Abstract

In human sperm, the steroid hormone progesterone changes the intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$). The progesterone-induced Ca^{2+} signals are biphasic: a rapid, transient increase is followed by a slow and sustained elevation of $[Ca^{2+}]_i$. This effect of progesterone is referred to as “non-genomic”, because the classical, genomic signaling pathway of steroids can be excluded. Most probably, the progesterone action on sperm is important for fertilization. The mechanism underlying the “non-genomic” action of progesterone is unknown. To elucidate the signaling pathway, I investigated the progesterone-induced Ca^{2+} signals using kinetic techniques. The experiments revealed that Ca^{2+} signals are caused mainly – if not exclusively – by Ca^{2+} influx. The progesterone-induced Ca^{2+} influx commences with virtually no latency. This indicates that progesterone interacts directly with plasma membrane proteins that regulate $[Ca^{2+}]_i$. I aimed to identify these target proteins in functional experiments and by using a progesterone-crosslinking approach. I could rule out several Ca^{2+} channels and Ca^{2+} transporters as target proteins for progesterone. Furthermore, I showed that human sperm express the putative progesterone-binding proteins PGRMC1 and PGRMC2. In binding studies, however, progesterone did not bind to the overexpressed, predicted steroid-binding domain of these proteins. For the progesterone-crosslinking approach I used a specifically engineered progesterone-crosslinking molecule. The rationale of the experiment was to analyze crosslinked proteins by mass spectrometry. The results are promising, although, equivocal. Nevertheless, progesterone-crosslinking is a powerful method to identify the target proteins of progesterone in human sperm. However, more synthesis work is needed to improve the progesterone-crosslinking molecules.