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Effect of FSH and progesterone on human spermatozoa cytosolic calcium

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ABSTRACT

Ejaculated spermatozoa must undergo a number of modifications before fertilizing the oocyte: among these the capacitation and the acrosome reaction. Calcium signals play an essential role in these functional and structural modifications. Mature spermatozoa have few organelles and a very small cytoplasmic volume but maintain the homeostasis of $[Ca^{2+}]_c$ with great accuracy. We study Ca^{2+} mobilization in human spermatozoa exposed to FSH and progesterone by measuring the $[Ca^{2+}]_c$ with the FURA-2AM method and report for the first time that the exposure to FSH (up to 98 ng/ml) produced an increase of $[Ca^{2+}]_c$ to an extent comparable to that observed with 1 μM progesterone. FSH and progesterone increase the spermatozoa $[Ca^{2+}]_c$ by acting primarily on calcium entry from the external medium. The effects of the two hormones on $[Ca^{2+}]_c$ were similar but not identical; the pre-treatment of progesterone blocks the effects of FSH, but not vice-versa. The increase of $[Ca^{2+}]_c$ due to FSH was more sensitive to nifedipine (VOCCs inhibitor) than that of progesterone. The effects of these hormones on calcium homeostasis may be relevant for sperm activation.

1. Introduction

Ejaculated human spermatozoa must be capacitated and undergo the acrosome reaction before fertilizing the oocyte [1,2]. The molecular mechanisms through which this is accomplished have been discussed amply [3].

Calcium plays a fundamental role in all the modification of sperm cells properties occurring after the ejaculation, such as motility, capacitation and the acrosome reaction [4,5]. Therefore, it is not surprising that spermatozoa maintain calcium homeostasis through the regulation of several types of calcium channels [6,7].

The discovery that progesterone may act on sperm through a mechanism that does not require the intervention of a genomic pathway has thrown some light on the activation of spermatozoa [8–10]. The effects of progesterone are mediated by: (a) the increase of $[Ca^{2+}]_c$, (b) the stimulation of the activity of phospholipases, (c) the phosphorylation of proteins and (d) the efflux of chloride [11–13]. Spermatozoa are sensitive to progesterone in a wide range of concentrations. The hormone increases $[Ca^{2+}]_c$ in capacitated and non-capacitated sperm [5,9,14–16].

The high concentration of progesterone (μmolar range) in the proximity of the cumulus oophorus [14,17] activates the entry of calcium through plasma membrane specific ion channels and may participate in triggering the acrosome reaction. Moreover, it has been reported that progesterone may act on intracellular Ca^{2+} stores [6].

Human FSH increases $[Ca^{2+}]_c$ in ovarian [18] and in Sertoli cells [19]. In addition, FSH receptor may act as a Ca^{2+} channel in cultured rat Sertoli cells [20]. FSH has been used as a follicular fluid marker of oocyte development potential [21]. However, the hormone has never been tested on ejaculated spermatozoa where it may contribute to maturation and favour the onset of the acrosome reaction. This prompted us to investigate whether such an effect may be present. If so, FSH may be used as an additional means to treat some forms of male infertility.

Dihydropyridines (such as nifedipine) block the entry of calcium through voltage-operated channels and hamper the acrosome reaction [22,23]. In spermatozoa, nifedipine selectively blocks the VOCCs channels and it has been used to study the complex mechanisms of calcium entry [24].

Therefore, we study the effects of progesterone and of FSH on the cytosolic Ca^{2+} homeostasis in human spermatozoa and use nifedipine to clarify the mechanisms of action of these hormones.

2. Materials and methods

2.1. Chemicals

α-Follitropin (Gonal-F 75) was a kind gift of Serono Pharma S.p.A., Bari, Italy. Bovine serum albumin (≥96%), Nifedipine, Progesterone (4-pregnene-3,20-dione), HEPES (N-[2-hydroxyethyl]-piperazine-N′-[2-ethanesulfonic acid]), EGTA (ethylene glycol-bis(β-aminoethyl ether)N,N,N′,N′-tetracetic acid), Earle's balanced

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salt solution with bicarbonate (EBSS, E2888) and FURA 2-AM were products of Sigma Chemical Co. (St Louis, MO). Before the use, progesterone (0.8 mM) and FURA 2-AM (2 mM) were dissolved in DMSO (dimethyl sulfoxide). Other reagents, all of reagent grade or better, were purchased from common commercial sources.

2.2. Preparation of spermatozoa

Fresh human semen was obtained from apparently healthy, normospermic [25] donors (25–35 years old) and was left 30–40 min at room temperature to liquefy. Spermatozoa were collected by the swim-up procedure described by Lopata et al. [26], partially modified. Briefly, Earle's balanced salt solution (1 ml) also containing 1% bovine serum albumin was stratified over 1 ml of seminal fluid. Samples were incubated for 60 min at 37 °C in a thermostated CO_2 chamber. The upper layer, rich in motile sperm, was collected, cells counted with a Burk's chamber and suspended in calcium-free HBSS buffer (140 mM NaCl, 5.3 mM KCl, 1 mM $MgCl_2$, 5 mM glucose, 25 mM HEPES, adjusted to pH 7.4) (about 10^7 cells ml^{-1}).

2.3. Determination of cytosolic calcium

Spermatozoa, prepared as described above, were mixed with FURA 2-AM to reach 4 μM FURA 2-AM and samples kept for 60 min at 37 °C in the dark. Then, sperm were harvested by centrifugation at 600 × *g* × 10 min and suspended in 10 ml of calcium-free HBSS buffer. Aliquots (about 2×10^6 cells) were centrifuged at 1000 × *g* × 10 min and suspended again in 1 ml of calcium-free HBSS buffer. When specified, the buffer also contained 1 mM $CaCl_2$.

$[Ca^{2+}]_c$ was determined as described [5,27]. Fluorescence was measured with a Perkin-Elmer spectrophotofluorimeter LS 50B, equipped for cytosolic calcium determination (ex 340 and 380 nm; em 510 nm; slit widths 7.5 nm, ex; 7.5 nm, em). Cytosolic calcium concentration was calculated as reported by Grynkiewicz et al. [28].

3. Results

The concentration of cytosolic Ca^{2+} ($[Ca^{2+}]_c$) was measured with the FURA-2AM method. To evaluate the effects of progesterone in our experimental settings, we added increasing amounts of progesterone to cells suspension. The addition of progesterone (0.1–1.0 μM) increased $[Ca^{2+}]_c$ in a dose-dependent fashion (Fig. 1). The increase was transient, reached a maximum at 50 s and levelled to a plateau 150 s after the addition of the hormone. For progesterone concentrations above 1 μM no further increases of $[Ca^{2+}]_c$ were observed (result not shown in Fig. 1). The ability of FSH (98 ng ml^{-1}) 300 s after progesterone to further increase the $[Ca^{2+}]_c$ was also tested but no effects have been found.

The possible actions of FSH were investigated by adding the hormone to cellular suspension. FSH (12–98 ng/ml) increased $[Ca^{2+}]_c$ in a dose-dependent way (Fig. 2). After the initial peak, $[Ca^{2+}]_c$ decreased very slowly. Concentrations of FSH above 98 ng/ml did not produce further increase of $[Ca^{2+}]_c$ (result not shown in Fig. 2).

The addition of progesterone (1 μM) 300 s after that of FSH produced an additional burst of $[Ca^{2+}]_c$ similar to the one that produced by 1 μM progesterone alone (Fig. 2). The increase of $[Ca^{2+}]_c$ due to the addition of progesterone decreased upon increasing FSH concentrations. If samples were pre-treated with 49–98 ng/ml FSH, the increase of $[Ca^{2+}]_c$ due to the subsequent addition progesterone was halved. Therefore, the pre-treatment with progesterone suppressed the increase of $[Ca^{2+}]_c$ due to the addition of FSH (Fig. 1) but the reverse was not true (Fig. 2). This may indicate that the effects of the two hormones did not add to each other. The experiments, whose profile is shown in Figs. 1 and 2, were repeated 10 times and similar results were always obtained. The highest difference between resting $[Ca^{2+}]_c$ values and $[Ca^{2+}]_c$ peaks ($\Delta[Ca^{2+}]$) was found about 50 s after the addition of the drugs. By expressing data as $\Delta[Ca^{2+}]$, the small variations due to different cell preparation disappeared and the coefficient of variation did not exceed 10%.

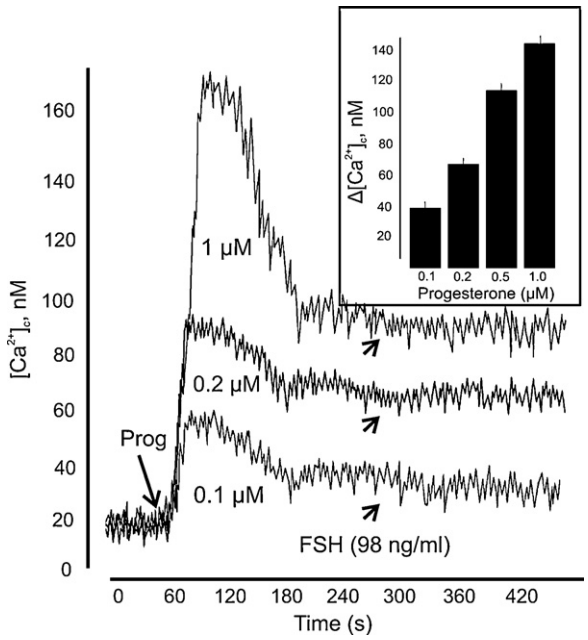

Fig. 1. Time-course of $[Ca^{2+}]_c$ produced by progesterone and the subsequent addition of FSH. Progesterone (at the indicated concentrations) was added when indicated by the arrow. FSH (98 ng ml^{-1}) was added when indicated. The figure reports the finding of a typical experiment. Sperm were suspended in HBSS buffer containing 1 mM Ca^{2+}.The experiment was repeated 10 times and a similar behaviour was always observed. If data are reported as the variation of calcium from the beginning of the experiment ($\Delta[Ca^{2+}]_c$) the coefficient of variation among various experiments did not exceed 10%.
Inset: Maximal increase of $[Ca^{2+}]_c$ ($\Delta[Ca^{2+}]_c$) after the addition of increasing amounts of progesterone. Data are the average of 10 experiments ± SEM.

To study the importance of extracellular calcium, we first omitted the ion from the incubation mixture and calcium was subsequently added. If Ca^{2+} was omitted from the incubation medium, the increase of $[Ca^{2+}]_c$ was dramatically reduced upon the addition of both hormones (Figs. 3 and 4).

Nifedipine was used to study the type of calcium channel connected to the action of the hormones. The effect of FSH (Fig. 3) was

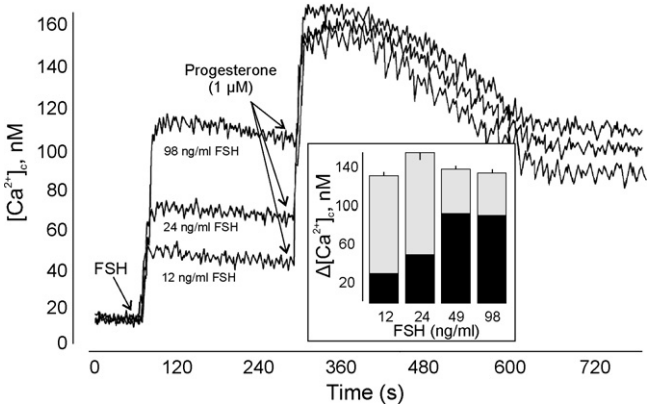

Fig. 2. Time-course of $[Ca^{2+}]_c$ produced by FSH and the subsequent addition of progesterone. FSH (at the indicated concentrations) was added when indicated and 1 μM progesterone was added subsequently. For further detail see Fig. 1.
Inset: Maximal increase of $[Ca^{2+}]_c$ ($\Delta[Ca^{2+}]_c$) after the addition of increasing amounts of FSH (black); the addition of 1 μM progesterone produced a further increase of $[Ca^{2+}]_c$ (grey). Data are the average of 10 experiments ± SEM.