Researchers identify regulator of human sperm cells

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UCSF researchers have identified an elusive molecular regulator that controls the ability of human sperm to reach and fertilize the egg, a finding that has implications on both treating male infertility and preventing pregnancy.

The team of biophysicists and molecular biologists also broke through a previous barrier to this research by developing a method to record electrical activity of a single human sperm cell through a process called patch-clamping.

The new findings shed light on a number of previous conundrums, including how sperm cells that are quiescent in the male reproductive system get activated in the female reproductive tract and why marijuana and zinc affect sperm motility and male fertility, the researchers say.

The findings are featured as the cover story in the February 5, 2010 issue of the journal "*Cell*".

The molecule in question, known as Hv1, operates as a pore in the outer membrane of the sperm cell that extrudes protons from the cell. Protons are positively charged particles in every atom that are also stable on their own in the form of the H+ hydrogen ion.

That extrusion process increases the pH of the internal cell environment, which makes it less acidic. Hv1 had previously been known to be primarily present in immune cells called phagocytes, but had not been

seen in other cells, including spermatozoa, according to Yuriy Kirichok, PhD, an assistant professor in the UCSF Department of Physiology who led the research.

Hv1 also was known to be inhibited by zinc and had what Kirichok called a "fingerprint" of unique behavior in terms of the properties of the transmembrane proton current it generates.

"For the first time ever, we had an opportunity to study the electrical activity of the human sperm cell and measure its ionic conductance," Kirichok said. "We realized there was a huge proton conductance at work. The activity looked just like Hv1 activity in phagocytes."

Hv1 also was a logical option because it is known to be activated by a rise in external pH and to be inhibited by extracellular zinc. The highest concentration of zinc in humans is found in the male reproductive tract, Kirichok said, which would inhibit an Hv1 channel and keep the spermatozoa in their quiescent state. The female reproductive tract is both more alkaline and distinctly lower in zinc levels.

By combining the skills and knowledge of biophysics, molecular biology, traditional pharmacology and biochemistry, the team was first able to record the proton current flowing across the sperm plasma membrane, then conduct a series of experiments to see whether it responded to the same external stimuli as Hv1: activation by membrane voltage, a rise in external pH and fatty acids, and inhibition by extracellular zinc.

When the results of those experiments mimicked Hv1, the UCSF team used a fluorescent antibody that is known to bind specifically with Hv1, and a test for the Hv1 messenger RNA that serves as a template for the gene that produces Hv1 to confirm presence of Hv1 in human sperm cells.

Each of those produced positive results and revealed an extremely high concentration of Hv1 in the flagellum of the human sperm cell.

Scientists have known for decades that sperm cells needed to become less acidic - or more alkaline - internally in order to be activated in the female reproductive tract and become able to fertilize the egg, but until now, they have not understood what caused the internal pH in sperm to rise, or alkalinize, due to an inability to measure proton currents across the human sperm membrane.

"The concentration of protons is extremely high at all times while the sperm are in the male reproductive tract, which makes the intracellular sperm environment acidic and inhibits the activity of the sperm cell," Kirichok said. "The way to activate the sperm cell is to enable the protons to leave the cell. Hv1 is what enables them to do that."

Kirichok explained that human spermatozoa are completely still in the male reproductive system, which is an acidic environment, and that an as-yet-unidentified proton pump keeps the proton levels even higher inside the cell. This makes the internal environment, with a pH of 6.0, more than 1,000 times more acidic than the female reproductive tract, which has a pH of 7.4.

"We know the Hv1 channel, when opened, can allow protons to exit, and activates a cascade of biochemical reactions that cause the spermatozoa to move, mature and prepare to fertilize the egg," he said. "In order to activate sperm cells, this ion channel must be activated."

Hv1 also is activated by endocannabinoid anandamide - a substance released by neurons and also the egg membrane. Kirichok said this could explain the confusing results that have been found in exposing sperm to cannabinoids, such as those contained in marijuana.

Marijuana use has long been associated with male infertility, but some studies have shown increased sperm activity in this exposure. Kirichok proposed that marijuana may mimic the endocannabinoid anandamide that is released by an egg cell, activating the Hv1 proton channel and causing the sperms to mobilize and burn out prematurely, while still in the male reproductive tract.

More information: http://www.cell.com

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