BSA-Mediated Rapid Calcium Uptake Initiates sAC Activation and cAMP Production During Mouse Sperm Capacitation

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Mammalian sperm require capacitation to fertilize oocytes while traveling through the female reproductive tract. Capacitation can also be induced *in vitro* by incubating in capacitation medium (CAP) containing energy sources, albumin, Ca^{2+}, and HCO_3^-. We have previously shown that only a subpopulation of mouse sperm increases the intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$) during the incubation in CAP. This rise became rapidly evident, within 1 min of incubation and it remained stable for at least 90 min.

Although previous reports have demonstrated that sAC requires both HCO_3^- and Ca^{2+} to be fully active, addition of each component alone produces a moderate increase in soluble adenylyl cyclase (sAC) activity. While HCO_3^- uptake has been studied, the mechanism for Ca^{2+} influx leading to the initial sAC activation is currently unknown. In the present work, we aimed to investigate early $[Ca^{2+}]_i$ changes required for the initial activation of sAC. We hypothesize that the Ca^{2+} ions required for activation of sAC are delivered by a CatSper-independent mechanism activated by bovine serum albumin (BSA). For that purpose, we analyzed early changes in $[Ca^{2+}]_i$ during capacitation in individual live sperm. Sperm were loaded with the Ca^{2+} dye Fluo-4 AM and analyzed by flow cytometry and single cell imaging. We observed that unlike the late capacitation-associated $[Ca^{2+}]_i$ increase, the rapid rise was independent from the CatSper channel complex, as CatSper1 KO sperm showed a significant rapid increase in $[Ca^{2+}]_i$ ($p=0.0001$ against CatSper1 KO 0 min; n=5) similar to the one from wild-type sperm ($p<0.0001$ against CatSper1 WT 0 min; n=5). This early capacitation-associated $[Ca^{2+}]_i$ increase originated in the sperm head followed by diffusion of Ca^{2+} to the flagellum. To determine which constituent of the CAP medium influences the initial Ca^{2+} uptake, $[Ca^{2+}]_i$ levels were measured in CatSper1 KO sperm after exposing them to either HCO_3^- or BSA. We observed that this Ca^{2+} increase is produced only in response to BSA ($p>0.05$ against CAP 1 min; n=5) and not to HCO_3^- ($p=0.04$ against CAP 1 min; n=5), and the direction of Ca^{2+} flow was like the one observed with CAP media.

To assess the activation of sAC by the rapid Ca^{2+} uptake, we monitored the intracellular cAMP levels by ELISA, in the absence of IBMX. After 1 min of incubation in non-capacitating media, sperm displayed only low levels of cAMP. Addition of BSA induced a significant increase in cAMP ($p=0.04$ against NC 1 min; $n>6$), similar to what is observed in CAP media ($p=0.01$ against NC 1 min; $n>6$). The levels of substrates phosphorylated by PKA in CatSper1 KO sperm, analyzed by immunoblotting using a specific antibody, behaved in agreement with these results, increasing after BSA exposure. Furthermore, the rapid $[Ca^{2+}]_i$ increase and the consequent intracellular cAMP rise were dependent on the presence of extracellular Ca^{2+} in the medium.

Altogether, our results suggest that a subpopulation of mouse sperm increases $[Ca^{2+}]_i$ very rapidly during capacitation due to a CatSper-independent influx of extracellular Ca^{2+}, induced by BSA. This rise in $[Ca^{2+}]_i$ is involved in the initial activation of sAC and consequently in cAMP production. These data provide critical insight into the molecular mechanisms behind sAC regulation that remained unknown and reveal the first stages that trigger the capacitation signaling pathways.

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