Expression of a rapidly inactivating Ca^{2+} channel in *Pleurodeles* oocytes during the resting season

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Abstract Two kinds of Ca^{2+} channel activities have been recorded in unfertilized *Pleurodeles* oocytes during the resting season: the previously described L-type and a transient one. The transient Ba current (I_{Ba-t}) exhibited a voltage threshold of -36 ± 7 mV, peaked at -18 ± 8 mV and reversed around +50 mV. It showed a fast monoexponential decay with an inactivation time course of 31.4 ± 1.7 ms at -20 mV. I_{Ba-t} was insensitive to nifedipine and ω-conotoxin-GVIA but blocked by Ni^{2+} (50 μM). Moreover, Cd^{2+} also reduced I_{Ba-t} but was less efficient than Ni^{2+}. When using Ca^{2+} as the charge carrier, the amplitude and the decay of I_{Ca-t} were largely similar to those of I_{Ba-t}. These data demonstrated that the population of Ca^{2+} channels could be seasonally modulated in *Pleurodeles* oocytes.

Key words: Transient Ca^{2+} channel; Resting season; *Pleurodeles* oocyte

1. Introduction

The diversity of Ca^{2+} channels and their possible coexistence in the same cell have been well documented [1–3]. Moreover, the presence of two types of Ca^{2+} channels was first demonstrated in marine animal oocytes [4]. Both T-type (a fast transient component activated at low potential; LVA) and L-type (a slow component activated at high potential; HVA) have been reported in starfish *Mediaster aequalis* oocytes [4], ascidian *Ciona intestinalis* oocytes and eggs [5,6]. Recently, we have described a HVA Ca^{2+} channel which has been attributed to the L-type on the basis of to its kinetics and pharmacological properties in *Pleurodeles* oocytes [7]. Interestingly, only the L-type current was observed during the breeding season. Indeed, the behavior of *Pleurodeles* oocytes is even more striking since oocyte maturation is regulated by two seasons: (i) the resting season from May to September when neither Ca^{2+} surge nor progesterone-induced maturation occurs (during this unresponsive resting period, oocytes display a highly negative membrane potential and a non-functional Na^+/K^+ pump) [8] and (ii) the breeding season when oocytes are responsive to progesterone in vitro. The responsive oocytes present a less negative resting potential as well as an electrogenic Na^+/K^+ pump [8].

In the present study, *Pleurodeles* oocytes were used during the resting season. Two kinds of Ca^{2+} channel activities have been recorded: the previously described L-type and a transient one that has not been shown before. Furthermore, during the resting season, the transient-type Ca^{2+} channel seemed to be predominant since some batches of oocytes did not even display the L-type current. These data led to the suggestion that the population of Ca^{2+} channels in *Pleurodeles* oocytes could be seasonally modulated.

2. Materials and methods

2.1. Oocytes preparation

All experiments were performed during the resting season, when *Pleurodeles* oocytes did not resume meiosis with progesterone treatment. Oocytes were dissected away from ovaries of female *Pleurodeles waltlii* (urodele amphibian, from Laboratoire de Physiologie Cellulaire, SN3, USTL) anesthetized with 1% MS 222 (tricaine methanesulfonate, Sandoz) and prepared as previously reported [7]. Stage VI oocytes were selected for electrophysiological measurements. Defolliculated oocytes were maintained for 2–6 days at 19°C in control solution (ND 96) containing in mM: 96 NaCl, 2 KCl, 2 $CaCl_2$ and 10 HEPES at pH 7.4 (NaOH) and supplemented with 50 μg/ml gentamicin. The incubation medium was changed every day.

2.2. Electrophysiological measurements

Electrophysiological measurements were performed using the standard two microelectrode voltage clamp technique with the TEV-200 amplifier (Dagan Instrument, 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 electrodes (0.2–1 MΩ). To record Ca^{2+} channel activity, oocytes were tested in high barium methanesulfonate solution (BaMS, in mM): 2 CsOH, 50 TEAOH, 40 BaOH, 10 HEPES, pH adjusted to 7.2 with methanesulfonic acid. Moreover, 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) in the BaMS. In order to record Ca currents, EGTA was injected into the oocytes one day before use and tests were always performed in 40 mM Ca^{2+}.

Drugs were applied externally by addition to the superfusate (gravity driven superfusion).

Defolliculated oocyte maturation was induced, in vitro, by continuous treatment for 8 h with progesterone (1 μg/ml). The maturation was assessed by the occurrence of white spot.

All experiments were performed at room temperature.

2.3. Analysis

In all experiments, oocytes were depolarized every 20 s from -100 mV to different test potentials for 250 ms. Ba currents were measured at the maximal inward current. The inactivation of I_{Ba-t} as a function of membrane potential was studied by applying 250 ms conditioning pre-pulses from a holding potential of -100 mV to a potential between -90 and -10 mV. The test potential was stepped up to -20 mV, a voltage which almost maximally activated I_{Ba-t}. To obtain the half-maximal voltage ($V_{0.5}$) and the slope factor (k) values of steady-state inactivation of I_{Ba-t}, we fitted data by a Boltzmann function, $g_{Ca} = g_{Camax}/(1+\exp((V_c - V_{0.5})/k))$.

The data were expressed as the mean ± standard error of n experiments, n = number of oocytes tested.

2.4. Drugs

Cadmium (Cd^{2+}), nickel (Ni^{2+}), 4-aminopyridine, tetraethylammonium (TEA) (Sigma, France) and ω-conotoxin-GVIA (Calbiochem, France) were made up in water. Nifedipine (Calbiochem, France) was dissolved in 50% ethanol to make a concentrated stock solution at 10 mM. EGTA (Sigma, France) was dissolved in 10 mM HEPES-

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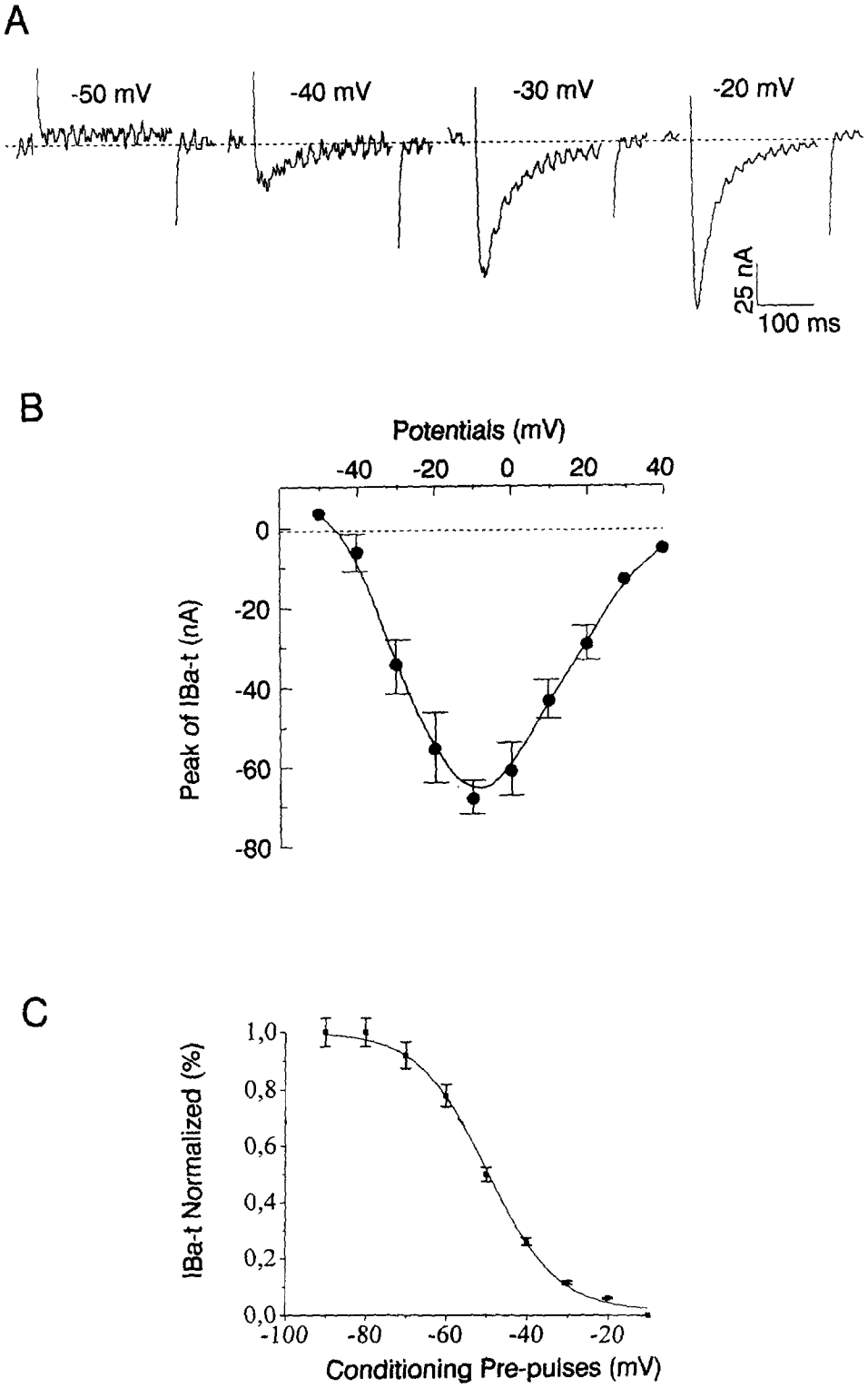

Fig. 1. Electrophysiological properties of a transient activating Ca current (using Ba^{2+} as charge carrier) in *Pleurodeles* oocytes. A: Typical waveform of I_{Ba-t} evoked from V_h of −100 mV to −50, −40, −30 and −20 mV. B: Mean current/voltage relationship of I_{Ba-t} measured from V_h of −100 mV. Data points were mean ± S.E. ($n = 10$). C: Steady-state inactivation relationship was obtained by measuring I_{Ba-t} elicited by depolarization to −20 mV at V_h −100 mV between −90 mV and −10 mV. Data points were mean ± S.E. ($n = 8$). Data were fitted by a Boltzmann function.

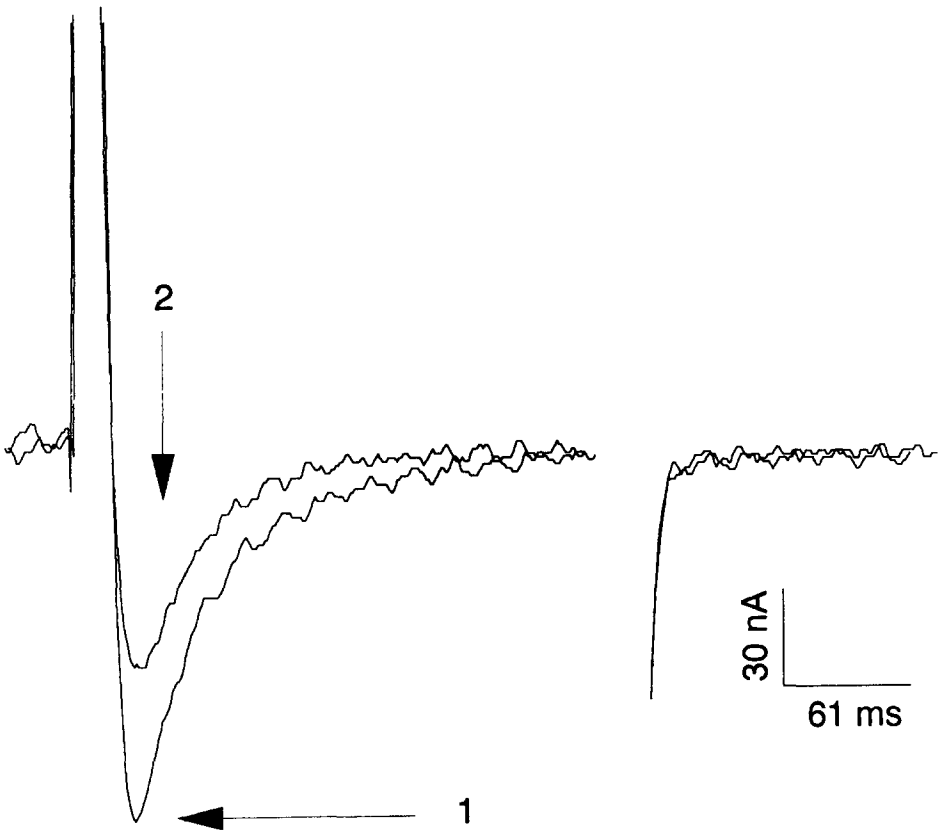

Fig. 2. Comparison of I_{Ba-t} and I_{Ca-t} waveforms. During depolarizations to −20 mV from a holding potential of −100 mV. Typical traces showing an inward transient current recorded (1) in BaMS solution (0 Cl, 40 mM Ba^{2+}) and (2) in CaMS solution (0 Cl, 40 mM Ca^{2+}). Experiments were performed in the same oocyte.

KOH water (pH 7.2). Final concentrations were obtained by appropriate dilution in BaMS solution.

Progesterone (Sigma, France) was dissolved in ethanol (1 mg/ml).

3. Results

3.1. Electrophysiological properties of the transient Ca^{2+} channel current

Voltage-dependent Ca^{2+} channel activity in *Pleurodeles* oocytes was recorded in BaMS solution. In six batches of oocytes, a depolarization from a holding potential of −100 mV to several test potentials resulted in a transient Ba current (I_{Ba-t}) that displayed fast activation kinetics (time to peak = 34 ± 6 ms, $n = 15$, Fig. 1A). The mean current/voltage relationship showed an activation threshold around −40 mV (−36 ± 7 mV, $n = 10$), a maximal amplitude around −20 mV (−18 ± 8 mV, $n = 10$) and a reversal potential located in the vicinity of +50 mV (Fig. 1B). Due to the difficulty of blocking the remaining outward current activated at +10 mV, we only estimated this reversal potential value. The mean amplitude of the peak of I_{Ba-t} was 64 ± 30 nA ($n = 10$). Fig. 1C shows the steady-state inactivation curve of I_{Ba-t}. Averaged half-inactivation potential ($V_{0.5}$) and the slope factor (k) values were −50.9 ± 2 mV ($n = 8$) and 7.8 ± 0.6 ($n = 8$) respectively. Ca^{2+} channels were fully available at less than −80 mV and were 90–100% inactivated at −20 mV (Fig. 1C). I_{Ba-t} was undoubtedly transient and its decay was best fitted by a single exponential function. The inactivation time constant was 31.4 ± 1.7 ms ($n = 15$, at −20 mV).

In some experiments, Ba^{2+} (40 mM) was replaced, on an equimolar basis, by Ca^{2+} (Fig. 2). Waveforms of both I_{Ba-t} and I_{Ca-t} currents were similar. In both cases, the current amplitude was not significantly changed (55 ± 15 nA, $n = 5$, for I_{Ca-t} and 64 ± 30 nA for I_{Ba-t}, $n = 10$, at −20 mV). In addition, the decay of I_{Ca-t} was not changed (the inactivation time constant was 30 ± 3 ms, $n = 5$, at −20 mV). Thus, this Ca conductance was no larger in Ba^{2+} than in Ca^{2+}.

Moreover, in four batches of oocytes, both transient and slow Ba currents were recorded. When the voltage was maintained at −100 mV (V_h) and transiently stepped at −20 mV, a typical Ba current, with both transient and slow components, was recorded (Fig. 3Aa). At V_h −40 mV, the transient component was almost totally inactivated and only the slow component remained (Fig. 3Ab). In contrast, during the breeding season, only the slow component was recorded in the same protocol conditions (Fig. 3Bab).

3.2. Pharmacological properties of I_{Ba-t}

The sensitivity of I_{Ba-t} to pharmacological agents shown to interact with Ca^{2+} channels was also investigated. Both the dihydropyridine antagonist nifedipine (10 μM, $n = 5$, Fig. 4A) and ω-conotoxin-GVIA (10 μM, $n = 6$, Fig. 4B) showed no significant effect on I_{Ba-t}. In contrast, I_{Ba-t} was sensitive to Ni^{2+}. Ni^{2+} reduced I_{Ba-t} in a dose-dependent manner (Fig. 4C,D). 20 μM Ni^{2+} reduced I_{Ba-t} by 20–30% ($n = 6$), 50 μM reduced I_{Ba-t} by 70% ($n = 7$) and 100 μM completely abolished I_{Ba-t} ($n = 10$). However, Cd^{2+} was less efficient in inhibiting I_{Ba-t} in *Pleurodeles* oocytes (Fig. 4E,F). I_{Ba-t} remained insen-

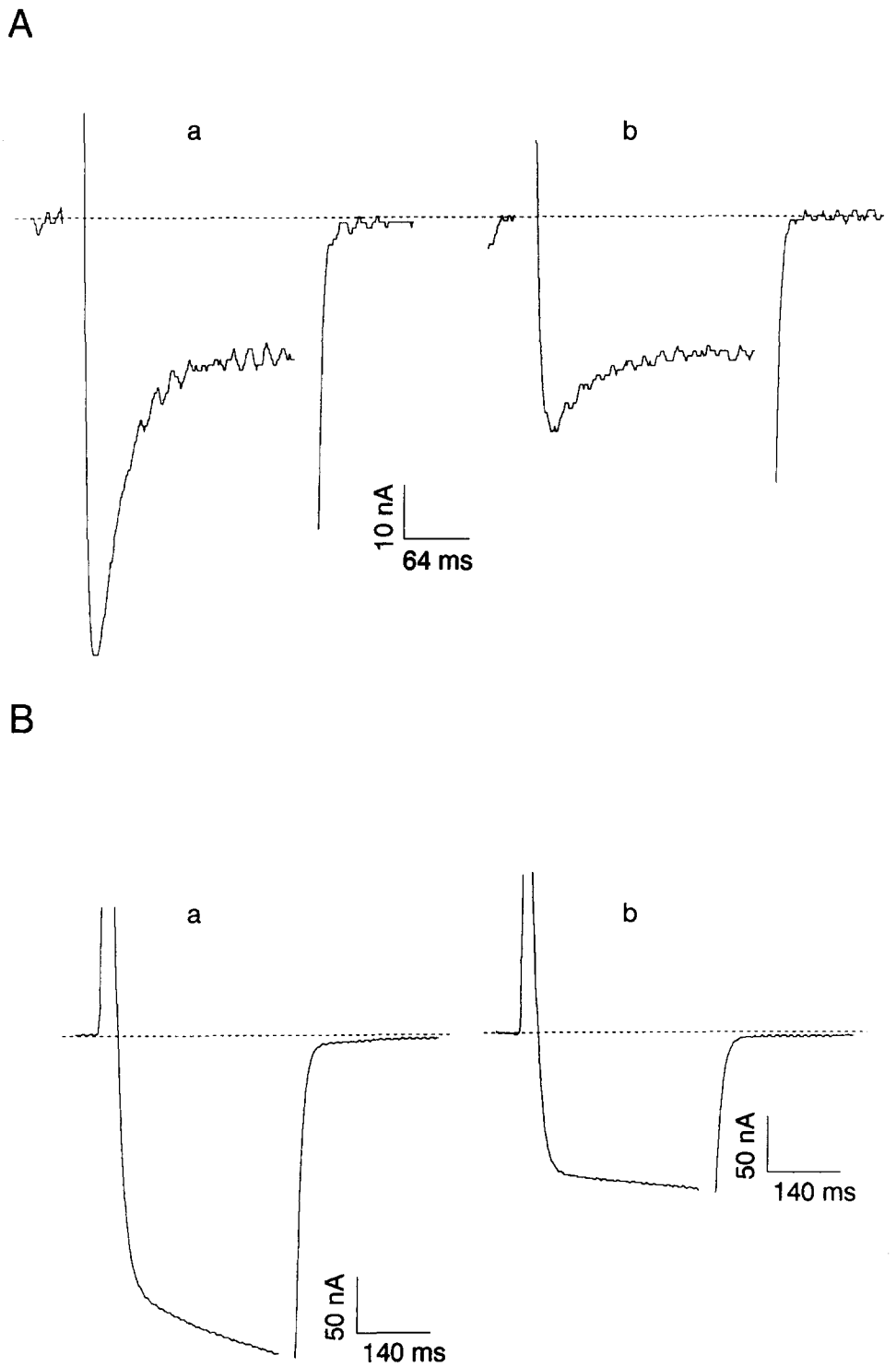

Fig. 3. I_{Ba} recorded in *Pleurodeles* oocytes during the resting and the breeding seasons. Ba currents were evoked by depolarizing to −20 mV from the holding potential of −100 mV (left panel, a) and −40 mV (right panel, b). A: During the resting season; B: during the breeding season. The duration of stimulation was 250 ms and 400 ms for I_{Ba} shown on A and B respectively.

sitive to Cd^{2+} at 50 μM ($n = 5$), but was reduced by 14 ± 5% ($n = 7$) and 51.9 ± 6% ($n = 8$) at 200 μM and 1 mM, respectively.

3.3. *Temporal distribution of the slow and the transient components of Ca^{2+} channels*

It is now well established that several types of Ca^{2+} channels display a variability with regard to their functional expression among tissues of several species [9,10]. The existence of such a phenomenon in *Pleurodeles* oocytes was investigated in this study. During the breeding season, progesterone induced the maturation of 95 ± 7% ($n = 60$) of the oocytes while during the resting season, only 11 ± 4% of 60 oocytes matured. Interestingly, the L-type Ba current was more frequently observed during the breeding season (about 90%, $n = 210$) while

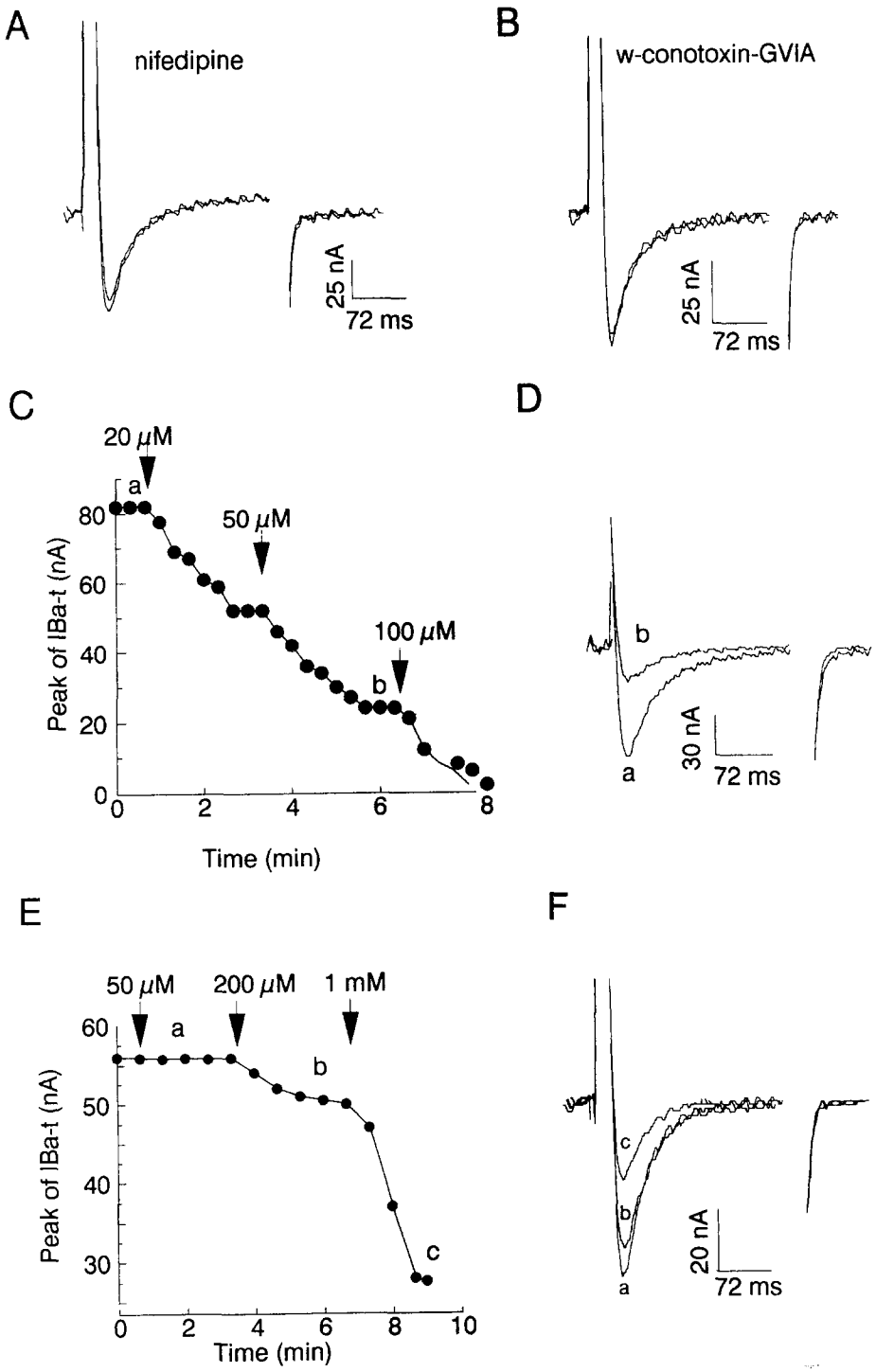

Fig. 4. Pharmacology of endogenous transient Ba current. I_{Ba-t} was induced by depolarization from −100 mV to −20 mV. Absence of the effect of (A) nifedipine (10 μM) and (B) ω-conotoxin-GVIA (10 μM). Traces before and after drug application are superimposed. C: Example of response of one oocyte to Ni^{2+}. The oocyte was superfused with several concentrations of Ni^{2+} (20 μM, 50 μM and 100 μM) as indicated. D: Individual Ba current traces induced by depolarizing pulse from −100 mV to −20 mV, (a) before and (b) after 50 μM Ni^{2+}. E: Effect of increasing concentrations of Cd^{2+} on I_{Ba-t}. The oocyte was initially exposed to the control solution. During the periods indicated, the oocyte was then exposed to 50 μM, 200 μM and 1 mM Cd^{2+}. F: Individual current traces obtained at the times indicated by the corresponding letters in graph E.

this transient type was only observed during the resting season with or without concomitant L-type activity depending on the batch of oocytes. In fact, in 60% of frogs, the transient type was observed alone, in the other 40% it was always observed in association with the L-type.

4. Discussion

The present study demonstrated that, in addition to the known L-type Ca^{2+} channel [7], *Pleurodeles* oocytes express a transient Ca^{2+} channel activity during the resting season, i.e.

when maturation does not naturally occur. The electrophysiological and the pharmacological properties of this transient current do not fit neatly into the known Ca^{2+} current classification.

The transient current described here appeared to be high-voltage activated (HVA); the peak current increased steeply over the range between −40 and −20 mV (Fig. 1). Inactivation was fast (31.4 ± 1.7 ms, n=15) and steady-state inactivation occurred at relatively negative potentials ($V_{0.5}$=−50 mV). Furthermore, I_{Ba-t} appeared resistant to nifedipine (10 μM, n=5) and ω-conotoxin-GVIA (10 μM, n=6), which specifically block L- and N-type Ca^{2+} currents respectively [1,11–14], but showed an unusual sensitivity to blockade by Ni^{2+}.

Regarding its threshold of activation (about −40 mV), rate of inactivation and sensitivity to Ni^{2+}, the transient current recorded in *Pleurodeles* oocytes was largely similar to the current referred to as T-type (or channel I) in ascidian *Ciona intestinalis* oocytes and eggs [5,6]. However, the transient current in ascidian *Ciona intestinalis* oocytes was less sensitive to Ni^{2+}. Indeed, high concentrations of Ni^{2+} (0.5 mM) were required to completely block this transient current [5].

Although I_{Ba-t} was inactivated rapidly and was quite sensitive to Ni^{2+}, like the T-type Ca^{2+} current, it was markedly different from the T-type reported in cardiac [15–17], smooth muscles [18] and neuronal cells [19,20], with regard to its voltage dependence, steady-state inactivation and rate of activation. Moreover, I_{Ba-t} was insensitive to dihydropyridine antagonists at high concentrations which have been reported to reduce the T-type Ca^{2+} current in various tissues [21–23].

Diverse forms of HVA Ca^{2+} channels have arisen from the existence of distinct α_1-subunits, alternative splicing [24,25] and possibly different assembly of auxiliary subunits such as the β-subunit [26,27]. Rat brain Ca^{2+} channel α_1-subunit (rbE-II), which is structurally related to HVA class A and B [28], displayed many properties of the LVA Ca^{2+} channels [28]. Moreover, doe-1, a Ca^{2+} channel cloned from the forebrain of *Discopyge ommata* [29], was a HVA Ca^{2+} channel but exhibited a high sensitivity to Ni^{2+} [30]. Thus, it appeared difficult, at this point of our investigations, to strictly classify the transient Ca^{2+} channels expressed in *Pleurodeles* oocytes. However, as this current was similar in magnitude when Ca^{2+} was used as charge carrier instead of the Ba^{2+} ions and as it was inactivated at V_h −40 mV, I suggest the existence of the T-like type Ca^{2+} channels in *Pleurodeles* oocytes.

The appearance of this transient Ca^{2+} conductance during the resting season of *Pleurodeles* oocytes raises the question of its physiological relevance. Previous studies have shown that changes in ionic permeability can occur in synchronicity with the onset of the resting season: (i) these changes lead to a more negative resting potential value, (ii) the Na^+/K^+ pump is not functional and (iii) during this season, the oocytes are not responsive to progesterone [8]. To my knowledge, however, the responsiveness to progesterone has not been clearly related to an alteration of any ionic conductance. It has been proposed that the alternative presence of transient and slow (L-type) Ca^{2+} channels could potentially serve in distinct biological functions. Further investigations concerning the involvement of the transient Ca^{2+} channel in the re-onset of meiosis (from prophase I to metaphase II) may help in the understanding of ionic conductance changes with regards to oocyte maturation.

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