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★ Abstract title:

Protein Kinase A modulation by nitric oxide during human sperm capacitation

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Study question:

To further examine how nitric oxide (NO) modulates Protein Kinase A (PKA) activity during the *in vitro* capacitation of human spermatozoa.

Summary answer:
We provide further evidence that NO plays a role in regulating specific phosphorylation events during human sperm capacitation.

What is known already:
Several studies have identified important factors involved in the regulation of sperm capacitation, a physiological process necessary to achieve the fertilization ability. Reactive Oxygen Species such as NO are generated during this process and are beneficial in low concentrations for its progress. It has been reported that NO can modulate PKA-dependent phosphorylation events linked to the capacitation in different species. NO can activate the sAC-cAMP-PKA pathway either directly or by increasing the cGMP levels. A rise in the cGMP concentration may inhibit cAMP degradation, which subsequently leads to PKA activation.

Study design, size, duration:
Semen samples were obtained from normozoospermic donors (n=7) by masturbation after 3–5 days of sexual abstinence. Spermatozoa were incubated for 4 hours in capacitating and non-capacitating conditions. The media were supplemented with 100 µM S-Nitrosoglutathione, a NO donor, and two inhibitors of NO synthesis: 10 mM N^G-Nitro-L-arginine Methyl Ester Hydrochloride and 10 mM Aminoguanidine hemisulfate salt. The experiments were performed in absence and presence of 10 mM L-Arginine monohydrochloride, the substrate for NO production.

Participants/materials, setting, methods:
The protein phosphorylation pattern on Serine and Threonine residues (i.e. PKA activity) was evaluated by Western blotting (WB). Proteins were separated by electrophoresis on 4-15% SDS-polyacrylamide gels and electrotransferred to PVDF membranes. The latter were treated with the following antibodies: rabbit monoclonal antibody anti-protein kinase A (1:2000) and goat anti-rabbit IgG-HRP (1:10000). The relative amount of signal in each membrane was quantified using the ImageQuant TL v8.1 software (GE Healthcare, Life Sciences, Buckinghamshire, UK).

Main results and the role of chance:
Our results indicated that in the presence of Nitric Oxide Synthase inhibitors, spermatozoa showed a lower Serine and Threonine phosphorylation pattern than those capacitated with the NO donor ($p < 0.05$) when quantifying the signal corresponding to the whole WB lane. Moreover, we observed a specific phosphorylation pattern for two PKA substrate species, ~ 87 and ~ 62 kDa, which showed a higher degree of phosphorylation in the presence of S-Nitrosoglutathione ($p < 0.05$). The inhibitory effect on PKA activity when blocking NO synthesis was again evident in the ~ 87 and ~ 62 kDa species.
Our data showed that the presence of L-Arginine had no significant effect when analyzing the signal corresponding to the whole WB lane. However, similarly to the experiment where L-Arginine was not used, the ~ 62 kDa species showed a lower amount of phosphorylation when using NOS inhibitors ($p < 0.05$).
These effects were not observed under non-capacitating conditions.

Limitations, reasons for caution:
The number of samples is small and should be increased. Also, the study should include infertile men in the future.

Wider implications of the findings:
We identified specific PKA substrates such as the species of approximately 87 and 62 kDa, which show a distinct Serine and Threonine phosphorylation pattern. These bands might include key proteins in modulating the events downstream of NO-mediated signaling and could be differently regulated in infertile men.

Trial registration number:
Not applicable.

Keywords:
nitric oxide
sperm
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capacitation