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L, P-/Q- and T-type Ca²⁺ Channels in Smooth Muscle Cells from Human Umbilical ArterySilvia Salemme¹, Alejandro Rebolledo¹, Francisco Speroni², Silvana Petruccelli² and Verónica Milesi¹

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Key Words

Ca²⁺ channels • Human umbilical artery • Patch clamp

Abstract

The electrophysiological and pharmacological properties of Ca²⁺ current (I_{Ca}) were determined by the whole-cell configuration of the patch-clamp technique in smooth muscle cells from human umbilical artery. Using 5 mM extracellular Ca²⁺, depolarizing step pulses from -60 to 50 mV from a holding membrane potential of -80 mV evoked an I_{Ca} which activated at membrane potentials more positive than -50 mV and exhibited a maximum current density in a range of 10-20 nA. Steady-state inactivation protocols using a V_{test} of 10 mV gave a voltage at one-half inactivation and a slope factor of -35.6 mV and 9.5 mV, respectively. Nifedipine (1 μM), an L-type Ca²⁺ channels antagonist, completely inhibited I_{Ca}, while the L-type Ca²⁺ channels agonist Bay-K 8644 (1 μM) significantly increased I_{Ca} amplitude. Moreover, the selective blocker of P-/Q-type Ca²⁺ channels ω-agatoxin IVA partially blocked I_{Ca} (about 40 % inhibition at +20 mV by 20 nM). These pharmacological results suggest that L- and P-/Q-type Ca²⁺ channels, both

nifedipine-sensitive, underlie the I_{Ca} registered using low extracellular Ca²⁺. The presence of the P-/Q-type Ca²⁺ channels was confirmed by immunoblot analysis. When I_{Ca} was recorded in a high concentration (30 mM) of extracellular Ca²⁺ or Ba²⁺ as current carrier, it was evident the presence of a nifedipine-insensitive component which completely inactivated during the course of the voltage-step (75 ms) at all potentials tested, and was blocked by the T-type Ca²⁺ channels blocker mibefradil (10 μM). Summarizing, this work shows for the first time the electrophysiological and pharmacological properties of voltage-activated Ca²⁺ currents in human umbilical artery smooth muscle cells.

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Introduction

Vascular smooth muscle tone is regulated by different mechanisms. One of the most important is the degree of Ca²⁺ permeability of the plasma membrane which is determined by the presence of several Ca²⁺ entry pathways. It has been demonstrated that low- and high-voltage-activated Ca²⁺ channels (LVA and HVA,

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respectively) are expressed in different kinds of vascular smooth muscle cells. Different pore-forming α subunits have been cloned and expressed in heterologous systems observing that alternative splicing exons and coexpression with $\alpha_2\delta$, γ and β subunits, play important regulatory roles in the properties of functional Ca^{2+} channels in native cells [1, 2].

Among the HVA Ca^{2+} channels family, the dihydropyridine-sensitive L-type Ca^{2+} channels are, by far, the most studied [3-6]. However, other members of the HVA Ca^{2+} channels family, the P-/Q-type Ca^{2+} channels, have been recently described in rat vascular smooth muscle cells, having a functional role in renal afferent arterioles [7]. Regarding the LVA Ca^{2+} channels, the presence of the T-type Ca^{2+} channels has been reported in several vessels [4, 8-11].

The study of the presence of different types of voltage-activated Ca^{2+} channels, and their electrophysiological and pharmacological properties, in human vascular smooth muscle cells is important to increase the knowledge of Ca^{2+} pathways that could be involved in contractile responses induced by changes in cell membrane potential or other vasoactive stimuli that can affect their activity. Nevertheless, the vast majority of reports in the literature regarding Ca^{2+} channels involve non-human vessels or cultured cells, and the information about human vascular smooth muscle is scant. In particular, P-/Q-type Ca^{2+} channels have not yet been described, to the best of our knowledge, in human vessels.

We focused our attention in Ca^{2+} channels of the human umbilical artery (HUA). In this vessel, there are some reports showing the effects of Ca^{2+} channels antagonist on arterial contractility [12, 13], and we have also reported that nifedipine induced a complete relaxation of HUA rings contraction induced by a depolarizing high K^+ solution [14]. However, there is no information regarding neither the electrophysiological characteristics nor the molecular structure of voltage-activated Ca^{2+} channels expressed in this artery.

In this work, we show for the first time the electrophysiological and pharmacological properties of voltage-activated whole-cell Ca^{2+} current in HUA smooth muscle cells, which suggest that L, P-/Q- and T-type Ca^{2+} channels are present in this vessel. Additionally, the presence of the α subunit of the P-/Q-type Ca^{2+} channels was confirmed by immunoblot analysis. L- and P-/Q-type Ca^{2+} channels, both sensitive to nifedipine 1 μM , underlie the majority of voltage-activated calcium current recorded with extracellular solutions containing 5 mM of Ca^{2+} , whereas the contribution of T-type Ca^{2+} channels could

be evidenced using a high extracellular Ca^{2+} or Ba^{2+} concentration.

Materials and Methods

Cell isolation procedure

Umbilical cords obtained after vaginal and cesarean delivery were placed in saline solution containing (in mM): 130 NaCl, 4.7 KCl, 1.17 KH_2PO_4 , 1.16 MgSO_4 , 24 NaHCO_3 , 2.5 CaCl_2 , pH 7.4 at 4°C and immediately transported to our laboratory and stored at 4°C. The arteries were dissected from the Wharton's jelly just before the cell isolation procedure. HUA smooth muscle cells were obtained by a method based on the one described by Klockner [15] and later modified in our laboratory in order to diminish the enzyme content in the dissociation medium (DM) [14]. Briefly, a segment of HUA was cleaned of any residual connective tissue, cut in small strips and placed for 15 min in a DM containing (in mM): 140 NaCl, 5 KH_2PO_4 , 5 MgCl_2 , 20 glucose, 5 HEPES, pH was adjusted to 7.4 with NaOH. The strips were then placed in DM with 2 mg/ml collagenase type I during 25 min, with gentle agitation, at 35°C. After the incubation period the strips were washed with DM and single HUA smooth muscle cells were obtained by a gentle dispersion of the treated tissue using a Pasteur pipette. The remaining tissue and the supernatant containing isolated cells were stored at room temperature (~20°C) until used.

Patch-clamp recordings

HUA smooth muscle cells were allowed to settle onto the coverglass bottom of a 0.5 ml experimental chamber. The cells were observed with a mechanically stabilized, inverted microscope (Telaval 3, Carl Zeiss, Jena, Germany) equipped with a 40X objective lens. The chamber was perfused for 15 min, at 0.5 $\text{ml}\cdot\text{min}^{-1}$ by gravity, with the extracellular saline solution (ESS, see composition later) before the patch-clamp experiment was started. Application of test solutions was performed through a multibarreled pipette positioned close to the cell investigated. After each experiment on a single cell, the experimental chamber was replaced by another one containing a new sample of cells. Only well-relaxed, spindle-shaped smooth muscle cells were used for electrophysiological recordings. Data were collected within 4-6 h after cell isolation. All experiments were performed at room temperature (~20°C).

The standard tight-seal whole-cell configuration of the patch-clamp technique [16] was used. Glass pipettes were drawn from WPI PG52165-4 glass on a two-stage vertical micropipette puller (PP-83, Narishige Scientific Instrument Laboratories, Tokyo, Japan) and pipette resistance ranged from 2 to 4 M Ω measured in ESS. Ionic currents were measured with an Axopatch 200A amplifier (Axon Instruments, Foster City, CA). Whole-cell currents were filtered at 2 kHz, digitized (Digidata 1200 Axon Instruments, Foster City, CA) at a sample frequency of 100 kHz, except in the case of the steady-state inactivation protocols in which the sampling frequency was 20 kHz. The experimental recordings were stored on a computer

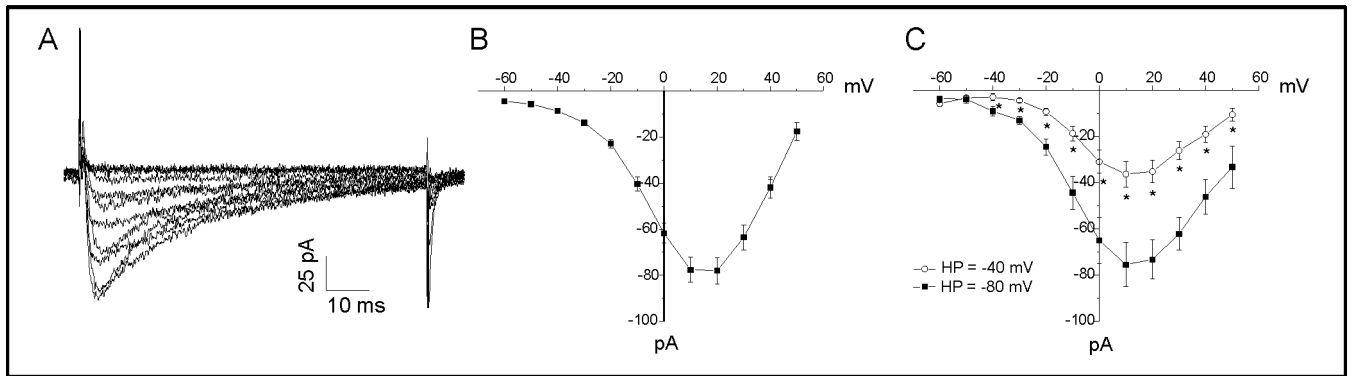


Fig. 1. Whole-cell Ca^{2+} current (I_{Ca}) in HUA smooth muscle cells. A: superimposed representative recordings of I_{Ca} elicited by voltage pulses between -60 and 50 mV from a membrane holding potential (HP) of -80 mV. B: mean I-V curve obtained from $n=50$ cells. C: mean I-V curves obtained using a membrane HP of -80 mV or -40 mV ($n=9$ cells); * indicates statistical difference from the current value obtained using a membrane HP of -80 mV ($p<0.05$).

hard disk for later analysis. Whole-cell capacitance was compensated electronically in most cells. In all cells whole-cell current was controlled applying test voltage steps during a short stabilization period discarding those in which the current amplitude did not remain constant with time.

Solutions

The ESS used for recording whole-cell ionic currents contained (in mM): 116 NaCl, 4.7 KCl, 5 CaCl_2 , 6 glucose, 5 HEPES, 10 tetraethylammonium (TEA); pH was adjusted to 7.4 with NaOH. In some experiments we used a CaCl_2 concentration of 30 mM while NaCl was adequately diminished to maintain the solution osmolarity. An identical ESS but containing 30 mM of BaCl_2 instead of CaCl_2 was also used.

The composition of the intracellular pipette solution (IPS) was (in mM): 120 CsCl, 5 Na_2ATP , 1 MgCl_2 , 10 glucose, 10 EGTA, 20 HEPES; pH was adjusted to 7.3 with CsOH.

SDS-PAGE and Immunoblot Analysis

Human umbilical arteries were isolated as described above. Endothelial layer was eliminated mechanically. Smooth muscle proteins were extracted with 20 mM Tris-HCl, 8 M Urea, 1% SDS, pH 7.5 and were resolved by sodium dodecylsulphate-polyacrylamide gel (6%) electrophoresis (SDS-PAGE) followed by transfer to nitrocellulose membranes (Schleicher & Schuell Bioscience, Inc., USA). The membranes were stained with Ponceau S to monitor protein loading prior to immunodetection. Nitrocellulose membranes were first blocked with 3 % (w/v) non-fat milk for 1 h at 37°C. After several washes, the membranes were incubated overnight at 4°C with rabbit antibodies against $\alpha 1\text{A}$ subunit of the P-/Q-type Ca^{2+} channel at a dilution of 1:500 (Alomone Labs, Jerusalem, Israel). Subsequently, the membranes were incubated with horseradish-conjugated anti-rabbit secondary antibody, and immunoreactive signals were detected with luminol followed by an exposure to X-ray film (Kodak, USA). The experiments were repeated after blocking the primary antibodies by pretreatment with a control peptide provided by the manufacturer.

Reagents

Collagenase type I (used for cell isolation), ethylene glycol-bis(β -aminoethyl ether) N,N,N',N' -tetraacetic acid (EGTA), CsCl, Na_2ATP , nifedipine, Ponceau S and luminol were purchased from Sigma-Aldrich (St Louis, MS, USA), and (\pm) Bay-K 8644 and ω -agatoxin IVA were purchased from Alomone Labs. Ltd. (Jerusalem, Israel). All other reagents were of analytical grade and purchased from local vendors. Stock solutions of nifedipine and Bay-K 8644 were prepared in DMSO while ω -agatoxin IVA and mibefradil were dissolved in water. Fresh aliquots of stocks solutions were added to the different extracellular solutions on the day of the experiment. The final maximal concentration of DMSO in the solutions used for current measurements was very low (0.05%), and data from the literature show that even greater concentrations (0.1%) of DMSO did not affect calcium currents [17, 18].

Statistics

The results are expressed as means \pm SEM. Paired or unpaired Student's t-tests were used to establish statistically significant differences between two groups. The level of statistically significant differences was set to $p<0.05$.

Ethical considerations

The collection of umbilical cords was authorized by the Ethics Committee of the Hospital General Interzonal de Agudos Gral. San Martin de La Plata, and the patients were informed that the umbilical arteries would be used for scientific research. The experimental protocols were approved by the Scientific Committee of our institution.

Results

To study the presence of voltage-activated Ca^{2+} channels in freshly dispersed smooth muscle cells from HUA, whole-cell currents were recorded using a high

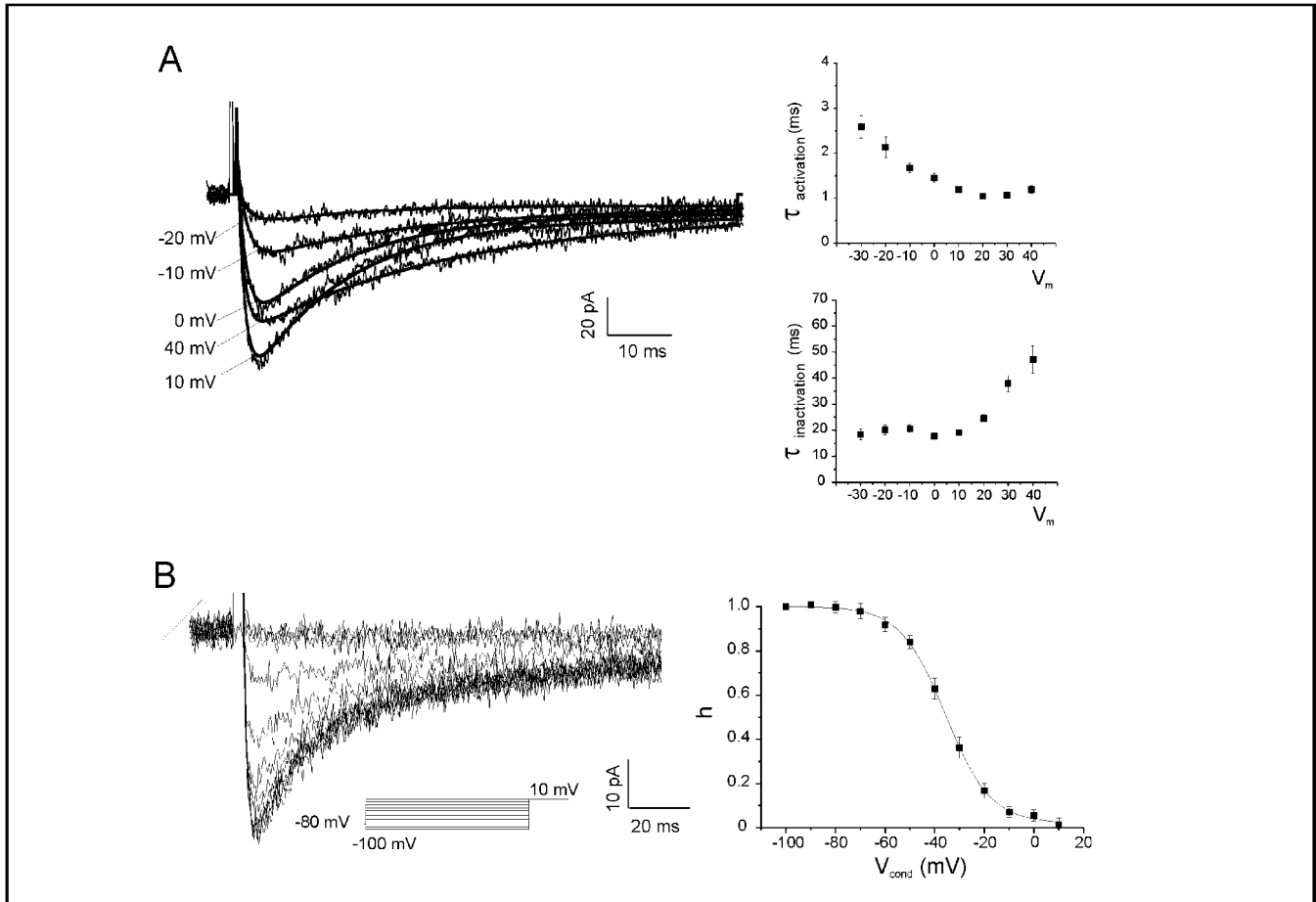


Fig. 2. Activation and inactivation properties of I_{Ca} . A: representative recordings of I_{Ca} with the fit curves superimposed, and the relationships between mean values of $\tau_{activation}$ and $\tau_{inactivation}$ plotted against membrane potential (V_m) ($n=34-36$ for each value). B: I_{Ca} registered by application of steady-state inactivation protocols using a V_{test} of 10 mV ($n=9$ cells) and its corresponding h-curve.

Ca^{2+} IPS, and 5 mM Ca^{2+} in the ESS. Figure 1A shows typical whole-cell Ca^{2+} currents (I_{Ca}) evoked by a voltage-clamp protocol applying 10 mV increase voltage-steps from -60 to 50 mV using a membrane holding potential (HP) of -80 mV. The mean peak current-voltage (I-V) relationship obtained from 50 smooth muscle cells of 18 different HUA samples is depicted in figure 1B. I_{Ca} begins to activate at membrane potential (V_m) values more positive than -50 mV (range -40 to -30 mV among different cells), it presents a maximum current density at a value between 10 and 20 mV, and an extrapolated reversal potential of approximately 60 mV. When I-V curves were obtained using a more depolarized membrane HP (-40 mV) the threshold of activation for I_{Ca} was around -20 mV, its maximum at membrane potentials between 10 and 20 mV, and its peak amplitude was significantly lower at all V_m tested compared to I-V curves obtained in the same cells using a membrane HP of -80 mV (figure 1C).

The I_{Ca} evoked by the different voltage-steps were well fitted with a double exponential function ($A_1e^{-(t-k)/\tau_{activation}} + A_2e^{-(t-k)/\tau_{inactivation}} + C$) obtaining two time parameters ($\tau_{activation}$ and $\tau_{inactivation}$) representative of activation and decay of inactivation kinetics, respectively. Figure 2A shows typical examples and the relationships between these parameters and the V_m , where it is possible to observe that while $\tau_{activation}$ decreased when V_m became more depolarized, $\tau_{inactivation}$ was voltage-independent up to 0 mV, while finally increased between 0 and 40 mV.

Steady-state inactivation of I_{Ca} was determined using a standard two-pulse protocol, with a membrane HP of -80 mV and a test potential (V_{test}) of 10 mV. 4-second-conditioning pulses from -100 mV to 10 mV (10 mV steps at 0.1 Hz) were applied before the test potential to assess I_{Ca} availability. Peak I_{Ca} amplitude elicited by the test potential, expressed as a proportion of the maximal I_{Ca} recorded, was plotted against the value of the conditioning potentials. A steady-state inactivation curve was obtained

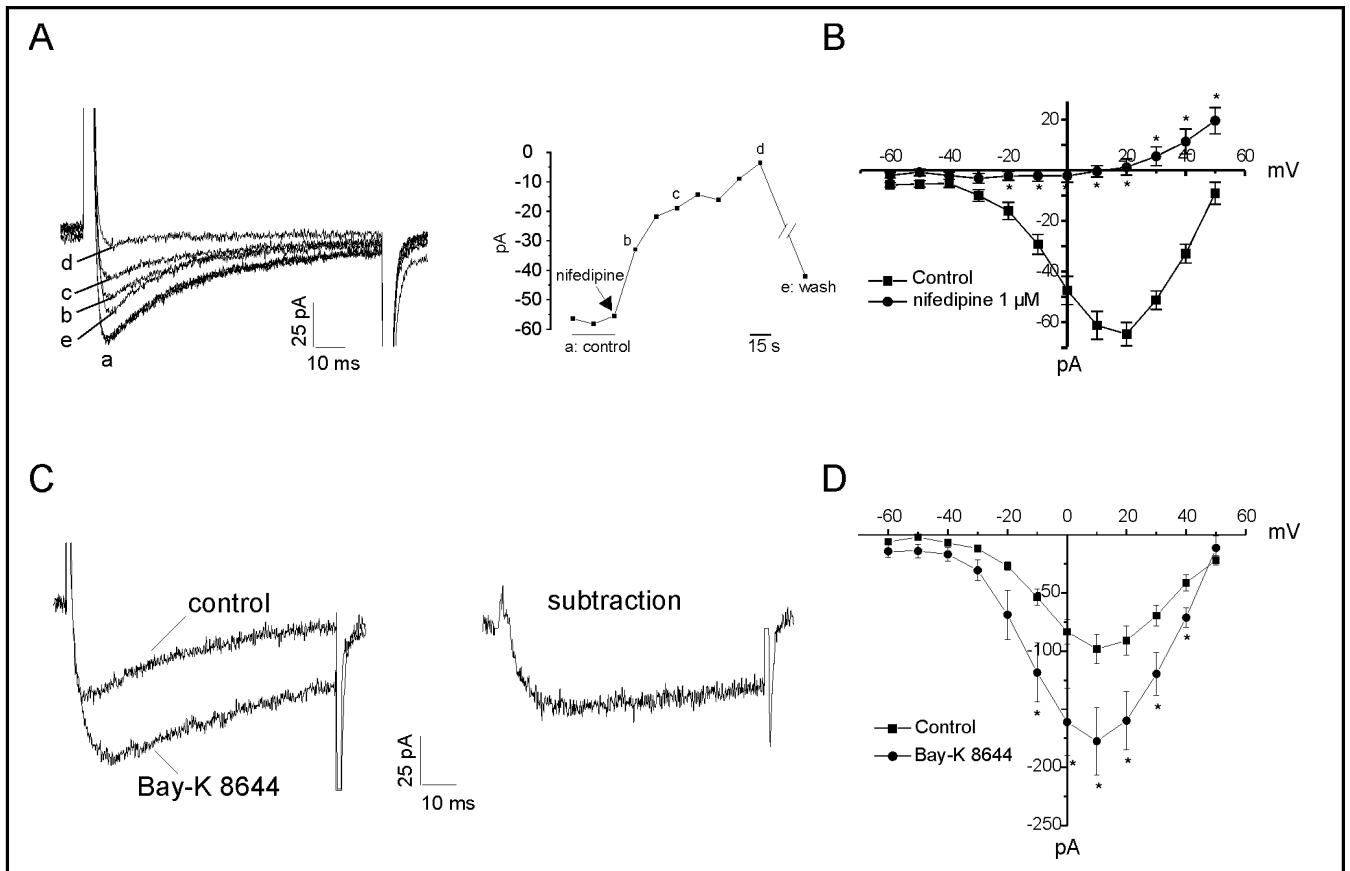


Fig. 3. Effect of nifedipine and Bay-K 8644 on I_{Ca} . A: Left panel: Typical recordings of I_{Ca} elicited by a voltage step from a membrane HP of -80 mV to 20 mV in (a) control conditions (3 superimposed recordings); after 15 s (b), 45 s (c) and 105 s (d) of addition of 1 μ M nifedipine; and after partial wash of nifedipine effects (e). Right panel: typical time course of blockade of peak I_{Ca} by 1 μ M nifedipine; the points (a) to (e) correspond to those shown in the left panel. B: mean I-V relationships in control conditions and after stable effect of 1 μ M nifedipine (n=7 cells). C: Typical recordings of I_{Ca} elicited by a voltage step from a membrane HP of -80 mV to 20 mV before and after Bay-K 8644 effect (superimposed), and the corresponding Bay-K 8644-induced I_{Ca} obtained by current subtraction. D: Mean I-V curves for control I_{Ca} and after enhancement of the current by Bay-K 8644 (n=6 cells). In B and D, * indicates statistical difference from the control value ($p < 0.05$).

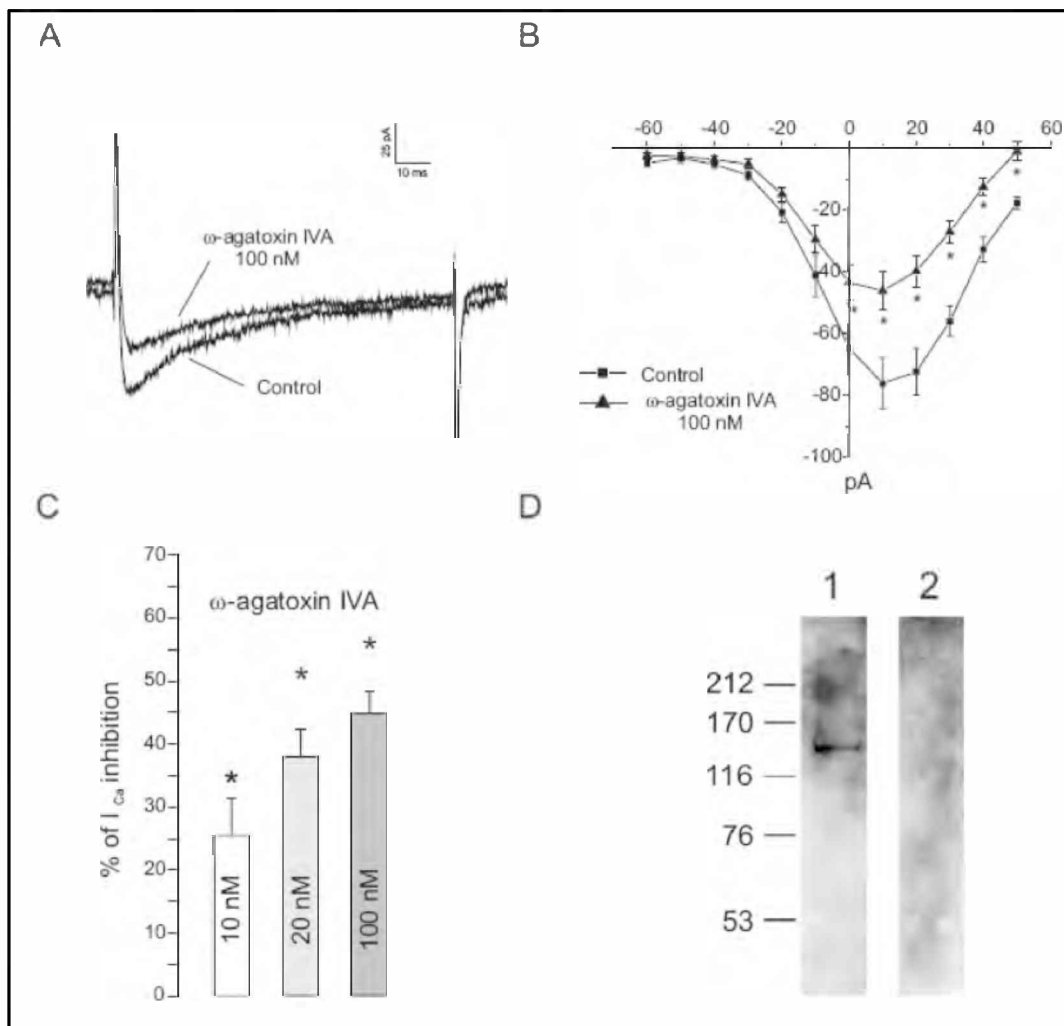
by fitting mean fraction current values with a standard Boltzmann equation: $h = \{1 + \exp[(V_m - V_{1/2})/k]\}^{-1}$, where h is the fraction of current inactivated, V_m is the membrane potential, $V_{1/2}$ is the voltage at one-half inactivation and k is the slope factor. The $V_{1/2}$ and k values obtained were -35.6 mV and 9.5 mV, respectively (Figure 2B), which is in agreement with the fact reported above (figure 1C) that when a membrane HP of -40 mV was used the peak currents obtained were about half of those registered using a membrane HP of -80 mV.

Nifedipine (1 μ M), the commonly used L-type Ca^{2+} channels antagonist, rapidly induced a complete inhibition of I_{Ca} (figure 3 A and B), while the L-type Ca^{2+} channel agonist Bay-K 8644 (1 μ M) significantly increased I_{Ca} amplitude. Although nifedipine, at a concentration commonly used as L-type Ca^{2+} channel blocker in vascular

smooth muscle cells, had a complete inhibitory effect on total Ca^{2+} current, suggesting that L-type Ca^{2+} channels underlie the total current, other members of the HVA Ca^{2+} channels family, in particular the P-/Q-type, have also been shown to be sensitive to nifedipine in the same range of concentration as L-type Ca^{2+} channels in other kinds of cells [19, 20]. Since P-/Q-type Ca^{2+} channels, extensively described in neurons, have been found only recently in vascular smooth muscle cells [7], we tested the effect of ω -agatoxin IVA, the selective blocker of P-/Q-type Ca^{2+} channels, on I_{Ca} in HUA smooth muscle cells observing that it was partially blocked: at 20 mV about 25% of the I_{Ca} was inhibited by 10 nM of toxin, and the inhibitory effect was higher for toxin concentrations of 20 or 100 nM (figure 4). The effect was reversible in most cells when the toxin was used at 10 nM, while at 20

Fig. 4. Effect of ω -agatoxin IVA on I_{Ca} and expression of P-/Q-type Ca^{2+} channel in human umbilical artery.

A: Typical recordings of I_{Ca} elicited by a voltage step from a membrane HP of -80 mV to 20 mV in control conditions and after a stable inhibition by 100 nM ω -agatoxin IVA. B: mean I-V curves obtained for control conditions and after stable effect of 100 nM ω -agatoxin IVA (n=5 cells); * indicates statistical difference from the control value (p<0.05). C: Bars represent average \pm SEM values of % of inhibition of I_{Ca} (at +20 mV) by ω -agatoxin IVA at three different concentrations: 10 nM (n=5), 20 nM (n=10) and 100 nM (n=5). * indicates statistical difference from zero (p<0.05). D: Immunoblot analysis with a specific antibody



against the $\alpha 1A$ subunit of the P-/Q-type Ca^{2+} channel on a sodium dodecylsulphate-polyacrylamide gel (6 %) electrophoresis (SDS-PAGE) of proteins extracted from human umbilical artery smooth muscle cells. Lane 1: antibody, lane 2: antibody pretreated with control peptide provided by the manufacturer. Molecular masses in kilodaltons are given on the left.

or 100 nM only in 4 out of 15 cells a partial wash could be observed.

To verify by a non-pharmacological approach that P/Q-type Ca^{2+} channel was present in human umbilical artery, immunoblots were performed using antibodies specific for mouse $\alpha 1A$ subunit of the P-/Q-type Ca^{2+} channel. Immunoblots of umbilical artery smooth muscle protein extracts gave rise to one band of the expected size [7], which did not appear when the antibody was pretreated with the control peptide provided by the manufacturer (Figure 4D). Other proteins present in the extract were not recognized by the antibody, therefore reaction conditions were specific. The immunoblots were repeated a total of three times, always with the same results. Moreover, similar results were also obtained (data not shown) using the same protocol with primary

antibodies purchased from two other chemical companies (Sigma-Aldrich, St Louis, USA; and Santa Cruz Biotechnology Inc., Santa Cruz, USA, which required an anti-goat secondary antibody).

Regarding the contribution of a LVA T-type Ca^{2+} current (I_{CaT}) to total I_{Ca} in HUA smooth muscle cells, in the present recording conditions (5 mM extracellular Ca^{2+}) we did not observe a T-type component in I_{Ca} neither by changing the membrane HP nor after nifedipine addition. First, and as it was shown in figure 1C, the change in membrane HP from -80 to -40 mV produced a decrease of the I_{Ca} elicited by all the membrane potentials tested, preventing the observation of a selective effect of membrane HP on a putative LVA Ca^{2+} current; and second, nifedipine completely blocked I_{Ca} .

However, Smirnov & Aaronson [4] found that in

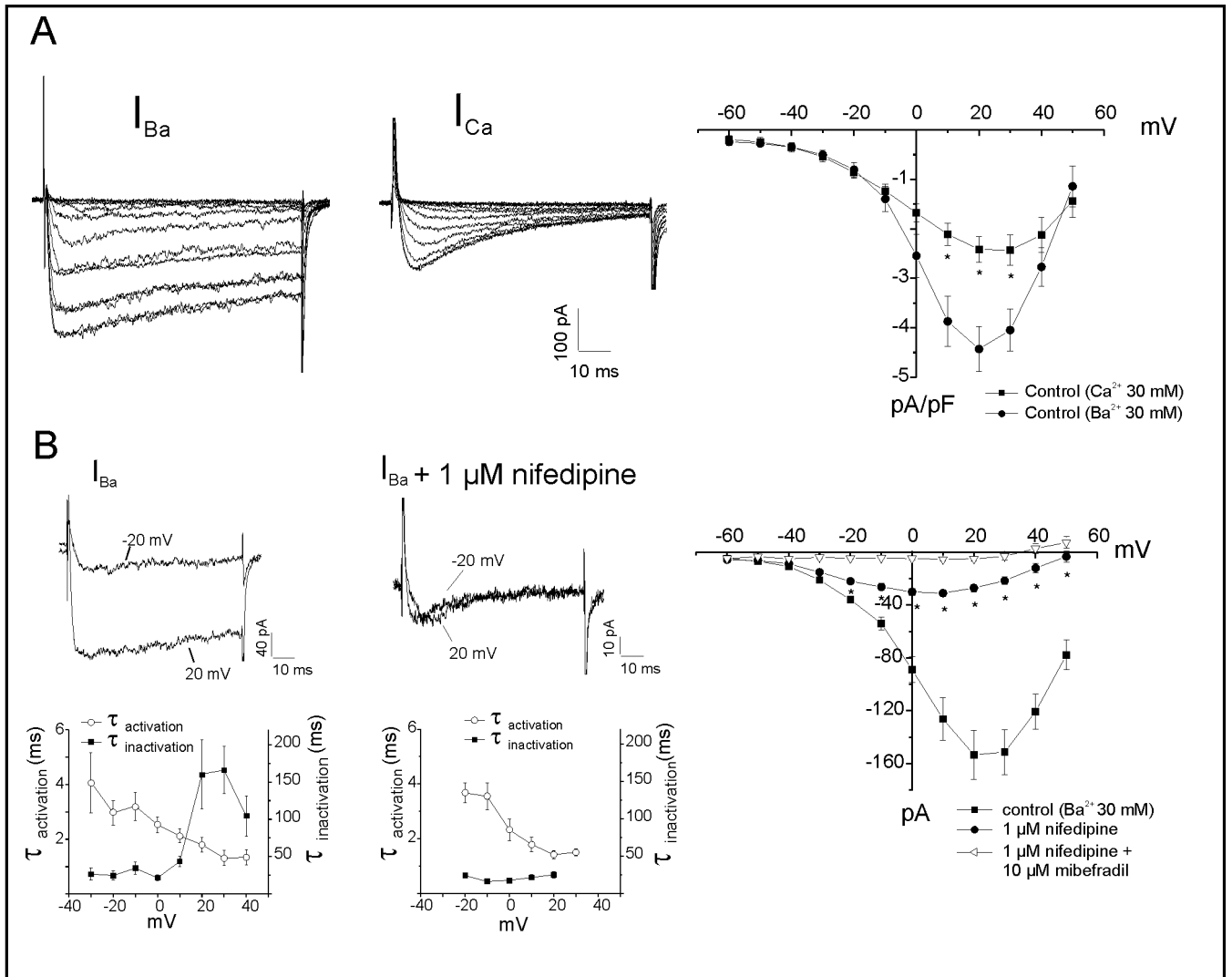


Fig. 5. Whole-cell currents recorded with high (30 mM) Ca²⁺ or Ba²⁺ extracellular concentration. A: Superimposed representative recordings of whole-cell currents elicited by voltage pulses between -60 and 50 mV from a membrane HP of -80 mV with Ba²⁺ as ion carrier (I_{Ba}, left panel) or Ca²⁺ (I_{Ca}, middle panel), and mean density I-V relationships obtained in such conditions (right panel, n=15 cells for I_{Ba} and n=13 cells for I_{Ca}); * indicates statistical difference from the values obtained for I_{Ba} (p<0.05). B: Typical recordings of I_{Ba} elicited from a membrane HP of -80 mV by voltage steps to -20 and 20 mV and the mean values of τ_{activation} and τ_{inactivation} plotted against V_m obtained in control conditions (left panel) and after a stable effect of 1 μM nifedipine (middle panel). In the right panel mean I-V curves found for I_{Ba} in control conditions (n=11 cells), after the effect of 1 μM nifedipine (n=8) and in presence of 1 μM nifedipine plus 10 μM of mibefradil (n=8 cells) are shown. * indicates statistical difference from the values obtained in control conditions (p<0.05).

human mesenteric artery cells there was a relatively small I_{CaT} which proved difficult to detect when cells were superfused with solutions containing low millimolar concentrations of divalent cation charge carrier, so we also tested the pharmacological profile of I_{Ca} using a high concentration (30 mM) of Ca²⁺ or Ba²⁺ in the extracellular solution with the aim of increasing the magnitude of I_{CaT}, if it were present. Figure 5A shows typical examples of I_{Ba} and I_{Ca} and the I-V relationships obtained by the same

voltage-protocol described in figure 1 but now using a high Ca²⁺ or Ba²⁺ concentration. I_{Ba} was significantly higher than whole-cell currents recorded in high Ca²⁺ in a range of membrane potentials between 10 to 30 mV, and inactivation time decay was slower than in high Ca²⁺ (τ_{inactivation} mean values at 20 mV: 160.6 ± 46.2 ms for I_{Ba}, n=9; and 24.7 ± 2.6 ms for I_{Ca}, n=11). Addition of 1 μM nifedipine partially inhibited I_{Ba}, leaving a current which inactivated completely during the time of the voltage step

(75 ms) at all V_m tested, and exhibited maximal amplitudes at V_m in the range of 0 to 10 mV (figure 5B). This nifedipine-insensitive current recorded in Ba^{2+} was completely blocked by 10 μM of mibefradil (figure 5B) in a reversible manner. Similar effects were observed on I_{Ca} recorded in high extracellular Ca^{2+} concentration, although the maximum value of its peak I-V relationship appeared at a more hyperpolarized V_m range (-20 mV to -10 mV, data not shown). The mean $\tau_{activation}$ and $\tau_{inactivation}$ values were obtained for I_{Ba} in control conditions and in presence of nifedipine, fitting the respective recordings with a double exponential function as done before. The values obtained were plotted as a function of the V_m and are shown in figure 5B.

Discussion

This study presents for the first time the electrophysiological and pharmacological characteristics of voltage-activated Ca^{2+} current in freshly isolated smooth muscle cells from HUA. The obtained results show that L-, P-/Q- and T-type Ca^{2+} channels are present in this vessel, constituting different voltage-activated Ca^{2+} pathways. Although L- and, to some extent, T-type Ca^{2+} channels were expected to appear, the presence of P-/Q-type Ca^{2+} channels is an important novelty, since, to the best of our knowledge, there are no previous reports of these channels in human vascular smooth muscle.

The electrophysiological and pharmacological results suggest that in HUA L- and P-/Q-type Ca^{2+} channels underlie the majority of I_{Ca} recorded with extracellular solutions containing 5 mM of Ca^{2+} , whereas the contribution of T-type Ca^{2+} channels could be evidenced using a high extracellular Ca^{2+} or Ba^{2+} concentration. As nifedipine (1 μM) completely blocked I_{Ca} , while ω -agatoxin IVA, the selective blocker of P-/Q-type Ca^{2+} channels, inhibited about 40% of total current, we can suggest that L- and P-/Q-type Ca^{2+} channels, both sensitive to dihydropyridines, elicited the total I_{Ca} . Dihydropyridine-sensitive P-/Q-type Ca^{2+} channels have been observed in other cells, such as rat melanotropic cells [19], and rat neurons [20]. Moreover, Hansen and coworkers [7] clearly described in a recent paper the presence and the functional role of P-/Q-type Ca^{2+} channels in rat vascular smooth muscle, and they discussed the fact that if dihydropyridines are able to inhibit P-/Q-type Ca^{2+} channels, there exists the possibility that the role of L-type Ca^{2+} channels has been overestimated and, as they

indicate, the effects of therapeutic concentrations of dihydropyridines could involve blockage of both types of Ca^{2+} channels. In agreement with this idea, our results show that in HUA smooth muscle cells nifedipine, used at a concentration commonly used to demonstrate the presence of L-type Ca^{2+} channels, blocked the whole of I_{Ca} , masking the presence of other types of channels, as the P-/Q-type, which were afterwards put in evidence using a specific toxin and whose expression was confirmed by immunoblotting. This could clearly lead to an overestimation of the role of L-type channels in this vessel.

When the concentration of divalent ion carrier was increased in the extracellular solution, it was possible to put in evidence a nifedipine-insensitive I_{Ca} , as other authors have reported in human vascular smooth muscle cells [4], which was completely blocked by 10 μM mibefradil. This current, likely a T-type Ca^{2+} current, is detectable at V_m positive to -50 mV and the maximum peak current variably lies between -20 and 10 mV depending on the ion current carrier. The electrophysiological characteristics of this I_{Ca} share some of the properties described in the literature for T-type Ca^{2+} currents in vascular and non-vascular smooth muscle cells [11]. In particular, the activation and inactivation parameters of this I_{Ca} were in the range of values described in the literature for T-type currents registered in arterial smooth muscle cells where generally the presence of a tiny LVA Ca^{2+} current has been reported [11].

The results obtained working at high extracellular Ca^{2+} or Ba^{2+} concentration, not only allowed us to observe a T-type Ca^{2+} current component, but were also useful to put in evidence some characteristics of HVA Ca^{2+} channels. The comparison of I-V curves in Ca^{2+} and Ba^{2+} showed that the current amplitude in Ba^{2+} was significantly higher than that in Ca^{2+} only at membrane potentials positive to 0 mV, the range of membrane potentials where the contribution of HVA Ca^{2+} channels is more important. This result is in accordance with the Ca^{2+} and Ba^{2+} permeability properties described for T- and L-type Ca^{2+} channels, namely, that they exhibit the same permeability to Ca^{2+} and are more permeable to Ba^{2+} than Ca^{2+} , respectively [11, 21]. The decay of inactivation in Ba^{2+} measured at 20 mV was much slower than in Ca^{2+} , suggesting the presence of a Ca^{2+} dependent-inactivation, as has been several times reported for L-type Ca^{2+} channels in different types of cells, and for P-/Q-type Ca^{2+} channels, at least in neurons [22] and chromaffin cells [23].

Physiological relevance of these results

As voltage-activated Ca^{2+} channels represent important pathways for Ca^{2+} influx in smooth muscle cells, and we are interested in the mechanisms of intracellular Ca^{2+} regulation in human umbilical artery, we first studied the properties of these channels with the aim of characterizing them to further study their role in the regulation of the contractile state of this vessel. Several contractile evidences have been reported with respect to dihydropyridine effects on HUA. Belford and coworkers [12] have reported that dihydropyridines were potent vasodilators of HUA rings precontracted with high- K^+ solutions, nifedipine being the most potent (it reached a maximum effect at a concentration of 1 μM). Nifedipine at 3 μM completely inhibited bupivacaine-induced twitches in HUA, while a tonic tone persisted in its presence [13]. Moreover, Gopalakrishnan and coworkers [24] showed that Bay-K 8644 caused a dose-dependent increase of tone in the HUA. Our results are in agreement with these findings, because recording in a low Ca^{2+} concentration (not so far from the physiological one) 1 μM nifedipine completely blocked, and Bay-K 8644 significantly increased, the macroscopic voltage-activated Ca^{2+} current in isolated smooth muscle cells from HUA. However, our results also show that nifedipine at 1 μM is able to block two different populations of Ca^{2+} channels, namely, L- and P-/Q-type Ca^{2+} channels. Data regarding the functional role of P-/Q-type Ca^{2+} channels in vascular smooth muscle cells are scant. Hansen and coworkers [7] have demonstrated in rat renal afferent arterioles that ω -agatoxin IVA induced a partial blocking effect on total whole-cell Ca^{2+} current and abolished high K^+ -induced contractions. It would be interesting to investigate in the future if these subtypes of HVA Ca^{2+} channels in HUA smooth muscle cells selectively participate in the regulation of different mechanisms that regulate the contractile state of this vessel.

In this work we show that in HUA smooth muscle cells a T-type Ca^{2+} current sensitive to mibefradil could be elicited by depolarization of membrane potential, although it was put in evidence in a high- Ca^{2+} or high- Ba^{2+} extracellular solution, a condition, nevertheless, used in several reports of Ca^{2+} channels in vascular smooth muscle [11]. At present, we can only speculate about their importance in HUA smooth muscle cells and further studies will be conducted to establish their possible function in this vessel. Up to now, T-type Ca^{2+} channels have been implicated in different roles in vascular smooth muscle, for instance, control of cell proliferation in human pulmonary artery [25], regulation of myogenic tone in rat skeletal muscle resistance arteries [10], and serving as Ca^{2+} entry routes required for activation of high conductance Ca^{2+} -activated potassium channels (BK_{Ca}) in mouse coronary arteries [26].

Taken together, our results open a field of future research about the role of the different Ca^{2+} channels in the regulation of contractility of HUA and consequently in the regulation of feto-placental circulation. They could constitute different targets for endogenous or exogenous agonists that modulate the intracellular Ca^{2+} concentration and hence the vessel tone.

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