Korean J Biol Sci 3: 53-58, 1999

Identification of Three Types of Voltage Dependent Ca^{2+}-Channels in Mouse Follicular Oocytes

In-Ha Bae*, Sook-Young Yoon, Yong-Dal Yoon[1], Moon Kyoo Kim[1], and Hae-Kwon Kim[2]

Department of Biology, College of Natural Sciences, Sungshin Women's University, Seoul 136-742, Korea;
[1]Department of Biology, College of Natural Sciences, Hanyang University, Seoul 133-791, Korea;
[2]Department of Biology, College of Natural Sciences, Seoul Women's University, Seoul 139-774, Korea

Key Words:
Mouse follicular oocyte
Ca^{2+}-channel
P/Q-type Ca^{2+}-channel
N-type Ca^{2+}-channel
L-type Ca^{2+}-channel

The immunocytochemical method was used to identify the existence of voltage-dependent Ca^{2+}-channels in mouse follicular oocytes. Three types of voltage-dependent Ca^{2+}-channels were shown to exist in the follicular oocytes for the first time, the P/Q-type Ca^{2+}-channel, the N-type Ca^{2+}-channel, and the L-type Ca^{2+}-channel. Among proven Ca^{2+}-channels, distributions of the P/Q-type Ca^{2+}-channel and L-type Ca^{2+}-channel showed localized staining (clustered pattern) on the oolemma. The distribution of the P/Q-type Ca^{2+}-channel showed all localized staining, and the range of localized staining was from 1 to 8 in staining intensity. As the staining intensity increased from 1 to 8, the number of localized staining decreased. The L-type Ca^{2+}-channel are homogeneously stained (29.4%-54.2%), while some of them (around 28.7%-44.1%) showed localized staining on the oolemma. However, the rest of them showed no staining at all (17.1%- 26.5%). On the contrary, the N-type Ca^{2+}-channel showed mostly homogeneous staining, while non-staining oocytes were around 33.8%. The rest showed localized staining (10%). However, staining intensity was much weaker than those of the P/Q-type and L-type Ca^{2+}-channel. In fact, the N-type Ca^{2+}-channel has been known to exist only in neurons (from ectoderm origin), but it is unknown how the N-type Ca^{2+}-channel exists in the follicular oocytes (from mesoderm origin). Further studies are needed to examine the expression of Ca^{2+}-channels during the developmental stages of the oocytes.

In most mammalian oocytes, meiosis is arrested in the ovary at the diplotene stage of the first meiosis. Oocytes in mature follicles resume meiosis in response to the preovulatory surge of gonadotropins. However, oocytes removed from the follicle with or without their surrounding cumulus cells resume meiosis spontaneously *in the absence of gonadotropic stimulation* (Edwards, 1965). However, Ca^{2+} has been found to be involved in germinal vesicle breakdown (GVBD) of both spontaneous oocyte maturation and *in vivo* gonadotropin induced maturation (Batta and Knudsen, 1980; Bae, 1981; De Felici and Siracusa, 1982; Bae and Channing 1985; Santella, 1998).

An increase in intracellular calcium has been shown to have a role in the re-initiation of meiosis in amphibian and mammalian oocytes (Kostellow and Morrill, 1980; De Felici et al., 1991; Kaufman and Homa, 1993).

Mattioli et al. (1990) found that cumulus-enclosed pig oocytes have a resting potential of -41.81 $\pm$ 0.61 mV; however, the removal of cumulus cells caused this potential to drop to -30.95 $\pm$ 0.43 mV. Adding LH to the culture media depolarized the potential of cumulus-enclosed oocytes to -32.90 $\pm$ 0.43 mV. In the meiosis-resumed oocytes *in vivo* (induced by hCG), the membrane potential of the oocytes was depolarized to -28.84 $\pm$ 1.01 mV.

Depolarization accelerated meiotic progression in pig oocytes under control conditions and triggered maturation in the majority of 1 mM dbcAMP arrested-oocytes (45% vs 96% GV). This means that oocyte depolarization is capable of triggering meiosis in pig oocytes (Mattioli et al., 1998). This suggests a linkage between depolarization and GVBD.

However, Yoshida (1982) found that the ovarian oocyte membrane of mice was found to be excitable. Ca^{2+}-dependent action potentials, which were blocked by Co^{2+}, indicated the existence of Ca^{2+}-channels. In addition, Na^{+}-dependent action potential was detected in the Ca^{2+}-free solution. These Na^{+} spikes were insensitive to tetrodotoxin (TTX) and were blocked by Co^{2+}, Cd^{2+}, or La^{3+}, suggesting that Na^{+} goes through the Ca^{2+}-channel instead of the Na^{+}-channel. It was concluded that both Na^{+} and Ca^{2+} pass through the Ca^{2+}-channels during excitation in mouse ovarian oocytes.

De Felici and Siracusa (1982) found that the mouse

* To whom correspondence should be addressed.
Tel: 82-2-920-7171, Fax: 82-2-927-5565
E-mail: ihbae@cc.sungshin.ac.kr

GV oocytes begin to degenerate in the Ca^{2+}-free medium in 1.5 h, and Bae and Channing (1985) found that *in vitro* matured pig oocytes (MII) begin to degenerate in a few hours in a Ca^{2+}-free medium. However, it was found that the cumulus cells surrounding the oocytes delay the degeneration of matured oocytes in a Ca^{2+}-free medium. However, this suggests that the external Ca^{2+} is required for the maintenance of a living state and survival and this kind of requirement can be satisfied by the influx of Ca^{2+} from the culture medium, suggesting that there are Ca^{2+}-channels in the mouse and pig oocytes.

The studies on the existence of the Ca^{2+}-channel in the mouse oocytes have been done, but the channel has not been identified (Yoshida, 1982, 1985). Similarly, Powers (1982) confirmed the existence of a Ca^{2+}-channel, but did not identify what type of channel it was. Blancato and Seyler (1990) also suggested the existence of a Ca^{2+}-channel by treatment with the Ca^{2+} channel blocker, diltiazem and the Ca^{2+}-antagonist, TMB-8, in the fertilization of mouse eggs and embryos, but have not identified the channel.

In the amphibian oocytes, Bourinet et al. (1992) identified the existence of a voltage-dependent Ca^{2+}-channel (L-type Ca^{2+}-channel) which is dihydropyridine sensitive and Charnet et al. (1994) also found the same type of Ca^{2+}-channel (L-type Ca^{2+}-channel) in *Xenopus* oocytes.

Different types of IP_3 receptors (Ca^{2+}release channels) were found in different cell types of an individual and immunocytochemical studies revealed a polarized distribution of IP_3R in the cytoplasm of the animal hemisphere and intensive localization in the perinuclear region of immature *Xenopus* oocytes (Kume et al., 1993). In addition, the distribution and the number of patches of the IP_3R changed depending on the developmental stage of the *Xenopus* oocyte (Kume et al., 1993). The overall structure of the *Xenopus* IP_3R is strikingly similar to that of the mouse IP_3R. The overall amino acid sequences of the *Xenopus* IP_3R shows 90% identity with that of the mouse IP_3R (Parys et al., 1992; Kume et al., 1993). Electrical properties of the cardiac muscles drastically change with development. The changes in the current density of ionic currents of cardiomyocytes are inconsistent among species (Satoh et al., 1996). Thus, it is the same in oocyte development.

Recently, Mattioli et al. (1998) confirmed the existence of a P-type Ca^{2+}-channel in pig follicular oocytes by using a confocal laser scanning microscope and a specific P-type Ca^{2+}-channel inhibitor (ω-agatoxin). This was the first time that the Ca^{2+}-channel was identifieds in mammalian oocytes.

Materials and Methods

Oocyte collection

Thirty-day-old female ICR mice were injected with 5 IU pregnant mares serum gonadotropin (PMSG) and follicle growth was induced. The mice were sacrificed by vertebrate dislocation and both sides of the ovaries were removed at post PMSG 45 h. After trimming the lipid tissues and blood clotting, and 3 washings, the ovaries were put in a watch glass which contained 2 ml of M2 medium with 100 μg/ml dbcAMP.

Under a stereomicroscope (Wild M5, Switzerland) Graafian follicles were ruptured with a 26G needle and cumulus-enclosed oocytes were released into the medium. Cumulus cells were removed by repeated suction and blown out with a small-diameter pasteur pipette with several repeatings. The medium was prepared with pH 7.3-7.4 and an osmolarity of 280 mOsm (Bae and Foote, 1980). The components of the M2 medium are as follows, NaCl, 94.66 mM; KCl, 4.78 mM; $CaCl_2$ 1.71 mM; KH_2PO_4, 1.19 mM; $MgSO_4$, 1.19 mM; Hepes, 20.85 mM; Na-lactate, 23.28 mM; Na-pyruvate, 0.33 mM; BSA (bovine serum albumin), 0.4% (w/v); penicillin G, 0.060 g/L; streptomycin, 0.050 g/L. Healthy looking oocytes with a germinal vesicle (GV) were selected. Selected oocytes were kept for a while until a large enough number of oocytes were collected in an incubator at 37℃, supplied with 5% CO_2 in air in which 100% humidity was maintained.

Ca^{2+}-channel antibodies and secondary antibody

Voltage-dependent Ca^{2+}-channel antibodies, 1) anti-α_{1A} subunit (P/Q-type Ca^{2+}-channel), 2) anti-α_{1B} subunit (N-type Ca^{2+}-channel), 3) anti-α_{1C} subunit (L-type Ca^{2+}-channel), 4) anti-α_{1D} subunit (L-type Ca^{2+}-channel) were bought from the Alomone labs (Israel). The anti-α_{1A} subunit is a polyclonal antibody raised in rabbits against a highly purified peptide (CNA1) corresponding to a residue of 865-881 of the α_{1A}-subunit of rat brain voltage-gated Ca^{2+}-channels, containing N-terminal lysine and tyrosine. The peptide was conjugated to a keyhole limpet hemocyanine with glutaraldehyde. The antibody was affinity-purified on immobilized CNA1. The anti-α_{1B} subunit (N-type voltage-gated Ca^{2+}-channel, corresponding to a residue of 851-867 of α_{1B}), anti-α_{1C} subunit (L-type voltage-gated Ca^{2+}-channel, corresponding to a residue of 818-835 of α_{1C} subunit) and an anti-α_{1D} subunit (L-type voltage-gated Ca^{2+}- channel, corresponding to a residue of 809-825 of α_{1D} subunit) prepared as in anti-α_{1A} as above, were used for identification of the Ca^{2+}-channel.

A biotin-labeled goat anti-rabbit antibody, ABC (avidin-biotin-peroxidase) complex, DAB (diaminobenzidine tetrahydrochloride), and normal goat serum were bought from the Vector lab (Burlingame).

Immunostaining

In this immunocytochemical study, a whole cell mounting method was adopted (Hsu et al., 1981; Middendorff et al., 1996). Follicular oocytes from Graafian follicles were fixed in 4% paraformaldehyde after removal of

the cumulus cells surrounding oocytes in 0.1 M phosphate buffer (pH 7.4) at room temperature for 30-40 min. Oocytes were treated with normal goat serum for 60 min at room temperature to block the endogeneous peroxidase activity and to reduce the nonspecific background staining. Then, primary antibodies were treated at 4℃ in a refrigerator for 16 h but not in the control. All the samples were kept at room temperature for 1 h after they were taken out of the refrigerator. The biotin-labeled goat anti-rabbit antibody (secondary antibody) was treated on the samples for 1 h and then, the ABC (avidin-biotin-peroxidase) complex was treated on the sample for 1 h. Finally, a 0.05% diaminobenzidine tetrahydrochloride (pH 7.4) was treated to stain the samples on the ice for 5-30 min.

To determine the level of nonspecific staining the samples were incubated with: 1) nonimmune goat serum as the first layer and 2) without primary antibodies.

All the samples were examined and photographed with a microscope of Leitz Laboverts (10X, 25X, 40X) equipped with Wild microphot MPS05 (Switzerland).

Results and Discussion

It was found in the preliminary experiment that both the freezing section and paraffin section were not a good method for oocytes and embryos. Therefore, the whole cell mounting method was adopted for this study. The ABC method in the present study showed very good localized staining and homogeneous staining without any difference between the antibody dilutions, 1:60- 200 and 1:120-400. The ABC method has been found to be a valuable tool for both routine histopathology and research. Permanence of the reaction product and usefulness in the fixed tissue sections make the immunoperoxidase method the technique of choice in histopathology at the present time. Several immunoperoxidase staining methods have been described. Among them, the ABC method is the most commonly used method not only because of its high sensitivity, but also because reliable reagents are commercially available. Since the staining intensity of the immunoperoxidase reaction is a function of peroxidase activity, it would be advantageous to bring more than three peroxidase molecules to one secondary antibody to further increase the intensity. Use of the avidin-biotin interaction in immunoenzymatic techniques produces intense staining as well as increased sensitivity (Middendorff et al., 1996).

Recently, significant progress has been made towards the molecular characterization of voltage-gated calcium channels from the skeletal muscle, smooth muscle, heart, and brain (Tsien et al., 1991). After this, it has been found that *Xenopus* oocytes possess a variable pool of voltage-dependent calcium-channels, the previously described T-, L-, N-, and P/Q-type calcium channels and PKA and PKC dependent Ca^{2+}-channels (Bourinet et al., 1992).

Very recently, Mattioli et al. (1998) proved the existence of a P-type calcium channel in pig follicular oocytes for the first time in mammals. In the present study of mouse follicular oocytes in which the immunocytochemical method was adopted, three voltage-dependent Ca^{2+}-channels (P/Q-type, N-type, and L-type) have been shown to exist in the oocytes for the first time. As for the control, there was no staining reaction at all in any part of the oocyte (Fig. 1 and Table 1). Control studies indicated that no nonspecific binding was derived from the biotin-labeled antibody used alone and no nonspecific background problem was ever found practically. Immunocytochemical studies revealed very polarized distributions in the *Xenopus* IP_3R (*Xenopus* IP_3 receptor, Ca^{2+}-release channel of calcium store) and in mouse oocytes IP_3R (Furuichi et al., 1990; Kume et al., 1993). However, the distributions of the P/Q-type calcium channel on the plasma membrane has never been known even in the *Xenopus* oocytes Less is known about α_{1A} (P/Q-type Ca^{2+}-channel subunit), the first DHP-insensitive Ca^{2+}-channel to be cloned, sequenced, and expressed (Mori et al., 1991), with information about its biophysical or pharmacological properties (Sather et al., 1993). In the anti-α_{1A} (P/Q-type Ca^{2+}-channel) treated group of the present studies, it was found that there are 2-3 very clear

Table 1. Number of localized staining and staining intensity of immunostained follicular oocytes

Antibody/ Total No. of oocytes	No staining	Number of localized staining								Homogeneous staining	
		8	7	6	5	4	3	2	1	weak	strong
Control/61	61 (100)										
Antiα_{1A} (1:120)/74	9 (12.2)	1 (1.4)		4 (5.4)	4 (5.4)	6 (8.0)	9 (12.2)	9 (12.2)	21 (28.3)	7 (9.5)	4 (5.4)
Antiα_{1B} (1:400)/77	26 (33.8)							2 (2.6)	6 (7.8)	23 (29.9)	20 (25.9)
Antiα_{1C} (1:400)/68	18 (26.5)			1 (1.5)	3 (4.4)	6 (8.8)	1 (1.5)	9 (13.2)	10 (14.7)	16 (23.5)	4 (5.9)
Antiα_{1D} (1:300)/70	12 (17.1)		1 (1.4)					8 (11.5)	11 (15.8)	19 (27.1)	19 (27.1)

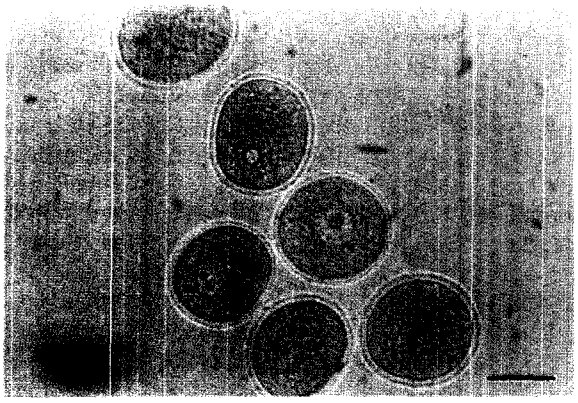

Fig. 1. No staining is found in this control. In this control group, primary antibody treatment was not done and the other procedures are same as in the experimental groups. Scale bar=50 μm.

localized stainings on the oocytes (Fig. 2 and Table 1). This P/Q-type calcium channel was densely enriched in a few sites and to the maximum, 5-6 sites on the oocytes. On the contrary, there were some oocytes which showed homogeneous staining all over the oocytes. However, there were a few oocytes which have not been immunopositively stained at all in the anti-α_{1A} treated group. Any difference in the staining intensity of immunopositively stained oocytes has never been observed in the two dilutions (1:60, 1:120) of the antibody in the present studies.

Of the four major types of Ca^{2+}-channels described in vertebrate cells (designated T, L, N and P/Q), N-type Ca^{2+}-channels are unique in that they are found specifically in neurons, have been correlated with the control of neurotransmitter release, and are blocked by ω-conotoxin, a neuropeptide toxin. However, recently isolated cDNA clones predicted two or more size forms of N-type α_1 subunits with C-terminal ends of different length in the rat and human (Dubel et al., 1992;

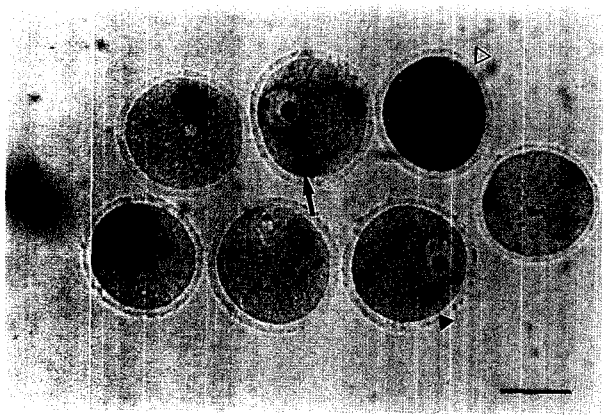

Fig. 2. P/Q-type Ca^{2+}-channels show localized stainings on the oolemma (➡), whereas one oocyte shows no staining at all (▲). Another oocyte shows homogeneous staining (△) rather than localized staining. Scale bar=50 μm.

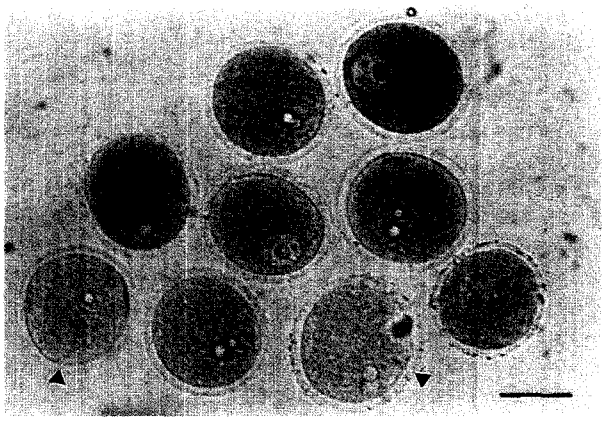

Fig. 3. N-type Ca^{2+}-channels show homogeneous staining all over the oolemma. Two oocytes are not stained (▲) as in the control oocyte, but others show stained oolemma. Scale bar=50 μm.

Williams et al., 1992). Two forms (α_{1B-1} and α_{1B-2}) were identified in human neuroblastoma (IMR32) cells and in the central nervous system, but not in the skeletal muscle or aorta tissue (Williams et al., 1992). N-type Ca^{2+}-channels are distinct in that they have been described only in neurons (Dubel et al., 1992). However, how these N-type Ca^{2+}-channels are present in the mesoderm derived oocytes in the present study has been unknown.

As for the anti-α_{1B} (N-type Ca^{2+}-channel) most of them show homogeneous staining reaction all over the surface of oocytes, and the staining intensity was less weak in comparison to those of localized staining as seen in the P/Q type Ca^{2+}-channel and L-type Ca^{2+}-channel. Around 33% of the oocytes were not stained at all in the anti-α_{1B} (N-type Ca^{2+}-channel) treated group. In this respect, weak staining seems to be a typical characteristic of the N-type Ca^{2+}-channel in mouse oocytes (Fig. 3 and Table 1). There seems to be two types of staining intensity in the anti-α_{1B} (N-type Ca^{2+}-channel). However, it is unclear whether this indicates two different N-types in the present study. There were just a few oocytes as well (10.4%) which showed localized staining as seen in the P/Q type Ca^{2+}-channel in this group, too.

Hell et al. (1993) described that the class C L-type Ca^{2+}-channels are strikingly clustered rather than smoothly distributed along the surface in the rat brain tissue and identified two size forms of the class C α_1 subunit. Immunoblotting revealed two size forms of the class C L-type α_1 subunit, L_{C1} and L_{C2}.

It has been found that 70% of L-type Ca^{2+}-channels could be precipitated with a saturating amount of anti-CNC1 and 20% by the highest available concentration of anti-CND1 (Hell et al., 1993). Emerging evidence suggests that generation of protein products of multiple sizes from a gene encoding individual calcium channels, α_1 subunit, is a common mechanism for the generation of calcium channel diversity. Two different

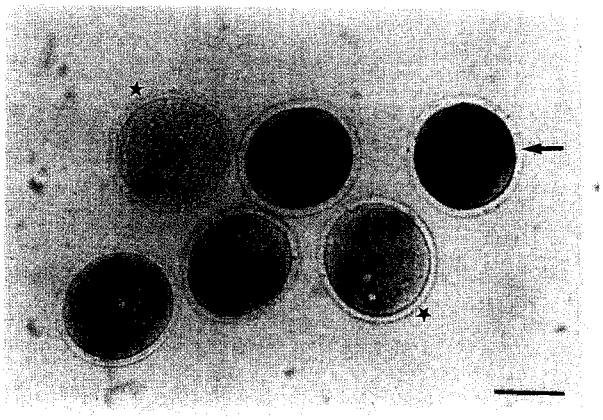

Fig. 4. L-type Ca^{2+}-channels (anti-α_{1C}) show localized stainings (➡) on the oolemma as shown in P/Q-type C2+-channels. Four oocytes show relatively strong staining, whereas two are weakly stained (★). Scale bar=50 μm.

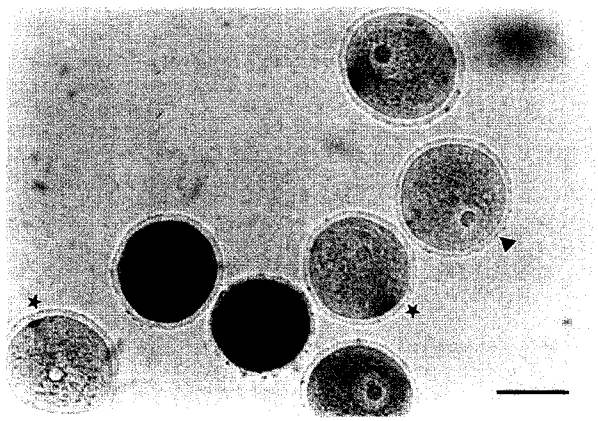

Fig. 5. L-type Ca^{2+}-channels (anti-α_{1D}) also show localized stainings on the oolemma. One oocyte appears not to be stained at all (▲). Two oocytes show very weak localized staining (★). Scale bar=50 μm.

size forms of the skeletal muscle L-type calcium channel α_1 subunit with apparent molecular masses of 190 and 210 kD were described in purified preparations (De Jongh et al., 1991) and in intact skeletal muscle cells and neurons (Lai et al., 1990; Hell et al., 1993). In the staining reaction with anti-α_{1C} (L-type Ca^{2+}-channel, Fig. 4, and Table 1) most of the oocytes showed 2-3 very clear, localized stainings as seen in the anti-α_{1A} (P/Q type Ca^{2+}-channel) and, in addition, some of them showed as many as 4-5 localized stainings. However, a variable number of localized stainings were found in this group. There are even some oocytes which showed no staining at all as seen in the control.

Hell et al. (1993) identified two size forms of the class D L-type α_1 subunit, L_{D1}. and L_{D2}. Class C (α_{1C}, L-type Ca^{2+}-channel) calcium channels were concentrated in clusters, while class D (α_{1D}, L-type Ca^{2+}-channel) calcium channels were generally distributed in the cell surface membrane of cell bodies and proximal dendrites (Hell et al., 1993).

As for anti-α_{1D}, there were also three different patterns of staining (Fig. 5 and Table 1); some showed 1-2 clear localized staining (28.7%), whereas others (54.2%) showed homogeneous staining all over the oocyte surface. Finally, 17.1% of oocytes were not stained at all in this group. All the oocytes do not show the same staining pattern with the antibody in the present study. This means that there is individuality in the number of Ca^{2+}-channel among oocytes. This kind of variable distribution and staining of voltage dependent Ca^{2+}-channels is also a common phenomenon in the nervous system (Cohen et al., 1991; Mills et al., 1994).

However, the anti-α_{1C} showed a more localized pattern of immunoreactivity compared to the anti-α_{1D} antibody.

In our unpublished data it is found that the number of localized staining of the P/Q type Ca^{2+}-channel is the highest in ovulated oocytes, intermediate in follicular oocytes and the lowest in the fertilized ovum. This has two different meanings; one is that the number of Ca^{2+}-channel change depending on the developmental stages, and the other is that the formation and disappearance of Ca^{2+}-channels take place at different developmental stages of oocytes.

Further studies on calcium channels in mouse follicular oocytes are needed in view of developmental changes to the preimplantational stages to the uterus.

Acknowledgements

This paper was supported by the Basic Science Research Institute Program from the Ministry of Education, BSR1-98-4437, a grant for professors of Sungshin Women's University, 1996-1998 and a grant from the Ministry of Science and Technology (97-N6-04-01-A-3).

References

Bae IH and Foote RH (1980) Maturation of rabbit follicular oocytes in a defined medium of varied osmolality. *J Reprod Fertil* 59: 11-13.

Bae IH (1981) Role of calcium in resumption of meiosis of cultured porcine cumulus-enclosed oocytes. In: 14th Annual Meeting, Society for the Study of Reproduction, Oregon State University, Corvallis, Oregon, Abstract 139.

Bae IH and Channing CP (1985) Effect of Ca^{2+} on pig follicular oocyte maturation *in vitro*. *Biol Reprod* 33: 79-87.

Batta SK and Knudsen JF (1980) Calcium concentration in the cumulus enclosed oocytes of rats after treatment with pregnant mare serum. *Biol Reprod* 22: 243-246.

Blancato JK and Seyler DE (1990) Effect of calcium-modifying drugs on mouse *in vitro* fertilization and preimplantation development. *Int J Fertil* 35: 171-176.

Bourinet E, Fouinrer F, Nargeot J, and Charnet P (1992) Endogeneous *Xenopus* oocyte Ca^{2+} channels are regulated by protein kinase A and C. *FEBS Lett* 299: 5-9.

Cohen MW, Jones OT, and Angelides KJ (1991) Distribution of Ca^{2+}-channels on frog motor nerve terminals revealed by fluorescent ω-conotoxin. *J Neurosci* 11: 1032-1039.

Charnet P, Bourinet E, Dubel SJ, Snutch TP, and Nargeot J (1994) Calcium currents recorded from a neuronal alpha 1C L-type calcium channel in *Xenopus* oocytes. *FEBS Lett* 344: 87-90.

De Felici M and Siracusa G (1982) Survival of isolated fully grown mouse ovarian oocytes is strictly dependent on external Ca^{2+}. *Dev Biol* 92: 539-543.

De Felici M, Dolci S, and Siracusa G (1991) An increase of intracellular free Ca^{2+} is essential for spontaneous meiotic resumption by mouse oocytes. *J Exp Zool* 260: 401-405.

De Jongh, Warner KS, Warner C, Colvin AA, and Catterall WA (1991) Characterization of the two size forms of the α_1 subunit of skeletal muscle L-type calcium channels. *Proc Natl Acad Sci USA* 88: 10778-10782.

Dubel ST, Starr TVB, Hell J, Ahlyanian MK, Enyeart JJ, Catherdl WA, and Sunth TP (1992) Molecular cloning of the α1 subunit of an ω-conotoxin-sensitive calcium channel. *Proc Natl Acad Sci USA* 89: 5058-5062.

Edwards RG (1965) Maturation of mouse, sheep, cow, pig, rhesus monkey and human ovarian oocytes. *Nature* 208: 349-351.

Furuichi T, Shiota C, and Mikoshiba K (1990) Distribution of inositol 1,4,5-triphosphate receptor mRNA in mouse tissues. *FEBS Lett* 267: 85-88.

Hell JW, Westenbrock RE, Warner C, Ahlijanian MK, Prystay W, Gilbert MM, Snutch TP, and Catterall WA (1993) Identification and differential subcellular localization of the neuronal class C and class D L-type calcium channel α_1 subunits. *J Cell Biol* 123: 949-962.

Hsu SM, Raive L, and Fanger H (1981) Use of avidin-biotin peroxidase complex (ABC) in immunoperoxidase technique: a comparison between ABC and unlabeled antibody (PAP) procedures. *J Histochem Cytochem* 29: 577-580.

Kaufman ML and Homa ST (1993) Defining role for calcium in the resumption of meiosis in the pig oocyte. *J Exp Zool* 265: 69-76.

Kostellow AB and Morrill GA (1980) Calcium dependence of steroid and guanine 3',5' monophosphate induction of GVBD in *Rana pipiens* oocytes. *Endocrinology* 106: 1012-1019.

Kume S, Muto A, Aruga J, Nakagawa T, Michikawa T, Furuichi S, Nakade S, Okano H, and Mikashiba K (1993) The *Xenopus* IP_3 receptor: structure, function and localization in oocytes and eggs. *Cell* 73: 555-570.

Lai Y, Seager MJ, Takahashi M, and Catterall WA (1990) Cyclic AMP dependent phosphorylation of two size forms of α1 subunits of L-type calcium channels in rat skeletal muscle cells. *J Biol Chem* 265: 20839-20848.

Mattioli M, Barboni B, Bacci ML, and Seren E (1990) Maturation of pig oocytes: observations on membrane potential. *Biol Reprod* 43: 318-322.

Mattioli M, Barboni B, and Gioia L (1998) Membrane depolarization triggers maturation in meiotically arrested pig oocytes by activating P-type Ca^{2+}-channels on the oolemma. In: 21st Society for the Study of Fertility Annual Conference, Glasgow, England, Abstract 28.

Middendorff R, Maronde E, Paust HJ, Miiller D, Davidoff M, and Olcese J (1996) Expression of C-type natriuretic peptide in the bovine pineal gland. *J Neurochem* 67: 517-524.

Mills LR, Niesen CE, So AP, Carlen PL, Spigelman I, and Jones OT (1994) N-type Ca^{2+} channels are located on somata, dendrites, and a subpopulation of dendritic spines on live hippocampal pyramidal neurons. *J Neurosci* 14: 6815-6824.

Mori Y, Friedrich T, Kim MS, Mikami A, Nakai J, Ruth P, Bosse E, Hofmann F, Flockerzi V, Furuichi T, Mikoshiba K, Imoto K, Tanabe T, and Numa S (1991) Primary structure and functional expression from complementary DNA of a brain calcium channel. *Nature* 350: 398-402.

Parys JB, Sernett SW, Delisle S, Snyder PM, Welsh MJ, and Campbell KP (1992) Isolation, and characterization of the inositol 1,4,5-triphosphate receptor protein in *Xenopus laevis* oocytes. *J Biol Chem* 267: 18776-18782.

Powers RD (1982) Changes in mouse oocyte membrane potential and permeability during meiotic maturation. *J Exp Zool* 221: 365-371.

Santella L (1998) Breakthroughs and views. The role of calcium in the cell cycle: facts and hypotheses. *Biochem Biophys Res Commun* 244: 317-324.

Sather WA, Tanake T, Zhang JF, Meri Y, Adams ME, and Tsien RW (1993) Distinctive biophysical and pharmacological properties of class A (B1) calcium channel α_1 subunits. *Neuron* 11: 291-303.

Satoh H, Sada H, Tohse N, and Shigenobu K (1996) Developmental aspects of electrophysiology in cardiac muscle. *Nippon Yakurigaku Zasshi* 107: 213-223.

Tsien RW, Elliner PT, and Horne WA (1991) Molecular diversity of voltage-dependent calcium channels. *Trends Pharmacol Sci* 12: 349-354.

Williams ME, Brust BF, Feldman, Patthi S, Simerson S, Maroufi A, Mccue AF, Velicelebi G, Ellis SB, and Harpold MM (1992) Structure and functional expression of an ω-contoxin-sensitive human N-type calcium channel. *Science* 257: 389-395.

Yoshida S (1982) Na^+ and Ca^{2+} spike produced by ions passing through calcium channels in mouse ovarian oocyte. *Plugers Arch* 395: 84-86.

Yoshida S (1985) Action potentials dependent on monovalent cations in developing mouse embryos. *Dev Biol* 110: 200-206.

[Received December 23, 1998; accepted January 19, 1999]