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FEBS Letters 344 (1994) 87–90

FEBS 13972

FEBS LETTERS

Calcium currents recorded from a neuronal α_{1C} L-type calcium channel in *Xenopus* oocytes

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Received 17 March 1994

Abstract

Xenopus oocytes expressing neuronal α_{1C}, α_2 and β_{1b} calcium channel subunit cDNAs were used in this study. During two-electric voltage clamp recording the oocyte was injected with 10–20 nl of a 100 mM BAPTA solution. Under these conditions, the endogenous Ca-activated Cl current was completely suppressed resulting in an α_{1C} Ba current free from Cl current contamination. BAPTA injection also allowed α_{1C} currents with different permeating ions, including Ca, to be examined. Compared to Ba and Sr, α_{1C} whole cell Ca currents were smaller in magnitude and showed kinetic and voltage-dependent properties more similar to those for L-type Ca currents recorded in native cells. That Ca-dependent inactivation occurs in BAPTA-buffered cells suggests that the Ca-binding site involved in this type of inactivation is very close to the pore of the channel.

Key words: Calcium-activated chloride current; Ca-dependent activation; BAPTA activation

1. Introduction

During the last few years significant progress has been made towards the molecular characterization of voltage-gated calcium (Ca) channels from skeletal muscle, smooth muscle, heart, and brain (reviewed in [1,2]). Expression studies have shown that Ca channel α_1 subunits are able to form functional channels with the expected pharmacology, and that the kinetic and voltage-dependent properties of hte $\alpha 1$ subunit are modulated by coexpression with ancillary Ca channel subunits [3–16]. Much of the work on exogenous expression of Ca channels has taken advantage of the powerful *Xenopus laevis* oocyte system [17,18]. While the oocytes possess relatively few endogenous expressed ion channels and receptors (see [18]), the examination of Ca currents is hampered by the presence of a small endogenous Ca current and a significant Ca-activated chloride (Cl) current [18–20]. The problem of the contaminating Ca-activated Cl conductance has traditionally been minimized by replacing Ca with barium (Ba) and omitting the Cl in the external solution [21]. In spite of this, the Ca-activated Cl current can still be activated by Ba, a problem especially apparent when large Ba currents are obtained by expression of cloned Ca channel subunits. In addition to affecting the amplitude of the whole cell Ba current, the contaminating Ba-activated Cl current affects Ca channel kinetics. Moreover, the recording of pure whole cell Ca currents through cloned Ca channels has not been proven possible in oocytes.

Neely and co-workers [12] have recently reported Ca and Ba currents through a mutant cardiac α_{1C} L-type channel using the cut-open oocyte technique. In this report we show that intraoocyte injection of the divalent ion chelating agent BAPTA completely suppresses the contaminating endogenous Cl current allowing the detection of pure whole cell Ba currents through newly synthesized neuronal α_{1C} L-type Ca channels [22]. BAPTA-injection also buffered intracellular Ca sufficiently to allow the whole cell L-type Ca current to be recorded in *Xenopus* oocytes. Compared to Ba currents, the α_{1C} L-type Ca currents were smaller in magnitude, showed an increased rate of inactivation and the current–voltage relation was shifted to more positive potentials.

2. Materials and methods

2.1. Expression plasmids for calcium channel subunits

The rat brain Ca channel cDNAs α_{1C}, β_{1b}, and α_2 have been previously described [15,22]. For nuclear injection into *Xenopus* oocytes each of the cDNAs was introduced into the PMT2 vertebrate expression vector [15,23].

2.2. Nuclear injection of Xenopus oocytes and electrophysiological recording

Oocytes were surgically removed from anaesthetized (0.1% MS222) adult *Xenopus laevis*. Follicular cell-free oocytes were obtained by enzymatic isolation using collagenase (type 1A, 1 mg/ml, 2–3 h) in Ca-free medium (in mM: Na, 82; KCl, 2; $MgCl_2$, 1; HEPES, 5; titrated at pH 7.2 with NaOH) and allowed to recover for 24 h in Ca-containing saline

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SSDI 0014-5793(94)00357-2

(in mM: NaCl, 98; KCl, 2; $MgCl_2$, 1; $CaCl_2$, 1.8; HEPES, 5; Na-pyruvate, 5; gentanycin, 50 μg/ml). Nuclear injections were made using a pneumatic injector. Approximately 5–15 nl of a mix containing cDNAs for α_{1C} + α_2 + β_{1b} (at ratio 1:1:1) were injected in the pigmented animal pole at a final concentration of 0.3 μg/μl. Oocytes were incubated at 19°C under gentle shaking with the saline solution renewed daily.

Two microelectrodes voltage-clamp experiments were performed after 3 to 7 days using an Axoclamp 2A amplifier (Axon Instruments Burlingame, CA). Voltage command and current recording were made using an Olivetti computer interfaced with the TM100 labmaster to the amplifier using the pClamp version 5.5 software (Axon Instruments). Microelectrodes were filled with 3 M CsCl, 10 mM HEPES, 10 mM EGTA, pH 7.2 (CsOH) and had a typical resistance of 0.5–1.5 MΩ. The bath was connected to the clamp circuit via an 3 M KCl-agar bridge. The 10 mM Ba solution contained: BaOH, 10; NaOH, 50; CsOH, 2; TEAOH, 20; *N*-methyl-D-glucamine, 20; HEPES, 5; titrated at pH 7.2 with methanesulfonic acid. Ca currents were recorded using a 10 mM Ca solution in which $Ba(OH)_2$ was replaced with $Ca(OH)_2$. At the beginning of each experiment ~10–30 nl of a solution containing 100 mM BAPTA (free acid) and 10 mM HEPES (pH 7.2 with CsOH) was injected in the oocyte via a third microelectrode (the final concentration of BAPTA into the oocyte was estimated to be 2–5 mM). Current–voltage curves were obtained by stepping from an holding potential of −80 mV for 400 ms, every 15 s, from −50 mV to + 60 mV (5 mV increments).

3. Results and discussion

Using either $BaCl_2$ or Ba-methanesulfonate based recording solutions to examine expression of a cloned neuronal α_{1C} L-type Ca channel we noticed that the current waveform differed depending upon the recording solution. In external solution containing Ba-methanesulfonate, voltage steps to elicit the α_{1C} Ba current often show a slowly developing inward current (Fig. 1A, trace 1). In contrast, in recording solutions containing $BaCl_2$ the α_{1C} Ba current display an apparent slow rate of inactivation (Fig. 1B, trace 1). We hypothesized that the different waveforms may reflect activation of the endogenous Ca-activated Cl current by either Ba entry or by Ca release from intracellular stores. In order to test this possibility we attempted to buffer the oocyte cytoplasm by injection of the divalent ion chelating agent, BAPTA. Intraoocyte injection of BAPTA resulted in whole-cell Ba currents with dramatically altered waveforms (Fig. 1A and 1B, traces 2). In Ba-methanesulfonate solution, BAPTA injection suppresses the slow inward current (Fig. 1A). Similarly, in $BaCl_2$ solution BAPTA injection removes the apparent inactivation of the α_{1C} current (Fig. 1B). In both cases the slow inward tail currents recorded during repolarization after a test pulse to + 10 mV were also suppressed (Fig. 1A and 1B). Taken together, these results suggest that a contaminating Cl conductance has been removed: in methanesulfonate an outward flow of Cl ions (inward current), and in $BaCl_2$ an inward flow of Cl ions (outward current).

The buffering of cytosolic divalent ions by BAPTA injection was highly effective, allowing the α_{1C} L-type channels expressed in *Xenopus* oocytes to be compared using Ba, Sr and Ca as permeating ions (Figs. 1C and 2).

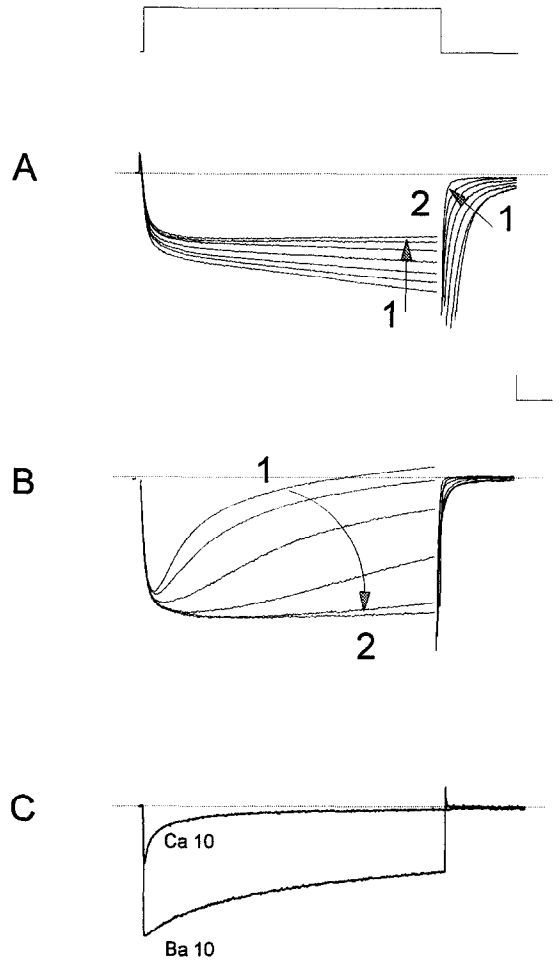

Fig. 1. Currents recorded from oocytes injected with a combination of plasmids coding for the α_{1C} (class C), α_2 and β_{1b} subunits. The holding potential was −80 mV, and the membrane was step to + 10 mV every 15 s. (A) Effect of BAPTA injection (see section 2) on the Ba current in a 10 mM Ba-methanesulfonate solution (arrows: 1, before BAPTA injection; 2, steady-state effect of BAPTA injection). (B) Same pulse protocol and BAPTA injection as in A, but using a 10 mM $BaCl_2$ recording solution. (C) Ba and Ca currents recorded using a 2-s test pulse at + 10 mV from a BAPTA-injected oocyte. Bath solutions were 10 mM Ba methanesulfonate and 10 mM Ca methanesulfonate. Scale bar for A and B panels: 200 nA and 50 ms; for panel C 200 nA and 500 ms.

Comparison of the Ba, Sr and Ca currents through α_{1C} shows that they differ in a number of respects. Firstly, the magnitude of the α_{1C} whole cell current is larger with Ba or Sr as the charge carrier compared to Ca (relative permeabilities Ca/Sr/Ba: = 1:2.1:3.4). Secondly, in Ba or Sr recording solutions the α_{1C} current show very little time-dependent inactivation and the current remaining after a 400 ms test pulse to + 10 mV constitutes greater than 80% of the peak current (Figs. 1C and 2). In contrast, the α_{1C} current in Ca solution decays rapidly and after a 400 ms test pulse represent, less than 50% of the peak inward current (Figs. 1C and 2). The decay of the α_{1C} Ca current shows a biexponential time course and the two time constants ($\tau 1$ and $\tau 2$) were weakly voltage-

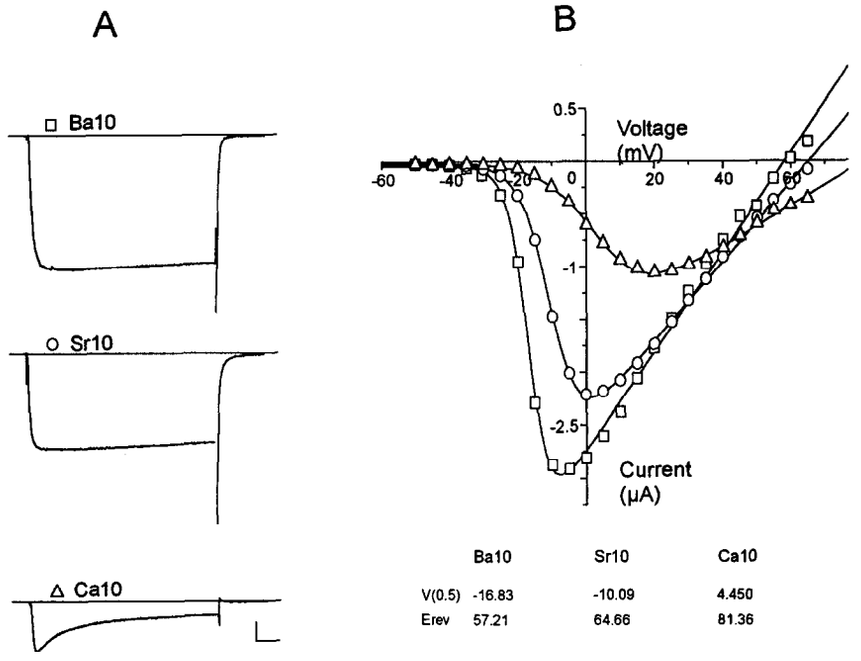

Fig. 2. (A) Typical currents recorded from BAPTA-injected oocytes in 10 mM Ba, Sr and Ca methanesulfonate solutions (from top to bottom). Scale bar: 100 nA and 50 ms. (B) Current–voltage curve obtained under conditions similar to A, and using step potential from −50 to + 70 mV (5 mV increments). See text for $V_{0.5}$ and reversal potential.

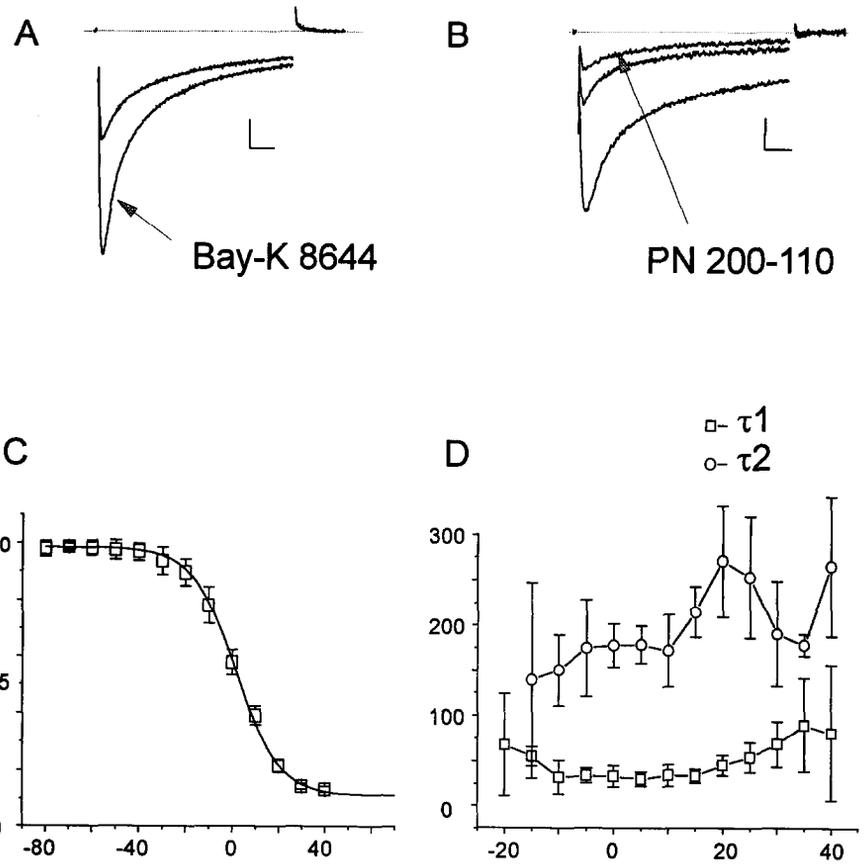

Fig. 3. Effect of Bay-K 8644 (5 μM, A) and PN200-110 (5 μM, B) on α_{1C} Ca currents. (C) Steady-state inactivation curve recorded in 10 mM Ca solution. The smooth line represent the best fit using the equation $I/I_{max} = A/\{1 + \exp[(V-V_{0.5})/k]\} + B$, with $A = 0.9$, $B = 0.1$, $V_{0.5} = 1.7$ mV and $k = 9.3$ mV (mean of 3 cells). (D) Voltage dependence of the two time constants of inactivation.

dependent (see Fig. 3D), similar to that obtained for L-type Ca currents recorded in isolated cardiac myocytes [24]. Thirdly, the potential for half activation $V_{0.5}$ was shifted depending upon the permeating ion. Fig. 2 shows that the potency for shifting $V_{0.5}$ to more positive potential was Ba < Sr < Ca ($V_{0.5}$ = −17 mV, −10 mV and + 4.5 mV, respectively), an effect related to the ability of these divalent ions to screen surface charges and consistent with previous studies on native L-type currents [25].

Also consistent with native L-type Ca currents, α_{1C} activity recorded with Ca as the charge carrier was sensitive to dihydropyridine agonist Bay K 8644 (5 μM, Fig. 3A) and the antagonist PN200-110 (5 μM, Fig. 3B). Examination of the steady-state inactivation properties in Ca solution shows that 2 second prepulses to various prepotentials induces a marked voltage-dependent inactivation od α_{1C} (Fig. 3C). The voltage for half inactivation was + 2 ± 0.5 mV (n =3). At prepulse potentials reaching + 30 mV, currents are almost completely inactivated (> 90%).

Injection of a high concentration of the chelating agent, EGTA (final concentration ~ 5–10 mM) did not prevent the development of contaminating currents during test-pulses, suggesting that the submembrane Ca or Ba concentrations were not efficiently buffered with EGTA (not shown).

In conclusion we find that the neuronal α_{1C} L-type Ca currents can be recorded in *Xenopus* oocytes preloaded with BAPTA. Compared to previous studies using Ba as the charge carrier, the α_{1C} Ca currents recorded are more typical of L-type Ca currents from native cells. The results indicate that the *Xenopus* oocyte system will be useful for structure and function studies examining the regulation and permeation of cloned Ca channels using Ca as the permeant ion. Of particular interest, since α_{1C} currents display Ca-dependent inactivation in the presence of high concentration of intracellular BAPTA these results suggest that the Ca-binding site involved in channel inactivation [26–28] is very close to the inner mouth of the pore.

Acknowledgements: The authors would like to thank Dr. Anthony Stea for comments on the manuscript and Dr. J.L. Galzy for helpful discussion on BAPTA injection. J.N. is supported by grants of l'Association Française contre les Myopathies and NATO (CRG 890374). E.B. is supported by INSERM and EMBO postdoctoral fellowships. T.P.S. is supported by a research fellowship from the Alfred P. Sloan Research Foundation and by grants from Medical Research Council of Canada and the Howard Hughes Medical Institute International Research Scholars Program.

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