PROTEIN KINASE A MODULATION BY NITRIC OXIDE DURING HUMAN SPERM CAPACITATION

Staicu, FD.[1,2]; Martinez-Soto, JC.[2,3]; Matas, C.[1,2*]

[1]Department of Physiology, Veterinary Faculty, University of Murcia, International Excellence Campus for Higher Education and Research (Campus Mare Nostrum), Murcia, Spain, [2]Institute for Biomedical Research of Murcia (IMIB), Murcia, Spain, [3]IVI-RMA Global, Murcia, Spain.
*e-mail: cmatas@um.es

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WHAT IS ALREADY KNOWN

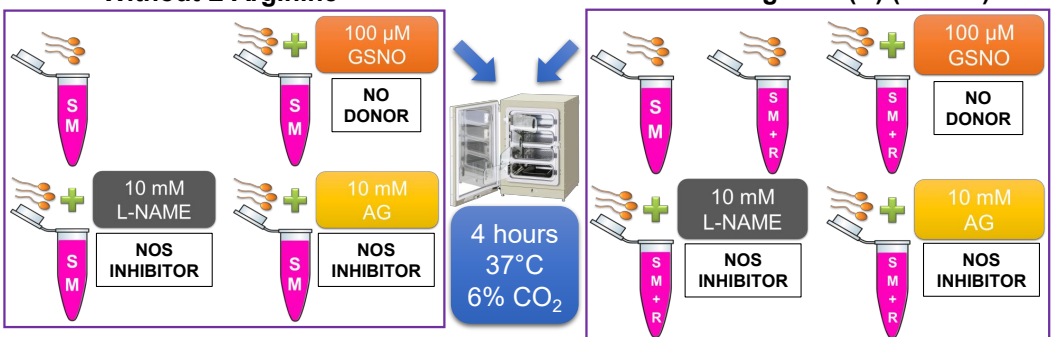
Several studies have identified important factors involved in the regulation of sperm capacitation. Reactive Oxygen Species such as Nitric Oxide (NO) are generated during this process and are beneficial in low concentrations for its progress. It has been reported that NO can modulate PKA-dependent phosphorylation 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.

OBJECTIVE

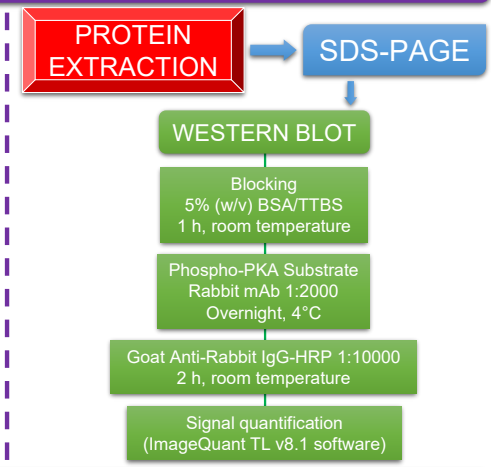
The aim of this study was to further examine how NO modulates PKA activity during the *in vitro* capacitation of human spermatozoa.

MATERIALS AND METHODS

7 normozoospermic samples from donors (3-5 days sexual abstinence)



The incubations were performed with **Sperm Medium (SM)**.



RESULTS

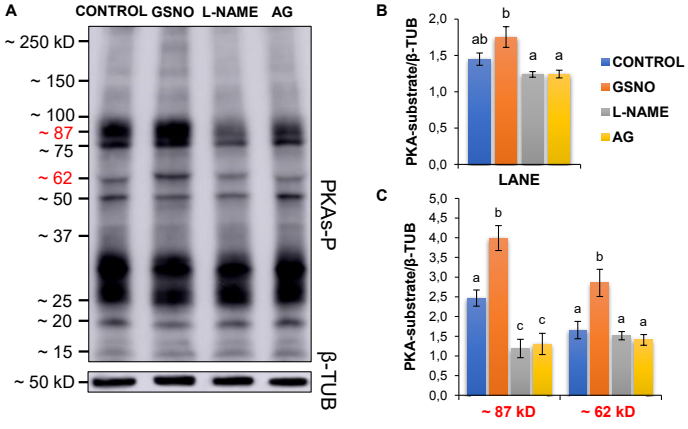


Figure 1. (A) Effect of GSNO, L-NAME and AG on PKA substrates phosphorylation (PKAs-P) in the absence of L-Arginine. (B,C) Relative optical density of PKA substrates. Data are shown as mean ± SEM. Different letters (a,b,c) indicate significant statistical differences between groups ($p < 0.05$). One-way ANOVA and Tukey's multiple comparisons test were performed.

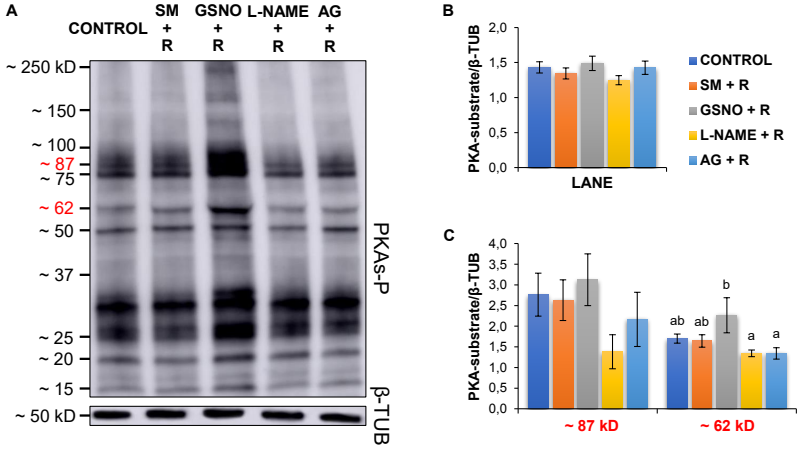


Figure 2. (A) Effect of GSNO, L-NAME and AG on PKA substrates phosphorylation (PKAs-P) in the presence of L-Arginine (R). (B,C) Relative optical density of PKA substrates. Data are shown as mean ± SEM. Different letters (a,b) indicate significant statistical differences between groups ($p < 0.05$). One-way ANOVA and Tukey's multiple comparisons test were performed.

When capacitated in the presence of NOS inhibitors, spermatozoa showed a lower Serine and Threonine phosphorylation pattern than those capacitated with the NO donor when quantifying the signal corresponding to the whole lane (Fig. 1B).
Moreover, we observed a specific phosphorylation pattern for two PKA substrate species, ~ 87 and ~ 62 kD, which showed a higher degree of phosphorylation in the presence of GSNO (Fig. 1C). The inhibitory effect on PKA activity when blocking NO synthesis was again evident in the ~ 87 and ~ 62 kD species (Fig. 1C).
The presence of L-Arginine had no significant effect when analyzing the signal corresponding to the whole lane (Fig. 2B). However, similarly to the experiment where L-Arginine was not used, the ~ 62 kD species showed a lower amount of phosphorylation when using NOS inhibitors (Fig. 2C).

CONCLUSIONS

We identified specific PKA substrates such as the species of approximately 87 and 62 kD, which show a distinct Serine and Threonine phosphorylation pattern. These bands might include key proteins in modulating the events downstream of NO-mediated signaling.

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