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Developmental Expression and Regulation of the Gap Junction Protein and Transcript in Rat Ovaries

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ABSTRACT The extensively developed network of cell-to-cell communication, generated by gap junctions, mediates transmission of small molecules between the cells of the ovarian follicle. Our study aimed at the analysis of the ontogeny and regulation of connexin43 (Cx43) the ovarian gap junction protein and its gene expression throughout folliculogenesis. Developmental analysis was performed using ovaries of immature rats at different ages and selected ovarian follicles of sexually mature female rats at different phases of their estrous cycle. In order to establish the effect of hormones involved in regulation of folliculogenesis on Cx43 modulation, the experimental animal model of sexually immature female rats administered with exogenous gonadotropins was employed. Developmental and hormonal modulations of Cx43 protein and its mRNA expression were studied by Western and Northern blot analysis, respectively. We found that Cx43 was undetectable in ovaries of rats on the first postnatal day, with a low level of this protein observed in 11-day-old rats ovaries. Some increase in the amount of Cx43 was observed at the age of 25 days with a dramatic elevation accompanied by phosphorylation of this protein that was specific to large antral follicles of sexually mature proestrous rats. Elimination of the protein was observed at estrus and could be prevented by cancellation of the preovulatory surge of luteinizing hormone (LH). This pattern of Cx43 modifications was mimicked by exogenous administration of hormones as follows: Pregnant mare's serum gonadotropin (PMSG) increased the Cx43 protein expression with a concurrent induction of its phosphorylation while a further human chorionic gonadotropin (hCG) injection resulted in a decrease of the signal. Analysis of the Cx43 mRNA showed a direct correlation between the Cx43 protein level and its gene expression. We conclude that: 1) At early folliculogenesis the ovarian gap junction protein Cx43 and its gene are developmentally regulated; and 2) After antrum formation, transcription, translation, and posttranslational modifications of Cx43 are regulated by gonadotropins. *Mol. Reprod. Dev. 47:231–239, 1997.*

Key Words: gap junctions; Cx43; ovarian follicle; gonadotropins

INTRODUCTION

Throughout folliculogenesis the oocyte grows and the follicular cells proliferate and differentiate. Several studies have demonstrated a strict dependency of oocyte growth and development upon the supply of nutrients transmitted from the follicle cells via gap junctions (Eppig, 1979; Heller et al., 1981; Brower and Schultz, 1981). Gap junctions are specialized regions in closely opposed membranes of neighboring cells that mediate cell-to-cell communication (Gilula and Reeves, 1972; Lowenstein, 1981). Specifically, they enable the cells to exchange small molecules, up to 1 kDa, coordinating their metabolic and electrical activities (Pitts and Simms, 1977; Bennet and Goodenough, 1978). Several types of gap junction proteins, referred to as connexins, have been identified in different tissues; the ovary predominantly expresses the 43 kDa gap junction protein designated as connexin43 (Cx43; Risek et al., 1990).

In addition to transmission of nutrients from the follicle cells to the oocyte, junctional communication in the ovarian follicles also regulates the meiotic status of the oocyte. This action is mediated by transmission of cAMP, the regulatory signal that maintains the oocyte in meiotic arrest (reviewed by Dekel, 1987, 1988a). Resumption of meiosis, also referred to as oocyte maturation occurs prior to ovulation in sexually mature rats and is induced by the preovulatory surge of LH. This hormone interrupts cell-to-cell communication within a selected population of ovarian large antral follicles, leading to a drop in intraoocyte concentrations of cAMP followed by oocyte maturation (reviewed by Dekel, 1988b). Downregulation of the ovarian Cx43 following the preovulatory LH surge has been previously demonstrated (Wiessen and Midgley, 1993). The mechanism of LH action on the ovarian gap junction protein and its gene expression has been characterized by us recently in isolated large antral follicles incubated in vitro with this gonadotropin (Granot and Dekel, 1994).

The ovulatory changes in the ovarian follicles are strictly regulated by gonadotropins. However, the early

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stages of folliculogenesis seem to be independent of these hormones (Greenvald and Roy, 1994). Our present study analyzes the developmental regulation of Cx43 in rat ovaries. Specifically, Cx43 gap junction protein modifications as well as its gene expression throughout folliculogenesis were initially investigated in ovaries of juvenile rats at different postnatal days. Further gonadotropin-induced modifications of the ovarian gap junctions were studied in a selected populations of follicles of sexually mature rats throughout their estrous cycle. Gonadotropin-treated immature rats were used for confirmation of results obtained in sexually mature rats.

MATERIALS AND METHODS

Materials and Agents

Highly specific anti-Cx43 antibodies and the cDNA probe, T7/T3 α19, containing insert sequence encoding to the mRNA for the Cx43 gene, were kindly provided by Dr. N.B. Gilula, The Scripps Research Institute, La Jolla, CA. These anti Cx43 antibodies and the cDNA probe were employed by us previously for the analysis of rat ovarian Cx43 protein and gene expression (Granot and Dekel, 1994). The following hormones and agents have been used: pregnant mare's serum gonadotrophin (PMSG, Gestyl, Organon, Oss, The Netherlands) human chorionic gonadotrophin (hCG, Pregnyl, Organon), and Nembutal (Ceva, Paris, France).

Animals and Tissue Collection

For developmental analysis, 1-, 11-, and 25-day-old Wistar female rats and sexually mature female rats at the morning of each of the 4 days of their estrous cycle were used. Among the sexually mature rats, only those that demonstrated at least two consecutive regular estrous cycles were employed. The different phases of the estrous cycle were identified by daily analysis of the vaginal cell population. The presence of ovulated eggs in the oviduct was further employed for confirmation of the estrous day. Animals were sacrificed by cervical dislocation and their ovaries were removed. In the immature rats, intact ovaries that contained a homogeneous population of follicles were used for analysis. However, ovaries of sexually mature rats contain a heterogeneous population of follicles at different developmental stages. Therefore, in these rats, individual follicles representing a specific stage characteristic for each day of the estrous cycle, were selected as follows: in proestrous rats, only large antral preovulatory follicles were recovered for analysis. On estrus, the selection was limited to young corpora lutea. On the other two days of the cycle the follicular samples included small antral follicles. The individual follicles were separated under a dissecting microscope, as described previously (Dekel and Sherizly, 1983).

In order to block the preovulatory surge of LH, female rats were administered with nembutal (35 mg/kg body weight) at 2 p.m. of proestrus. This agent inhibits the release of the hypothalamic GnRH, leading to a subsequent abolishment of LH secretion preventing ovulation (Daane and Parlow, 1971). One group of nembutal-treated rats was sacrificed on 6 p.m. of proestrus (the time of the preovulatory LH surge in our rat colony). Serum LH levels in these rats were determined and compared to those in rats injected with the vehicle alone. LH was determined by radioimmunoassay (Daane and Parlow, 1971) using NIDDK-r-LH-RP-3 as reference. Reagents were supplied by the National Hormone and Pituitary Program. Another group of nembutal treated rats was sacrificed on the morning of estrus and their oviducts examined for the presence of ovulated eggs. Only rats that failed to ovulate were further employed for this experiment. The ovaries of these rats were removed and the large antral preovulatory follicles were recovered.

To further establish the specific effect of FSH and LH on the ovarian gap junction protein we used the experimental animal model of gonadotropin-treated sexually immature rats (Gilula et al., 1978). Specifically, 23-day-old female rats were administered with either PMSG (10 IU) alone or with PMSG followed by hCG (5 IU) 48 hr later. This treatment protocol induces in our rat colony an ovulatory response of 35 ± 7 eggs/animal. The treated rats were sacrificed at the indicated time points after PMSG or hCG. The ovaries were removed and individual follicles were selected as follows: large antral preovulatory follicles and young corpora lutea in PMSG alone and PMSG/hCG-treated rats, respectively.

For an overall analysis of the follicular populations, intact ovaries from rats at the different ages and treatments mentioned above were fixed in Bouin's solution and further processed for histological examination.

Membrane Preparation

Membranes from intact ovaries and isolated follicles were prepared as described previously (Granot and Dekel, 1994). Tissues were homogenized (Homogenizer Heidolph, Type RZR 1, Germany) for 30 sec in homogenization buffer (20 mM Tris, pH 7.5, 250 mM sucrose), supplemented with 10 mM dithiothreitol (DTT), 2 mM EDTA, 5 mM [Ethylenebis-(oxyethylenenitrilo)]tetraacetic acid (EGTA), pepstatin (1 µg/ml), leupeptin (1 µg/ml), and 1 mM phenylmethylsulfonyl fluoride (PMSF, Sigma, St. Louis, MO). Large tissue particles were removed by slow centrifugation (250 *g*, Sorvall, Type GLC-2) for 5 min. The supernatant was recovered and membranes were isolated by ultracentrifugation for 1 hr at 4°C (45000 rpm Beckman Ultracentrifuge, Type L2-65B, Rotor Type 50 TI). The pellet was resuspended in 100–200 µl homogenization buffer, and protein was determined (Bradford, 1976).

Western Blot Analysis

Samples of isolated membranes were dissolved in Laemmli sample buffer (Laemmli, 1970), boiled, and

loaded (30 μg protein/lane) on 12.5% SDS-polyacrylamide gel. After electrophoresis, the proteins were transferred to a nitrocellulose membrane (Hybond-C super, Amersham). Detection of the Cx43 protein was performed by immunoblot analysis using specific anti-Cx43 antibodies, as described previously by Milks et al. (1988). Detection of the Cx43 protein-antibodies complexes was performed by using protein A, horseradish peroxidase linked (Amersham) and ECL detection reagents (Amersham), according to the manufacturer's protocol.

RNA Extraction and Northern Blot Analysis

The modulations of Cx43 gene expression were evaluated by northern blot analysis as described previously (Granot and Dekel, 1994). cDNA probe T7/T3 α19 which encodes Cx43 (Risek et al., 1990) was labeled with [α-^{32}P]dCTP (Amersham) by random priming, using a kit obtained from Boehringer (Mannheim, Germany). Total RNA was extracted by the acid-guanidinium-phenol-chloroform method (Chomczynski and Sacci, 1987).

Aliquots of RNA (5 μg) were electrophoresed through a 1% Agarose gel containing 6% formaldehyde. The RNA was blotted onto Hybond-N membrane (Amersham, U.K.) and hybridization was carried out at 42°C in buffer containing 50% formamide, 5 × STE (10 mM Tris, pH 8.0, 100 mM NaCl and 1 mM ethylenediaminetetraacetate, EDTA), 1% sodium dodecyl sulfate (SDS), 1 × Denhard's solution, and 20 mM Na-phosphate, pH 6.8. [^{32}P]-labeled cDNA probe was added to a final radioactivity of 2×10^6 cpm/ml. Hybridization with a rRNA cDNA probe was performed under the same conditions. The filter was washed according to the manufacturer's protocol, followed by autoradiography.

Densitometric Analysis

Western and Northern blot autoradiograms were quantitated by densitometric analysis (Computing Densitometer, Molecular Dynamics, Model 300 A). Results of Northern blots were normalized according to rRNA. Each set of experiments was repeated 3–6 times, and similar results were obtained. One representative example of each set of experiments is shown under Results.

RESULTS

Morphological Analysis of the Ovaries Examined

An overall analysis of the follicular populations in the ovaries examined for their Cx43 protein and gene expression throughout this study was performed by histology. Ovaries recovered on the first postnatal day consisted of clusters of primary follicles composed of an oocyte surrounded by one layer of somatic cells (Fig. 1a). In 11-day-old rat ovaries, many secondary follicles in which the oocyte is surrounded by multilayers of follicular cells could be observed (Fig. 1b). At the age of 25 days, the ovaries are populated by many small antral follicles, the somatic component of which is differentiated into the cumulus that is attached to the oocyte and the granulosa cells that compose part of the follicular wall (Fig. 1c).

Unlike the ovaries of the juvenile rats, the ovaries obtained from sexually mature cycling rats contain a heterologous population of follicles at different stages of development, among which primary, secondary, various sizes of antral follicles, and corpora lutea could be observed (Fig. 1d–f). Antral follicles similar in their dimensions represent ovaries of metestrous and diestrous rats (Fig. 1d). The size of the antrum on the proestrous day is significantly expanded (Fig. 1e). The presence of postovulatory follicles transformed into young corpora lutea characterizes ovaries recovered from estrous rats (Fig. 1f).

PMSG treatment of 23-day-old juvenile rats dramatically increased the size of the follicles as a result of their antrum expansion (Fig. 2a). Administration of hCG resulted in the release of the cumulus-oocyte complexes at ovulation and the transformation of the ovulating follicles into young corpora lutea (Fig. 2b).

Developmental Analysis of Cx43 Protein and Gene Expression

Our initial experiments were designed for the analysis of the pattern of the postnatal expression of the ovarian Cx43 protein and its mRNA. For this purpose ovaries of rats at different ages were analyzed. The autoradiogram in Figure 3 shows that the Cx43 protein expression increased with age. While ovarian Cx43 protein was undetectable on the first postnatal day, a low level of this protein was observed in ovaries of 11-day-old rats with some further increase in the amount of Cx43 at the age of 25 days.

The amount of Cx43 protein in ovarian follicles of sexually mature rats was substantially higher than that demonstrated in the juvenile rats. This increase was accompanied by the appearance of two additional forms of the protein with a slightly retarded electrophoretic mobility. The two somewhat larger proteins recognized by the highly specific anti Cx43 antibodies have been previously shown by us to represent phosphorylated forms of Cx43 (Granot and Dekel, 1994). The gradual elevation in the amount of the protein observed in large antral follicles between metestrus and proestrus was followed by almost total elimination of the signal in young corpora lutea isolated on the estrous day of the cycle. The reduction in the amount of the Cx43 protein observed on estrus was completely inhibited in rats that have been administered with nembutal on proestrus. This treatment resulted in cancellation of the preovulatory surge of LH (27.0 ± 4.7 and 0.58 ± 0.04 ng/ml in saline and nembutal-injected rats, respectively) associated with a total block of ovulation.

Developmental analysis of the Cx43 mRNA (Fig. 4) shows a high correlation between the Cx43 protein level

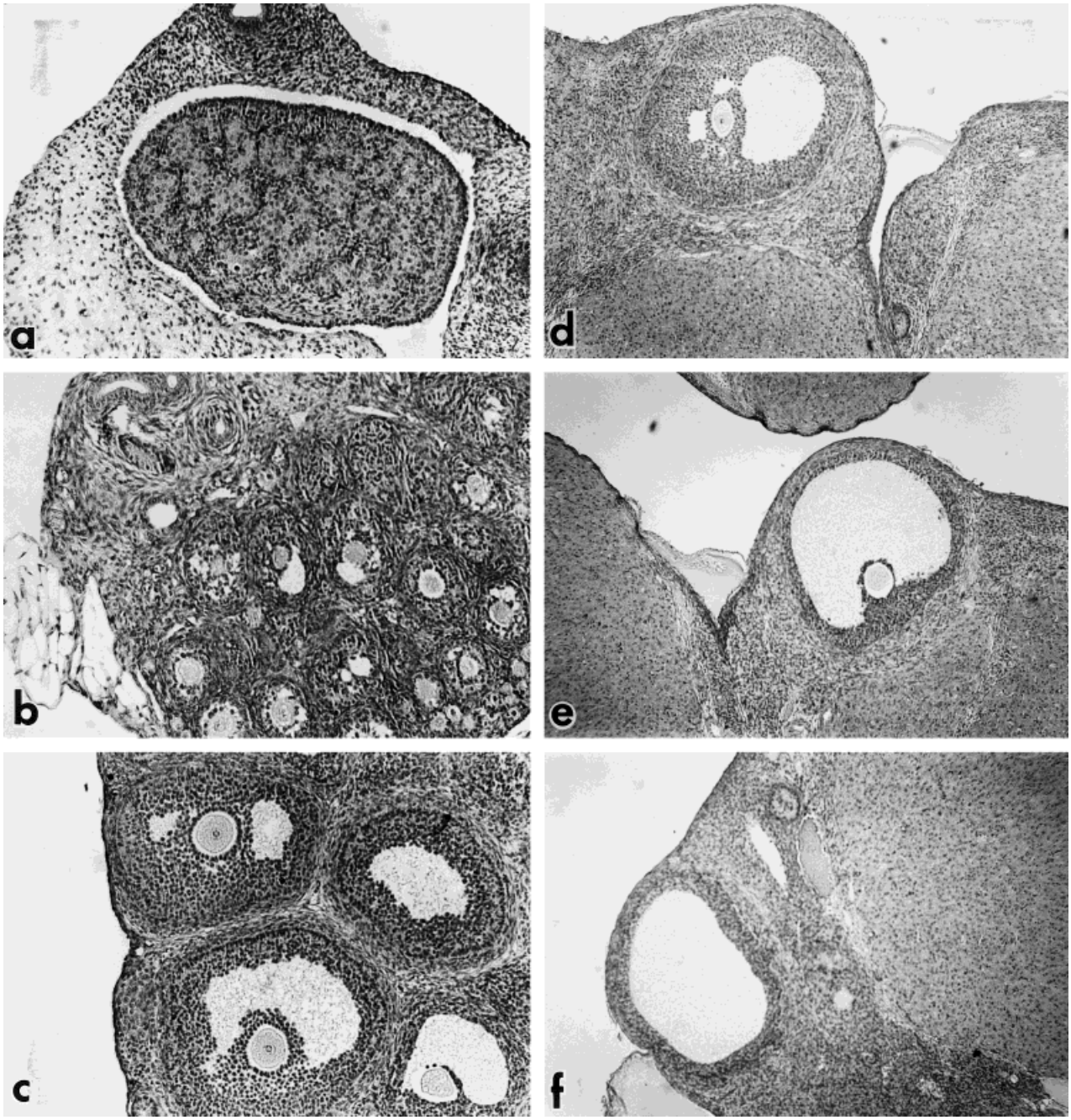

Fig. 1. Histological analysis of developmental changes in rat ovarian follicles throughout folliculogenesis. Ovaries and follicles were isolated from: (**a**) a 1-day-old rat, (**b**) a 11-day-old rat, (**c**) a 25-day-old rat, (**d**) either metestrous or a diestrous rat, (**e**) a proestrous rat, (**f**) an estrous rat (a–c X 1000; d–f ×650).

and its gene expression. The ovarian Cx43 mRNA was not detectable on the first postnatal day. A low level of this transcript was observed in ovaries of 11-day-old rats followed by a further increase in its amount at the age of 25 days. Similar to the results obtained in the protein analysis, we observed an increase in the Cx43 mRNA level in large antral follicles of sexually mature animals on proestrus. This increase in the mRNA level was followed by a sharp reduction in the amount of the Cx43 transcript in postovulatory follicles on estrus.

The Role of Gonadotropins in Regulation of Cx43 Expression

The experiments described above demonstrated the modulations of the ovarian Cx43 in large antral follicles upon the transition from the preovulatory to the post-

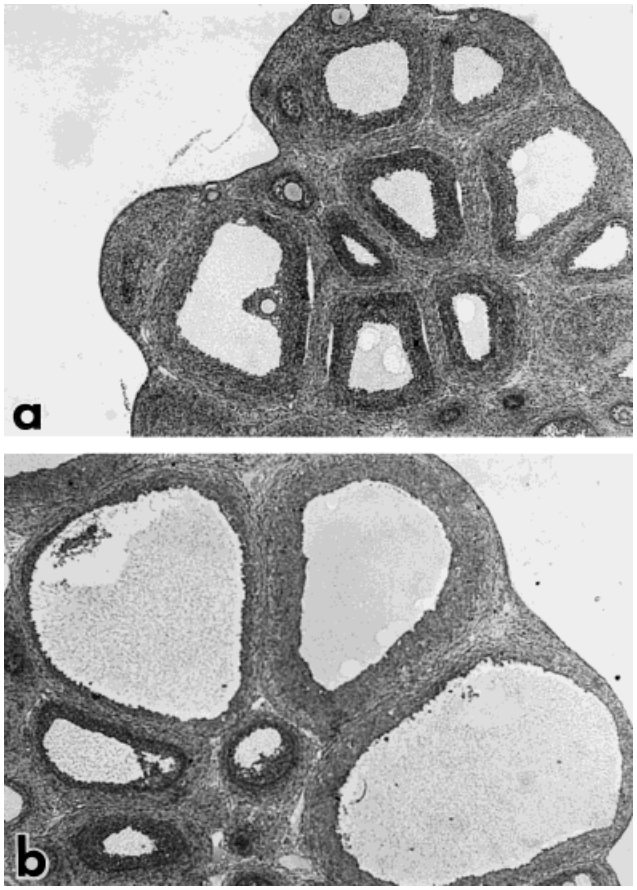

Fig. 2. Histological analysis of ovaries from gonadotropin-treated sexually immature rats. follicles were isolated from: (**a**) a 25-day-old PMSG-treated rat, (**b**) a 25-day-old PMSG/hCG-treated rat, (×400).

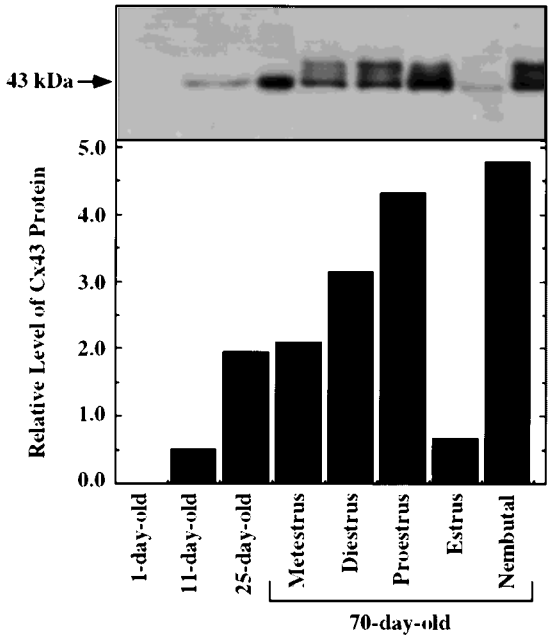

Fig. 3. Developmental modulation of Cx43 protein level. Intact ovaries from 1-, 11-, and 25-day-old rats, and individual follicles selected on each day of the estrous cycle representing specific stages of development, were removed, as described under Materials and Methods. Membranes were isolated and subjected to SDS-PAGE, followed by Western blot analysis using rabbit anti-Cx43 antibodies. Densitometric analysis was performed as described under Materials and Methods.

ovulatory stage of development. Our following experiments aimed at further establishment of the specific effect of each of the two gonadotropins, FSH and LH on the ovarian Cx43. For that purpose, the commonly used experimental model of sexually immature female rats administered with either PMSG alone, or with PMSG followed by an hCG injection was employed. These two hormones represent the effects of FSH and LH, respectively. This treatment protocol that attempts to mimic the sequence of gonadotropin secretion during the estrous cycle, results in the development of large antral follicles in numbers dependent on the dose of PMSG followed by their ovulation. We found that the changes in both the amount and phosphorylation state of Cx43 protein observed at proestrus and estrus were indeed mimicked by the exogenous administration of the hormones as follows: PMSG substantially increased the Cx43 protein expression with a concurrent induction of its phosphorylation while the additional injection of hCG resulted in a decrease of the signal detected at 8 h followed by its elimination at 24 hr (Fig. 5).

The effect of these gonadotropins was also tested on the Cx43 mRNA. Northern blot analysis (Fig. 6) revealed that PMSG increased the Cx43 mRNA level while the additional hCG injection resulted in a significant decrease in the amount of this transcript.

DISCUSSION

Our study demonstrates that the amount of the ovarian gap junction protein Cx43 and its transcript are developmentally regulated. We also show an estrous cycle dependent pattern of Cx43 gene expression, protein translation and post translational modifications. We further provide evidence that the estrous cycle dependent modifications of Cx43 protein and mRNA are regulated by the gonadotropic hormones.

The ontogeny of the ovarian gap junctions has been previously demonstrated by morphological examinations. Using transmission electron microscopy, Merk et al. (1972) described the initial appearance of gap junctions in 18-day-old rat ovarian follicles. Later, more detailed ultrastructural studies (Albertini and Anderson, 1974; Anderson and Albertini, 1976) showed that plaques of gap junctions in rabbit follicle cells are initially detected around the time of antral formation and grow rapidly during expansion of the antrum, attaining their maximal size and frequency in the mature preovulatory follicle.

The use of more sensitive methods such as immunocytochemistry allowed recently detection of rat ovarian Cx43 protein that is limited to the surface epithelium of

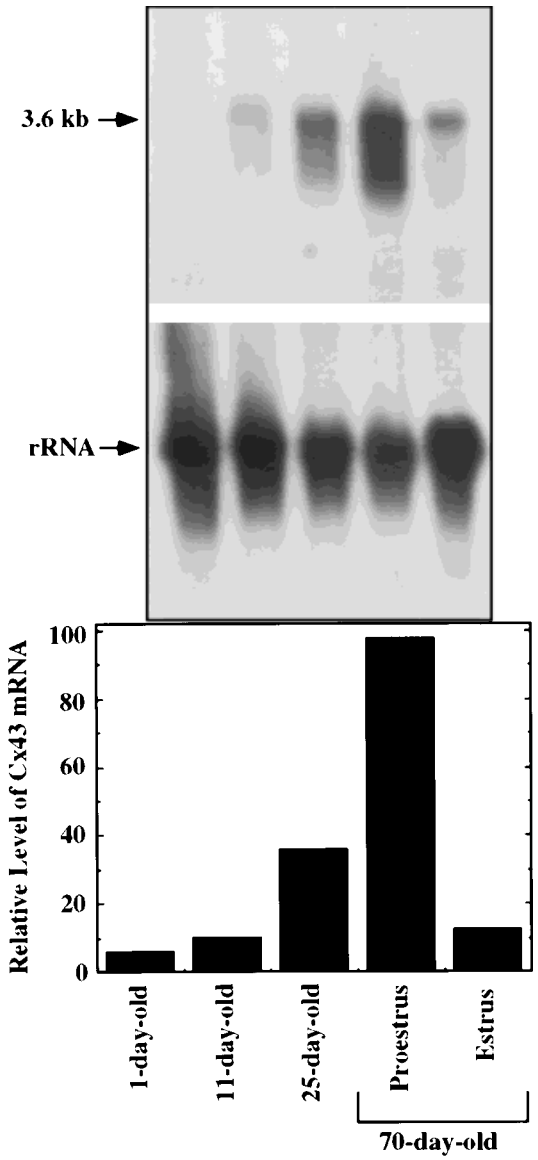

Fig. 4. Developmental analysis of Cx43 gene expression. Intact ovaries from 1-, 11-, and 25-day-old rats and selected follicles from sexually mature rats on proestrus and estrus, as described under Materials and Methods, were analyzed. Total RNA was extracted and the specific Cx43 mRNA was detected by Northern blot analysis using the relevant cDNA probe. Densitometric analysis was performed and normalized according to rRNA, as described under Materials and Methods.

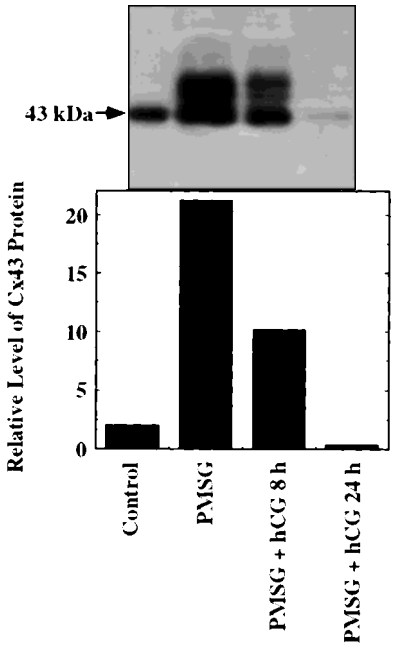

Fig. 5. Gonadotropin-induced modulations of Cx43 protein in ovarian follicles of juvenile rats. Sexually immature female rats administered with either PMSG alone or with PMSG followed by hCG 48 hr later were employed. For analysis, individual follicles were selected as described under Materials and Methods. Membranes were isolated and subjected to SDS-PAGE, followed by Western blot analysis using rabbit anti-Cx43 antibodies. Densitometric analysis was performed as described under Materials and Methods.

the ovary on the 2nd postnatal day (Mayerhoffer and Garfield, 1995). The expression of this protein increased in a direct correlation with follicular development and its presence became prominent at about 20 days of age. Using immunoblot analysis our present study confirms these findings demonstrating an age-related increase in the levels of the ovarian Cx43 gap junction protein. The increase in the amount of the Cx43 protein detected by both, immunoblotting and immunocytochemistry, possibly accounts for the enlargement of the gap junction plaques observed in the previously mentioned ultrastructural studies (Albertini and Anderson, 1974; Anderson and Albertini, 1976).

In addition to our findings on the developmental regulation of the ovarian Cx43 protein, we herein demonstrate, for the first time, that the expression of the gene that encodes for this protein is modified in an age-dependent pattern. The correlation shown in our study, between the amounts of the protein and its mRNA indicates that the developmental regulation is at the level of gene expression.

Modifications of the Cx43 gap junction protein and gene in sexually mature rats throughout the estrous cycle have been studied by Wiesen and Midgley (1993), using immunocytochemistry and in situ hybridization. Their study could not detect changes in the amount of the Cx43 protein between metestrus and proestrus, but showed a decrease in the level of Cx43 protein and mRNA in the granulosa cells of the large preovulatory follicles at late proestrus and early estrus. Our present study demonstrates a sharp decline of the Cx43 protein and its transcript in the young corpora lutea on the morning of estrus and presents clear evidence that this decrease in the Cx43 is specifically related to the LH surge by administration of nembutal. This treatment that inhibits the hypothalamic GnRH secretion, abolishing the hypophyseal LH release and the consequent

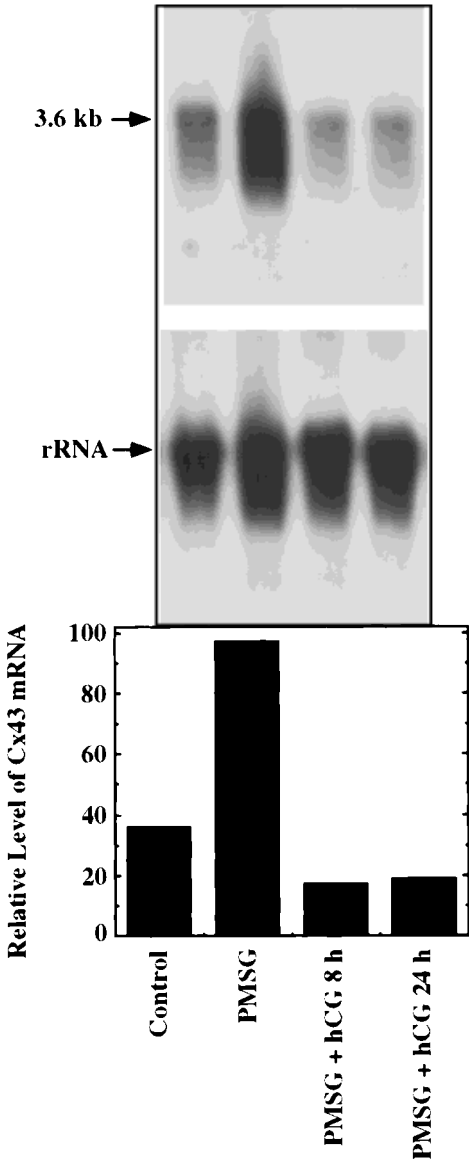

Fig. 6. Effect of gonadotropins on Cx43 mRNA level in ovarian follicles of juvenile rats. Sexually immature female rats administered with either PMSG alone or with PMSG followed hCG were employed. For analysis, individual follicles were selected as described under Materials and Methods. Total RNA was extracted and the specific Cx43 mRNA was detected by Northern blot analysis. Densitometric analysis was performed and normalized according to rRNA.

ovulatory response, totally blocked the reduction in Cx43 levels. Moreover, the use of Western blot analysis enabled us to quantitate our results demonstrating a gradual increase in Cx43 levels between metestrus and proestrus that was not detectable by immunocytochemistry.

The results of our analysis of the Cx43 protein agree with the anatomical examination performed earlier using electron microscopy. These studies showed that exposure of preovulatory rat and rabbit follicles to an ovulatory dose of hCG, leads to a significant reduction in the amount of gap junctions in the granulosa cell membranes. Similarly, a significant decrease in gap junction area was observed soon after the ovulatory stimulus in rat cumulus-oocyte complexes (Larsen et al., 1981, 1986, 1987).

The gonadotropic hormone hCG has also been shown to affect gap junction permeability. Gilula et al. (1978) showed decreasing levels of electrical coupling in rat cumulus-oocyte complexes after hCG injection. Administration of hCG has also been shown to have a negative effect on metabolic coupling in both sheep and rat ovarian follicles (Moor et al., 1980; Sherizly et al., 1988). The hCG-induced down-regulation observed at the functional level correlates with the decrease in the number of gap junctions observed in the above mentioned ultrastructural analysis (Larsen et al., 1981; Larsen et al., 1986; Larsen et al., 1987). Taken together, the modification in both these parameters presumably reflects the reduction in the Cx43 protein expression observed after administration of hCG in our present study. Furthermore, our demonstration of hCG-induced down-regulation of Cx43 mRNA suggests that the hormonal regulation of the ovarian gap junctions is at the level of its gene expression. These in vivo findings fully agree with our previous results (Granot and Dekel, 1994) showing an in vitro LH-induced down regulation of the Cx43 protein and its mRNA in isolated intact rat ovarian follicles.

Most of the studies on the hormonal modulations of the ovarian gap junctions at ovulation focused on the effect of hCG. The only study that investigated the effect of PMSG was performed by Merk et al. (1972). This study showed an increased number of gap junctions per cell associated with follicular development, that was induced, in that case, by exogenous administration of PMSG. Our data demonstrate for the first time the effect of PMSG on the ovarian gap junction protein and its gene expression. The increase in the ovarian Cx43 protein and mRNA level following PMSG treatment obtained in our present study, may account for the elevated number of the gap junctions observed previously by Merk et al. (1972). PMSG possesses intrinsic activities of both gonadotropins, FSH and LH. However, since the granulosa/cumulus cells of ovarian follicles at that developmental stage do not express receptors for LH (Zeleznik et al., 1974), it is unlikely that the Cx43 protein and gene modulation by PMSG is related to its LH activity. Therefore, these results seem to suggest that the increase in the Cx43 protein and mRNA that was observed in juvenile PMSG-treated as well as in sexually mature rats on proestrus may represent the response of the ovarian granulosa/cumulus to FSH.

It is not only the PMSG-induced increase of Cx43 protein and gene expression but also the effect of this gonadotropin on post-translational modifications of the gap junction protein that is herein initially reported. The appearance of phosphorylated forms of Cx43 was also observed in sexually mature rats, suggesting that

PMSG action in the juvenile rat model may resemble such effect of FSH throughout the estrous cycle. The appearance of phosphorylated forms of the Cx43 protein that is correlated with development is apparently not a unique feature of the ovary. Traub et al. (1994) demonstrated that Cx43 in adult mouse heart appears to be phosphorylated to a higher extent than that in embryonic heart. Changes in Cx43 phosphorylation that are induced by LH have been reported by us previously (Granot and Dekel, 1994). This and other studies describe modulations in gap junctions permeability that correlate with phosphorylation/dephosphorylation of Cx43 (Crow et al., 1990; Musil et al., 1990b; Saez et al., 1990; Swenson et al., 1990; Berthoud et al., 1993; Godwin et al., 1993).

In conclusion, the combined results of our present and previous studies are compatible with the following sequence of events: At the early postnatal period the ovarian Cx43 gene expression is gradually elevated resulting in an age-related increase in the protein level. Follicular development that seems to be gonadotropin-independent at these early stages (Greenwald and Roy, 1994) is strictly regulated by these hormones at later stages of folliculogenesis. In the large antral follicles, cell-to-cell communication becomes quite intensive due to FSH-induced elevation in Cx43 gene expression and protein translation. This gonadotropin also stimulates post-translational modifications, manifested as Cx43 phosphorylation that may have a role in regulation of gap junction function. The proestrous LH-surge induces a reduction in the Cx43 transcript that results in elimination of the protein at estrus. Under these circumstances intercellular communication within the ovarian follicle is terminated. Down-regulation of gap junction permeability in rat ovarian follicles has been previously shown to promote oocyte maturation (Piontkewitz and Dekel, 1993).

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