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Role of Phosphodiesterase 3A in Regulation of Diplotene Arrest of Mammalian Oocytes

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Abstract

Cyclic nucleotide phosphodiesterases (PDEs) are a group of enzymes that regulate cyclic nucleotides such as cyclic adenosine 3', 5' monophosphate (cAMP) and cyclic guanosine 3', 5' monophosphate (cGMP) level in mammalian oocytes. Among all subtypes of PDEs family, PDE 3A is specifically located in the cytoplasm of oocyte. The cAMP is generated in the granulosa cells as well as in oocyte whereas cGMP is produced only in the granulosa cells of preovulatory follicles. PDE 3A hydrolyzes cAMP with great affinity than cGMP in the oocyte. Decrease of oocyte cAMP level initiates downstream pathway to destabilize maturation promoting factor (MPF) that finally results in meiotic resumption from diplotene arrest. On the other hand, specific PDE inhibitors such as cilostamide, milrinone, ORG 9935 and cilostazol reversibly inhibit enzyme activity and prevent cAMP hydrolysis. High level of intraoocyte cAMP inhibits spontaneous meiotic resumption and maintains diplotene arrest in follicular oocyte. Indeed, PDE inhibitors are choice to prevent spontaneous meiotic resumption both *in vivo* as well as under *in vitro* culture conditions to study the meiotic cell cycle regulation in mammalian oocytes.

Keywords: PDE 3A inhibitors; Cyclic nucleotides; Oocyte meiosis; Diplotene arrest

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Introduction

In mammals, oocyte is surrounded by several layers of granulosa cells in follicular microenvironment. These encircling granulosa cells provide nourishment, growth factors, signal molecules and meiosis regulatory factors to the oocyte within the follicle [1]. Inside the follicle, oocyte is not allowed to resume meiosis and remain arrested at diplotene stage for few months to several years depending on mammalian species [2,3]. Maintenance of diplotene arrest is achieved in the follicular microenvironment due to high level of cyclic nucleotides in oocytes [4]. Synthesis and transfer of adenosine 3', 5' monophosphate (cAMP) as well as cyclic guanosine 3', 5' monophosphate (cGMP) from encircling granulosa cells to the oocyte result in the maintenance of diplotene arrest [5] **(Figure 1).** On the other hand, gonadotropin surge increases phosphodiesterase (PDE) activity in the granulosa cells [6,7] and disrupts gap junctions signaling pathway in granulosa cells and between granulosa cells to oocyte [8,9]. Disruption of gap junctions interrupts the transfer of both cyclic nucleotides to the oocyte leading to a transient decrease of their levels in the oocyte [10]. The decrease of cyclic nucleotides level initiate downstream pathway to destabilize maturation promoting factor (MPF). The destabilized MPF triggers meiotic resumption from diplotene arrest [5,7].

Literature Review

Oocyte has an ability to synthesize cAMP sufficient to maintain diplotene arrest [1,11,12]. However, removal of encircling granulosa cells causes a transient decrease of oocyte cAMP level and results in spontaneous meiotic resumption from diplotene

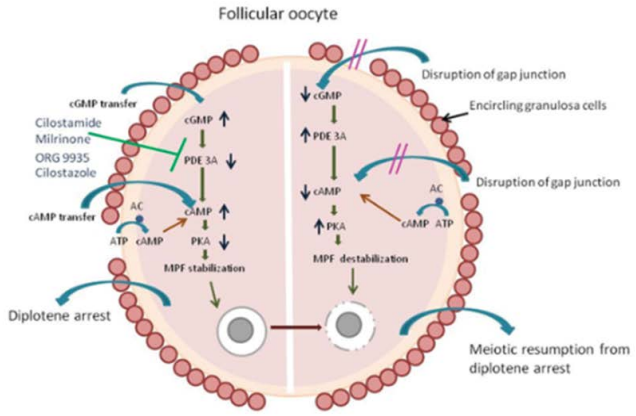

Figure 1 A schematic diagram showing role of PDE 3A in the regulation of cAMP and thereby meiotic arrest at diplotene stage in mammalian oocyte. Transfer of cAMP as well as cGMP from encircling granulosa cells maintains their high level in the oocyte. Increased level of cGMP inhibits PDE 3A activity. Several PDE 3A inhibitors (cilostamide, milrinone, org 9935 and cilostazole) inhibit enzyme activity thus elevating intraoocyte cAMP level. High level of cyclic nucleotides prevents MPF destabilization and maintains high level of stabilized MPF and thereby meiotic arrest at diplotene stage. On the other hand, disruption of gap junction and interruption in the transfer of cyclic nucleotides from encircling granulosa cells to the oocyte cause a transient decrease in intraoocyte cAMP level. The decrease of oocyte cAMP level initiates downstream pathways to destabilize MPF. MPF destabilization finally triggers meiotic resumption from diplotene arrest.

arrest [4,11]. Several drugs that can elevate intraoocyte cAMP level have been used to prevent spontaneous meiotic resumption in mammalian oocytes. Adenylate cyclase activators such as forskolin [13] and broad range PDE inhibitors such as theophylline and IBMX [14] have been used to inhibit spontaneous resumption of meiosis in oocytes. Several drugs that specifically inhibit granulosa cell PDEs mimic the action of gonadotropin and maintain meiotic arrest in mammalian oocytes [6,15]. Among all eleven subtypes of PDE, PDE 3A is located in oocyte and plays a major role in the regulation of cAMP in oocyte.

PDE 3A activity has been demonstrated in several mammalian species including rat [16], mouse [17], bovine [14], cow [18], pig [19], monkey [20] and human oocytes [17,21]. The 80% of the total PDE activity has been reported in bovine oocyte [22] and its activity increases by its phosphorylation through protein kinase A (PKA) [23]. PDE 3A can hydrolyze both cAMP as well as cGMP but affinity towards cAMP is greater than cGMP [24]. In PDE 3A deficient mouse oocyte, the high level of cAMP blocks MPF activation and inhibits meiotic resumption [24,25]. PDE 3A activation decreases oocyte cAMP during meiotic resumption in mouse oocytes [17].

Both, cAMP and cGMP regulate meiotic cell cycle by modulating MPF activity. cGMP elevates intraoocyte cAMP level by inhibiting PDE 3A activity, which is responsible for the cAMP hydrolysis [26]. Elevated cAMP level leads to phosphorylation of cyclin-dependent kinase 1 (CDK1) that triggers MPF stabilization and maintains meiotic arrest at diplotene stage [9,27] **(Figure 1).** A decrease in cGMP level by its specific PDEs relieves the PDE 3A inhibition in the oocyte [8,15] and reduces cAMP level. Decreased level of cAMP inactivates PKA, destabilizes MPF and triggers meiotic resumption in rat oocytes [9,28].

Successful fertilization and embryo development depend on the nuclear as well as cytoplasmic maturation of the oocyte. PDE 3A inhibitors could be useful as a strategy to promote the oocyte cytoplasmic maturation by maintaining the meiotic arrest for short period of time *in vitro* in mouse [29], monkey [20] and bovine [30]. Specific PDE 3A inhibitors, such as cilostamide [13], milrinone [31], ORG 9935 [32] and cilostazol [33] improve oocyte maturation and developmental competency in pig, lamb, monkey and mouse oocytes. The PDE 3A is sufficient to hydrolyze oocyte cAMP and trigger meiotic resumption from diplotene arrest in several mammalian species [7]. Hence to study a complex process of meiosis in follicular oocytes, we describe the use of several drugs that reversibly inhibit oocyte meiosis under *in vitro* as well as *in vivo* conditions.

Cilostamide

Cilostamide, N-cyclohexyl-N-methyl-4-[(2-oxo-1H-quinolin-6-yl) oxy] butanamide, is one of the most studied inhibitors of PDE 3A in mammals. Cilostamide treatment maintains meiotic arrest at diplotene stage in mouse [34], pig [35], ovine [36], bovine [19] and buffalo [37] oocytes cultured *in vitro*. Cilostamide along with forskolin delay spontaneous meiotic resumption from diplotene arrest in pig [13], ovine [38] and human oocytes cultured *in vitro* [39]. Temporary nuclear arrest at diplotene stage by cilostamide is beneficial for spindle/chromosome configurations, improves cytoplasmic maturation and allows better synchronization between cytoplasmic and nuclear maturation in pig [13] and human oocytes [40]. Long-term culture with cilostamide is harmless to oocyte growth, differentiation and maturation in mouse oocyte [21].

Milrinone

Milrinone, 6-methyl-2-oxo-5-pyridin-4-yl-1H-pyridine-3-carbonitrile, is another oocyte specific PDE 3A inhibitor [18,41]. Milrinone treatment maintains diplotene arrest and improves developmental competency of bovine [41] and lamb oocytes *in vitro* [31]. Unlike other PDE 3A inhibitors, milrinone is not found suitable under *in vivo* conditions with several side effects such as fatal arrhythmias; a condition of irregular heart beat [42].

ORG 9935

ORG 9935, 3-(5, 6-dimethoxy-1-benzothiophen-2-yl)-4-methyl-4, 5-dihydro-1H-pyridazin-6-one, is a carboximidamide derivative and specific PDE 3A inhibitor. It blocks spontaneous meiotic resumption from diplotene arrest in mouse [29], monkey [20] and human oocytes *in vitro* [43]. ORG 9935 inhibits gonadotropin-induced meiotic resumption from diplotene arrest in monkey [44] without affecting ovulation *in vivo* [32]. In some experiments, poor tolerance of ORG 9935 has been observed in monkey *in vivo* [45].

Cilostazol

Cilostazol (CLZ), 6-[4-(1-cyclohexyl-1H-tetrazol-5-yl) butoxy]-3,4-dihydro-2-(1H)-quinolinone, is another specific inhibitor of PDE 3A. CLZ prevents pregnancy in naturally cycling mouse [46] and swine [47] and induces ovulation of immature (diplotene or M-I) oocytes depending on the time, dose and frequency of administration [48]. It inhibits meiotic resumption in mouse oocytes both *in vivo* as well as *in vitro* [33]. However, CLZ does not inhibit meiotic resumption in rhesus monkey *in vivo* [49]. CLZ is safer than ORG 9935 [50] and milrinone [44]. Contraceptive and safety effects of CLZ are proved in experimental mouse treated with this drug [50]. CLZ could be used as a safe contraceptive drug if its correct dose is identified and to improve pregnancy outcome in infertile women undergoing *in vitro* fertilization (IVF) [33].

Conclusion

The PDE 3A play a major role in the hydrolysis of oocyte cAMP level. The decreased intraoocyte cAMP level results in MPF destabilization and then meiotic resumption from diplotene arrest in mammalian oocytes. On the other hand, PDE 3A inhibitors reversibly inhibit PDE 3A activity and elevate intraoocyte cAMP level. The increased intraoocyte cAMP level prevents MPF destabilization. The high level of MPF heterodimer maintains meiotic arrest in follicular oocytes. These properties of PDE 3A inhibitors make them as first choice to be used during *in vitro* analysis of meiotic cell cycle regulation in mammalian oocytes.

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