that form the actual channel pore complex with other subunits, such as CatSper1, CatSper3, and CatSper4 (12).

Isolation of Ejaculated Spermatozoa. Protocols for the human sperm studies were approved by the Committees on Human Research at the University of California, Berkeley and 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 the artificial human tubal fluid solution: 98 mM NaCl, 4.7 mM KCl, 0.3 mM KH_2PO_4, 2 mM $CaCl_2$, 0.2 mM $MgSO_4$, 21 mM Hepes, 3 mM glucose, 21 mM lactic acid, 0.3 mM sodium pyruvate, pH 7.4 (adjusted with 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 (22). Freshly ejaculated sperm samples obtained from the *CatSper2*-deficient patient were also allowed to liquefy for 30–60 min at 22 °C, followed by purification

by a spin-down procedure as indicated elsewhere (22), because of the inability to purify such samples using a standard swim-up technique (human *CatSper2*-deficient spermatozoa were asthenozoospermic).

Patch-Clamp Recording. Gigaohm seals between the patch pipette and human spermatozoa were formed at the cytoplasmic droplet. 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 containing 130 mM NaCl, 5 mM KCl, 1 mM $MgSO_4$, 2 mM $CaCl_2$, 5 mM glucose, 1 mM sodium pyruvate, 10 mM lactic acid, 20 mM Hepes, pH 7.4 adjusted with NaOH, 320 mOsm/L. 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 25–30 MΩ and 50–70 MΩ for Cs^+/K^+-based and NMDG-based intracellular solutions, respectively. 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 130 mM cesium methanesulfonate, 70 mM Hepes, 3 mM EGTA, 2 mM EDTA, 0.5 mM Tris·HCl, pH 7.4 adjusted with CsOH. DVF solution for recording of monovalent CatSper currents contained 140 mM cesium methanesulfonate, 40 mM Hepes, 1 mM EDTA, pH 7.4 adjusted with CsOH. HS solution was used to record baseline current while measuring monovalent CatSper currents (Ca^{2+} in HS solution inhibits monovalent CatSper currents and causes Ca^{2+}-dependent inactivation of CatSper channels). Proton currents were recorded in DVF solution as described by Lishko et al. (22). Potassium KSper currents were recorded with nominal free bath solution containing 140 mM $KMeSO_3$, 20 mM Hepes, pH 7.4. Pipettes were filled with 130 mM $KMeSO_3$, 4 mM KCl, 5 mM Hepes, 1 mM EDTA, 5 mM EGTA, pH 7.4. To inhibit the monovalent potassium current through CatSper channels, which can conduct potassium in the absence of other ions, 1 mM Ca^{2+} was added to the bath solution (as indicated). Some KSper recordings from spermatozoa from the *CatSper2*-deficient patient were performed with intracellular and bath solutions containing 135 mM $KMeSO_3$, 4 mM KCl, 20 mM Hepes, 1 mM EDTA, 8 mM EGTA, pH 7.4. Osmolarities of all of the mentioned solutions were ∼321 mOsm/L and 335 mOsm/L for bath and pipette solutions, respectively. All electrophysiology experiments were performed at 24 °C. Data were analyzed with Origin 7.0 and Clampfit 9.2 software. Statistical data were calculated as the mean ± SEM (SEM), and *n* indicated number of experiments.

ACKNOWLEDGMENTS. We thank Dr. D. Babcock for critical reading of the manuscript. We also thank Biology of Reproduction Laboratory of The Centre for the Study and Conservation of Human Ova and Sperm (CECOS) at Cochin Hospital in Paris, for sperm evaluation of the *CatSper2*-deficient patient, and Dr. Alexandre Pariente from Pau Hospital for his generous help. This work was supported by National Institute of Child Health and Human Development (NICHD) Grant R01HD068914 (to Y.K.), by a National Institutes of Health/NICHD K12 award (to J.F.S.), and by a Winkler Family Foundation Fellowship (to P.V.L.).

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