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Meiosis Reinitiation of Mussel Oocytes Involves L-Type Voltage-Gated Calcium Channel

Martine Tomkowiak, Pierre Guerrier, and Slavica Krantic*

Laboratoire de Biologie Moléculaire et Cellulaire, UMR 49, Centre National de la Recherche Scientifique et Ecole Normale Supérieure de Lyon, 69364 Lyon Cédex 07, France

Abstract In the present work we assessed the involvement of L-type voltage opening Ca^{2+} channels in KCl-induced meiosis reinitiation of metaphase-arrested blue mussel (*Mytilus galloprovincialis*) oocytes by performing binding assays with a tritiated dihydropyridine analog (+)PN 200110. Our data reveal the existence of a single class of dihydropyridine receptors in plasma membrane-enriched rough microsome preparations of mussel oocytes. The apparent affinity (K_d) of characterized receptors equals 1.32 ± 0.21 µM while their maximal binding capacity (B_{max}) is 620 ± 150 pmol/mg protein. The comparison of the rank order of potency of analogs tested to: 1) inhibit [(+)-[^{3}H]PN 200110 specific binding and 2) block KCl-induced meiosis reinitiation pointed to the pharmacological profile similar to but not identical with those previously described for mammalian dihydropyridine receptors. The efficiencies of all antagonists tested are linearly related ($r = 0.995$) in binding- (inhibition of [(+)-[^{3}H]PN 200110 specific binding) and biological (inhibition of meiosis reinitiation) assays thus arguing for functional involvement of L-type Ca^{2+} channels in oocyte activation. Reversibility of antagonist actions on meiosis reinitiation and dependence of receptor binding characteristics on a membrane polarization state further suggested such a role. J. Cell. Biochem. 64:152–160.

Key words: dihydropyridine receptor; pharmacology; metaphase-arrested oocytes; KCl-induced meiosis reinitiation

Different types of voltage-sensitive Ca^{2+} channels have been described in neurons and cardiac and skeletal muscle cells: they were classified according to the specific biophysical and pharmacological properties [Tsien et al., 1991]. One of the most studied voltage-sensitive Ca^{2+} channels is that inhibited by 1,4-dihydropyridines known also as a dihydropyridine (DHP) receptor [Triggle and Rampe, 1989]. Development of Ca^{2+} channel blockers that are more selective than "classical" DHP compounds (nitrendipine and nifedipine) such as (+)PN 200110 permitted receptor purification and subsequent cloning [Jan and Jan, 1989]. Molecular biology data revealed that the DHP receptor consists of five subunits (α1, α2, β, γ, δ) of which only α1 contains a DHP analog binding site [Campbell et al., 1988] and serves a dual function as a voltage sensor and as an L-type Ca^{2+} channel [Nargeot, 1991; Beam et al., 1992].

Calcium channel blockers that are relatively specific for voltage-opening Ca^{2+} channels inhibit fertilization-dependent oocyte activation in bivalve molluscs [Dubé, 1988; Guerrier et al., 1993]. In these species, mature oocytes (oocytes that can be fertilized and are able to initiate and direct embryonic development after fertilization) are arrested at either prophase (e.g., *Spisula, Barnea*) or metaphase (e.g., *Ruditapes, Mytilus*) of the first meiotic division. Fertilization leads universally to oocyte activation and reinitiation and completion of meiosis. The first visible oocyte response to activation consists in germinal vesicle breakdown (for those arrested in prophase) or in first polar body emission (for those arrested in metaphase), followed by second polar body extrusion and pronucleus formation. The onset of oocyte activation depends (entirely or partly according to the species) on the increase of Ca^{2+} influx and subsequent increase of free Ca^{2+} ions within the cytoplasm [Dubé and Guerrier, 1982; Dubé, 1988; Krantic et al., 1991; Abdelmajid et al., 1993]. These ionic events are generally preceded by rapid depolarization of oocyte plasma membrane [Jaffe, 1976; Gould-Sommero et al., 1979; Cross and Elinson, 1980].

In addition to fertilization, activation of bivalve mollusc oocytes can be triggered by vari-

*Correspondence to: Dr. S. Krantic, Lab. Biol. Mol. Cell., ENS, 46 Allée d'Italie, 69364 Lyon Cédex 07, France.

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ous chemical stimuli [Allen, 1953; Dubé and Guerrier, 1982; Osanai and Kuraishi, 1988; Dubé and Dufresne, 1990]. In bivalves, a rise in extracellular potassium (50 to 100 mM KCl) is commonly used in experimental conditions to depolarize plasma membrane and activate oocytes via increasing Ca^{2+} influx [Dubé and Guerrier, 1982; Dubé, 1988; Guerrier et al., 1993; Abdelmajid et al., 1993]. It has been reported that in *Mytilus* oocytes a KCl-induced activation cannot occur in the absence of extracellular Ca^{2+} [Abdelmajid et al., 1993]. Moreover, removal of external Ca^{2+} triggers a decrease in intracellular Ca^{2+} concentrations during meiosis reinitiation induced by either KCl or fertilization [Deguchi and Osanai, 1994]. However, the precise nature of relevant, plasma membrane-associated voltage-opening Ca^{2+} channels has not yet been examined.

DHP-sensitive or L-type Ca^{2+} channels mediate long-lasting Ca^{2+} currents in response to membrane depolarization and hence ensure long-term Ca^{2+} replenishment [Reuter, 1983]. Putative Ca^{2+} channels involved in oocyte activation should have precisely these characteristics. However, a possible role of DHP-sensitive Ca^{2+} channels in oocyte activation has not been assessed as yet.

The aim of the present work was to study the involvement of DHP receptors in KCl-induced meiosis reinitiation of *Mytilus galloprovincialis* oocytes. Our detailed biochemical and pharmacological analysis of specific DHP analog, (+)PN 200110, binding to oocyte preparations revealed the existence of such receptors on oocyte membranes. We assessed their functional involvement in meiosis reinitiation by: 1) comparing the efficiencies of different Ca^{2+} channel blockers in the inhibition of [(+)-[^{3}H]PN 200110 specific binding with their efficiencies in the inhibition of meiosis reinitiation triggered by KCl and 2) studying their regulation in different membrane polarization conditions.

METHODS

Materials

[(+)[Methyl-^{3}H]PN 200110 (88 Ci/mmol; 1 Ci = 3.7 GBq) was purchased from Amersham (Les Ulis, France). (+)Methyl PN 200110 was a generous gift from Sandoz Ltd. (Basel, Switzerland). Other DHP analogs were ordered from RBI (Bioblock, Lyon, France). All other chemicals and drugs were obtained from standard commercial sources.

Handling of Oocytes and Quantification of Their Response to KCl and DHP Analogs

Adult specimens of the blue mussel *Mytilus galloprovincialis* were collected at Roscoff (France) and kept in running sea water tanks until used. Animals were induced to spawn in individual finger bowls filled with warm (35°C) artificial seawater as previously described [Dufresne-Dubé et al., 1983]. Spawn oocytes were filtered and washed three times with artificial seawater. The gamete suspensions thus obtained consisted of oocytes arrested in metaphase of first meiotic division. They were then treated for 20 min at room temperature with either 50 mM KCl added to artificial seawater (by replacement of NaCl) or with 50 mM KCl and various Ca^{2+} channel antagonists. All antagonists assayed were freshly prepared as 10 mM solutions in either methanol (nimodipine, PN 200110, and flunarizine) or DMSO (nitrendipine, nifedipine). These stock solutions were then diluted in artificial seawater and tested at concentrations ranging from 0.1 to 50–100 μM. The final concentration of either vehicle (methanol and DMSO) did not exceed 0.05% and had no effect on spontaneously occurring and KCl-induced polar body emission.

The inhibition of KCl-induced extrusion of first polar body by DHP analogs was quantified on oocyte samples previously fixed and stained with DNA-specific fluorescent probe (Hoechst 33342). The quantification consisted in determination of chromosome meiotic stage among 200 randomly chosen oocytes per condition.

Oocytes Membrane Preparations

Whole, intact oocytes were homogenized with a Potter Teflon homogenizer in 20 volumes of buffer (320 mM sucrose, 50 mM Tris-HCl, pH 7.5) by 10 hand strokes. To prepare the oocyte fractions enriched in plasma membranes, homogenates were centrifuged (45000×*g*, 15 min at 4°C). Resulting pellets were washed in the same buffer and centrifuged (45000×*g*, 15 min) again. The rough microsome preparations obtained were suspended in a small volume of buffer and either used immediately (for estimation of maximal binding capacity) or frozen in liquid nitrogen and kept at −80°C until used.

Na/K-ATPase activity was assayed by a method of Post and Sen [1967] in order to estimate a quantitative index of plasma membrane content [Lingrel and Kuntzweiler, 1994]

in oocyte homogenates and rough microsome preparations as previously described [Krantic et al., 1993].

Binding Assays

Binding assays were performed by a modification of the isotopic dilution method [Borsotto et al., 1984; Pinçon-Raymond et al., 1985]. For equilibrium binding studies, oocyte preparations (100 µl containing 50–100 µg of protein) were incubated for 20 min in 50 mM Tris-HCl pH 7.5 with increasing concentrations of [(+)-[^{3}H] PN 200110 in a final volume of 1 ml. The non-specific component of total binding was measured in the presence of 100 µM nonradioactive (+)PN 200110.

The incubation of [(+)-[^{3}H]PN 200110 with homogenates of *Mytilus* oocytes gave a weak (20%) specific binding signal (data not shown). In contrast, in rough microsome preparations, the specific binding represented about 50% of total binding at [(+)-[^{3}H]PN 200110 concentrations approaching K_d (Fig. 1). Analysis of membrane-associated Na/K-ATPase activity showed the two-fold enrichment of this marker in the latter preparations (Table I) when compared to oocyte homogenates.

Membrane-enriched rough microsome preparations were incubated with [(+)-[^{3}H]PN 200110 at 4°C and at room temperature. Signal-to-noise ratio was better (data not shown) when the incubation was performed at 4°C: this temperature was chosen for further experiments.

Specific [(+)-[^{3}H]PN 200110 binding increased with increasing incubation time: the steady-state equilibrium was reached between 10–15 min and maintained for up to 30 min (data not shown). A 20-min period was therefore chosen for further binding assays.

After the end of incubation, bound and free ligands were separated by filtration of the incubation mixture over Whatman GF/C glass fiber filters (presoaked for 2 h at room temperature in 50 mM Tris-HCl buffer, pH = 7.5). Filters

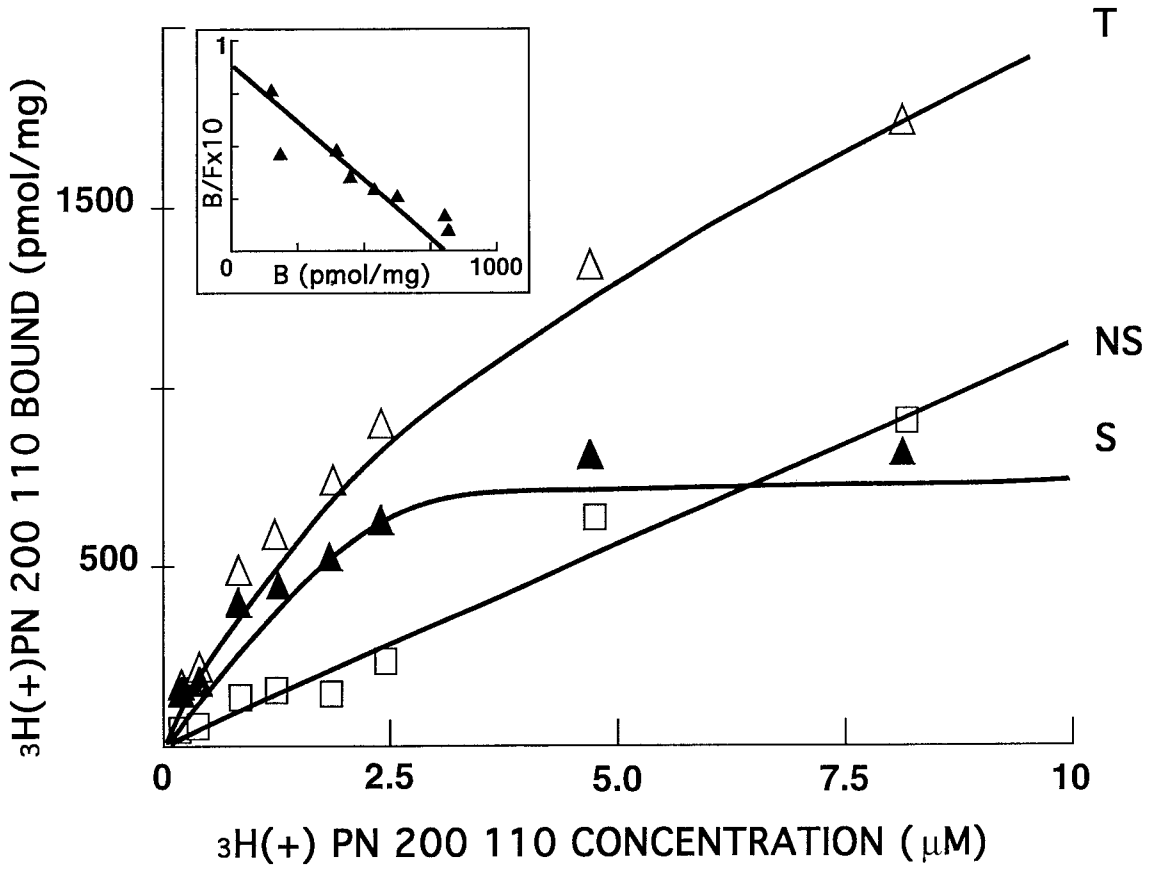

Fig. 1. Saturation of [(+)-^{3}H]PN 200110-specific binding by increasing concentration of free [(+)-^{3}H]PN 200110 in rough microsome preparations. Specific binding ("S," closed triangles) has been determined by subtraction of non-specific binding ("NS," open squares) from total binding ("T," open triangles). Inset represents the Scatchard linearization of specific binding data. Each value is the mean of triplicate determinations (SEM values were less than 5%) from one representative experiment in which K_d and B_{max} were respectively, 1.14 µM and 780 pmol/mg of protein.

TABLE I. Na/K-ATPase Activity in *Mytilus* Oocyte Preparations

	Total ATPase (A) (µmol Pi/mg prot/h)	Total ATPase + Ouabain (B) (µmol Pi/mg prot/h)	Na/K-ATPase (A–B) (µmol Pi/mg prot/h)	F[a]
Homogenate	3.55 ± 0.10	1.70 ± 0.20	1.85 ± 0.25	1
"Mb" Fraction[b]	10.52 ± 0.60	7.05 ± 0.15	3.53 ± 0.70	1.89 ± 0.38

[a]F = enrichment factor.
[b]"Mb" fraction corresponds to the plasma membrane enriched rough microsome preparations (for details, see Methods).

were rapidly washed with 15 ml (3 × 5 ml) of ice-cold 50 mM Tris-HCl pH 7.5, dropped into 6 ml of Non Aqueous Biodegradable Counting Scintillant (Amersham) and shaken vigorously. After at least 2 h, radioactivity was assayed by liquid scintillation counting with 60% efficiency.

In competition experiments, oocyte rough microsome preparations were incubated with 0.6 µM of [(+)-[^{3}H]PN 200110 in the presence of various concentrations of different calcium channel ligands. Experiments were carried out in duplicate and repeated at least three times independently. Stock solutions of analogs tested in binding experiments were prepared as indicated for polar body emission assays except that they were diluted in 50 mM Tris-HCl, pH 7.5 buffer. The final concentration of either vehicle (methanol and DMSO) did not exceed 0.05% and had no effect on [(+)-[^{3}H]PN 200110 specific binding.

Binding data were expressed in picomoles of [(+)-[^{3}H]PN 200110 bound per mg of protein. Protein concentration was measured according to the method of Lowry et al. [1951].

Data Analysis

Equilibrium saturation and drug competition data were treated by iterative curve-fitting using Lundon computer program based on non-linear least squares regression analysis (Lundon Software Inc.). Hill coefficients (n_H) were determined by using McPherson's modified EBDA-LIGAND program [McPherson, 1983]. Differences between data were statistically assessed using one-way analysis of variance (ANOVA) and considered significant at $P < 0.05$.

RESULTS

Characterisation of [(+)-[^{3}H]PN 200110 Binding Sites

The specific component of the [(+)-[^{3}H]PN 200110 binding was saturable (Fig. 1). Scatchard plots of equilibrium binding data were linear and yielded K_d of 1.32 ± 0.21 µM and B_{max} of 620 ± 150 pmol/mg of protein. Non-linear regression analysis (Lundon Software Inc.) of experimental versus computed data for experimental points showed that they fit better to a one- rather than to a two-site model ($P < 0.05$). Hill coefficients (n_H) were close to unity ($n_H = 0.93 \pm 0.10$).

Pharmacological Profile of [(+)-[^{3}H]PN 200110 Binding Sites

All Ca^{2+} channel blockers of DHP type tested were able to inhibit [(+)-[^{3}H]PN 200110 specific binding (Fig. 2A). All non-related compounds (dopamine, norepinephrine, serotonin) had no effect on [(+)-[^{3}H]PN 200110 specific binding when tested in concentration range from 0.1 to 50 µM (data not shown). The class IV Ca^{2+} channel antagonist of the piperazine type such as flunarizine was completely inefficient (Fig. 2A).

The inhibition curves obtained for all efficient compounds were monophasic and did not extend over more than two orders of magnitude (Fig. 2A). All efficient compounds were able to inhibit [(+)-[^{3}H]PN 200110 specific binding completely (Fig. 2A).

The rank order of potency of active analogs for inhibition of [(+)-[^{3}H]PN 200110 specific binding was determined by comparison of their IC_{50} concentrations (pharmacologically defined as the analog concentration necessary to inhibit 50% of [(+)-[^{3}H]PN 200110 specific binding). It was: nimodipine = nitrendipine = (+)PN200110 > nifedipine.

Oocyte Response to DHP Analogs In Vivo

All DHP analogs tested inhibited KCl-induced polar body emission in a dose-dependent manner (Fig. 2B). The inhibition was total with IC_{50} (pharmacologically defined as the analog concentration necessary to inhibit 50% of KCl-

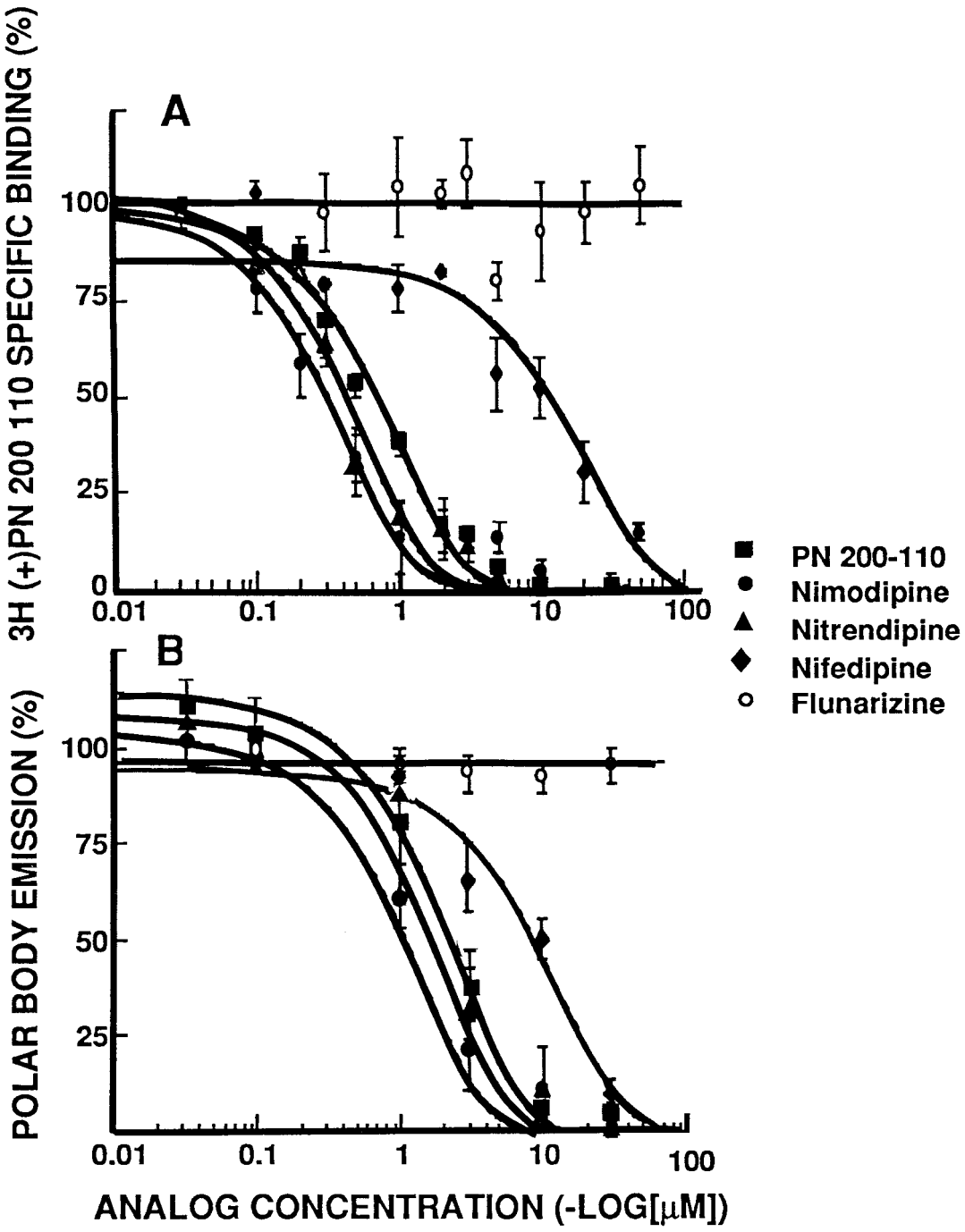

Fig. 2. DHP-related compounds inhibit both [(+)-^{3}H]PN 200110 specific binding in oocyte rough microsome preparations (**A**) and polar body emission in *Mytilus* oocytes (**B**). The DHP inhibition is expressed as a % of maximal response, i.e., [(+)-^{3}H]PN 200110 specific binding in (A) and polar body emission in (B) determined in the absence of DHP compounds. In experiments presented in A, the reference 100% value for specific binding is 484 ± 26 pmol/mg of protein (corresponding total- and non-specific binding are respectively 735 ± 49 pmol/mg and 250 ± 26 pmol/mg of protein; non-specific binding corresponds therefore to 34 ± 2% of total binding). For experiments presented in B, 100% value corresponds to 170–200 oocytes with extruded polar body out of 200 oocytes examined per condition. Each value in A and B corresponds to the mean ± SEM of five independent experiments each done in triplicate.

induced response) concentrations of 11 ± 6 μM, 1.27 ± 0.54 μM, 0.74 ± 0.34 μM, and 0.52 ± 0.14 μM for nifedipine, nitrendipine, (+)PN 200110, and nimodipine, respectively. Neither of these compounds could trigger meiosis reinitiation when tested in the absence of KCl thus excluding any partial agonist effect (data not shown). In contrast, flunarizine was inefficient even at the highest (50 μM) concentration tested (Fig. 2B).

Comparisons between the efficiencies (as determined by respective IC_{50} values) of active compounds in the inhibition of [(+)-[^{3}H]PN 200110 specific binding and their efficiencies to inhibit polar body extrusion revealed the existence of a linear relation (r = 0.995) between these two parameters (Fig. 3).

Regulation of [(+)-[^{3}H]PN 200110 Binding Sites

The affinity of [(+)-[^{3}H]PN 200110 binding sites increased significantly ($P < 0.05$) with membrane polarization. It was 0.49 ± 0.20 μM for depolarized state (50 mM KCl added to artificial seawater) versus 1.06 ± 0.08 μM and 1.14 ± 0.14 μM for polarized state (0 and 5 mM KCl added to artificial seawater, respectively). In contrast, in depolarized conditions, B_{max} values were not significantly different from the values determined for polarized conditions (Table II). Non-linear regression analysis (Lundon Software Inc.) revealed a single population of receptors in both polarized and depolarized conditions.

Reversibility of DHP Analog Action on Inhibition of Meiosis Reinitiation

The inhibitory effect of dihydropyridine analogs on KCl-induced polar body emission was reversible: after a wash of Ca^{2+} channel blockers, 50 mM KCl could still induce polar body emission (Fig. 4). However, this reversibility was partial for (+)PN 200110 actions (Fig. 4).

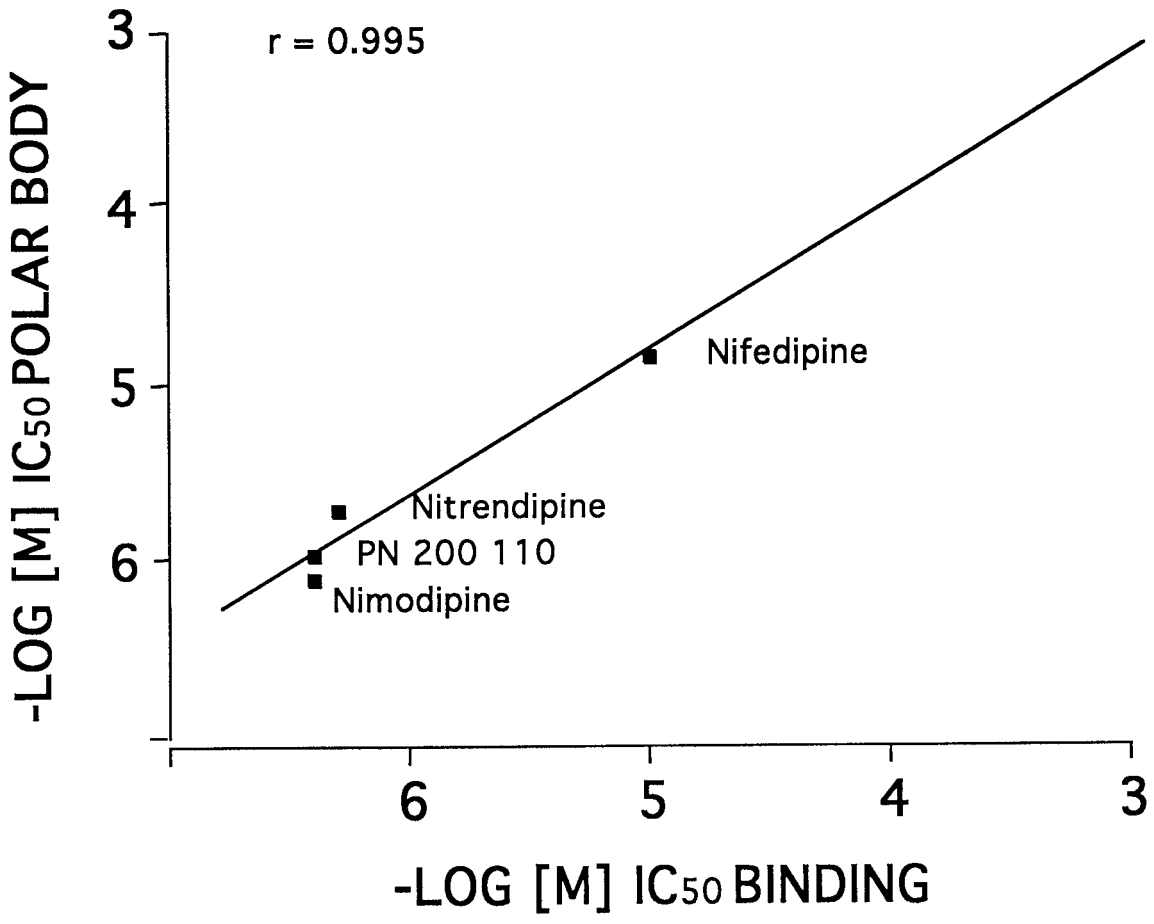

Fig. 3. Linear relationship (r = 0.995) existing between the efficiencies (as determined by evaluation of IC_{50} values) of DHP-related compounds to: 1) inhibit polar body emission (y-axis) and 2) inhibit [(+)-[^{3}H]PN 200110-specific binding (x-axis). IC_{50} values were taken from the data presented in Figure 2.

TABLE II. Equilibrium Binding Constants of [(+)-[^{3}H]PN 200110 Binding Sites Depend on Membrane Polarization State

KCl concentration (mM)	K_d (μM)	B_{max} (pmol/mg)
0	1.06 ± 0.08	741 ± 73
5[a]	1.14 ± 0.14	674 ± 62
50[a]	0.49 ± 0.20[b]	461 ± 33

[a]Oocyte suspensions were exposed to KCl for 2 h, then washed twice with artificial seawater and processed for rough microsome preparation as described in Methods.
[b]$P < 0.05$ versus 0 mM KCl ($F = 18.58$ for table F value of 10.13).

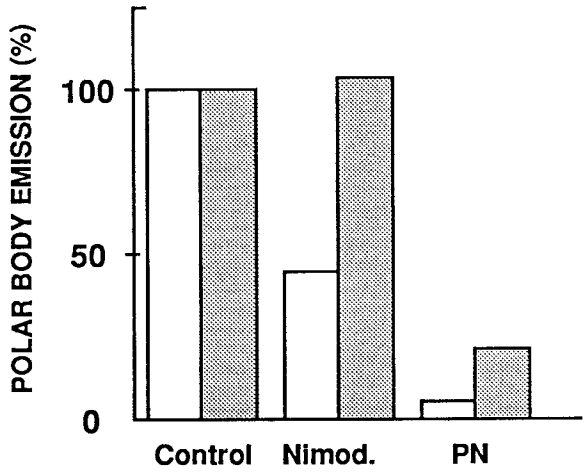

Fig. 4. DHP analogs inhibition of meiosis reinitiation is reversible. The same oocyte suspension was divided into different batches which were then treated with either KCl (50 mM added to artificial seawater) alone (control) or simultaneously with KCl (50 mM) and various DHP compounds at 10 μM concentration. An aliquot of every batch was fixed and stained for polar body quantification (empty columns). The rest was washed twice and restimulated with artificial seawater containing 50 mM KCl for 20 min. Polar body scores after second stimulation with KCl are given by hatched columns. Values represent the means of duplicate determinations (SEM values were less than 5%) obtained in one out of three different experiments in which similar results were obtained. Indicated percentages of polar body emission have been normalized against control values taken as 100%. According to the experiment, 100% corresponded to 170–200 out of 200 oocytes examined per condition.

DISCUSSION

The present study provided biochemical and pharmacological data strongly suggesting that plasma membrane DHP receptors of *Mytilus galloprovincialis* oocytes involved in meiosis reinitiation are L-type Ca^{2+} channels.

The equilibrium binding analysis of *Mytilus* oocyte binding sites based on monolinear Scatchard plot and non-linear regression analysis favorizing the one-site model (Lundon Software Inc.), revealed that the oocyte membrane fractions studied contain a single class of DHP receptors. Moreover, the monophasic inhibition curves that did not extend over more than two orders of magnitude, obtained for competition between active compounds and [(+)-[^{3}H]PN 200110 and Hill coefficients (n_H) close to unity, further confirmed the homogeneity of DHP receptors in oocyte preparations. Our data agree with results obtained for DHP-related inhibition of specific binding measured with some [nitrendipine: Schmid et al., 1984] but not all [DMBodipyDHP: Schmid et al., 1984] DHP ligands in muscle cells. By contrast, in other excitable cells (neurons, skeletal, and cardiac muscle cells) DHP binding to homogenates or purified fractions appears more complex than simple interaction with a single binding site [for review, see Triggle and Rampe, 1989].

The pharmacological profile of DHP receptors reported here is very similar to those described for plasma membranes of mammalian muscle cells in pharmacological [Schmid et al., 1984; Galizzi et al., 1986; Knaus et al., 1992] and electrophysiological [Hess et al., 1984] studies. However, all efficient compounds were 100- to 1000-fold less potent on *Mytilus* oocyte DHP receptors. Hence, these receptors appear similar but are not identical to mammalian DHP receptors. Besides, it has been reported that, despite structural analogies with mammalian receptors, *Drosophila* DHP receptors have particular pharmacological and immunological properties [Pauron et al., 1987].

Our biochemical data raised the question of the functional role of *Mytilus* DHP receptors, in particular because it has been reported that low affinity and high capacity DHP binding sites may not be related to Ca^{2+} channel functions [for review, see Triggle and Rampe, 1989; Janis and Triggle, 1991]. However, the K_d value of [(+)-[^{3}H]PN 200110 binding sites in *Mytilus* oocyte membrane estimated as 1.32 ± 0.21 μM is a value consistent with the (+)PN 200110 concentration that is effective in inhibition of KCl-induced meiosis reinitiation in this species. Moreover, as we reported in the present study, the pharmacological profile of [(+)-[^{3}H]PN 200110 binding sites was almost identical to the pharmacological profile obtained for inhibition of meiosis reinitiation via DHP-sensitive Ca^{2+} channels: the efficiencies of compounds tested upon inhibition of meiosis reinitiation are linearly related ($r = 0.995$) with their efficiencies in the inhibition of [(+)-[^{3}H]PN 200110

specific binding. This confirms and further extends the previous observations concerning the absolute dependence of KCl-induced meiosis reinitiation on extracellular Ca^{2+} that have initially pointed to the involvement of plasma membrane Ca^{2+} channels [Abdelmajid et al., 1993; Deguchi and Osanai, 1994]. Moreover, our data show that KCl-induced oocyte activation is the most suitable model for the study of L-type Ca^{2+} channels involvement in meiosis reinitiation. Indeed, in this model system, a perfect correlation exists between the inhibition of plasma membrane L-type Ca^{2+} channels and inhibition of meiosis. Such a correlation cannot be found during fertilization-triggered meiosis reinitiation since this latter requires both membrane Ca^{2+} channels and internal Ca^{2+} stores for the increase of intracellular Ca^{2+} concentrations [Deguchi and Osanai, 1994].

The additional argument for the functional involvement of *Mytilus* oocyte DHP receptors in meiosis reinitiation came from our data about their regulation. Binding parameters of functional DHP receptors depend on membrane potential: numerous biochemical and electrophysiological studies have reported that in different cell types, membrane depolarization triggers two- to twenty-fold increases in DHP receptor affinity; maximal binding capacity is either unaltered or decreased [for review, see Triggle and Rampe, 1989; see also Liu et al., 1994]. As expected for functional DHP receptors, our data revealed two- to three-fold increase in ligand affinity in depolarized conditions.

Finally, if characterized DHP receptors act as functional Ca^{2+} channels in oocyte plasma membrane, then after the wash of DHP antagonist which allows membrane repolarization, restimulation by KCl (50 mM) should depolarize oocyte membrane and consequently trigger meiosis reinitiation. Such a reversible effect of the DHP antagonists on the oocyte Ca^{2+} channel was exactly what we observed. However, it remains unclear why (+)PN 200110-dependent inhibition of KCl-induced first polar body emission was not totally reversible. Indeed, the reason cannot be the residual presence of (+)PN 200110 since an analogous wash in nimodipine experiments prior to secondary KCl application led to the complete recovery of the polar body emission.

In conclusion, our data strongly suggest the presence of a single class of DHP receptors in membrane-enriched fractions prepared from *Mytilus* oocytes. They apparently act as functional Ca^{2+} channels involved in oocyte activation by mediating the activation-associated first transient $[Ca^{2+}]i$ increase. Relevantly, this transient increase does not occur if KCl is applied in the absence of the external Ca^{2+} [Deguchi and Osanai, 1994]. In addition, the Ca^{2+} channels described in this study appear essential for polar body emission where they are probably mediating the sustained elevation of $[Ca^{2+}]i$. Indeed, the withdrawal of extracellular Ca^{2+} only 30 seconds after KCl-induced *Mytilus* plasma membrane depolarization completely blocked the elevation of $[Ca^{2+}]i$ and prevented the polar body emission [Deguchi and Osanai, 1994]. Further studies combining pharmacological and electrophysiological approaches are needed in order to definitively demonstrate that the Ca^{2+} channels assessed by Deguchi and Osanai and those characterized in our study represent identical functional entities. However, since meiosis reinitiation triggered either by fertilization or by KCl displays similar sensitivity to removal of external Ca^{2+} [Deguchi and Osanai, 1994] and DHP inhibition (present study) and since our pharmacological data revealed the existence of a single class of DHP receptors, the same L-type Ca^{2+} channels appear to be involved in meiosis resumption of *Mytilus* oocytes induced by both stimuli.

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REFERENCES

Abdelmajid H, Leclerc-David C, Moreau M, Guerrier P, Ryazanov A (1993): Release from the metaphase I block in invertebrate oocytes: possible involvement of Ca^{2+}/calmodulin-dependent kinase III. Int J Dev Biol 37:279–290.

Allen RD (1953): Fertilization and artificial activation in the egg of the surf clam *Spisula solidissima*. Biol Bull 105:213–239.

Beam KG, Adams BA, Niidome T, Numa S, Tanabe T (1992): Function of a truncated dihydropyridine receptor as both voltage sensor and calcium channel. Nature 360:169–171.

Borsotto M, Barhanin J, Norman RI, Lazdunski M (1984): Purification of the dihydropyridine receptor of the voltage-dependent Ca^{2+} channel from skeletal muscle transverse tubules using (+)3H PN 200110. Biochem Biophys Res Comm 122:1357–1366.

Campbell KP, Leung AT, Sharp AH (1988): The biochemistry and molecular biology of the dihydropyridine-sensitive calcium channel. TINS 10:425–430.

Cross NL, Elinson RP (1980): A fast block to polyspermy in frogs mediated by changes in the membrane potential. Dev Biol 75:187–198.

Deguchi R, Osanai K (1994): Repetitive intracellular Ca^{2+} increases at fertilization and the role of Ca^{2+} in meiosis reinitiation from the first metaphase in oocytes of marine bivalves. Dev Biol 163:162–174.

Dubé F (1988): The relationships between early ionic events, the pattern of protein synthesis and oocyte activation in the surf clam *Spisula solidissima*. Dev Biol 126:233–241.

Dubé F, Dufresne L (1990): Release of metaphase arrest by partial inhibition of protein synthesis in blue mussel oocytes. J Exp Zool 256:323–332.

Dubé F, Guerrier P (1982): Activation of *Barnea candida* (Mollusca, Pelecypoda) oocytes by sperm or KCl, but not by NH4Cl, requires a calcium influx. Dev Biol 92:408–417.

Dufresne-Dubé L, Dubé F, Guerrier P, Couillard P (1983): Absence of a complete block to polyspermy after fertilization of *Mytilus galloprovincialis* (Mollusca, Pelecypoda) oocytes. Dev Biol 91:246–256.

Galizzi J-P, Fosset M, Romey G, Laduron P, Lazdunski M (1986): Neuroleptics of the diphenylbutylpiperidine series are potent calcium channel inhibitors. Proc Natl Acad Sci USA 83:7513–7517.

Gould-Somero M, Jaffe LA, Holland L (1979): Electrically-mediated fast polyspermy block in eggs of the marine worm, *Urechis caupo.* J Cell Biol 82:426–440.

Guerrier P, Leclerc-David C, Moreau M (1993): Evidence for the involvement of internal calcium stores during serotonin-induced meiosis reinitiation in oocytes of bivalve mollusc *Ruditapes philippinarum*. Dev Biol 159:474–484.

Hess P, Lansman JB, Tsien RW (1984): Different modes of Ca channel gating behaviour favored by dihydropyridine Ca agonists and antagonists. Nature 311:538–544.

Jaffe LA (1976): Fast block to polyspermy in sea urchin eggs is electrically mediated. Nature 261:68–71.

Jan LY, Jan YN (1989): Voltage-sensitive ion channels. Cell 56:13–25.

Janis RA, Triggle DJ (1991): Drug acting on calcium channels. In Hurwitz L, Patridge LD, Leach JK (eds): "Calcium Channels: Their Properties, Functions, Regulation and Clinical Relevance." Boca Raton: CRC Press, pp 195–249.

Knaus H-G, Moshammer T, Friedrich K, Kang HC, Haugland RP, Glossmann H (1992): In vitro labeling of L-type Ca^{2+} channels by fluorescent dihydropyridines: evidence for a functional, extracellular heparin-binding site. Proc Natl Acad Sci USA 89:3586–3590.

Krantic S, Dubé F, Quirion R, Guerrier P (1991): Pharmacology of the serotonin-induced meiosis reinitiation in *Spisula solidissima* oocytes. Dev Biol 146:491–498.

Krantic S, Guerrier P, Dubé F (1993): Meiosis reinitiation in surf clam oocytes is mediated via a 5-hydroxytryptamine5 serotonin membrane receptor and vitelline envelope-associated high affinity binding site. J Biol Chem 268:7983–7989.

Lingrel JB, Kuntzweiler T (1994): Na^+,K^+-ATPase. J Biol Chem 269:19659–19662.

Liu J, Bangalore R, Triggle DJ (1994): Modulation of L-type Ca^{2+} channels in clonal rat pituitary cells by membrane depolarization. Mol Pharmacol 45:1198–1206.

Lowry OH, Rosebrough NJ, Farr AL, Randall RJ (1951): Protein measurement with Folin phenol reagent. J Biol Chem 193:265–275.

McPherson GA (1983): A practical computer-based approach to the analysis of radioligand binding experiments. Comput Prog Biomed 17:107–114.

Nargeot J (1991): Mixing and matching calcium. Current Biology 1(6):350–352.

Osanai K, Kuraishi R (1988): Response of oocytes to meiosis-inducing agents in pelecypodes. Bull Mar Biol Stn Asamushi, Tôhoku Univ 18:45–56.

Pauron D, Qar J, Barhanin J, Fournier D, Cuany A, Pralavorio M, Berge JB, Lazdunski M (1987): Identification and affinity labelling of very high affinity binding sites for the phenylalkylamine series of Ca^{2+} channel blockers in the *Drosophila* nervous system. Biochemistry 26:6311–6315.

Pinçon-Raymond M, Rieger F, Fosset M, Lazdunski M (1985): Abnormal transverse tubule system and abnormal amount of receptors for Ca^{2+} channel inhibitors of the dihydropyridine family in skeletal muscle from mice with embryonic muscular dysgenesis. Dev Biol 112:458–466.

Post RL, Sen AK (1967): Sodium and potassium-stimulated ATPase. Methods Enzymol 10:762–768.

Reuter H (1983): Calcium channel modulation by neurotransmitters, enzymes and drugs. Nature 301:569–574.

Schmid A, Renaud J-F, Fosset M, Meaux J-P, Lazdunski M (1984): The nitrendipine-sensitive Ca^{2+} channel in chick muscle cells and its appearance during myogenesis in vitro and in vivo. J Biol Chem 259:11366–11372.

Triggle DJ, Rampe D (1989): 1,4-dihydropyridine activators and antagonists: Structural and functional distinctions. TIPS 10:507–511.

Tsien RW, Ellinor PT, Horne WA (1991): Molecular diversity of voltage-dependent Ca^{2+} channels. TIPS 12:349–354.