Potassium Channels in Human Umbilical Artery Cells

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OBJECTIVE: *To identify K^+ channels of smooth muscle of human umbilical artery using the patch-clamp technique and to study their effect on resting tone of umbilical artery rings.*

METHODS: *Whole-cell and single-channel patch-clamp recordings in enzymatically isolated smooth muscle cells were made. Measurements of developed isometric force were performed on intact tissue.*

RESULTS: *Delayed rectifier K^+ channels (K_{DR}) and large-conductance Ca^{2+}-activated K^+ channels (BK_{Ca}) contribute to the whole-cell voltage- and time-dependent outward K^+ current, as it was specifically inhibited by 5 mM 4-aminopyridine (4-AP; K_{DR} blocker) (92 ± 4% at 0 mV, n = 7), by 1 mM tetraethylammonium (TEA; BK_{Ca} blocker) (71 ± 4% at +60 mV, n = 4), and by 200 nM iberiotoxin (BK_{Ca} blocker) (64 ± 7% at +60 mV, n = 4). In outside-out patches, BK_{Ca} channels had a single-channel conductance of 132 ± 4 pS (n = 24) in asymmetric K^+ conditions and 216 ± 4 pS (n = 4) in a symmetric K^+ gradient. The activity of the BK_{Ca} channels was significantly augmented by 1 μM Ca^{2+} in the inside-out configuration. 4-AP had no effect on resting tone of intact arterial rings. TEA produced contraction of arterial rings whereas phloretin, an activator of BK_{Ca}, relaxed them, which means that BK_{Ca} channels are functional in intact tissue and are involved in the maintenance of resting tone in this human vessel.*

CONCLUSION: *The identities of K^+ channels in the human umbilical artery were shown using the patch-clamp technique, and the physiologic effect of K^+ channels on resting tone was documented. (J Soc Gynecol Investig 2003;10:339–46)*

KEY WORDS: BK_{Ca} currents, K_{DR} currents, human umbilical artery, smooth muscle cells.

Potassium channels are expressed diffusely in the plasma membrane of different kinds of vascular smooth muscle cells, and they have been involved in the regulation of membrane potential, an important factor involved in excitation-contraction coupling. Activation of potassium channels in smooth muscle cells produces hyperpolarization and vasorelaxation, whereas their inhibition induces depolarization and contraction. Therefore, the activity of potassium channels plays an important role in the maintenance of resting tone and in the active responses to endogenous or exogenous vasoactive substances. Concerning the functional role of large-conductance Ca^{2+}-activated K^+ channels (BK_{Ca}) in human vessels, we previously reported that BK_{Ca}-carried currents were involved in the control of resting membrane potential and vascular tone of the saphenous vein.[1] K_{Ca} channels also regulate human pial artery tone[2] and the contractile response to nitrovasodilators in human coronary arteries.[3]

In the human umbilical artery (HUA), one of the vessels involved in fetoplacental circulation, the mechanisms that regulate the contractile state of smooth muscle cells are very important in order to achieve optimal gas and nutrients exchange between the fetus and the placenta. Using K^+ channels blockers on human umbilical artery rings, Lovren and Triggle have recently reported that nitric oxide-induced relaxation involves the activation of delayed rectifier K^+ channels (K_{DR}) and K_{Ca} channels.[4] But to the best of our knowledge, there are no reports that study the characteristics of K^+ channels in smooth muscle cells from these arteries with electrophysiologic techniques. In order to characterize the composition of K^+ currents in freshly enzymatically dispersed HUA smooth muscle cells, we present in this paper whole-cell currents showing the presence of K_{DR} and BK_{Ca} channels, and single channel data where BK_{Ca} properties are studied. We also present evidence that these channels are functional in intact tissue and are implicated in the maintenance of the resting vascular tone.

METHODS

Cell Isolation Procedure

Umbilical cords were obtained from normal, term pregnancies after vaginal and cesarean deliveries, placed in saline solution (NaCl 0.9%) at 4C and immediately transported to our laboratory and stored at 4C. The arteries were dissected from the

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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.[5] Briefly, a segment of HUA was cleaned of any residual connective tissue, cut in small strips, and placed for 15 minutes in a dissociation medium containing 140 mM NaCl, 5 mM KH_2PO_4, 5 mM $MgCl_2$, 20 mM glucose, and 5 mM HEPES (pH 7.4). The strips were then placed in dissociation medium with 2 mg/mL collagenase and 0.5 mg/mL protease type I for 25 minutes at 30C, with gentle agitation. After the incubation period, the strips were washed with dissociation medium, 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 until used.

Patch-Clamp Recording

HUA smooth muscle cells were allowed to settle onto the cover glass bottom of a 0.5-mL experimental chamber. The cells were observed with a mechanically stabilized, inverted microscope (Telaval 3; Zeiss, Jena, Germany) equipped with a 40× objective lens. The chamber was perfused by gravity for 15 minutes with standard extracellular saline solution (SESS, see composition later) at 0.5 mL/minute before the patch-clamp experiment was started. Test solutions were applied through a multibarreled pipette positioned near the cell being 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 electrophysiologic recording. Data were collected within 4–6 hours after cell isolation. All experiments were performed at room temperature (approximately 20C).

The standard tight-seal configurations of the patch-clamp technique were used.[6] 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 MΩ measured in SESS. 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) at a sample frequency of 40 kHz, and stored on a computer hard disk for later analysis. Series resistances ranged from 10 to 15 MΩ, and whole-cell capacitance was compensated for electronically.

Single-channel currents were recorded in inside-out and outside-out configurations, filtered at 2 kHz, and digitized at 16 kHz. Voltage-clamp 30–60-second recordings were obtained at different membrane potential values. Open probability is expressed as NPo, where N is the number of single channels present in each patch. NPo values were calculated using the following expression[7]:

$$NPo = (A_1 + 2A_2 + 3A_3 + \ldots + nA_n)/(A_0 + A_1 + A_2 + \ldots A_n),$$

where A_o is the area under the curve of the amplitude histogram which corresponds to the closed state, and A_1, A_2. . . A_n are the areas corresponding to the n open-state levels of current. The parameters of the histogram were obtained from least-squares Gaussian fits of experimental data.

Solutions

The standard extracellular saline solution (SESS) used for recording whole-cell and outside-out ionic currents contained (in mM) 150 NaCl, 5.4 KCl, 1.2 $MgCl_2$, 1 $CaCl_2$, 20 glucose, and 5 HEPES (pH was adjusted to 7.4 with NaOH). The composition of the intracellular pipette solution (IPS) was (in mM) 130 KCl, 5 Na_2ATP, 1 $MgCl_2$, 10 glucose, 0.1 ethylene glycol-bis(b-aminoethyl ether)N,N,N′,N′-tetraacetic acid (EGTA), and 20 HEPES (pH was adjusted to 7.3 with NaOH).

Bath and pipette solutions for single-channel experiments in symmetric inside-out configurations contained (in mM) 140 KCl, 0.5 $MgCl_2$, 1 EGTA, 10 HEPES, and 10 glucose and 140 KCl, 0.5 $MgCl_2$, 1 $CaCl_2$, 10 HEPES, and 10 glucose, respectively; in both solutions pH was adjusted to 7.4 with NaOH.

Tension Measurements of Intact Tissue

HUA rings (2–3 mm) were suspended between two stainless steel wires that could be separated with a micrometer to obtain the desired passive force. One of the wires was tied rigidly to an isometric force transducer (Letica TRI-201; Letica Scientific Instruments, Barcelona, Spain) whose output signal was amplified and recorded using a data acquisition board (DT16EZ, Data Translation Inc., Marlboro, MA) and software (Labtech Notebook Pro; Laboratory Technology Corp., Wilmington, MA) and stored for further analysis. The preparations were then submerged in a glass chamber containing SESS at 37C bubbled with O_2. A passive tension of 2 g was applied by turning the micrometer, and the rings were allowed to equilibrate at this applied force for 60 minutes with washing and readjustment of passive tension every 20 minutes. Tetraethylammonium (TEA), a blocker of BK_{Ca} channels,[8] or 4-aminopyridine (4-AP), a blocker of K_{DR} channels,[8] was added after the stabilization period. The force developed was expressed as gram force (gF) per gram wet tissue (gW).

Reagents

TEA, 4-AP, iberiotoxin (IBTX), phloretin, EGTA, apamin, CsCl, $BaCl_2$, Na_2ATP, and all the enzymes used for cell isolation were purchased from Sigma Chemical Co. (St. Louis, MO). All other reagents were of analytical grade. Fresh aliquots of stock aqueous solutions of TEA, IBTX, or apamin were added to the extracellular solutions on the day of the experiment. 4-AP solution was prepared by dissolving 4-AP in an SESS solution where 5 mM of NaCl was omitted to maintain the solution's osmolarity. The pH of the solution was adjusted to 7.4 with HCl.

Statistics

The results are expressed as mean ± standard error of the mean. Paired or unpaired Student *t* tests were used to establish

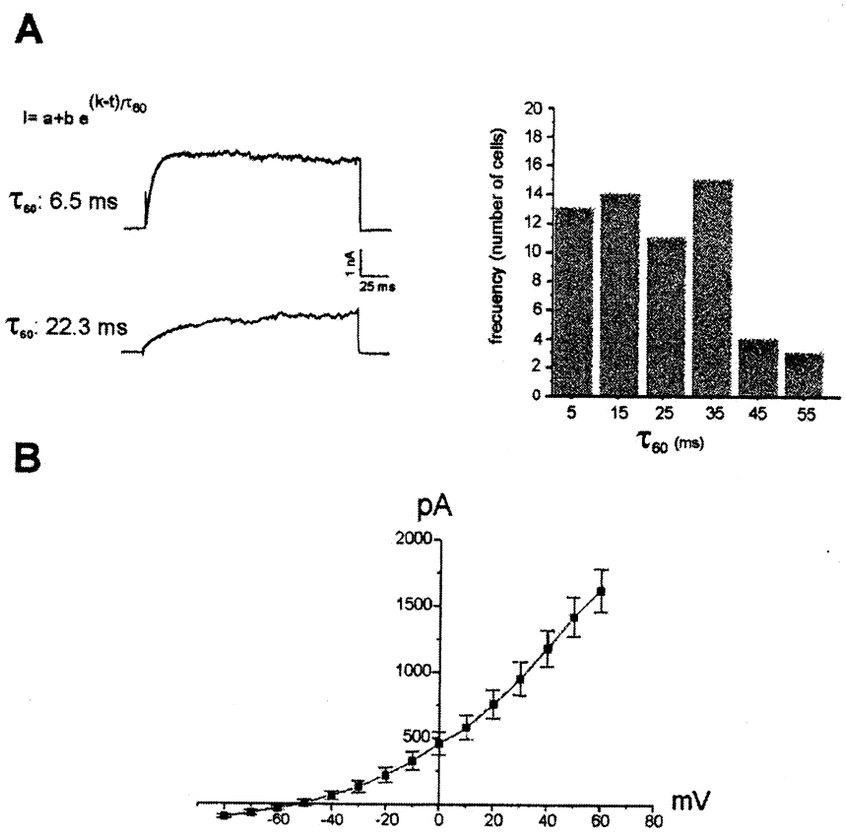

Figure 1. Macroscopic K^+ currents in smooth muscle cells from HUA. A) Left panel, typical recording traces evoked by a potential step from −60 to +60 mV in two different cells showing a fast and a slow time course of activation. Also shown are the time constants of activation obtained from a first order exponential function fit. Right panel, histogram of the distribution of time constants (n = 61). B) Mean I-V relationship considering all tested cells (n = 61).

statistically significant differences with a P value lower than .05.

RESULTS

To characterize macroscopic ionic currents in HUA smooth muscle cells, we performed voltage-clamp whole-cell recordings using isolated cells bathed in SESS and dialyzed with an intracellular pipette solution containing 0.1 mM EGTA. A series of hyperpolarizing and depolarizing test potentials ranging from −80 to +60 mV were applied in 10-mV increments from a holding potential of −60 mV. In response to this protocol, HUA smooth muscle cells evoked a family of voltage- and time-dependent outward currents at membrane potential values positive to −40 mV. To quantify their kinetic of activation, a first-order exponential function was used to fit the first 130 milliseconds of the total current evoked by a voltage step from −60 to +60 mV. The time constant (τ_{60}) of this fit was variable (Figure 1A), even in cells from the same sample of umbilical artery, showing values in a range of 1 to 57.6 milliseconds (n = 61, from 26 umbilical cords). However, we did not observe any difference in cell capacitance values (32.7 ± 1.2 pF), which would permit us to describe different types of smooth muscle cells. Figure 1B shows the mean current-voltage relationship (I-V curve) obtained from all tested cells. The estimated membrane potential value (zero current point) measured from the mean I-V curve shown in Figure 1 was −51 mV.

The most important ion carrier of voltage- and time-dependent outward currents was the K^+ ion, because the total current was almost completely blocked by the K channels in specific blockers (20 mM TEA, 82.1 ± 6.8% block, n = 6 at +60 mV, $P < .05$; and 1 mM $BaCl_2$, 86 ± 5% block, n = 5 at +60 mV, $P < .001$) in SESS. Furthermore, a very small current was evoked when the same voltage protocols were performed using an intracellular pipette solution in which KCl was replaced by CsCl (end-current measured at +60 mV: 176 ± 38 pA versus 2101 ± 615 pA, n = 11; $P < .05$).

No evidence was obtained for any inactivating transient outward K^+ current when membrane potential was held for 1 second at different conditioning potentials between −100 and −20 mV in 20-mV increments, followed by test steps to +60 mV.

To study which kind of K^+ channels were responsible for the total current, we determined the pharmacologic profile of whole-cell currents by assaying different K^+ channel blockers that are commonly used as inhibitors of K^+ channel currents on vascular smooth muscle cells.[8] The K_{DR} channel blocker 4-AP was first tested on whole-cell currents elicited by a series of hyperpolarizing and depolarizing test potentials ranging from −80 to +60 mV applied in 10-mV increments from a holding potential of −60 mV. We did not observe any effect of 4-AP at concentrations of 0.3 and 1 mM, whereas the effect of 5 mM 4-AP (a concentration that is commonly used to block K_{DR} current in smooth muscle cells) was variable among

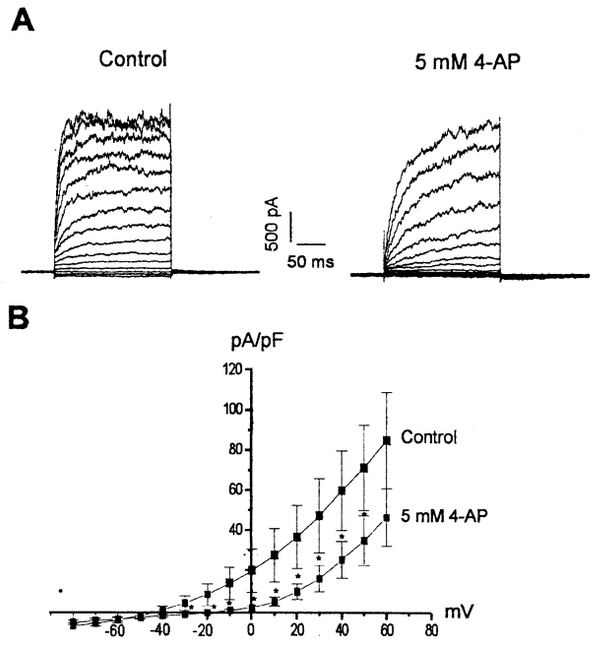

Figure 2. Inhibitory effects of 5 mM 4-AP on outward net currents. A) Families of membrane currents evoked by hyperpolarizing and depolarizing steps from −80 mV to +60 mV from a holding potential of −60 mV in controls (SESS) and in the presence of 5 mM 4-AP. B) Mean I-V relationships for early current measured at 65 milliseconds before and after 4-AP (n = 7,*P < .05 by Student paired t test).

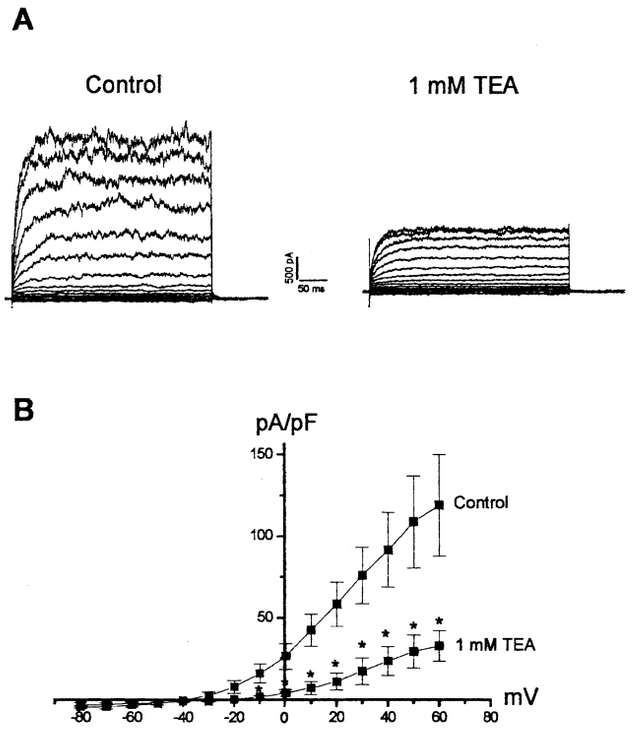

Figure 3. Effects of 1 mM TEA on outward net currents. A) Families of membrane currents evoked by hyperpolarizing and depolarizing steps from −80 to +60 mV from a holding potential of −60 mV in controls (SESS) and in the presence of 1 mM TEA. B) Mean I-V relationships for end current measured at 235 milliseconds before and after 1 mM of TEA (n = 4,*P < .05 by Student paired t test).

the tested cells. In seven cells having total currents with fast activation kinetics (mean τ_{60} value of 12.8 ± 3.2 milliseconds), 5 mM 4-AP significantly inhibited the total net current evoked by voltage steps in a range of −30 to +60 mV (92 ± 4% block at 0 mV, P < .05). Figure 2 shows the inhibitory effect of 5 mM 4-AP on a typical whole-cell recording and on the mean I-V curve. Conversely, in cells with a slower activation kinetic (mean τ_{60} value of 27.2 ± 5 milliseconds, n = 10; P < .05 compared with the first group), 5 mM 4-AP did not block but significantly increased the current amplitude evoked by membrane potential values between +10 and +60 mV (108 ± 40% of current increase at +60 mV, P < .05; n = 8).

TEA at a low concentration (1 mM) is used as a BK_{Ca} channel blocker in vascular smooth muscle cells.[8,9] In HUA cells it reduced net current by 71 ± 4% at +60 mV (n = 4 cells, P < .05) (Figure 3), whereas a lower concentration (0.1 mM) did not inhibit the currents. The more selective BK_{Ca} channel blocker IBTX (used at 100 nM) significantly reduced whole-cell current by 32 ± 9% in four of seven tested cells, but in the other three cells no effects were observed. Additional experiments were performed in which IBTX concentration was increased from 100 nM to 200 nM in cells first exposed to 5 mM 4-AP, in order to test whether an increase in the toxin concentration would be more effective in blocking the currents. In fact, IBTX at 200 nM reduced the net current by 64 ± 7% at +60 mV (P < .05, n = 4, Figure 4).

We also tested the presence of the low conductance K_{Ca} channels in HUA smooth muscle cells by using the selective blocker apamin at a concentration of 100 nM. The results showed that apamin did not produce any effects on net whole-cell currents (data not shown, n = 7).

Single-Channel Recordings

To confirm that BK_{Ca} channels underlie the TEA- and IBTX-sensitive whole-cell current observed in HUA smooth muscle cells, we performed additional experiments using outside-out patches in the same conditions as those used for whole-cell recordings. In this asymmetric K^+ gradient condition (130 mM inside and 5 mM outside), most of the patches showed a large single-channel current level at membrane potential values between −20 and +40 mV. At zero voltage membrane potential these currents were different from zero, as expected for currents carried through K^+ channels. Figure 5A shows a representative continuous recording of an outside-out patch held at +20 mV in the asymmetric K^+ gradient, where it is possible to observe the open level of unitary current. After measuring the current values from this kind of recording obtained at different membrane potential values, we constructed the mean I-V curve. The slope conductance value, obtained by a linear fitting of the all points on the I-V curve, was 124 ± 4 pS (n = 111, 24 patches) (Figure 5B). We also tested the effects of TEA in these kinds of experiments and found that extracellular application of 1 mM TEA reduced the

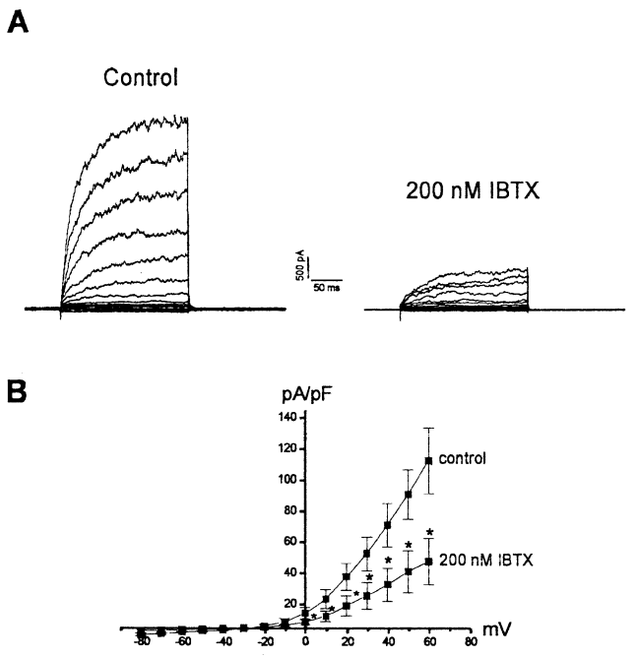

Figure 4. Effects of 200 nM IBTX on outward net currents in cells first exposed to 4-AP 5 mM. A) Families of membrane currents evoked by hyperpolarizing and depolarizing steps from −80 mV to +60 mV from a holding potential of −60 mV in controls (SESS + 5 mM 4-AP) and in the presence of IBTX 200 nM + 4-AP 5 mM. B) Mean I-V relationships for end current measured at 235 milliseconds before and after 200 nM IBTX, both in the presence of 5 mM 4-AP (n = 4, $^*P < .05$ by Student paired t test).

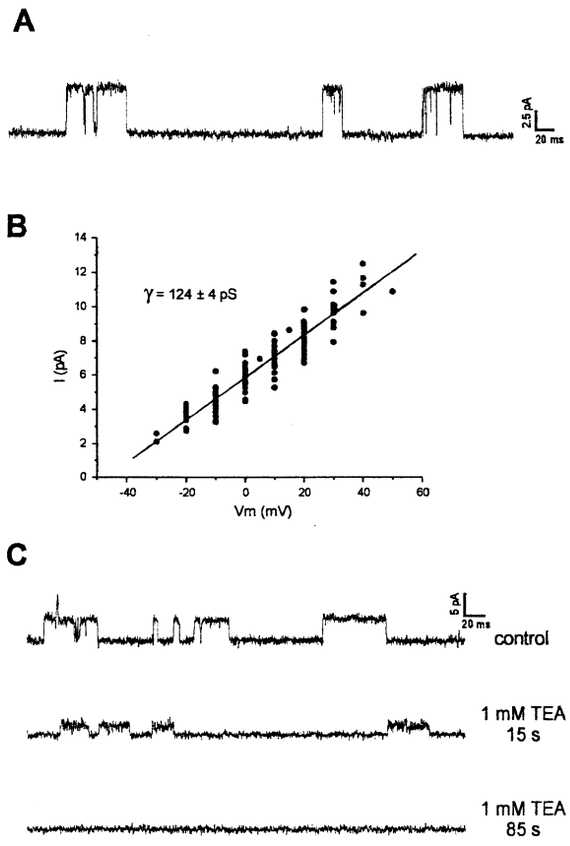

Figure 5. A) Representative continuous recording in the outside-out configuration. B) All points I-V curve for the 124 pS channel (n = 111, from 24 patches). C) Typical records showing that in the outside-out configuration, 1 mM TEA first reduces the current's amplitude and finally blocks the 124 pS channel. This result was observed in a total of four HUA patches.

unitary current, first increasing the channel noise, as fast block drugs do, and finally completely inhibiting the current (n = 4 patches) (Figure 5C). These properties, the conductance value, and the TEA sensibility are in accordance with the characteristics of the BK_{Ca} channels described in the literature. Several reports showed that BK_{Ca} channels present a lower conductance value in a physiologic asymmetric K^+ gradient compared with the typical value of approximately 270 pS reported for this channel in symmetric inside-out conditions.[10] To study this channel characteristic, we obtained outside-out patches in SESS and then changed the bath solution to another solution where K^+ concentration was increased to 130 mM; this allowed us to record in the same patch the current through the channel, first in an asymmetric condition and then in a symmetric condition. Figure 6 shows direct evidence of the change in current level when the patch was exposed to a symmetric K^+ gradient coming from the asymmetric one; the conductance value obtained for such a condition was 216 ± 4 pS (n = 16, four patches). When these patches were exposed to 1 mM of TEA the current was also completely inhibited (n = 4, data not shown).

When recorded in the inside-out configuration in a symmetric K^+ gradient, the channel had a conductance value of 274 ± 7 pS (n = 47, eight patches), and it showed sensitivity to the calcium concentration. When inside-out patches were perfused with a bath solution containing an elevated Ca^{2+} concentration (1 μM) the NPo was significantly augmented from a value of 0.000625 ± 0.000498 (n = 8) in control conditions (1 mM EGTA in the bath solution) to 0.424 ± 0.137 (n = 7) with 1 μM of Ca^{2+} at +20 mV. Figure 7 shows a typical recording of channel activation by calcium.

Tension Measurements in HUA Rings

K^+ channel blockers (TEA and 4-AP) and a BK_{Ca} channel activator (phloretin) were tested on intact tissue. We observed that 1 mM of TEA did not affect HUA resting tone (0.5 ± 1.2 gF/gW, n = 14), whereas increasing TEA concentration to 2 and 5 mM significantly contracted all tested rings in a concentration-dependent manner (8.5 ± 3.1 gF/gW and 22.3 ± 5.2 gF/gW, respectively n = 9, $P < .05$). Additionally, we tested the effects of 50 μM of phloretin on resting tone of HUA rings in order to check a possible vasorelaxant effect (−21.8 ± 5.6 gF/gW, n = 7 $P < .05$) resulting from an increase in the activity of BK_{Ca} channels (Figure 8). The K_{DR} blocker 4-AP, which was tested at a concentration of 5 mM, did not have any effect on HUA resting tone (−5.3 ± 6.6 gF/gW, n = 12).

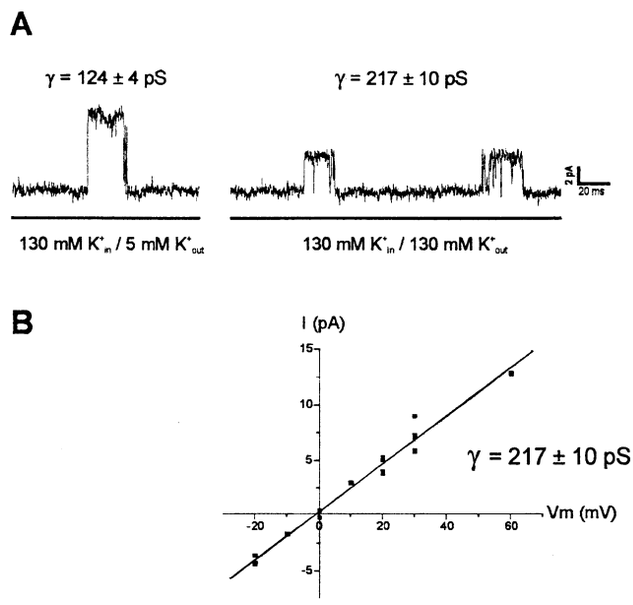

Figure 6. A change in the bath concentration of K^+ from 5.4 mM to 130 mM (symmetric K^+ gradient) reduces the amplitude of the unitary current and the channel conductance in outside-out patches. A) Typical recordings at +20 mV in control bath solution (asymmetric K^+ gradient) and in a 130 mM K^+ solution (symmetric K^+ gradient). B) All points I-V curve in a 130 mM K^+ solution.

DISCUSSION

This study presents direct electrophysiologic evidence of K^+ channel expression in human umbilical artery smooth muscle cells. Our results show that two different currents, namely IK_{DR} and IBK_{Ca}, underlie almost all of the macroscopic whole-cell current. Additionally, the fact that apamin 100 nM did not produce any effect on the total current suggests that the low conductance K_{Ca} channels are not present in these cells. The presence of a rapid inactivating I_A-like potassium current is also unlikely.

The macroscopic current was inhibited almost completely by the nonselective K^+ channel blockers. The inhibitory effect produced by specific blockers allowed us to conclude that in our experimental conditions, HUA smooth muscle cells have as major components of whole-cell current a K_{DR}-4-AP–sensitive fast current, which was activated at membrane potentials positive to −40 mV, and a TEA-IBTX-sensitive slower BK_{Ca} current, which was activated at membrane potentials positive to −10 mV. However, the 4-AP effects show that, unlike the BK_{Ca} component, the K_{DR} component is not present in all cells. The variability of the 4-AP effects can be accounted for by the different patterns of voltage-dependent control currents. This K^+ channel blocker inhibited a component of the total current only in cells that had a faster activation time constant, which is compatible with the pres-

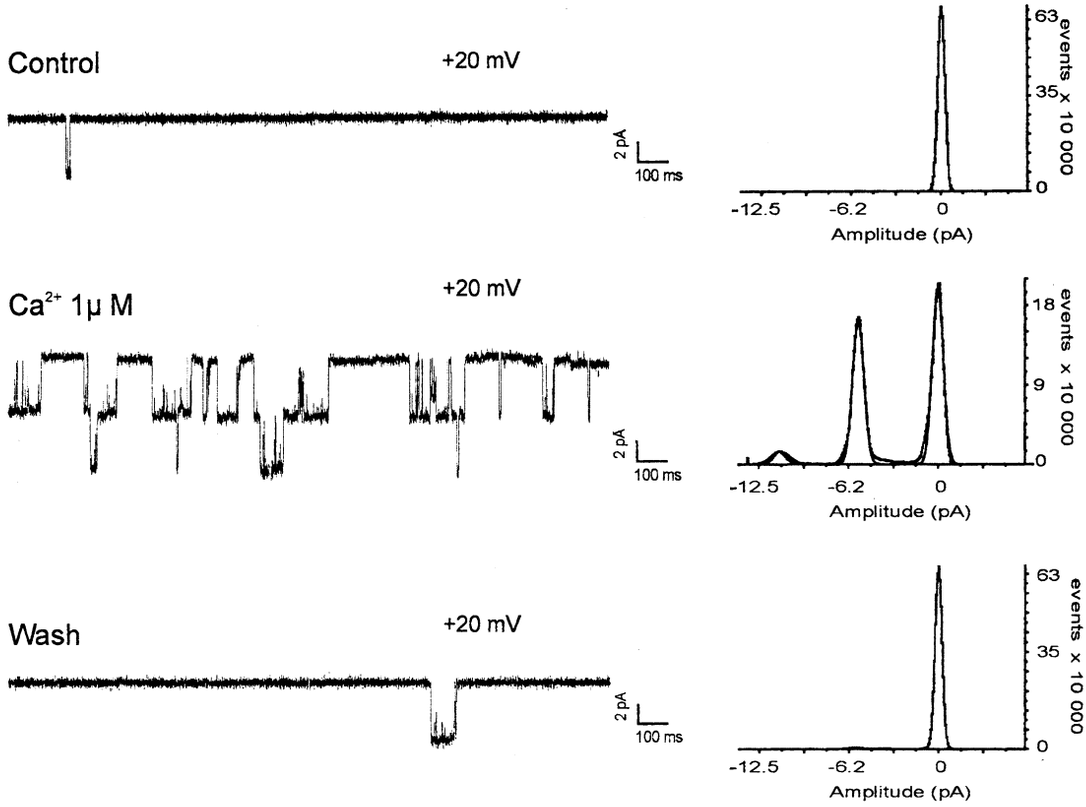

Figure 7. In the inside-out configuration, an increase in the Ca^{2+} concentration of the bath solution (facing the intracellular side of the plasma membrane) increases the open probability of 274 pS channel. Shown are representative recordings (left) and corresponding amplitude histograms (right).

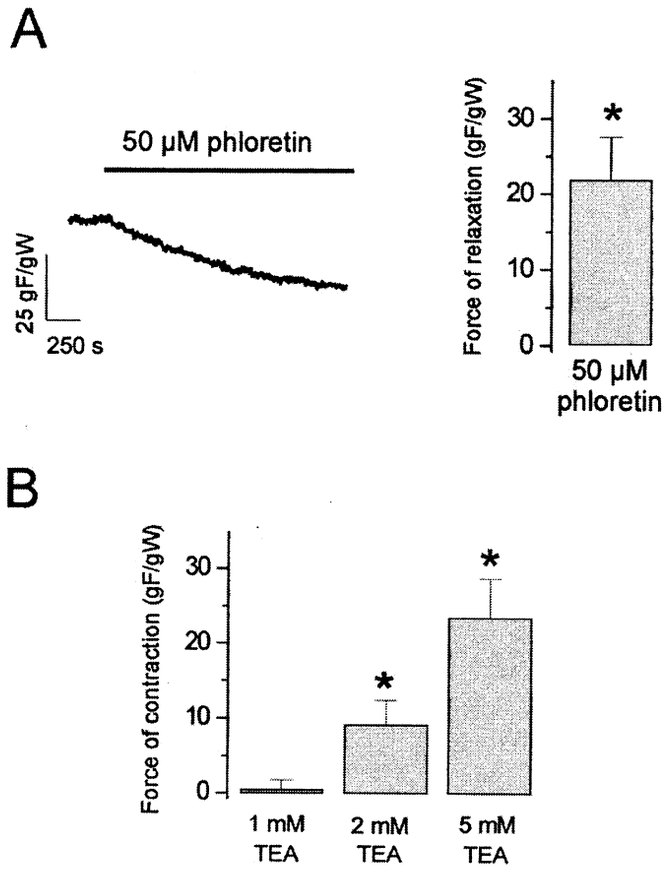

Figure 8. Effects of activation and blockage of BK_{Ca} channels on resting tone of intact HUA rings. A) Left panel, representative recording of phloretin relaxation of unstimulated HUA intact rings; right panel, mean relaxation by phloretin in seven rings. B) Effects of 1, 2, and 5 mM TEA on resting tone of HUA rings (n = 14, 9, and 9, respectively).

ence of fast K_{DR} currents, but did not inhibit cells with slower activation kinetics, which presumably lack the K_{DR} component. The increase in net current induced by 5 mM 4-AP at depolarizing membrane potentials in this latter group of cells may be related to indirect activation of BK_{Ca} currents by the drug as a result of intracellular alkalinization or sarcoplasmic reticulum calcium release or both, induced by 4-AP, as previously reported.[11,12]

The single-channel results show that the BK_{Ca} channel properties are in accordance with the reported data for this channel in other types of vascular smooth muscle cells. Additionally, we present direct evidence of how the conductance of this channel was reduced by almost 50% when the transmembrane K^+ gradient was changed from a symmetric, nonphysiologic condition to an asymmetric, physiologic one, as it was described by other authors in vascular smooth muscle cells.[13]

The mechanical results show that BK_{Ca} channels are functional in intact tissue and are involved in the maintenance of resting tone, because the K^+ channel blocker TEA (2 mM) elicited contractions in unstimulated HUA rings. The concentration of TEA that produced a statistically significant contraction was slightly higher than that which blocked BK_{Ca} in the patch-clamp experiments (1 mM TEA); this difference might reflect the need for higher doses of blockers in intact tissue than in isolated cells. The half-block dose for K_{DR} channels is 10 mM,[8] so there would be little, if any, inhibition of these channels at 2 mM. Conversely, we did not observe contractile effects of 4-AP on resting tone of HUA rings; however, based on 4-AP effects on whole-cell currents, we think that this may be related to opposite effects of the drug, ie, the inhibition of K_{DR}-carried current causing depolarization and the stimulation of a BK_{Ca} hyperpolarizing current in different cells of the same vascular segment.

BK_{Ca} channels characterized in HUA could also participate in the mechanism of cellular signals that transduce variations in cytosolic Ca^{2+} concentration into changes in cell membrane potential. Local or blood-borne agents involving BK_{Ca} in their action mechanisms could have a relevant role in the control of HUA, a vessel that has no autonomic innervation.[14] Thus, the results of the present study may have important implications for our knowledge of basic physiology of umbilical-placental blood flow.

Moreover, the relaxing effect of the BK_{Ca} activator phloretin on resting tone shows that BK_{Ca} can be further activated in these conditions. In agreement with this result, Lovren and Triggle[4] recently reported that BK_{Ca} and K_{DR} channels participate in nitric oxide-induced relaxation of precontracted HUA rings. Furthermore, Rosenfeld et al[15] reported that in ovine uterine arteries estrogen-induced vasodilatation is mediated in part by activation of BK_{Ca} channels. Because we

established the presence of these channels in HUA, it is possible to speculate that estrogens may be one of the factors underlying basal HUA tone that could have BK_{Ca} as a membrane target to elicit HUA relaxation.

In summary, we identified K_{DR} and BK_{Ca} currents in HUA smooth muscle cells that could be targets for different vasoactive endogenous or pharmacologic substances related to the regulation of fetoplacental circulation. Additionally, this study presents single-channel properties of BK_{Ca} in human vascular tissue, which has not been previously studied with the patch-clamp technique. Further experiments will be performed to assess the regulation of these channels.

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