Effects of gamma-amino butyric acid, progesterone and oestradiol on human spermatozoa acrosome reaction

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Summary

The acrosome reaction (AR) is influenced by the action of several molecules such as GABA, P₄, and E₂. Objective: to determine the interaction between GABA / E₂, and GABA / P₄ in the AR. Methods: Washed human spermatozoa capacitated for 2 h were incubated in the following conditions: BWW medium alone (control) or BWW plus: P₄, E₂, GABA, GABA+ P₄ or GABA+ E₂. The % of AR was determined by FITC-PSA. Results: Spermatozoa incubated with GABA or P₄ showed higher % of AR; additionally, separate and simultaneous P₄ and GABA incubations showed no significant difference in the % of AR. GABA plus E₂ incubation showed lower % of AR than GABA only incubated spermatozoa. Conclusions: The AR would be modulated by inductors such as GABA and P₄; there is not synergistic interaction between these molecules on AR induction; besides, there are inhibitory hormones such as E₂, whose effect prevails in a combined GABA/E₂ incubation. The localisation of these compounds would determine timely AR occurrence.

Introduction

Spermatozoa acrosome reaction (AR) is a fundamental process in mammalian fertilisation. Only reacted spermatozoa will be able to penetrate the zonae pellucidae (ZP) and fuse with the oocyte (Yanagimachi et al., 1994; del Rio et al., 2007). The acrosome is a vesicle located in the spermatozoon head which contains a variety of enzymes (e.g., acrosin, hyaluronidase) which degrade the ZP so that spermatozoa can pass through it. Fusion between the spermatozoon plasma membrane and the outer acrosomal membrane takes place during the AR. This generates the formation of vesicles exposing the acrosomal content to the extracellular space (Barros et al., 1967). After
The passage of the spermatozoon through the ZP, the equatorial and post-equatorial segment will be able to fuse with the oocyte plasma membrane (Vigil, 1989).

The onset of the AR can result from the interaction of a ligand with a sperm membrane receptor. Steroidal and proteical – such as ZP3 protein – ligands have been identified. Stimulation of the AR by progesterone (P<sub>4</sub>) has been described to be dose-dependent in capacitated spermatozoa (Calogero <i>et al</i>., 1999). On the other hand, oestradiol (E<sub>2</sub>) has been described as an AR inhibitory hormone, reducing the % of AR observed. In a combined administration of E<sub>2</sub> and P<sub>4</sub>, the inhibitory effect of E<sub>2</sub> prevails (Vigil <i>et al</i>., 2008). In addition, gamma-amino butyric acid (GABA) is a well-known inhibitory neurotransmitter within the central nervous system, and it has been reported to be a dose-dependent inductor of the AR (Shi <i>et al</i>., 1997).

The purpose of the present study was to determine the effect of joint administration of E<sub>2</sub> and GABA in the % of AR, and at the same time, to characterise the interaction between GABA and P<sub>4</sub> over the AR.

**Materials and Methods**

The following protocol was approved by the Bioethics Committees of the Pontificia Universidad Católica de Chile and the Fundación Médica San Cristóbal. Human seminal samples were collected from normozoospermic individuals according to World Health Organisation parameters (WHO, 1999). The samples were obtained following 3-5 days of sexual abstinence, and once emitted were incubated at 37 ºC (WHO, 1999). Later, they were washed in a Percoll gradient (84 % lower; 58 % upper), and the pellet was resuspended in BWW medium supplemented with 4 % bovine serum albumin (BSA). The spermatozoa were capacitated for 2 h at 37 ºC in an atmosphere of 5% CO<sub>2</sub>, at a concentration of 10 × 10<sup>6</sup> cells/mL.

The following incubations were performed from a stock of capacitated spermatozoa: control (BSA 4 %); P<sub>4</sub> (10.1 µmol/L); E<sub>2</sub> (840 pmol/L); GABA (10.1 µmol/L); P<sub>4</sub> (10.1 µmol/L) + GABA (10.1 µmol/L) and E<sub>2</sub> (840 pmol/L) + GABA (10.1 µmol/L). Three replicates were performed for each incubation, at 0, 120 or 240 min of exposure to the studied compounds. An aliquot of 100 µL was taken of each replicate, which was stained with Hoechst (1.9 µmol/L) for 15 min (37 ºC, 5 % CO<sub>2</sub>); subsequently, the aliquot was centrifugated for 5 min at 800 g in a solution of PVP + PBS. The obtained pellet was resuspended in 99.8 % ethanol and stored in a freezer for 30 min. Then, a 20 µL aliquot was obtained, placed on a glass plate and incubated in a covered humid chamber with 20 µL FITC-PSA (100 µg/mL) for 15 min. Next, the excess stain was removed by washing it with ultrapure water. Finally, mounting fluid was applied and the aliquot was placed under a cover glass and observed with a fluorescence microscope, determining the % of AR corresponding to each incubation by characterising the spermatozoa acrosomes.

In order to analyse the data obtained, an ANOVA was used for each incubation time. Subsequently, a Tukey’s <i>a posteriori</i> test was performed to determine the groups which evidenced significant difference from the rest. Significance was determined at <i>p</i> < 0.05.
Results

The values of % AR for the different performed incubations show significant difference for 120 and 240 min intervals (one way ANOVA; \( p < 0.05 \); figure 1, figure 2). Tukey’s \textit{a posteriori} test indicated that, for both incubation times, spermatozoa treated with inductors such as \( P_4 \) and GABA showed significantly higher % of AR values than control (\( p < 0.05 \), figure 1, figure 2). Similarly, GABA incubation % of AR was higher than GABA + \( E_2 \) incubation, both for 120 and for 240 min (figure 1). Finally, % of AR values for \( P_4 \), GABA and GABA + \( P_4 \) incubations did not present significant difference for any of the studied times (figure 2).

Discussion

Our research group previously published the interactions between \( P_4 \) and \( E_2 \) at the onset of the AR (Vigil \textit{et al}., 2008), suggesting a hormonal non-genomic regulation of the AR. During the migration of the spermatozoon through the female reproductive tract, these are exposed to different concentrations of steroidal hormones. The mucus secreted by the cells of the cervical crypts contains a high \( E_2 \) concentration in the periovulatory period that would be inhibiting a premature occurrence of the AR; at the same time, the follicular fluid present at ovulation has high \( P_4 \) concentrations that would induce the AR at the moment of fertilisation. The present study was performed...
using the physiological periovulatory concentrations of these hormones (Adamopolous et al., 2000) making it possible to elucidate the physiological interaction between these molecules. The presence of GABA at the level of the uterus has been reported; however, the physiological variations of this compound and its exact location in the female reproductive tract are not known (Erdő et al., 1989).

**Conclusions**

On the basis of the aforementioned results, we suggest that the hormone variations that take place *in vivo* during the menstrual cycle could regulate the AR favouring the timely occurrence of this process. At the cervix, the E₂ contained in the periovulatory cervical mucus would have an inhibitory effect on the occurrence of the AR. Then, the distal third of the Fallopian tube presents high concentrations of P₄ coming from the follicular fluid, which would favour the AR at the moment of the encounter of the spermatozoon and the oocyte. Other compounds, such as GABA, whose presence in the female reproductive tract has been identified, could also play a significant role in the AR. The absence of synergistic interaction between P₄ and GABA suggests there is a mechanism shared by both inductors in the AR regulation. Finally, these non-genomic pathway interactions of GABA, P₄ and E₂ might not be exclusive of the spermatozoon, and be present, as non-genomic events, in other somatic cells as well.
References


