FEBS Letters 351 (1994) 58–62

FEBS 14429

Endogenous DHP-sensitive Ca^{2+} channels in *Pleurodeles* oocytes

H. Ouadid[a,*], E. Browaeys-Poly[b], J.P. Vilain[b], P. Guilbault[a]

[a]*Laboratoire de Physiologie Cellulaire and* [b]*Laboratoire de Biologie du Développement, SN3, Université des Sciences et Technologies, 59655 Villeneuve d'Ascq Cedex, France*

Received 21 June 1994

Abstract

The double electrode voltage-clamp technique was used to study voltage-dependent Ca^{2+} channels in *Pleurodeles* oocytes. From a holding potential of −80 mV, Ba-current (I_{Ba}) (recorded in Cl-free solution, Ba^{2+} = 40 mM) activated at −36.7 ± 4 mV, peaked at −11.6 ± 4 mV and reversed at 55 ± 7 mV (n = 24). This current activated slowly (rise time was 0.98 ± 0.2 s; n = 14 at −10 mV) and was not inactivated. Cadmium (Cd^{2+}, 500 μM) completely inhibited I_{Ba}. The effect of Cd^{2+} was dose-dependent (EC_{50} = 37 ± 5 μM; n = 5). Moreover, I_{Ba} was insensitive to ω-conotoxin (10 μM) but interestingly this I_{Ba} displayed dihydropyridine (DHP) sensitivity. Bay K 8644 (5 μM), a DHP activator, increased the peak current amplitude in a dose-dependent manner (EC_{50} = 5.9 ± 0.6 μM; n = 10) and shifted the threshold and the maximum of current/voltage relationship towards negative potentials by −10 mV. Nifedipine (5 μM), a DHP antagonist, decreased I_{Ba} by 80% at HP of −80 mV (EC_{50} = 1.2 ± 0.2 μM; n = 6). We concluded that *Pleurodeles* oocytes possess High-Voltage Activated Ca^{2+} channels with properties similar to L-type Ca^{2+} channels.

Key words: *Pleurodeles* oocytes; Dihydropyridine; Ca^{2+} channel; Voltage-clamp

1. Introduction

A variety of oocytes produce action potentials when their membrane is depolarized. In invertebrates oocytes, the action potentials result from the activation of both Ca^{2+} and Na^{+} currents while in vertebrate oocytes, purely calcium or sodium dependent spikes are observed [1,2,3]. Ca^{2+} channel multiplicity has been extensively demonstrated in a variety of cell types [4,5] including oocytes and eggs [1,6]. In ascidian eggs, two different Ca^{2+} channels have been identified: one, the 'high-voltage-activated' (HVA), so-called L-type Ca^{2+} channel (DHP-sensitive); the other one is a 'low-voltage-activated' (LVA), so-called T-type Ca^{2+} channel [7]. In the immature oocytes from the starfish *Mediaster*, both HVA and LVA Ca^{2+} channels coexist [8]. However, only HVA Ca^{2+} channels have been recorded in *Xenopus* oocytes [9]. To date, there have been no electrophysiological studies on Ca^{2+} channels in *Pleurodeles* immature oocytes, while a rise of intracellular Ca^{2+} activity has been observed during progesterone induced maturation [10]. With Ba^{2+} ions as charge carriers, we found HVA Ca^{2+} channel currents with very slower kinetics of activation and inactivation. Interestingly, these currents are sensitive to dihydropyridines, agonist (Bay K 8644) and antagonist (nifedipine).

2. Materials and methods

2.1. Oocyte reparation

All experiments were performed during the breeding season (September to May) when *Pleurodeles* oocytes resumed meiosis with progesterone treatment. Oocytes were dissected away from ovary of female *Pleurodeles waltlii* (urodele amphibian, from Laboratoire de Biologie du Développement, USTL, France) anaesthetized with 1% MS 222 (tricaine methanesulfonate; Sandoz) and prepared as reported previously [11]. Stage VI oocytes (diameter of 1.6 mm) were selected for electrophysiological measurements. Defolliculated oocytes could be maintained for 2–6 days at 19°C in control solution containing (ND 96; in mM): NaCl 96, KCl 2, $CaCl_2$ 2 and HEPES [4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid] 10, pH 7.2 with NaOH and supplemented with 50 μg/ml gentamicin. Incubation medium was renewed daily.

2.2. Electrophysiological measurements

Electrophysiological measurements were performed using the standard two microelectrode voltage-clamp technique with TEV-200 amplifier (Dagan Instruments, Minneapolis, MN). Stimulation of the preparation, data acquisition and analysis were performed using the pCLAMP software (ver. 5.5, Axon Instrument, Burlingame, CA). Oocytes were placed in a recording chamber (300 μl) and impaled with 3 M CsCl-filled microelectrodes (0.2–1 MΩ).

Drugs were applied externally by addition to the superfusate (gravity driven superfusion). We used a Cl free solution (CaMS; in mM): NaOH 96, KOH 2, CaOH 2 and HEPES 10, pH 7.2 (methane sulfonic acid) in order to block the Cl currents. The main part of the outward K current was inhibited by using Cs instead of K in the recording electrodes and 4-aminopyridine (10 mM) was added in the CaMS and in the BaMS solutions. No fast Na current was detected in these oocytes. However, all extracellular Na was replaced by tetraethylamonium and tetrodotoxin (10 μM) was added. Thus, to record Ca^{2+} channel activity, oocytes were tested in the high barium methane sulfonate solution (BaMS, in mM): TEAOH 50, CsOH 2, BaOH 40 and HEPES 10, pH adjusted to 7.2 with methane sulfonic acid.

2.3. Drugs

Cadmium, 4-aminopyridine, tetraethylamonium and tetrodotoxin were purchased from Sigma (St. Louis, MO, USA) and were dissolved in water. The DHP agonist Bay K 8644 and antagonist nifedipine (Calbiochem, France) were dissolved in 50% ethanol to make concentrated stock solutions (10 mM) and stored at −20°C. ω-Conotoxin was purchased from Calbiochem (France) and was dissolved in water to make stock solution (10 mM). All final concentrations were obtained by appropriate dilution in BaMS solution.

2.4. Analysis

Ba-current was measured as the difference between the maximal inward current amplitude and the zero current level. Most data are expressed as the means (S.E.M.) (n = number of experiments).

* Corresponding author. Fax: (33) 20 43 40 38.

SSDI 0014-5793(94)00816-7

3. Results

The mean membrane potential from immature oocytes was −63 ± 10 mV (n = 10) and the membrane capacitance was 0.19 ± 0.04 μF (n = 16).

3.1. Electrophysiological properties of the Ba-current (I_{Ba})

In a normal physiological bathing medium (ND 96), depolarization to +20 mV from a holding potential of −80 mV elicited an outward current (Fig. 1A$_1$). The perfusion of a Cl-free solution (CaMS; Ca^{2+} = 2 mM) reduced this outward current (Fig. 1A$_2$). This result suggests that a portion of outward current is carried by Cl ions. The perfusion of the high Ba^{2+} solution (BaMS; Ba^{2+} = 40 mM) blocked completely the remaining outward current and elicited an inward Ba-current (Fig. 1A$_3$). Moreover, *Pleurodeles* oocytes developed a Ca^{2+} activated K^+ current which activated about −40 mV. Fig. 1B shows the Ca^{2+} activated K^+ current elicited from a holding potential of −80 mV to −10 mV during exposure to high Ca^{2+} solution (CaMS; Ca^{2+} = 20 mM). An inward current was elicited when Ca^{2+} (20 mM) was replaced by Ba^{2+} (20 mM) (Fig. 1B). However, even in BaMS solution, a small outward current remained at above + 30 mV.

This paper is based on recordings Ca^{2+} channel activity in *Pleurodeles* oocytes. Fig. 1C shows typical waves of I_{Ba} evoked for various depolarizations between −40 to +30 mV from a holding potential of −80 mV. The peak I_{Ba} magnitudes were normalized for variation in oocytes size using oocytes membrane capacitance. The mean of I_{Ba} density records during a test pulse to −10 mV from a holding potential of −80 mV was 0.86 ± 0.21 μA/μF (n = 24). I_{Ba} density/voltage relationship is shown in Fig. 1D. For test pulses of a 2 s duration, I_{Ba} activated at −36.7 ± 4 mV (n = 24), peaked at −11.6 ± 4 mV (n = 24) and reversed at 55 ± 7 mV (n = 24). However, in few oocytes no inward Ba-current could be recorded except in the presence of the DHP agonist (Bay K 8644) (data not shown). Thus, only a HVA Ca^{2+} channel is evident in the *Pleurodeles* oocytes. Using a 2 or 6 s pulses, we were unable to observe any inactivation of I_{Ba} (data not shown). Furthermore, I_{Ba} showed extremely slow inactivation requiring 3 min pulse duration (Fig. 1E).

Further characterization of these Ca^{2+} channels has been focussed on the effect of various classical Ca^{2+} channel inhibitors or activators used to differenciate between the different types of HVA Ca^{2+} channels.

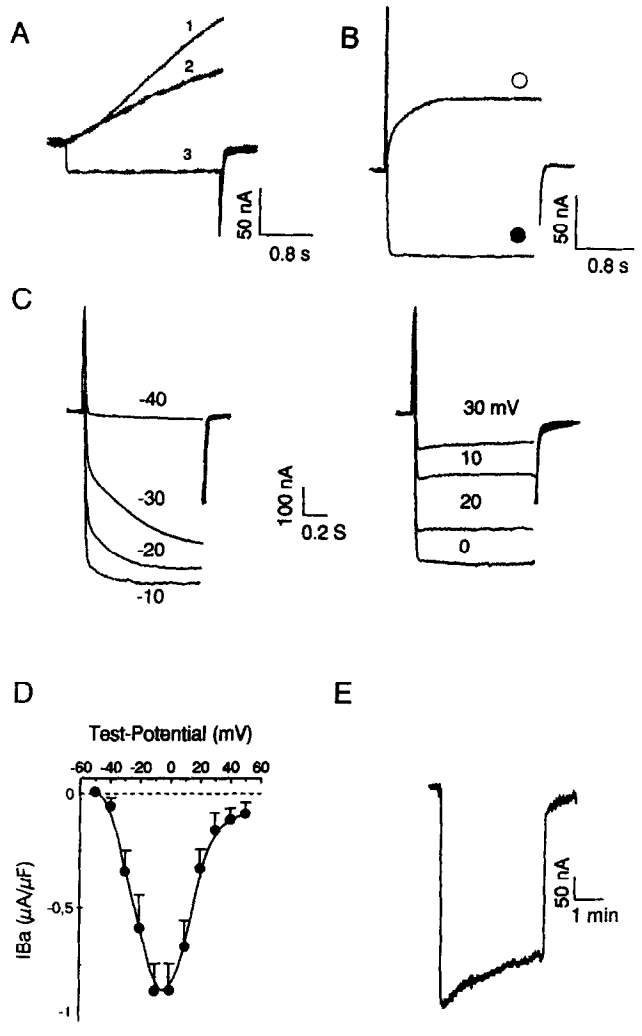

Fig. 1. (A–E) Electrophysiological properties of 'high-voltage activated' calcium channel currents (using Ba^{2+} as charge carrier) in *Pleurodeles* oocytes. (A) Typical traces showing an outward current recorded: (1) in normal physiological medium (ND 96); (2) in the free Cl solution (CaMS; Ca^{2+} = 2 mM); and (3) the recovery of I_{Ba} when superfused with high BaMS solution (0 Cl, 40 mM Ba^{2+}). The holding potential (V_h) was stepped from −80 mV to + 20 mV for 2 s. (B) Outward current elicited from Vh of −80 mV to −10 mV (○) in the high CaMS solution (0 Cl, 20 mM Ca^{2+}). The substitution of external Ca^{2+} (20 mM) by Ba^{2+} (20 mM) elicited an inward I_{Ba} (●). (C) Typical waveforms of I_{Ba}. From V_h of −80 mV, I_{Ba} was evoked by test pulses to depolarization between −40 mV and −10 mV (left panel) and between 0 mV and +30 mV (right panel) presented in 10 mV increment. (D) Current/voltage relationship of the peak current normalized by the membrane capacitance. Mean I_{Ba} density (μA/μF) are plotted as a function of depolarization ± S.E.M. (n = 24). (E) Kinetics of activation and inactivation of I_{Ba}. The waveforms of I_{Ba} evoked at −10 mV from V_h of −80 mV for 3 min duration.

3.2. Effect of Ca^{2+} on I_{Ba}

Among the many potent inorganic Ca^{2+} channel blockers, Cd^{2+} is of particular interest in a number of preparations. Fig. 2A shows effect of different concentrations of Cd^{2+} on HVA Ba-current activated from −80 mV to −10 mV. The reduction of I_{Ba} by Cd^{2+} was dose-dependent with an EC_{50} of 37 ± 5 μM (n = 5; Fig. 2B). A complete block of I_{Ba} was obtained in the presence of 500 μM Cd^{2+} (Fig. 2A,B,C). Fig. 2C shows peak I/V relationship determined in the absence and the presence of 500 μM Cd^{2+}.

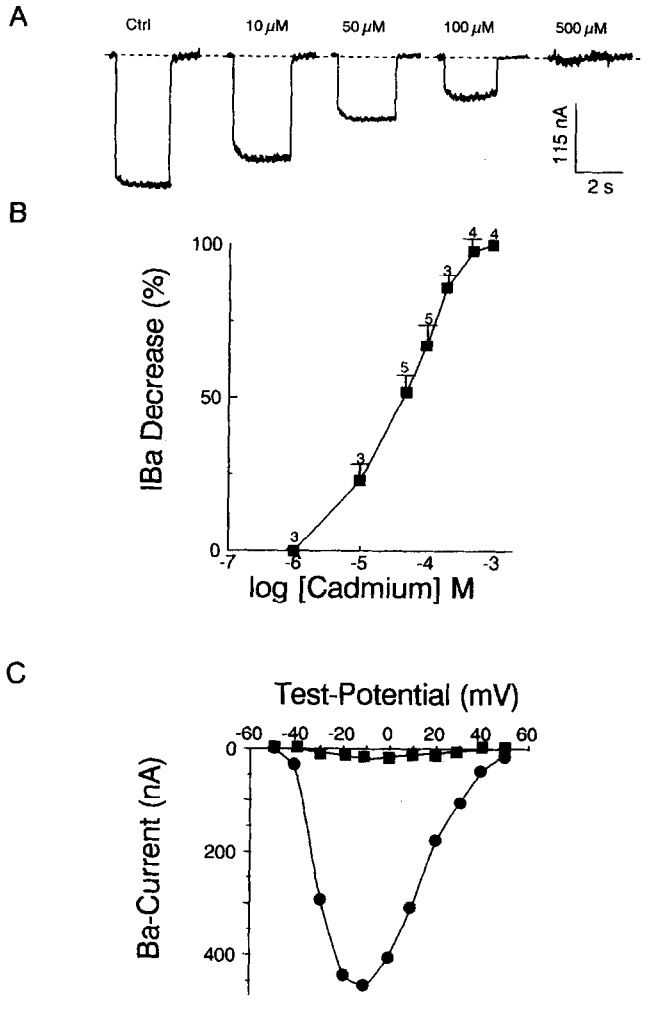

Fig. 2. (A–C) Effects of various concentrations of Cd^{2+} on I_{Ba}. (A) Effect of Cd^{2+} (10, 50, 100, 500 μM) on I_{Ba}. The holding potential was stepped from −80 mV to −10 mV for 2 s (Ctrl = control). (B) Cumulative dose/response relationship of the inhibition of I_{Ba} by Cd^{2+}. Each point is expressed as mean ± S.E.M., n = number of oocytes. The dose of 50% inhibition is 37 ± 5 μM (n = 5). (C) Current/voltage relationship of I_{Ba} (●) before and (■) after perfusion of Cd^{2+} (500 μM). V_h was −80 mV, test pulses 2 s in duration and 10 mV in increment were applied every 40 s.

3.3. *Effect of ω-conotoxin (ω-cgTx) and dihydropyridines (DHP) on* I_{Ba}

In many tissues, the entry of Ca^{2+} ions can be modulated by diverse classes of organic compounds that have proved their efficiency as ligands to purify and characterize Ca^{2+} channels.

Sensitivity to ω-cgTx and 1–4 dihydropyridines has been used to identify different subtypes of Ca^{2+} channels. ω-cgTx has no effect on L-type Ca^{2+} channels in smooth, cardiac and skeletal muscle but reduces the N-type Ca^{2+} channels [12]. When ω-cgTx was applied in concentrations with a saturating block in other preparations (10 μM), in *Pleurodeles* oocytes, no effect on I_{Ba} was observed (n = 12, data not shown).

The primary site of action of 1–4 dihydropyridines is thought to be L-type Ca^{2+} channels [13]. Bay K 8644, a dihydropyridine agonist, has been shown to promote Ca^{2+} current [14,15]. External application of Bay K 8644 (5 μM) increased the peak inward current at test potential from −80 mV to −20 mV (Fig. 3A). The effect of Bay K 8644 on I_{Ba} was dose-dependent with an EC_{50} of 5.9 ± 0.6 μM (n = 10) (Fig. 3B). The threshold and the maximum of the I/V relationship (Fig. 3C) shifted about −10 mV in the negative direction consistent with the shift in voltage dependence of activation seen in heart [16,17], neurones [18] and skeletal cells [19].

The effect of the DHP antagonist nifedipine on I_{Ba} is shown in Fig. 3. The peak inward current was significantly reduced by nifedipine (5 μM) at test potential −20 mV (Fig. 3D). The effect of nifedipine on I_{Ba} was dose-dependent with an EC_{50} of 1.2 ± 0.2 μM (n = 6) (Fig. 3E). By contrast to the effect of Bay K 8644, no leftward shift in the I/V curve was obtained with nifedipine (Fig. 3F).

4. Discussion

The main result of our study is the existence of HVA Ca^{2+} channel DHP-sensitive in the *Pleurodeles* oocytes. These HVA Ca^{2+} channels, like others found in various preparations [5,13] can be distinguished by the activation voltage (≈ −30 mV) and their sensitivity to the inorganic blocker Cd^{2+} (EC_{50} = 37 ± 5 μM, Fig. 2). Moreover, I_{Ba} reported here was modulated by DHP agonist and antagonist. Bay K 8644, a DHP activator, enhances the peak I_{Ba} and induces a left shift by about −10 to −15 mV of both threshold and the maximum of the I/V curve. Nifedipine, a DHP antagonist, reduces I_{Ba} by ≈ 80% at HP of −80 mV and induces no leftward shift in the I/V curve (Fig. 3D–F). Taken together, these results agree well with those obtained on cardiac [16,17] and skeletal L Ca^{2+} channels [19,20]. However, some discrepancies can be observed between our results and those derived from cardiac and skeletal cells: (i) the DHP concentrations used to obtained the half maximum effect in our experiments are 100–1000-fold higher than those reported in cardiac (≈ 10^{-8} M for both nifedipine and Bay K 8644 [14,21] and in skeletal cells (≈ 10^{-9} M) [19]. However, it should be noted that these (EC_{50}) from cardiac and skeletal cells were obtained in the presence of the normal external calcium while in our experiments we use a 40 mM external Ba^{2+} concentration; (ii) in skeletal muscle, Bay K 8644 shifted only the threshold of I/V curve [19] while in our study both the threshold and the peak of the I/V curve are shifted in the presence of Bay K 8644.

So, what types of channel mediates I_{Ba} in *Pleurodeles* oocytes? In terms of the voltage-dependent properties and sensitivity to DHP (agonist and antagonist), this channel resembles the L-type Ca^{2+} channel reported in

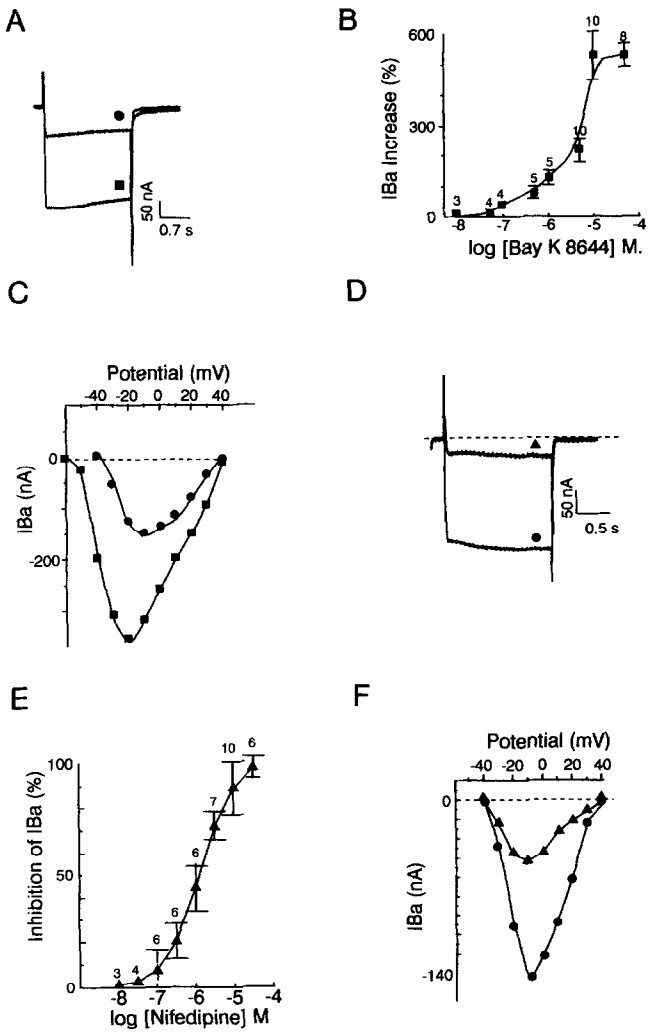

Fig. 3. (A–F) Regulation of I_{Ba} by DHPs agonist (Bay K 8644) and antagonist (nifedipine). (A) Individual Ba-current traces evoked by a depolarizing pulse from −80 mV to −20 mV; (●) I_{Ba} recorded in the control solution and (■) maximal increase of I_{Ba} induced by perfusion with the same solution containing Bay K 8644 (5 μM). (B) Cumulative dose/response relationship of increase I_{Ba} by Bay K 8644. The dose of 50% increase is 5.9 ± 0.6 μM (n = 10). Each point is expressed as mean ± S.E.M., n = number of oocytes. (C) Current/voltage relationship traced from V_h −80 mV (●) in absence and (■) in presence of Bay K 8644 (5 μM). The threshold and the maximum of I_{Ba} were shifted by about −10 mV towards negative potentials. (D) Individual Ba-current traces recorded from −80 mV to −20 mV, (●) I_{Ba} control, (▲) I_{Ba} in the presence of nifedipine (5 μM). (E) Cumulative dose/response relationship of decrease I_{Ba} by nifedipine. The dose of 50% decrease is 1.2 ± 0.2 μM (n = 6). Each point is expressed as mean ± S.E.M., n = number of oocytes. (F) Current/voltage relationship traced from V_h −80 mV (●) in absence and (▲) in presence of nifedipine (5 μM).

various tissues: cardiac [17,22], neuronal [18,23] and skeletal [20,24]. In terms of the activation kinetic (time to peak, 0.98 ± 0.2 s at −10 mV), I_{Ba} closely resembles the slow activating DHP-sensitive Ca^{2+} channel reported in skeletal muscle [24,25]. In terms of inactivation kinetics (non-inactivating I_{Ba}), I_{Ba} is similar to the fast rising non-inactivating DHP Ca^{2+} channel reported in dysgenic skeletal muscle [26].

Recently, molecular biological studies have revealed that the HVA Ca^{2+} channel is a protein complex containing α_1, α_2/δ, β and γ subunits [27,28]. Recent studies have revealed a diversity among L-type Ca^{2+} channels arising from: (i) multiples genes encoding the pore forming α_1-subunit; and (ii) various genes (at least 4) encoding the auxilliary β-subunit. While it is already known that the α_1-subunit plays a major role in determining the activation kinetics [27,28], the auxilliary subunits, in particular β-subunit plays a major role in determining both the kinetics and voltage dependence of the inactivation process. In L-cells, stable expression of skeletal muscle α_1 alone was sufficient to generate a slowly activating and inactivating Ca^{2+} channel [29,30]. The coexpression of skeletal muscle β with skeletal muscle α_1 markedly accelerate both the activation and inactivation kinetics [29,30]. As reported here I_{Ba} was activated slowly and did not inactivate may indicate that the L-type Ca^{2+} channels in *Pleurodeles* oocytes contain α_1-subunit (DHP sensitive) but a few and/or no β-subunit.

Our study argues an heterogenecity in Ca^{2+} channel types between amphibian oocytes and more generally between vertebrate and invertebrate oocytes: (i) T and L Ca^{2+} channels in ascidian eggs and starfish oocytes [7,8]; (ii) HVA Ca^{2+} channels different from the four subtypes known in *Xenopus* oocytes [9,31,32] and (iii) a DHP-sensitive HVA Ca^{2+} channel subtype in the *Pleurodeles* oocytes. These Ca^{2+} channels (DHP-sensitive) in the *Pleurodeles* oocytes might have a physiological involvement in the regulation of free internal Ca concentration during meiosis, fertilization and development.

References

[1] Moreau, M., Guerrier, P. and Vilain, J.P. (1985) in: Biology of Fertilization. I. Model Systems and Oogenesis (C.B. Metz and A. Monroy, eds.) pp. 299–345, Academic Press, New York.
[2] Schlichter, L.C. (1983) Dev. Biol. 98, 47–59.
[3] Baud, C., Kado, R.T. and Marcher, K. (1982) Proc. Natl. Acad. Sci. USA 79, 3188–3192.
[4] Hullin, R., Biel, M., Flockerzi, V. and Hofmann, F. (1993) TCM 3, 48–53.
[5] Tsien, R.W., Ellinor, P.T. and Horne, W.A. (1991) Trends Pharmacol. Sci. 12, 349–354.
[6] Dascal, N. (1987) CRC Crit. Rev. Bioch. 22, 317–387.
[7] Dale, B., Talevi, R. and Defelice, J. (1991) Exp. Cell Res. 192, 302–306.
[8] Hagiwara, S., Ozawa, S. and Sand, O. (1975) J. Gen. Physiol. 65, 617–644.
[9] Bourinet, E., Fournier, F., Nargeot, J. and Charnet, P. (1992) FEBS Lett. 299, 5–9.
[10] Moreau, M., Vilain, J.P. and Guerrier, P. (1980) Dev. Biol. 78, 201–214.
[11] Rodeau, J.L. and Vilain, J.P. (1987) Dev. Biol. 120, 481–493.
[12] McCleskey, E.W., Fox, A.P., Feldman, D., Cruz, L.J., Olivera,

B.M., Tsien, R.W. and Yoshikami, D. (1987) Proc. Natl. Acad. Sci. USA 84, 4327–4331.
[13] Pelzer, D., Pelzer, S. and McDonald, T.F. (1990) Rev. Physiol. Biochem. Pharmacol. 114, 107–207.
[14] Kokubun, S. and Reuter, H. (1984) Proc. Natl. Acad. Sci. USA 81, 4824–4827.
[15] Schramm, M., Thomas, G., Towart, R. and Franckowiak, G. (1983) Arzneimittelforsch. 33, 1268–1272.
[16] Hess, P., Lansman, J.B. and Tsien, R.W. (1984) Nature 311, 538–544.
[17] Ouadid, H., Seguin, J., Richard, S., Chaptal, P.A. and Nargeot, J. (1991) J. Mol. Cell Cardiol. 23, 41–54.
[18] Fox, A.P., Nowycky, M.C. and Tsien, R.W. (1987) J. Physiol. 394, 149–172.
[19] Cognard, C., Romey, G., Galizzi, J.P., Fosset, M. and Lazdunski, M. (1986) Proc. Natl. Acad. Sci. USA 83, 1518–1523.
[20] Beam, K.G. and Knudson, C.M. (1988) J. Gen. Physiol. 91, 799–815.
[21] Hess, P., Lansman, J.B. and Tsien, R.W. (1985) in: Cardiovascular Effects of Dihydropyridine-type Calcium Antagonists and Agonists (Z. Fleckenstein, C. Van Breemen, R. Gross and F. Hoffmeister, eds.) Bayer Symposium IX, pp. 34–35, Spinger, Berlin/Heidelberg/New York.
[22] Bean, B.P. (1989) Annu. Rev. Physiol. 51, 367–384.
[23] Nowycky, M.C., Fox, A.P. and Tsien, R.W. (1985) Proc. Natl. Acad. Sci. USA 82, 2178–2182.
[24] Cognard, C., Lazdunski, M. and Romey, G. (1986) Proc. Natl. Acad. Sci. USA 83, 517–521.
[25] Almers, W. and Palade, P.T. (1981) J. Physiol. 312, 159–176.
[26] Adams, B.A. and Beam, K.G. (1989) J. Gen. Physiol. 94, 429–444.
[27] Lory, P., Varadi, G. and Schwarz, A. (1991) News In Physiological Sciences 6, 277–281.
[28] Nargeot, J., Dascal, N. and Lester, H.A. (1992) J. Membrane Biol. 126, 97–108.
[29] Lacerda, A., Kim, H.S., Ruth, P., Perez-Reyes, E., Flockerzi, V., Hofmann, F., Birnbaumer, L. and Brown, A.M. (1991) Nature 352, 527–530.
[30] Varadi, G., Lory, P., Schulz, D., Varadi, M. and Schwartz, A. (1991) Nature 352, 159–162.
[31] Dascal, N., Snutch, T.P., Lubbert, Davidson, N.R. and Lester, H.A. (1986) Science 231, 1147–1150.
[32] Lory, P., Rassendren, F.A., Richard, S., Tiaho, F. and Nargeot, J. (1990) J. Physiol. 426, 95–112.