Cell Calcium 53 (2013) 68–75

Contents lists available at SciVerse ScienceDirect

Cell Calcium

journal homepage: www.elsevier.com/locate/ceca

Ca^{2+} mobilization in cumulus cells: Role in oocyte maturation and acrosome reaction

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ARTICLE INFO

Article history:
Received 3 October 2012
Received in revised form 2 November 2012
Accepted 3 November 2012
Available online 4 December 2012

Keywords:
Ca^{2+} mobilization
Cumulus cells
Oocyte maturation
Acrosome reaction

ABSTRACT

The cumulus mass is a group of cells surrounding the oocyte. Cumulus cells are known to play a role in a number of reproductive events including oocyte maturation and acrosome reaction. Calcium mobilization in cumulus cells has been implicated in the process of oocyte maturation and acrosome reaction; however, its exact role remains elusive. In this review, we summarize the findings on Ca^{2+} mobilization in cumulus cells, with particular focus on (1) the gonadotropin/paracrine factors-induced cumulus Ca^{2+} mobilization and its downstream signaling pathways in oocyte maturation and (2) the sperm-derived factors-induced cumulus Ca^{2+} mobilization in the cross-talk between cumulus and sperm and thus acrosome reaction. The evidence suggests that as an important signaling event, Ca^{2+} mobilization in cumulus cells is important to germ cell development and function, and thus essential to fertilization and reproduction.

1. Introduction

In mammals, the oocyte is enclosed by layers of cells called granulosa cells in the follicles. As the follicle develops to a tertiary follicle, the granulosa cells differentiate into two types. Those layers sticking to the follicle wall are called mural granulosa cells, while those layers surrounding the oocyte are named cumulus cells. After the ovulation, the oocyte with the surrounding cumulus layers is expelled from the follicle into the fallopian tube. The cumulus cells, or cumulus oophorus are defined as the cluster of cells that surround the oocyte both before and after ovulation [1]. In the tertiary follicle, the cumulus cells are compacted. The cumulus cells are very closely linked with one another and with the oocyte. Therefore they are often described as the cumulus–oocyte complex (COC). This linkage is not only a physical connection, but also a close cell–cell communication via the gap junctions existing between the cumulus cells and between the cumulus cells and the oocyte, through which signals diffuse [2]. However, at the time shortly before ovulation (preovulatory phase), the ovulation signals, FSH/LH surge, stimulate the cumulus to undergo a form of expansion that transforms the layers of cells into a loose and sticky network due to the secretion of a hyaluronic acid-rich matrix [1,3].

The cumulus cells are thought to be important to oocyte maturation and fertilization. It is known that maturation signals do not directly act on the oocyte, but act on the granulosa/cumulus cells, which transmit the signals to the oocyte and initiate the maturation process [4]. At the time of fertilization, sperm encounter the cumulus cells before they meet the oocyte. Recent studies indicate a cross-talk between cumulus cells and sperm, which is involved in acrosome reaction [5]. In both oocyte maturation and acrosome reaction, a number of signaling pathways are involved and interplay with each other, among which Ca^{2+} and the Ca^{2+}-related pathways are thought to play a critical role [6]. This review will summarize the findings in the past decades on the Ca^{2+} mobilization and the related signaling pathways in cumulus cells, and discuss its role in oocyte maturation and acrosome reaction.

2. Ca^{2+} mobilization in cumulus cells and oocyte maturation

2.1. Oocyte maturation

Oocyte maturation is the process involving the development of the immature oocyte into a mature ovum. It is accompanied with two stages of meiosis: meiosis I and meiosis II. Meiosis I starts at fetal stage and halts at the prophase until puberty. At this stage, the nucleus of the oocyte, which is called germinal vesicle (GV), is still intact and the oocyte at this stage is called GV oocyte. When puberty arrives, the hypothalamus–pituitary system secretes hormones, such as GnRH, FSH and LH, which activate the follicle pool with a number of follicles starting to grow in each ovarian cycle. In the middle of the cycle, Meiosis I resumes upon the FSH/LH surge. The resumption of meiosis is initiated by the breakdown of the GV (GVBD). Then, the oocyte splits into a secondary oocyte and a polar body. Immediately afterwards, Meiosis II begins. The oocyte arrests at the metaphase of Meiosis II (MII) until fertilization. A few

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http://dx.doi.org/10.1016/j.ceca.2012.11.007

hours later, the MII oocyte (also called the egg) with the expanded cumulus mass is extruded from the follicle and enter the fallopian tube. The MII oocyte (egg) is a matured oocyte ready for fertilization [4,7,8].

Oocyte maturation can also be achieved in vitro. Isolated COCs or antral follicles can undergo oocyte maturation in the presence of gonadotropins or growth factors. Although oocyte can undergo spontaneous maturation in culture medium in the absence of gonadotropins if the surrounding granulosa/cumulus cells are removed [9], the maturation rate and developmental capability of the oocyte obtained from spontaneous maturation is significantly lower than that from induced maturation with an intact cumulus mass, suggesting the importance of the interaction between the cumulus and oocyte in oocyte maturation [10].

2.2. Involvement of Ca^{2+} in oocyte maturation

Accumulating evidence has indicated that Ca^{2+} is required for oocyte maturation. Early studies investigated the involvement of Ca^{2+} in oocyte maturation by depleting the extracellular Ca^{2+} or adding Ca^{2+} ionophore, Ca^{2+} chelator, Ca^{2+} channel blocker to the maturation medium. Depletion of intracellular Ca^{2+} store or chelation of intracellular free Ca^{2+} by BAPTA-AM did not affect GVBD [11]. Calcium channel blockers, verapamil and tetracaine or depletion of extracellular Ca^{2+} could inhibit the formation of polar body of denuded mouse oocytes without affecting GVBD process [12,13]. In contrast to mice, BAPTA-AM was reported to inhibit GVBD of denuded pig oocyte [14]. For gonadotropin or growth factor-induced maturation of COC or follicle-enclosed oocyte, Maruska et al. and Fan et al. reported that Ca^{2+} chelators, EDTA or BAPTA-AM, Ca^{2+} channel blockers or depletion of extracelluar Ca^{2+} could remarkably inhibit meiosis I progression of bovine [15,16] and pig COCs [14], but without affecting GVBD or cumulus expansion, suggesting that Ca^{2+} is dispensable for GVBD and cumulus expansion, but is required for completion of the meiosis. However, Goren et al. showed that ionophores, such as A23187 or ionomycin, could induce GVBD in rat COCs [15,16]. Inhibition of intracellular IP_3-sensitive Ca^{2+} store by neomycin or LiCl, or chelation of intracellular Ca^{2+} by BAPTA-AM also produced an inhibitory effect on the gonadotropin-induced GVBD in mouse COCs [11], suggesting a positive role of Ca^{2+} in gonadotropin-induced GVBD. The discrepancy between species suggests that although Ca^{2+} is important for oocyte maturation in different species, the stage at which Ca^{2+} signals are required and the mechanism of Ca^{2+} mobilization may be species-specific. Although these studies indicate an indispensible role of Ca^{2+} in oocyte maturation, the questions as to how the Ca^{2+} signal is triggered and whether cumulus Ca^{2+} signal plays a role in this process remain unclear.

2.3. Maturation signals acting on cumulus cells

In physiological situation, maturation of the oocyte is induced by the action of hormonal [7,17–20] and paracrine [21–26] factors, as shown in Table 1. However, it is generally thought that gonadotropin LH is the most important factor that initiates the maturation process [7]. In mice, the oocyte undergoes GVBD, the initial event of maturation, 3 h after injection of hCG, which is an analog of LH [27]. In the in vitro maturation, LH can induce maturation of the fully grown competent oocyte in the follicles. However, LH receptors (LHR) have not been found in oocytes, but found in the granulosa cells in large follicles [28,29]. Although the expression of LHR in the cumulus is still controversial, it has been detected in the cumulus cells of some species, including human, bovinae and pig [28,30,31]. Low expression of LHR has also been detected in rat and mouse cumulus cells [32–35], although others reported negative finding [29].

FSH receptor is expressed in both granulosa cells and cumulus cells, but not in the oocyte [36]. Although FSH is thought be to more important in follicle development, it also plays a role in oocyte maturation in combination with LH or growth factors [17,18].

Beside gonadotropins, other hormones, such as growth hormone (GH), and paracrine factors, such as the EGF family, are also known to play a role in oocyte maturation. GH is found to have beneficial effect on oocyte maturation when supplemented to COC maturation medium with the presence of gonadotropins [19,20]. EGF and EGF-like factors, including amphiregulin, epiregulin and beta-cellulin are secreted by mural granulosa cells and cumulus cells in preovulatory follicles after gonadotropin surge [37,38]. Their receptors are mainly distributed in cumulus cells of the COC [39,40]. EGF family are reported to mediate or propagate the effect of LH and FSH in oocyte maturation. Both FSH and LH stimulate the expression of EGF-like factors in cumulus or granulosa cells [21–23], which in turn activate the EGF receptor and the downstream ERK1/2 signaling [41]. EGF-like factors can induce oocyte maturation in vitro in the absence of gonadotropins [21]. Moreover, inhibition of EGF receptor by antiserum or inhibitor (AG1478) is able to inhibit the LH- or FSH-induced maturation [21,39,42], demonstrating that EGF receptor is involved in the process of oocyte maturation.

2.4. Ca^{2+} mobilization in cumulus cells induced by maturation signals

Since receptors for gonadotropins or EGF-like factors are expressed in the cumulus cell, but not in the oocyte, it is believed that maturation signals from gonadotropins or EGF-like factors are detected and transduced by the cumulus cells, then transmitted into the oocyte either to input maturation-stimulating signals or to remove the maturation-inhibiting signals in the meiotic arrested oocyte.

When FSH/LH binds to their receptors, which can couple to Gαs, Gαq or Gαi signaling, in the cumulus cells, it activates two main signals corresponding to two types of second messengers: cAMP and Ca^{2+}. Interestingly, cAMP and Ca^{2+} are the most important intracellular signals in oocyte maturation.

The most well-characterized signal is cAMP. cAMP is known to be an inhibitory signal in the oocyte to maintain the meiosis arrest, but a positive signal for maturation in cumulus cells. It is thought that before the gonadotropin surge, cumulus cells transmit their cAMP to the oocyte through the gap junctions, which maintains the meiosis arrest by keeping a high cAMP concentration in the oocyte. However, in the preovulatory phase, increase in cAMP level in the

Table 1
Hormonal and paracrine factors involved in oocyte maturation.

Type of factors	Name of factors	Source	Localization of actions	References
Gonadotropins	FSH	Pituitary	GC and CC	[17,18]
	LH	Pituitary	GC and CC	[7,28–35]
Other hormones	Growth hormone (GH)	Pituitary	CC	[19,20]
Pacaracrine factors	EGF-like factors (amphiregulin, epiregulin, β-cellulin)	GC	CC	[21–23,37–40]
	IGF-1	GC	CC or oocyte	[24,25]
	BDNF, GDNF	GC	CC or oocyte	[25,26]