Inhibitory peptides help identify receptor complex vital to male fertility

Review Article

The acrosome reaction is critical for the sperm to penetrate the female egg and fertilize it. Progesterone plays a key role in the acrosome reaction, but how this works has been unclear since spermatozoa lack classical progesterone receptors. Wenming Xu and collaborators at Sichuan University, China, The Chinese University of Hong Kong, and the Australian National University, have shown that the progesterone-induced Ca^{2+} influx required for the acrosome reaction involves the progesterone receptor or modulator, gamma-aminobutyric acid type A ($GABA_A$) receptor delta subunit (GABRD), in combination with the $P2X_2$ receptor. This work included dissecting the activity of specific $GABA_A$ receptors with peptides that mimic critical protein interaction motifs, making them useful tools in competitive inhibition studies.

During fertilization, a sperm fuses with the plasma membrane of the female egg and penetrates to fertilize it. Penetration involves the 'acrosome reaction', where the acrosome is a cap-like structure over the anterior half of the sperm's head. This reaction is initiated by a rise in intracellular Ca^{2+} in the head and neck region of the spermatozoa in response to progesterone, which is known to play a key role in fertilization. The acrosome can then fuse with the plasma membrane of the egg cell, leading to penetration of the egg cell and fertilization.

There has been a lot of debate about the nature of the receptor or protein complex responsible for the progesterone-induced influx of Ca^{2+} into the spermatozoa. Several studies have suggested that $GABA_A$-like receptors play a role in this process, and $GABA_A$ channel blockers and antagonists do inhibit the progesterone-induced influx of Ca^{2+} and the acrosome reaction. The complication is that $GABA_A$ ion channels are anion channels, which means that a cation channel must also be involved. Wu and collaborators therefore determined which components of the pentameric $GABA_A$ receptor are involved in the progesterone-induced influx of Ca^{2+}, and also which Ca^{2+} channel or receptor the $GABA_A$ receptor interacts with to achieve this.

Peptides used to dissect receptor structure and function

Investigating the role of the $GABA_A$ receptor meant determining which subtype of this pentameric structure, which is made up of different assemblies of multiple subunits, is involved. To do this, the researchers impeded signaling by using synthetic peptides that mimic critical protein interaction motifs and whose competitive inhibition reduces $GABA_A$ ion channel signaling. These peptides were highly specific, in contrast to conventional inhibitory drugs that inhibit all forms of $GABA_A$ receptor. Initial western blot analysis indicated that mouse spermatozoa expressed a $GABA_A$ variant called GABRD, which consists of δ-subunits, so the researchers focused on peptides directed against a portion of this subunit by using an inhibitory peptide called $\delta_{(392\text{-}422)}$.

Peptide synthesis

$GABA_A$ competitor peptides were synthesized using standard fluorenylmethyloxycarbonyl (Fmoc) chemistry on a Symphony® X peptide synthesizer, purified by C_{18} reverse-phase HPLC and their identity confirmed by mass spectrometry. To facilitate the uptake of peptides into spermatozoa, the peptides were coupled with the cell-penetrating peptide penetratin. All peptides contained an additional cysteine at the N-terminus and disulfide bonds were formed between each $GABA_A$ peptide and penetratin under non-reducing conditions and purified by C_{18} reverse-phase HPLC.

The inhibitory peptide reduces progesterone-induced Ca^{2+}influx

The first confirmation that delta subunits were involved in the Ca^{2+}influx came with an experiment that demonstrated that increased intracellular levels of Ca^{2+} in spermatozoa after progesterone stimulation were suppressed by the $\delta_{(392-422)}$ peptide but unaffected by a scrambled δ control peptide or a Y_2-peptide (Figure 1).

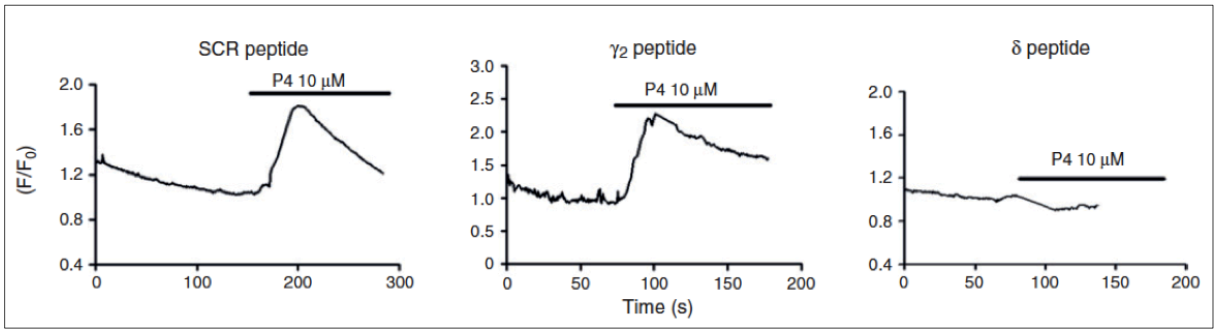

Figure 1. The $\delta_{(392-422)}$ inhibitory peptide reduces progesterone-induced Ca^{2+}influx in spermatozoa (shown by a fluorescence signal, F/F_0) in contrast to a scrambled δ control peptide (SCR) and a Y_2-peptide. From Figure 3b, Xu et al.

A P2X cation channel receptor is also involved

The researchers noted that recent studies had indicated that $GABA_A$ receptors interact with $P2X_2$ receptors, which are ATP-gated cation channels to modulate agonist-induced changes in intracellular Ca^{2+} in neurons. Not only that, $P2X_2$ receptors are expressed in spermatozoa and mediate ATP-induced Ca^{2+} increases. These observations made $P2X_2$ receptors clear candidates for involvement in the progesterone-induced Ca^{2+} response in spermatozoa.

This possibility was tested using a P2X receptor antagonist and the $\delta_{(392-422)}$ inhibitory peptide. It was discovered that the P2X receptor inhibitor reduced a progesterone-induced increase in Ca^{2+}. Adding $\delta_{(392-422)}$ inhibitory peptide separately also blocked the Ca^{2+} increase to the same extent. Additionally, removing extracellular Ca^{2+} also reduced the progesterone-induced Ca^{2+} increase. The researchers also observed that both $\delta_{(392-422)}$ inhibitory peptide and P2X receptor antagonist had the same effect on the progesterone-induced acrosome reaction.

Immunofluorescence staining and co-immunoprecipitation confirmed that both $GABA_A$ δ- and α-subunits and $P2X_2$ receptor co-localized in the acrosome region of spermatozoa and form a complex. The research team could also show that progesterone inhibited the interaction between GABRD and $P2X_2$ receptors, which could be reversed by adding the $\delta_{(392-422)}$ inhibitory peptide, suggesting that the modulation of GABRD and its interaction with $P2X_2$ receptors are essential for progesterone action in mouse spermatozoa.

The role of GABRD and $P2X_2$ in the human acrosome reaction is confirmed

The insights gained so far into the progesterone-induced control of Ca^{2+} influx had been made using the mouse as an animal model, so it was of particular interest to see if GABRD and $P2X_2$ receptors were involved in a similar way in humans. Staining and co-immunoprecipitation studies showed that the two receptors are expressed in the midpiece of human spermatozoa where they interact directly. The researchers could also show that levels of the δ-subunit were significantly lower in infertile men compared to fertile men, and that reduced expression levels of the δ-subunit could be directly correlated with low acrosome reaction levels.

A valuable insight into human infertility

By applying a range of techniques, including peptide-based inhibition studies, the researchers could show that GABRD is a novel progesterone receptor or modulator that interacts with the $P2X_2$ receptor to generate the progesterone-induced Ca^{2+} influx into spermatozoa to initiate the acrosome reaction that is crucial for egg fertilization. Insights such as this could lead the way in the effort to understand and potentially treat human male infertility.

Reference

Sperm gamma-aminobutyric acid type A receptor delta subunit (GABRD) and its interaction with purinergic P2X2 receptors in progesterone-induced acrosome reaction and male fertility. Xu W, et al. Reprod Fertil Dev. 2017 Sep;29(10):2060-2072. doi: 10.1071/RD16294.

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