The ovarian gap junction protein connexin43: regulation by gonadotropins

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The major role of the ovarian follicle is the timely production of a mature fertilizable oocyte. This mission is accomplished by a gonadotropin-regulated, gap junction-mediated alteration between established and interrupted cell-cell communication. Recent studies have revealed that gonadotropin action on ovarian gap junctions is elicited at the transcriptional, translational and post-translational levels. Here, we review the existing information generated on the molecular mechanisms employed by the gonadotropins to elicit their effect on the ovarian gap junction protein Cx43.

The primordial ovarian follicle, which is formed during prenatal development or shortly after birth, comprises a prophase-arrested oocyte and one layer of granulosa cells. Throughout folliculogenesis, the oocyte grows and acquires the competence to resume meiosis, whereas the granulosa cells proliferate and develop specific steroidogenic capacities. The formation of the primordial follicles is independent of gonadotropin influence and the involvement of the gonadotropic hormones in the early stages of follicle growth is also uncertain. However, later stages of follicular growth and differentiation are heavily dependent on follicle-stimulating hormone (FSH) and luteinizing hormone (LH). Specifically, FSH is the main promoter of follicular maturation, enhancing granulosa cell proliferation and regulating estradiol production. The control of FSH on steroidogenesis is synergized by LH, which also plays a role in the more advanced stages of follicular development, stimulating oocyte maturation, ovulation and luteinization.

The ovarian follicle functions as a single physiological unit. The coordinated function of its different compartments, the oocyte and the cumulus/granulosa cells, is mediated by cell-cell communication that is generated by gap junctions [1]. Early studies have shown that oocyte growth and development are strictly dependent upon the supply of nutrients transmitted from the follicle cells [2–4]. Later studies demonstrated that the meiotic status of the oocyte is also subjected to regulation by the somatic compartment of the ovarian follicle. This control is mediated by the transmission of cAMP from follicle cells, which maintains the oocyte in meiotic arrest [1,5]. Interruption of cell-cell communication in the ovarian follicle, which occurs in response to the preovulatory surge of LH, leads to a drop in intraoocyte concentrations of cAMP, followed by oocyte maturation [6]. More recently, it was postulated that the oocyte does not only receive but also provides regulatory signals, which control the development and differentiation of the follicle cells [7–10]. The most recent studies have provided evidence that the ovarian gap junctions play an indispensable role in germ cell development and ovarian folliculogenesis [11–13].

Gap junctions are specialized regions in closely opposed membranes of neighboring cells [14], which allow the cells to exchange small molecules, thus coordinating their activities [15–16]. Each gap junction channel comprises two symmetrical hemispheres (termed connexons) derived from two neighboring cells. The connexon comprises a hexagonal arrangement of six protein subunits referred to as connexin (Cx). Connexins are encoded by members of a multigene family that are defined by their molecular weight and share high homology. Numerous types of Cxs (e.g. Cx26, Cx30.3, Cx32, Cx37, Cx40, Cx43 and Cx45) have been identified in ovarian tissues of different mammalian species [17]. Here, we summarize the gonadotropin regulation of Cx43, which is the most abundant Cx found in the ovarian follicle [18].

It is well established that cell–cell communication in the ovarian follicle is mediated by channels predominantly comprising Cx43 [11,18,19]. However, it has been suggested that localization of Cx43 is restricted to the cumulus/granulosa cells, whereas oocytes exclusively produce Cx37 [11]. By contrast, a more recent study has demonstrated the presence of mRNA encoding Cx43 and the protein itself in rat oocytes [20], with the protein localized to the inner side of the oocyte surface. Cattle oocytes have also been shown to produce Cx43 [21]. Collectively, these findings suggest that homotypic Cx43 gap junctions, in addition to heterotypic gap junctions that comprise both Cx37 and Cx43, might be present between the oocytes and their adjacent cumulus cells.

Gonadotropin-regulated production of Cx43

The ovary is a dynamic structure in which follicles are constantly developing from the primordial stage. The first growing follicles appear in mammalian ovaries around birth, but the process of follicular growth culminates in ovulation only with the initiation of reproductive cycles at the time of puberty.
Cx43 has been detected in primordial follicles and its accumulation has been shown to accompany follicular growth [22]. However, there is currently no evidence for the involvement of gonadotropins at this early stage. Regulation of the ovarian gap junction protein by gonadotropins was initially suggested by the correlation between changes in Cx43 synthesis throughout the estrous cycle and the profile of serum concentrations of FSH and LH. These studies found an increase in the amount of Cx43 in the large antral follicles between metestrus and the morning of proestrus, a stage at which serum concentrations of FSH are raised [22]. Conversely, the preovulatory surge of serum concentrations of LH was followed by a drop in the level of mRNA encoding Cx43 [19,22]. This effect of LH was accompanied by a substantial reduction in the membrane areas of gap junctions [23], and in the amount of the Cx43 protein [22]. Administration of nembutal to rats at early proestrus blocks the release of the hypothalamic gonadotropin releasing hormone and consequently inhibits the preovulatory surge of LH. This treatment prevented the reduction in Cx43 levels, indicating that the downregulation of this gap junction protein can be attributed to LH [22].

More direct evidence for the regulatory effects of LH and FSH on the synthesis of the ovarian gap junction protein was provided by the exogenous administration of these gonadotropins. An early ultrastructural study demonstrated that FSH injected into hypophysectomized rats promoted an increase in the ovarian gap junctional membrane [24], whereas a dose of human chorionic gonadotropin (hCG), which induces ovulation, led to a significant reduction in the quantity of gap junctions in granulosa cells [25]. Furthermore, the pattern of Cx43 modifications throughout the estrous cycle can be mimicked precisely by exogenous gonadotropins in the super-ovulation experimental rat animal model as follows: the FSH-like hormone, pregnant mare's serum gonadotropin, upregulated the expression mRNA encoding Cx43 and the synthesis of the Cx43 protein, whereas an additional injection of hCG resulted in a reduction in the mRNA encoding Cx43 and the disappearance of the protein [22].

The direct effect of LH and FSH on the synthesis of Cx43 in the ovarian follicle was tested in vitro and results that were similar to those mentioned above were obtained. Exposure of a rat granulosa cell line to porcine FSH resulted in an increase in mRNA encoding Cx43 [26]. By contrast, LH elicited an inhibitory effect on Cx43 synthesis in large preovulatory follicles in vitro [27]. This last study also demonstrated that ovarian Cx43 is present in multiphosphorylated forms, the relative abundance of which varies in response to LH. Specifically, short exposure to LH induced a transient phosphorylation of the protein. Elimination of the protein and a decrease, but not eradication, of mRNA encoding Cx43 was seen after a longer exposure to LH.

That a certain amount of mRNA encoding Cx43 can still be detected, whereas the protein is completely absent, suggests that, in addition to transcriptional, translational and post-translational modifications, LH might also have an effect on Cx43 at the level of protein degradation. Several studies have shown that the turnover of connexins is exceptionally rapid [28,29], and that degradation of Cx43 involves both the lysosome [30] and the proteasome pathways [30–32]. Phosphorylation, in most cases, is a prerequisite for ubiquitination that marks the protein for proteosomal destruction [33]. Therefore, it is plausible that LH-induced Cx43 phosphorylation could represent an event preceding its degradation.

In contrast to its negative regulation of Cx43 protein and mRNA in the preovulatory ovarian follicle, LH has been reported to have a stimulatory effect on Cx43 production in the baboon postovulatory follicle upon its development into a corpus luteum [34]. An increase in the non-phosphorylated form of the Cx43 protein was seen in the early luteal phase, whereas the phosphorylated form predominated in the mid-luteal phase.

**Gonadotropin-regulated gating of Cx43 gap junctions**

The effects of LH and FSH on Cx43 in the preovulatory ovarian follicle are also reflected at the level of gap junction permeability. In addition to upregulating Cx43 synthesis, FSH also induced an increase in electrical coupling in a rat granulosa cell line [26]. Conversely, LH and/or hCG have been shown to downregulate electrical coupling in rat cumulus-oocyte complexes [35] and decrease metabolic coupling in sheep [36], pig [37], hamster [38] and rat ovarian follicles [39]. The decrease seen in the rat temporally coincides with the immediate, LH-induced phosphorylation of the Cx43 protein demonstrated in a later study [27]. It has been speculated that this post-translational modification might induce conformational changes in the Cx43 protein that could account for the closure of the gap junction channels.

Phosphorylation of Cx43 has also been implicated as a regulatory mechanism for the gating of the gap junction channels in several non-ovarian systems [40,41]. Cx43 is usually phosphorylated on serine residues, and the presence of consensus sequences for several serine/threonine kinases, such as protein kinase A (PKA) and C (PKC), has been identified on the Cx43 molecule. Along this line, PKA activity has been shown to induce clustering of junctional channels into functional gap junction plaques [42,43]. Conversely, PKC-induced Cx43 hyperphosphorylation was associated with the closure of junctional channels [42,44,45]. Similarly, Cx43 phosphorylation by mitogen-activated protein kinase induced interruption of junctional communication [45–47]. Cx43 is also phosphorylated on tyrosine residues after activation of src kinase, leading to cellular uncoupling [48,49].

**Signaling pathways involved in gonadotropin action on ovarian Cx43**

It has been well established that the biological activity of both LH and FSH is transduced through
The involvement of sex steroids

FSH and LH are known to stimulate ovarian granulosa cells to produce estrogen and progesterone, respectively. A reasonable assumption would therefore be that the gonadotropin-induced modulations of Cx43 in the ovary could be mediated by these sex steroids. This idea is gaining support from accumulated information regarding the action of steroids on uterine myometrial Cx43. These studies clearly demonstrate that estrogen upregulates, whereas progesterone downregulates, the synthesis of Cx43 in rat myometrium. However, only a few studies on the effect of sex steroid hormones on gap junctions in ovarian follicles have been reported, and the currently available information in this regard is limited. It has been shown that follicular atresia in hypophysectomized rats is accompanied by a decrease in the number of gap junctions and that 17β-estradiol (E2), which rescued the follicles from undergoing atresia, inhibited this reduction [24]. Similarly, it has been shown that treatment of sexually immature rats with an E2 implant induced an increase in mRNA encoding Cx43 and Cx43 protein synthesis in the ovary, and that its withdrawal resulted in follicle atresia, which was accompanied by a reduction in the number of the gap junctions [55].

The mechanism by which the aforementioned steroidal modulates the synthesis of Cx43 in the ovarian follicle has not been investigated, but several studies with myometrial cells suggest that this regulation is elicited at the transcriptional level and is mediated through the Fos/Jun proteins (AP-1 complex), which bind to the promoter of the gene encoding Cx43. Specifically, progesterone has been shown to reduce c-fos and c-jun expression, leading to reduced expression of the mRNA encoding Cx43 and in protein levels [56]. Estrogen, however, induced an increase in c-fos and c-jun expression, leading to an increase in mRNA encoding Cx43 and protein synthesis [56].

Concluding remarks

The currently available information on the mode of gonadotropin regulation of cell–cell communication in the ovarian follicle is presented in Fig. 1. Recent studies directed at the molecular level of resolution suggest that FSH upregulates cellular communication by increasing both the expression of mRNA encoding Cx43 and protein levels. By contrast, LH stimulates a reduction in mRNA encoding Cx43 and leads to the elimination of the protein. This effect of LH is preceded by Cx43 hyperphosphorylation, the functional role of which is not clear. Future studies should be directed at identification of the sites on the Cx43 molecule that are phosphorylated in response to LH, and their possible involvement in regulation of the gating of the ovarian gap junction channels. Such information will also shed light on the specific kinases that mediate LH action on the ovarian Cx43.

the cAMP-dependent PKA biochemical pathway [50]. It is therefore suggested that FSH-induced Cx43 phosphorylation in rat ovarian follicles at proestrus [22] is catalyzed by PKA. Thus, the increase in intercellular communication in granulosa cells exposed to FSH [26] might represent the stimulatory effect of PKA on clustering of junctional channels into functional gap junctions [42,43].

However, the mediatory role of PKA in LH action on Cx43 does not appear to fit into this theory. This apparent contradiction could be solved by reports that, in addition to stimulation of adenylyl cyclase, LH binding to its receptors on the plasma membranes of ovarian cells leads to generation of diacylglycerol, which activates PKC [51,52]. This idea was confirmed by demonstrations that LH-induced progesterone production in rat granulosa cells is mediated by a dual signaling pathway that includes both PKA and PKC [53,54]. In agreement with these findings, it has been shown that LH-induced Cx43 phosphorylation in cultured preovulatory ovarian follicles was partially inhibited by staurosporine, a PKC inhibitor [27]. Taken together, these results suggest that LH-induced phosphorylation of Cx43 is mediated by both PKA and PKC. The identity of the signal transduction pathway that is involved in LH-induced breakdown of communication remains to be elucidated.

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