Single-channel recording of inositol trisphosphate receptor in the isolated nucleus of a muscle cell line

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ABSTRACT

Nuclear calcium appears to have an important role in the regulation of gene expression in many cells, but the mechanisms involved in controlling nuclear Ca^{2+} signaling are controversial and still poorly understood. We have described the presence of inositol 1,4,5 trisphosphate (IP_3) receptors in the nuclei of skeletal muscle cells. Now, we have characterized the properties of the IP_3 receptors channels present in the nuclei of the 1B5 cell line, which do not express any isoforms of the ryanodine receptor. Immunocytochemistry of isolated nuclei confirmed the presence of IP_3R in the nuclear envelope and fluorescence measurements in nuclei suspensions allowed us to document ATP-dependent calcium loading by the nucleus and its release upon IP_3 addition. Patch clamp of nuclear membranes was performed, and single-channel activity recorded was dependent on the presence of IP_3 in the pipette; single-channel conductance was in the range reported in the literature for these channels, and the open probability was shown to be dependent on IP_3 concentration. The presence of functional IP_3 receptors in the nuclear envelope membrane is likely to have an important function in the regulation of nucleoplasmic calcium concentration and consequently in the regulation of transcription in muscle cells.

Key terms: single channel, cell nucleus, IP3 receptor, muscle.

PROLOGUE

Probably the scene that best pictures Eduardo Rojas is the following: back in 1973, I (EJ) drove to the Marine Station at Montemar with several students on the eve of an international course on biophysics directed by Eduardo. Upon arrival, we were handed nails, hammers and saws and put to work building the facilities that successfully lodged the course for the following weeks. The course was, of course, very good (and, in my case, was probably my first contact with single muscle fibers), but the image of the professor teaching the students how to build the laboratory walls helped to create the right ambience as well as deep ties among all the participants.

My friendship with Eduardo had begun long before that moment and has remained strong over the years. Knowing of my interest in studying intracellular calcium, in 1992 and 1993 he invited me to learn how to use calcium-sensitive dyes and easily convinced Harvey Pollard that the study of cultured skeletal muscle cells (which I had to learn elsewhere at NIH) was important for the Institute of Diabetes, Digestive and Kidney Diseases. We published a paper together that described, for the first time, what we now call the slow calcium signal, which was later related to regulation of gene expression in muscle cells and whose involvement in diabetes still remains to be shown. The following paper describes ion channels involved in that particular calcium signal within the cell nucleus. That particular study had little impact in Eduardo's laboratory but allowed several productive research lines to be established in Chile, and many students have been

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trained as scientists in this area. This was what Eduardo wanted and what always was his vision of the scientist's role.

INTRODUCTION

Calcium is an important second messenger that mediates a wide variety of biological processes in cells, such as secretion, contraction, differentiation, proliferation, programmed cell death, and gene expression (for reviews, see Brini and Carafoli, 2000; Berridge et al., 2003). Ca^{2+} concentration changes in the cytoplasm and nucleus often are assumed to occur more or less simultaneously due to a lack of diffusion barriers between them (see Bootman et al., 2000, for review). However, several studies indicate that not withstanding the high permeability of nuclear pores, there is an active Ca^{2+} gradient between the nucleus and cytoplasm (Al-Mohanna et al., 1994; Leite et al., 2003). Moreover, several lines of evidence suggest that fluctuations of nuclear Ca^{2+} levels have specific biological effects that differ from those of cytosolic Ca^{2+} (Hardingham et al., 2001; Quesada et al., 2002). The mechanisms involved in controlling nuclear Ca^{2+} signaling are controversial and still poorly understood. The nucleus is surrounded by a double membrane, the nuclear envelope, which is structurally and functionally related to the endoplasmic reticulum (ER) (Lanini et al., 1992), and as such it is capable of accumulating Ca^{2+} inside its lumen (Gerasimenko et al., 1995). In addition, it shares with the ER other characteristics, for example, the presence of a Ca^{2+}-Mg^{2+}-ATPase in the external nuclear envelope (Kaprielian and Fambrough, 1987), Ca^{2+}-binding proteins such as calreticulin (Camacho and Lechleiter, 1995), calbidin-D_{28k}, and nucleolin (Malviya et al., 1990). All these properties confer to the nucleus the capacity to store Ca^{2+}. Also common to both organelles is the presence of IP_3 receptors (IP_3Rs) and ryanodine receptors (RyRs) presumably responsible for Ca^{2+} release to the nucleoplasm.

The IP_3Rs comprise a family of Ca^{2+} permeable channels, the three known isoforms differ in their amino acid sequence, affinity for IP_3, and modulation by Ca^{2+} (Joseph, 1996). Subcellular distribution of IP_3R isoforms also is different in many cell types, and particularly, the nuclear distribution remains unclear. Considering that the activation of these channels is a consequence of the activity of the IP_3 signaling pathway, the IP_3Rs in the nucleus could then be activated by the local generation of IP_3 due to the nuclear presence of phospholipase C (PLC), which hydrolyzes phosphatidylinositol 4,5 bisphosphate (PIP_2) (Irvine, 2000). Moreover, the nucleus has the other components needed for IP_3 metabolism (Maraldi et al., 1999). Thus, the nucleus has the potential to regulate nucleoplasmic Ca^{2+}, independently of cytoplasmic Ca^{2+} levels.

Our previous findings of IP3-mediated Ca^{2+} signals in skeletal myotubes kept in primary culture show an important nuclear component following depolarization (Jaimovich et al., 2000; Powell et al., 2001; Eltit et al., 2004), which also is present in muscle cell lines (Estrada et al., 2001). These results led us to consider the notion that IP_3Rs have a role in the generation of nuclear Ca^{2+} signals. Functionally, we already have suggested that IP_3-mediated Ca^{2+} increase relates to the regulation of early gene expression (Powell et al., 2001; Araya et al., 2003; Carrasco et al., 2003).

We obtained and characterized a fraction of purified nuclei from cultured rat skeletal myotubes. Both immunoblot and $[^3H]IP_3$ binding studies revealed an important concentration of IP_3 receptors in the nuclear fraction. Immunofluorescence and immuno-electron microscopy studies identified type-1 as well as type-3 IP_3 receptors in the nucleus; type-1 preferentially located in the inner nuclear membrane and type-3 mainly in the nucleoplasmic region, while the type-2 IP_3 receptor was confined to the reticulum (Cárdenas et al., 2005; Jaimovich et al., 2005). Isolated nuclei responded to IP_3 with rapid and transient $[Ca^{2+}]$ elevations, which were inhibited by known blockers of IP_3 signals. Nuclear Ca^{2+} increases triggered by IP_3 evoked phosphorylation of cAMP

response element binding protein with kinetics compatible with sequential activation (Cárdenas et al., 2005). These results support the idea that Ca^{2+} signals, mediated by nuclear IP_3 receptors in muscle cells, are part of a distinct Ca^{2+} release component that originates in the nucleus and is likely to participate in gene regulation mediated by cAMP response element-binding protein.

It is important to characterize the single-channel properties of the IP_3 receptors located in the nuclear membrane. The IP_3 receptor channels have been studied by patch-clamp techniques in many biological preparations, such as Xenopus oocytes, cell nuclei from transfected cells, and in cell nuclei from rat cerebellar Purkinje cells and granule neurons (Marchenko et al., 2005). In this work, we characterized the electrophysiological properties of the IP_3 receptors channels presents in the cell nuclei of 1B5 cells. In this cell line, the IP_3 receptor channel is particularly abundant (Estrada et al., 2001), and the fact that this cell line does not express any isoforms of the ryanodine receptor (Buck et al., 1997) makes it particularly suitable for electrophysiological recordings of nuclear IP_3 receptor activity.

METHODS

Cell cultures and immunostaining

Muscle cells in primary culture and myoblasts of the immortalized dyspedic mouse cell line 1B5 (kindly provided by Dr. Paul Allen, Brigham and Women Hospital, Boston, MA) were prepared as described previously (Jaimovich et al., 2000; Moore et al., 1998; Takeshima et al., 1994). Cells were grown in a mixture of equal parts of DMEM and F12 medium plus 20% of Fetal Bovine Serum. At 80% of confluence the differentiation was initiated, replacing the fetal bovine serum by horse serum at 5% the first day and 2.5% the second day.

Immunocytochemistry of isolated nuclei was performed essentially as previously reported (Powell et al., 1996). Briefly, nuclear monolayers placed in coverslips were fixed with methanol at -20ºC for 12 minutes. The blockade was in 1% BSA, and the incubation of the primary antibodies was overnight at 4ºC. Isolated nuclei were then washed and incubated with secondary antibody during one hour at room temperature. Coverslips were mounted in Vectashield (Vector Laboratories, Inc.) for confocal microscopy, and representative images were acquired (LSM Pascal 5, Carl Zeiss). Negative controls were performed applying only the secondary antibodies. For type-3 IP3R, the pre-immune serum also was used. The images reproduced herein were manipulated in Adobe Photoshop™ to improve clarity; no information was added or deleted by those adjustments.

Nuclear isolation

1B5 nuclei were isolated as previously described by Boehning et al. (2001). Briefly, the cells were washed twice in PBS and detached from the culture dish by exposure to 5 mM EDTA for 10 min at 37ºC. Then the cells were centrifuged (500 x g, 4ºC), washed once in PBS, and centrifuged again. The cells were resuspended in a lysis buffer (250 mM sucrose, 140 mM KCl, 10 mM Tris pH 7.5 1 mM PMSF, and a complete cocktail of proteases inhibitors). The resulting cell suspension was homogenized gently in a Dounce glass homogenizer by 25-30 strokes. Then, the homogenate was centrifuged (500 x g, 4ºC), and the pellet containing the nuclear fraction was resuspended using the same buffer. The homogenate was kept on ice and used the same day for patch-clamp experiments.

Fluorimetry for Ca^{2+}

Nuclei were pre-incubated in a resting solution containing: 125 mM KCl, 2 mM K_2HPO_4, 50 mM HEPES, 4 mM $MgCl_2$, and 0.1 mM EGTA (with < 5 nM [Ca^{2+}] free), plus 5.4 μM fluo-3/AM (Molecular Probes, Eugene, OR, USA) for 45 minutes at 4ºC. Then, they were incubated in a solution containing 140 mM KCl, 10 mM HEPES, 1mM $MgCl_2$, 100 μM EGTA, 75

μM $CaCl_2$ with 200 nM free [Ca^{2+}], pH = 7.4, T = 25°C, estimated using the Winmaxc program. Fluorimetric experiments were carried out in a suspension of nuclei previously loaded with fluo-3AM. Nuclei were then stimulated with 10 μM IP_3. Fluorescence was measured using a multi-label reader Mithras LB 940 (Berthold technologies, Bad Wildbad, Germany).

Patch-clamp recording solutions

The bath and pipette solution contained 140 mM KCl, 10 mM HEPES, and 300 nM [Ca^{2+}] free approximately and adjusted to pH 7.1 with KOH. The free Ca^{2+} concentration was achieved adding 100 μM EGTA and 50 μM $CaCl_2$ to the solution: these values were calculated with webmax (http: //www.stanford.edu/~cpatton/webmaxc/webmaxcS.htm). Single channel recordings were analyzed using Clampfit 9 and plotted using Origin 7. All recording were made at room temperature (25°C).

Electrophysiology

A 100 μl aliquot of the isolated cell nuclei was placed on a glass cover slip previously treated with polylysine and filled with 1 ml of recording solution. The patch pipette, normally filled with 9 μl of the recording solution, was perfused with 1 μl of recording solution with 100 μM IP_3 and allowed to diffuse.

We performed single-channel recording of IP_3 receptor on isolated nuclei of primary cultures and 1B5 cells. Pipettes of approximately 10 Mega Ohms resistance were constructed. Single-channel recordings were amplified using an Axopatch-1D amplifier and filtered at 2 KHz. Data was digitized at 5 KHz with the Axotape program and stored in the hard disk for off-line analysis. The seal was obtained in smoothed areas of the nuclei (membrane blebs, see Figure 3a) and typically had resistance values between 1 to 2 Giga Ohms. As suggested by the literature (Sackmann and Neher, 1995), gigaseals of 10 Giga Ohms are needed for single channel recording. We could not obtain these seal resistance values, possibly due to the lipid constitution of the nuclear envelope or the presence of partly closed or occluded nuclear pores in the nuclear envelope (Mazzanti et al., 2001). However, we were able to obtain single-channel recordings with relatively low levels of noise in our experimental conditions. Once obtained, the seals were excised and tested for ion channel activity at different voltages. If no ion channel activity was observed, the patch pipette was perfused with IP_3. This procedure assured us that the single-channel recording indeed corresponded to the activity of the IP_3 receptor channel.

The choice of 300 mM for free [Ca^{2+}] in the bath and in the pipette for the single-channel recordings was taken because it coincides with the peak of the bell-shaped curve of free(Ca^{2+}) vs. the open probability (P_0) reported for the IP_3 type-1 receptor channel and with the plateau for Po in the type 3 IP_3 receptor (Hagar et al., 1998). Although in these conditions we could not distinguish between types 1 or 3, we maximized the probability to observe functional IP_3 receptors channels.

The open probability P_o was calculated from the total amplitude histogram obtained from each record, taking the ratio of the areas corresponding to the open channel over the sum of the closed and open areas estimated from the 2 Gaussian fit of the histogram. The open-channel amplitude was calculated as the difference of the means obtained for the closed and open areas.

RESULTS

Figure 1 shows the immunolabeling of isolated nuclei using fluorescent probes and anti IP_3 receptor antibodies, there is a continuous labeling pattern in the nuclear envelope region for the type-1 IP_3 receptor (Fig. 1a). For the type-3 IP_3 receptor, the label also was seen in the nuclear envelope, but a distinct label of intra nuclear structures also can be seen (Fig. 1b). When nuclei were loaded with a calcium-sensitive dye that remains within the nucleoplasmic space, a decrease in fluorescence upon ATP

addition indicates calcium uptake by the nuclear envelope compartment (Fig. 2). The addition of 10 μM IP_3 to the nuclei bathing medium produced a fast increase in fluorescence that suggests calcium release from the nuclear envelope towards the nucleoplasm.

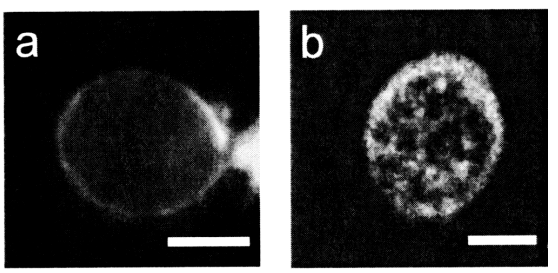

Figure 1. Immunofluorescence analysis of isolated nuclei from the 1B5 cell line.
a. Type-1 IP_3R shows a continuous labeling in the nuclear envelope region; **b.** Type-3 IP_3R shows both a continuous label in the nuclear envelope and important structured label in the nucleoplasm. Bar = 5 μm.

Figure 3 shows typical single-channel current traces corresponding to the IP_3 receptor activity from patches of nuclei obtained from rat primary myotubes in the inside-out configuration. The myonuclei were pre-incubated for 1 hour with 50 μM Ryanodine to eliminate ion channels activity corresponding to the Ryanodine receptor. The pipette voltage was held at -40 mV. After IP_3 was perfused into the recording pipette, the trace shows an 8-minute period without activity (Fig. 3b). Part of this period possibly corresponds to the time required for IP_3 diffusion to the tip of the pipette. As shown in Figure 3c, once IP_3 reached the minimal concentration to activate the channel, a typical single channel recording was observed. For each trace, the open probability P_o can be estimated, and the corresponding current amplitude histogram is depicted. The calculated P_o was 0.25 ± 0.01 at the onset of activity. As IP_3 diffusion progressed, it produced an increase in the open probability. As seen in figure 3d, 18 minutes after the IP_3 perfusion in the pipette, the open probability reached a maximum P_o of 0.84 ± 0.02.

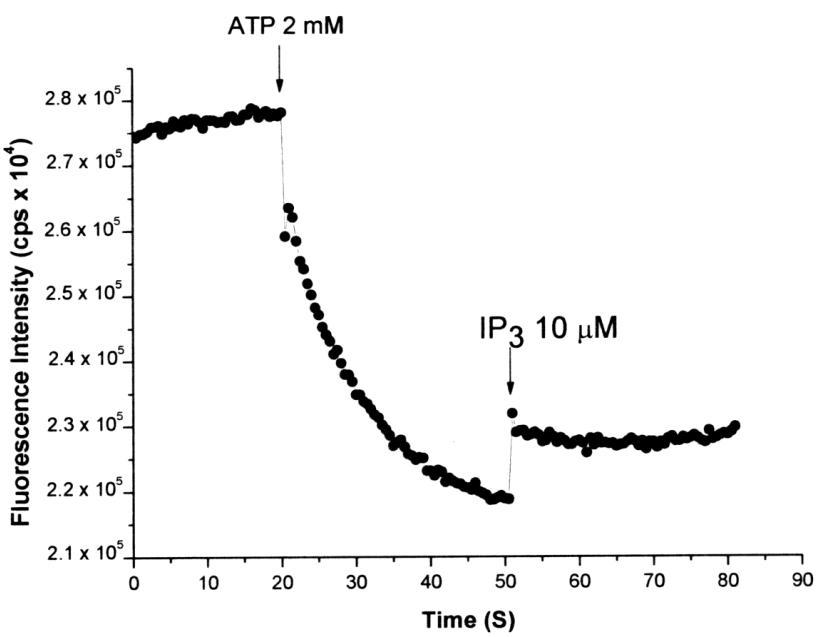

Figure 2. Calcium load and release in isolated nuclei from the 1B5 cell line.
Fluorescence measurement in a nuclei suspension loaded with Fluo-3AM, shows that addition of 2.5 mM ATP induced an important fluorescence decrease, while 10 μM IP_3 induced a fluorescence increase. Traces shown are representative of almost 35 different measures from 6 different preparations.

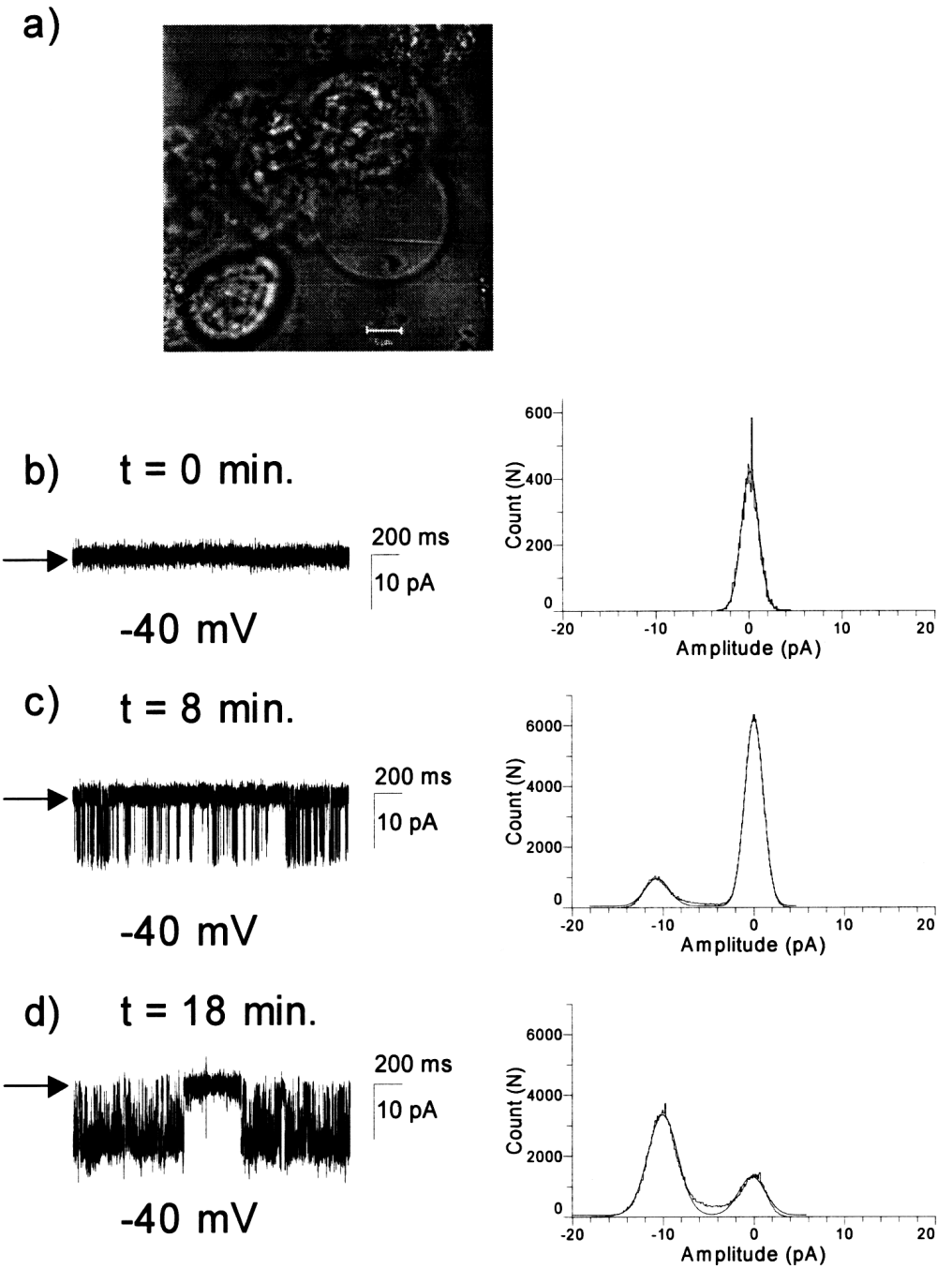

Figure 3. Detection of native IP_3R channels in isolated rat myonuclei.
Fig. 3a: Transmitted light image of an isolated nucleus showing a membrane bleb. The patch pipette always was attached to the bleb region of the myonucleus. The IP_3 dependence of gating was confirmed by direct perfusion of IP_3 in the pipette. **Fig. 3b** corresponds to the period of IP_3 diffusion in to the pipette; **Fig 3c**, 8 minutes later; and **Fig. 3d**, 18 minutes after IP_3 perfusion. The P_0 was 0.25 ± 0.02 at 8 minutes after IP_3 perfusion and reached a maximum of 0.84 ± 0.02 at 18 minutes. The pipette potential was -40 mV. Pipette solution contained 140 mM KCl, 300 nM Ca^{2+}, Hepes 10 mM, pH = 7.1, and the bath solution contained identical solution, except that IP_3 was not added, and in rat myonuclei, 50 μM Ryanodine was added. The arrow denotes the zero current level.

To further characterize the IP_3 receptor channel activity in 1B5 cells nuclei, we made recordings with a fixed initial concentration of IP_3 in the pipette (1 and 10 μM). These cells are devoid of Ryanodine receptors, so IP_3 receptors are probably the only calcium-release channels present. We observed IP_3 receptor activity in 10% of successful seals. As a control, we made 20 seals without IP_3 addition into the pipette. We could not distinguish ion channel activity from background noise in this condition. Figures 4a and 4b show two typical current traces obtained at 1 and 10 μM IP_3, respectively. The pipette potential in both cases was held at 30 mV. There is a marked tendency to find the channel open at the higher IP_3 concentration.

We calculated channel amplitude and P_o in equal samples of 2 seconds duration from the records obtained with 1 and 10 μM IP3 in the pipette. The mean channel amplitude was 6.4 ± 0.1 pA for 1 μM IP_3 and 7.4 ± 0.9 pA for 10 μM IP_3. The calculated P_o was 0.27 ± 0.01 for 1 μM IP_3 and 0.64 ± 0.01 for 10 μM IP_3. Figure 4c summarizes these properties for all tested voltages at both IP_3 concentrations. Again, there is an overall higher P_o at all voltages at 10 μM IP_3.

Figure 5a shows traces at different pipette voltages with 10 μM IP_3. We measured the single-channel current amplitude at each pipette voltage tested. Figure 5b collects these values plotted versus the applied voltage. The curve did not show rectification within the applied voltage range, the slope conductance for the channel varied between 183 and 289 pS, in agreement with the broad range of conductance values that has been reported in previous works (Mack and Foskett, 1998). Due to the variations in conductance values within the above-mentioned range, we could not ascertain a difference in conductance between the two concentrations of IP_3 used.

DISCUSSION

Several reports indicate the presence of IP_3R activity in the nucleus in different cell types. Direct electrophysiological measurements show IP_3-dependent receptor channel activity in isolated nuclei from *Xenopus Leavis* oocytes (Stehno-Bittel et al., 1995) or from mammalian nuclear envelope (Boehning et al., 2001). On the other hand, Gerasimenko et al. (1995), demonstrated using confocal microscopy that isolated nuclei from liver show an increase of nucleoplasm Ca^{2+} after IP_3-stimulation and determined that the source of Ca^{2+} was in the nuclear envelope. However, the source of nuclear Ca^{2+} remains a controversial issue. The presence of nuclear pores, with a diameter of 9 nm and a length of 15 nm, suggests that it should be permeable to all molecules up to 50 kDa (Stoffler et al., 1999) and implies the assumption that Ca^{2+} is able to equilibrate rapidly between the cytosolic and nuclear compartments by simple diffusion. In the neuroendocrine pancreatic β-cell, it was demonstrated that cytosolic Ca^{2+} oscillations induced by glucose, potassium, and carbachol enter the nucleus without restriction (Brown et al., 1997). No significant differences between nuclear and cytosolic Ca^{2+} concentration were observed in HeLa cells when monitoring Ca^{2+} inside the nucleus, using a chimeric cDNA encoding a fusion protein with the photoprotein aequorin and by a nuclear translocation signal derived from the rat glucocorticoid receptor (Brini et al., 1993). However, it has been demonstrated both in oocytes and in HeLa cells that is possible to induce an IP_3-dependent rise of nuclear Ca^{2+}, independently of the cytoplasm (Lui et al., 1998; Santella and Carafoli, 1997). Furthermore, the participation of IP_3Rs in the regulation of nuclear Ca^{2+} in skeletal muscle has been described only recently (Cárdenas et al., 2005).

The presence of IP_3 receptors in the nuclear envelope membrane from myotubes has been determined using immunocytochemistry, binding studies, and Western blots (Cardenas et al., 2005). Functional studies of IP3 receptor channel activity in the nucleus are scarce nevertheless (Marchenko et al., 2005). Lack of more detailed information is attributable partly to the difficulties in obtaining gigaohm seals in the nuclear membrane. The presence of the nuclear pore complex and that of attached endoplasmic reticulum

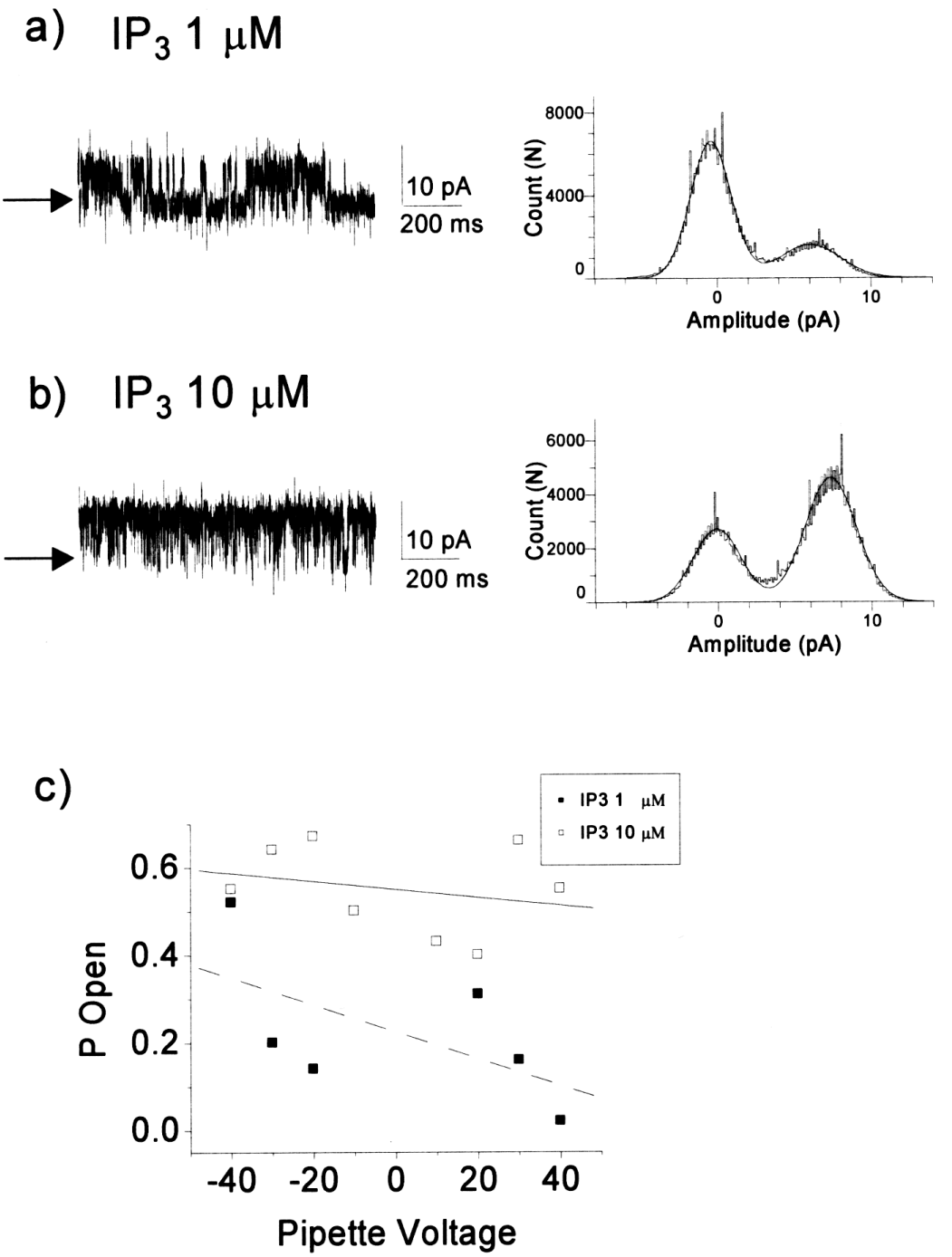

Figure 4. The IP_3 receptor open probability increases in an IP_3-dose dependent manner.
Fig. 4a and 4b: The pipette voltage was 30 mV. The bath and pipette solution contained 140 mM KCl, 10 mM HEPES, and 300 nM free [Ca^{2+}] and was adjusted to pH 7.1 with KOH. 1 and 10 μM IP_3 was added in the pipette solution in Fig. 4a and Fig 4b respectively. The corresponding current amplitude histograms are depicted in the right panel. The mean amplitude was 6.4 ± 0.1 pA for 1 μM IP_3 and 7.4 ± 0.9 pA for 10 μM IP_3. We estimated P_o using the area beneath Gaussian curve as: Open area/Total area. The calculated P_o was 0.27 ± 0.01 for 1 μM IP_3 and 0.64 ± 0.01 for 10 μM IP_3. **Fig. 4c:** Values for P_0 with 1 and 10 μM IP_3 at the different pipette voltages tested. P_0 was larger with 10 μM IP_3 than with 1 μM IP_3 at all voltages tested.

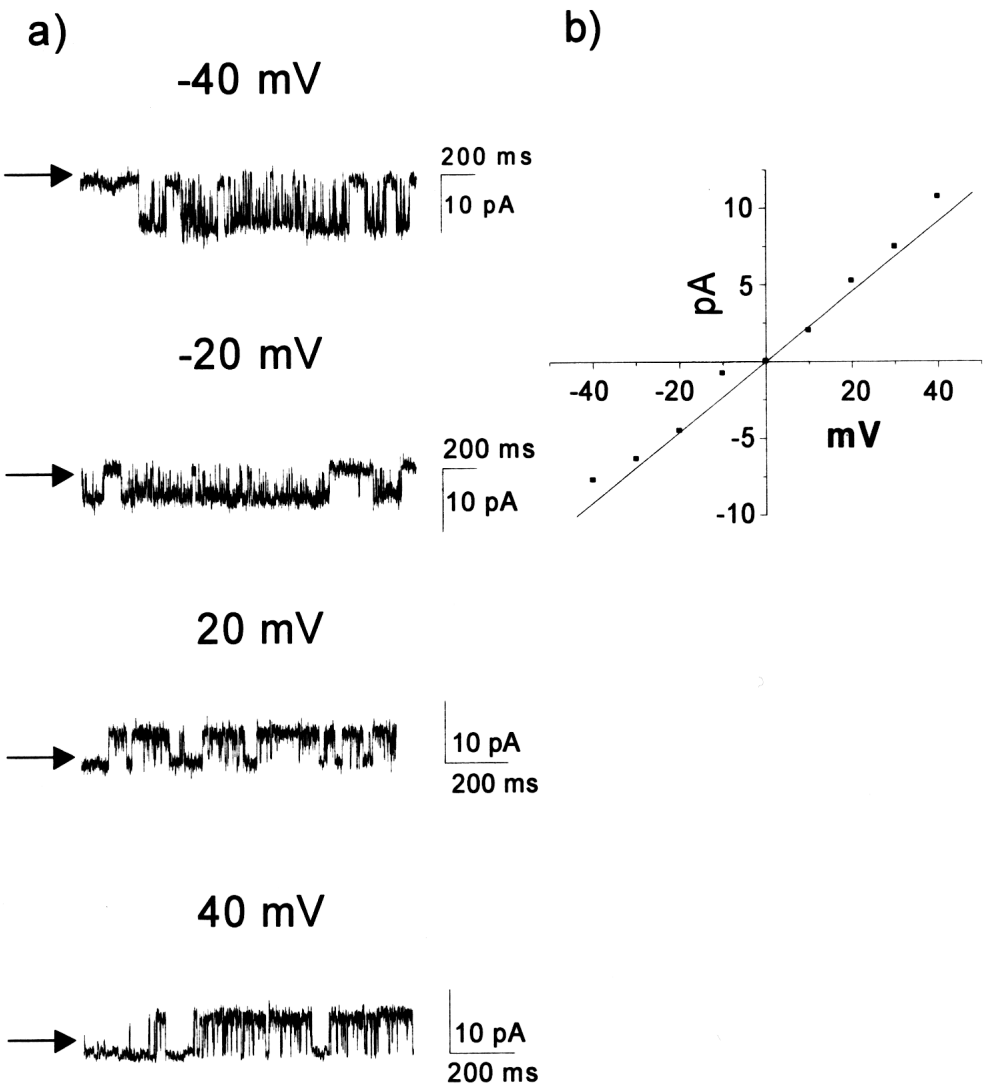

Figure 5 Voltage current relationship for IP_3-receptor channel is linear.
Fig. 5 a: Typical traces of single-channel recordings at different pipette voltages. The bath and pipette solution contained 140 mM KCl, 10 mM HEPES and 300 nM free [Ca^{2+}] and was adjusted to pH 7.1 with KOH. 10 μM IP_3 was added in the pipette solution. The arrow shows the zero current level. **Fig. 5b** shows an IV plot corresponding to the same experiment. The data points represent current amplitudes determined at holding potentials between -40 mV and 40 mV from traces shown in Fig. 5a. Solid lines, linear regressions for each dataset, the R coefficient was 0.99391 with $P < 0.0001$. The channel had a slope conductance of 228 ± 13 pS.

membranes have been offered as explanations (Mazzanti et al., 2001). We concur with the authors of previous works in the technical difficulties inherent to these preparations. We successfully patch clamped a reduced fraction of nuclei by attaching the patch pipette to large membrane blebs that come out of the muscle cell isolated nuclei. The presence of these blebs in the nuclear envelope region suggests that the membrane surface area of this structure is much larger than that corresponding to a sphere having the nuclear diameter. Thus, it is most likely that invaginations of the nuclear envelope are present (Cárdenas et al., 2005) and may be the source of these blebs.

The fact that we were able to record IP_3-sensitive channels by direct aspiration of the membrane into the pipette may indicate that we are patching the outer nuclear membrane in its right-side-out configuration (Fig. 6). Immunocytochemical studies and immunoelectron microscopy (Cárdenas et al., 2005) suggest that both type-1 and type-3 IP_3R are present in the nuclear envelope region, as well as in invaginations towards the nucleoplasm. Type-1 IP_3R co-localizes with an inner nuclear membrane marker, and for both types, immuno gold particles are much more abundant in the inner than in the outer nuclear membrane region (Cárdenas et al., 2005). Nevertheless, some particles of anti-type-1 IP_3R antibody were seen in the outer nuclear membrane region. Although unlikely, it is conceivable as well that during formation of the bleb, the inner nuclear membrane (and the IP_3-sensitive site) could get exposed to the external medium, though we cannot establish whether the channels we see are placed in the inner or outer nuclear membrane.

As for distribution of IP_3R in the nucleus, not much is known about their precise topology. Humbert et al. (1996) demonstrated that IP_3Rs are located in the inner nuclear membrane. However, it has been demonstrated recently that different IP_3R isoforms have different location in the nucleus; in HepG2 liver cells, type-2 IP_3Rs are expressed in the nucleus, mainly in the nuclear envelope and occasionally in the nucleoplasm, while the type-3 IP_3R only was expressed in the cytoplasm, and type-1 was absent in these cells (Leite et al., 2003). In addition, these authors measured Ca^{2+} signals induced by IP_3 and found different responses in the nucleus and in the cytoplasm, which were attributed to

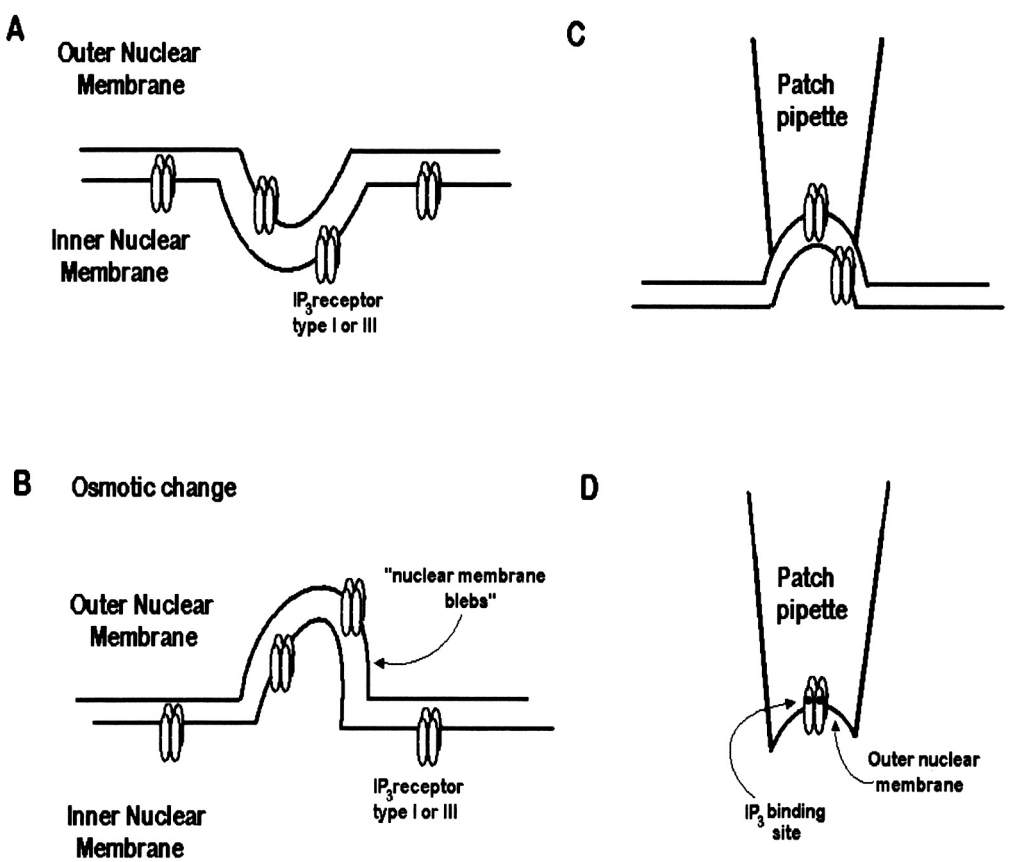

Figure 6. Schematic description of the possible location of IP_3 receptors in the nuclear envelope membranes.

different characteristics of IP_3R isoforms (Leite et al., 2003). In endothelial cells, type-2 IP_3R are distributed uniformly within the nucleus, but type-1 and type-3 IP_3Rs were absent from the nucleus (Laflamme et al., 2002). In neonatal rat cardiomyocytes, the distribution of receptors is similar to that presented here for myotubes (Ibarra et al., 2004), while in adult cardiac cells, type-2 IP_3R is present in the nuclear envelope (Bare et al., 2005).

The presence of tubular structures inside the nucleus was described in the C6 glioma cell line and in HeLa cells. These structures apparently correspond to prolongations of the nuclear envelope, enriched with IP_3R (Lui et al., 1998; Lui et al., 2003). The nucleoplasmic reticulum, a reticular network of nuclear Ca^{2+} store that is continuous with the ER and nuclear envelope, was proposed recently in SKHep1 epithelial cells (Echevarria et al., 2003) to be enriched in type-2 IP_3R and in minor proportion with type-3 IP_3R. Moreover, through photo release of nitrophenylethyl ester-caged IP_3 microinjected in the cell and confocal laser scanning microscopy IP_3-induced Ca^{2+} release from the nucleoplasmic reticulum was detected (Echevarria et al., 2003). We found that in skeletal muscle cells, all three isoforms of IP_3R are expressed with different intracellular distribution (Cárdenas et al., 2005). Type-2 IP_3R was distributed more or less homogeneously with a reticular pattern that probably represents sarcoplasmic reticulum membranes in undifferentiated myotubes. We previously have reported that type-1 IP_3R is expressed in the nuclear envelope as well as in the sarcoplasmic reticulum with a striated pattern (Powell et al., 2001). Furthermore, type-3 IP_3R was distributed mainly inside the nucleus, not exclusively associated to the nuclear membrane, as assessed both by minimal confocal co-localization using LAP2, and by electron microscopy using immuno-gold (Cárdenas et al., 2005).

Taken together, these data shows that IP_3R isoforms have a differential intracellular distribution in skeletal muscle cells and at least one of them functions as Ca^{2+}-release channel within nuclear compartments. This finding, together with the presence of Ca^{2+}-pump activity associated to isolated nuclei, suggest a role of the nuclear envelope and associated membrane structures in the regulation of nucleoplasmic Ca^{2+} concentration.

Local nuclear IP_3 production and local Ca^{2+} release are likely to play a mayor role in regulating both transcription factors and the transcription process of many genes; isolated nuclei from cultured muscle cells constitute a good model system to unravel such mechanisms.

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