Contribution of Kv Channels to Phenotypic Remodeling of Human Uterine Artery Smooth Muscle Cells

Eduardo Miguel-Velado, Alejandro Moreno-Domínguez, Olaia Colinas, Pilar Cidad, Magda Heras, M. Teresa Pérez-García, José Ramón López-López

Abstract—Vascular smooth muscle cells (VSMCs) perform diverse functions that can be classified into contractile and synthetic (or proliferating). All of these functions can be fulfilled by the same cell because of its capacity of phenotypic modulation in response to environmental changes. The resting membrane potential is a key determinant for both contractile and proliferating functions. Here, we have explored the expression of voltage-dependent K^+ (Kv) channels in contractile (freshly dissociated) and proliferating (cultured) VSMCs obtained from human uterine arteries to establish their contribution to the functional properties of the cells and their possible participation in the phenotypic switch. We have studied the expression pattern (both at the mRNA and at the protein level) of Kvα subunits in both preparations as well as their functional contribution to the K^+ currents of VSMCs. Our results indicate that phenotypic remodeling associates with a change in the expression and distribution of Kv channels. Whereas Kv currents in contractile VSMCs are mainly performed by Kv1 channels, Kv3.4 is the principal contributor to K^+ currents in cultured VSMCs. Furthermore, selective blockade of Kv3.4 channels resulted in a reduced proliferation rate, suggesting a link between Kv channels expression and phenotypic remodeling. **(*Circ Res*. 2005;97:1280-1287.)**

Key Words: Kv channel expression ■ vascular smooth muscle cells ■ cell proliferation ■ K^+ currents

The vascular smooth muscle cells (VSMCs) of mature animals are highly specialized cells whose main function is contraction. Although during vasculogenesis, the principal role of VSMCs is proliferation and synthesis of the matrix components of the vessel wall, differentiated VSMCs proliferate at an extremely low rate and express a unique repertoire of contractile proteins, ion channels, and signaling molecules required for contraction. However, VSMCs can undergo relatively rapid and reversible phenotypic changes in response to local environmental conditions. Accelerated proliferation of VSMCs is known to play a key role in atherosclerotic plaque formation and postangioplasty restenosis and is a common feature in hypertensive arteries.[1]

VSMCs express a large repertoire of ion channels and membrane receptors that vary widely among different vascular beds and are key determinants of the electrical and contractile responses of the cells.[2–5] Although release of intracellular Ca^{2+} is necessary for effective contraction, Ca^{2+} influx through voltage-activated Ca^{2+} (Cav) channels is responsible for initiating contraction, making resting membrane potential (E_m) a primary determinant of vascular smooth muscle tone.[4] Membrane depolarization opens Cav and raises $[Ca^{2+}]_i$, and cytosolic-free Ca^{2+} serves, in turn, as a critical signal transduction element in a variety of cell functions, such as contraction, migration, proliferation, and gene expression.[6–8]

There is increasing evidence showing that K^+ channels may have an important role in dedifferentiation and proliferation of VSMCs. Modulating E_m, K^+ channels can affect not only Ca^{2+} influx, a well-established factor influencing cell proliferation, but also the driving force for Na^+-dependent transport, the intracellular pH, and the regulation of cell volume, all factors that also participate in proliferation and apoptotic processes.[9,10] The role of K^+ channels on cell proliferation is a complex modulatory activity that only certain K^+ channels at specific times and locations can perform. This fact, together with the broad diversity of functional K^+ currents among different vascular beds, has ravelled the analysis of the participation of K^+ channels on VSMCs proliferation. At least 4 different types of K^+ channels have been identified in VSMCs: inward rectifier, voltage-gated (Kv), ATP-gated, and Ca^{2+}-gated K^+ (BKCa) channels.[2,4] The expression of these 4 types has been reported to vary among vascular beds as well as with vessel size.[5,11] However, Kv and BKCa channels are present in virtually all vascular myocytes and strongly influence contractile respons-

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From the Departamento de Bioquímica y Biología Molecular y Fisiología e Instituto de Biología y Genética Molecular (E.M.-V., A.M.-D., O.C., P.C., M.T.P.-G., J.R.L.-L.), Universidad de Valladolid y Consejo Superior de Investigaciones Científicas, Facultad de Medicina, Valladolid, Spain; and Institut de Malalties Cardiovasculars (M.H.), Hospital Clinic, Institut d'Investigacions Biomediques August Pi i Sunyer, Universidad de Barcelona, Barcelona, Spain.

Correspondence to José Ramón López-López, Departamento de Bioquímica y Biología Molecular y Fisiología, Facultad de Medicina, Universidad de Valladolid., C/ Ramón y Cajal 7, 47005 Valladolid, Spain. E-mail jrlopez@ibgm.uva.es

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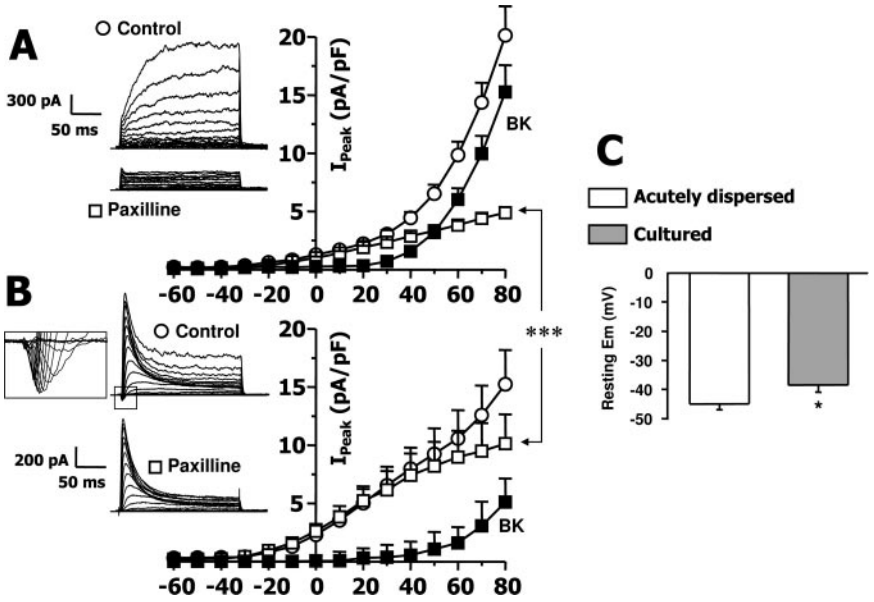

Figure 1. Relative contribution of BKCa and Kv to K^+ currents of acutely dissociated (A) and cultured (B) VSMCs. I/V relationships were obtained from a holding potential of −80 mV in 10-mV depolarizing steps from −60 to +80 mV in control conditions (○, total K^+ current) and in the presence of 500 nmol/L paxilline to block the BK component (□, Kv current). The contribution of BK currents is the difference between control and paxilline (■). Representative traces from a cell in each group are shown on the left. The inset in B shows the presence of Na^+ currents in cultured cells. ***$P<0.001$. C, Mean values of resting E_m obtained in 12 cells in each group in perforated-patch conditions. *$P<0.05$.

es.[2] Moreover, several Kv channels have been described to participate in the proliferation process in pulmonary artery smooth muscles cells (SMCs).[12,13]

The goals of the present study were to characterize the expression profile of Kv channels in VSMCs from human uterine artery in contractile and proliferating phenotype and to evaluate their possible contribution to phenotypic modulation. Enzymatic dispersion of small pieces of uterine arteries provide VSMCs in contractile phenotype, which were studied within the same day, whereas proliferating VSMCs were obtained from explants of uterine arteries kept in culture for several passages. The molecular identification of the functional Kvα subunits in both preparations shows that whereas Kv1 members are the main contributors to the Kv currents in contractile VSMCs, Kv3.4 expression is upregulated under proliferating conditions and represents the largest proportion of the Kv current in dedifferentiated VSMCs. Furthermore, selective blockade of Kv3.4 channels decreased proliferation rate, suggesting a direct relationship between channel function and uterine artery SMCs proliferation.

Materials and Methods

Uterine arteries were obtained from patients undergoing hysterectomy at the Clinic Hospital of Barcelona, with protocols approved by the Human Investigation Ethics Committee of the Hospital. Details of the materials and methods used in this study are in the online data supplement available at http://circres.ahajournals.org.

Results

Kv Currents in Freshly Dissociated and Cultured VSMCs

K^+ currents were studied in acutely dissociated cells and in cultured cells obtained from human uterine arteries. After establishing the whole-cell configuration, current–voltage (I/V) relationships for K^+ currents were obtained every 2 minutes. After several records of I/V curves in control conditions, BKCa currents were subtracted by using the selective blocker paxilline (500 nmol/L).[14,15] Figure 1 shows average I/V curves and sample records (insets) obtained in acutely dispersed cells (Figure 1A) and in cultured VSMCs (Figure 1B) before and after paxilline application. As previously reported,[16] cultured cells exhibited tetrodotoxin-sensitive inward Na^+ currents, which were absent in acutely dispersed cells (Figure 1B, inset). Depolarization elicited outward currents with an apparent threshold for activation positive to −40 mV, and paxilline decreased current amplitudes at potentials positive to +30 mV. This effect was more evident in acutely dispersed cells. Cell capacitance was 40.21±2.45 pF in fresh VSMCs (n=28) and 44.58±6.17 pF in cultured cells (n=18). Current density was not significantly different between the 2 groups (19.04±2.76 versus 15.11±2.72 pA/pF at +80 mV), but BKCa current represents more than 70% of the outward K^+ current in the freshly dissociated VSMCs and only 20% in the cultured cells. Furthermore, we consistently found a kinetic change with a larger proportion of inactivating K^+ currents in cultured VSMCs (see traces in Figure 1). Resting E_m measurements under perforated-patch conditions showed that contractile VSMCs had, on average, more hyperpolarized potentials than proliferating VSMCs (−45.0±1.9 mV versus −38.4±2.4 mV respectively, $P<0.05$; Figure 1C). Resting E_m was not modified by paxilline in either of the 2 groups (data not shown). These observations indicate that the proliferation of human uterine artery VSMCs is associated with a decrease in the expression of BKCa currents and an increase in the contribution of Kv currents.

Pharmacological Characterization of Kv Currents

Whereas downregulation of BKCa currents with the phenotypic switch has been described previously,[3,17] changes in Kv currents are not well characterized. Therefore, we characterized pharmacological Kv currents in both phenotypes after blocking BKCa currents with paxilline. Correolide was used to selectively block Kv1 currents,[18] tetraethylammonium (TEA) sensitivity allowed the identification of Kv2 and Kv3 currents, and TEA- and correolide-insensitive current was attributed to Kv4 currents.[19] The latter were also identified by

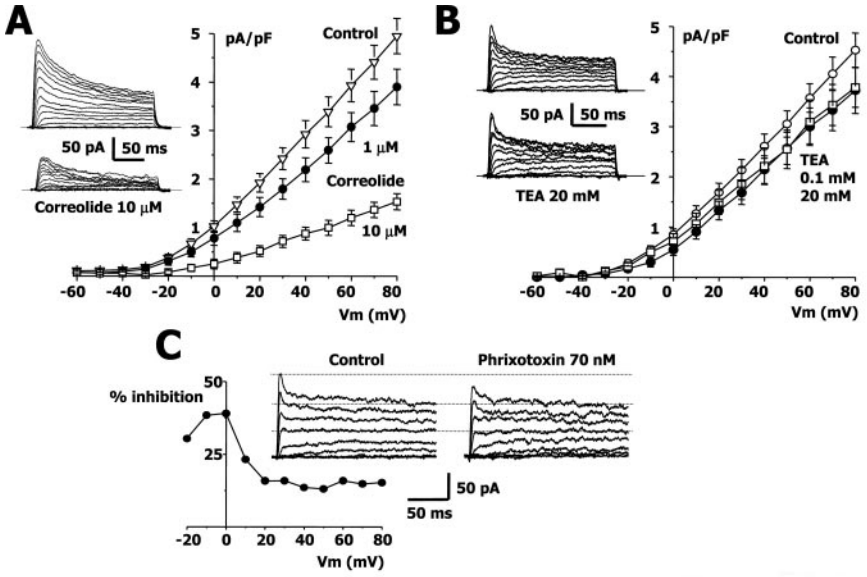

Figure 2. Pharmacological characterization of Kv currents in acutely dissociated VSMCs. A, Effect of 1 and 10 μmol/L correolide on the peak current amplitude at different voltages (n=14 cells). Representative traces from a cell in control conditions and after 10 μmol/L correolide are also shown. B, TEA did not have a significant effect at concentrations up to 20 mmol/L. The mean peak current density in control conditions (○) and in the presence of 0.1 mmol/L TEA (□) and 20 mmol/L TEA (●) are shown. Data are mean±SEM of 10 cells. C, Effect of 70 nmol/L phrixotoxin-1. Representative traces from −60 to +80 mV in 10-mV steps in a cell in control solution and in the presence of phrixotoxin. The plot shows the voltage dependence of the effect of the drug in the same cell. Phrixotoxin-1 effect was studied in 3 more cells. Vm indicates membrane potential.

their sensitivity to block by toxins such as heteropodatoxin[20] and phrixotoxin.[21]

In acutely dispersed VSMCs (Figure 2), Kv1 currents were the predominant component of the K^+ currents (Figure 2A). Correolide reduced Kv current amplitude by 21% or 70% at +80 mV when applied at 1 or 10 μmol/L, respectively. At 10 μmol/L, the effect was statistically significant at voltage values above −30 mV. TEA was applied at low (micromolar) and high (millimolar) concentrations to dissect the contribution of high- and low-TEA–sensitive Kv channels. There was a small reduction in the current amplitude in the presence of 100 μmol/L TEA (15% inhibition at +80 mV; Figure 2B), which did not significantly increase after raising TEA up to 20 mmol/L. The onset of TEA inhibition appeared at more positive potentials than that of correolide (compare Figure 2A and 2B). The effect of TEA was the same on the total Kv current as on the correolide-insensitive fraction of the current, suggesting that Kv1 channels in acutely dispersed VSMCs are not sensitive to TEA (data not shown). The contribution of Kv4 channels to Kv currents was studied with specific blockers. Figure 2C shows an example in which 70 nmol/L phrixotoxin was used. The effect of the drug was voltage dependent (see inset). On average, phrixotoxin induced a 15% inhibition on the current amplitude at +80 mV, similar to the estimated proportion of TEA- and correolide-insensitive current (12.0±3.5%).

When the same pharmacological study was performed in cultured VSMCs, a clearly different pattern was apparent (Figure 3). First, correolide (Figure 3A) had only a very modest effect (11.5±6.3% inhibition at +40 mV). Second, the predominant fast-inactivating current observed in these cells was almost completely abolished by low TEA (up to 1 mmol/L; Figure 3B). Finally, blockers of Kv4 channels had almost no effect (Figure 3C). Therefore, the predominant Kv current in cultured VSMCs is a transient current with high-TEA sensitivity, pointing to some members of the Kv3 subfamily, particularly Kv3.4. This suggestion was confirmed by exploring the effect of BDS-1, a selective blocker of Kv3.4 channels. BDS-1 (2.5 μmol/L) decreased the current amplitude by ≈40% to 50% at potentials of −20 mV or greater (Figure 3D). Taken together, these functional data suggest an extensive remodeling of Kv channels associated with the phenotypic modulation, with a predominant role of Kv1 in acutely dispersed and of Kv3 in cultured VSMCs, as indicated by the summary data of Figure 3E.

Expression Profile of Kvα mRNA

To study the molecular correlates of the Kv currents, real-time PCR was performed in both contractile and proliferating VSMCs. The results, normalized to RPL18 mRNA amount and corrected for the amplification efficiency of each reaction (see online data supplement), are plotted in Figure 4A. Unexpectedly, Kv4 channels represent the largest amount in both preparations, accounting for 67% and 80% of the total Kvα mRNA (see online data supplement). We did not detect expression of Kv3.1, Kv3.2, or Kv1.1 in any preparation. With the exception of Kv4.2 and Kv3.4 subunits, which undergo an increased expression in cultured VSMCs, proliferation induced a downregulation of mRNA of all Kvα subunits present in fresh tissue. Figure 4B shows the magnitude of the changes in the expression of Kvα subunit mRNA from proliferating VSMC, taking the levels of mRNA in contractile VSMCs as the calibrator. In general, there is a correlation between changes in mRNA levels and changes in functional expression when comparing contractile and proliferating VSMCs (compare Figures 4B and 3E): whereas Kv1 channels are downregulated, Kv3.4 channels upregulate their expression and their functional contribution in cultured VSMCs.

Expression and Subcellular Location of Kvα Proteins

The above data show, nevertheless, some discrepancies between the mRNA levels of a given Kvα subunit and its contribution to the total Kv current, which could be attributable to the absence of correlation between mRNA and protein levels or, alternatively, to altered membrane trafficking of the protein. To distinguish between these 2 possibilities, we

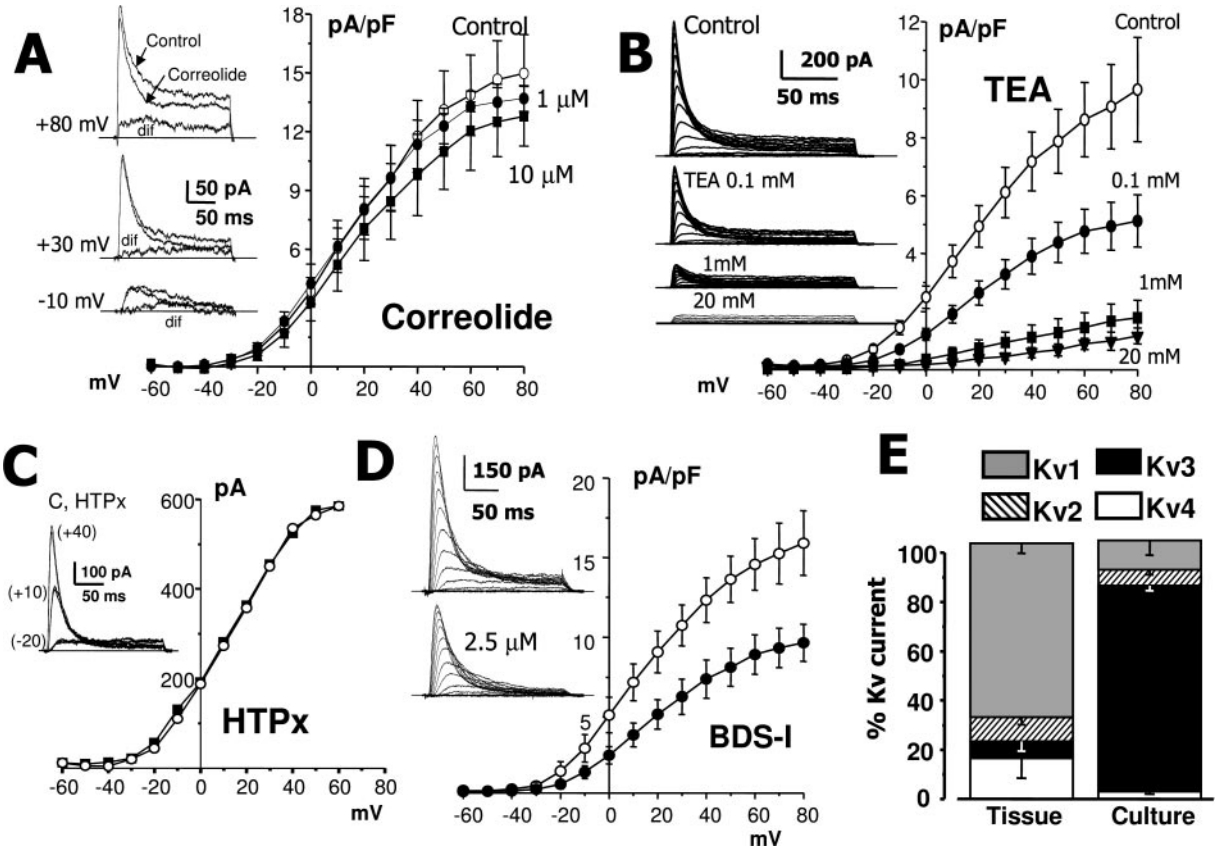

Figure 3. Pharmacological characterization of Kv currents in cultured VSMCs. A, Effect of correolide on cultured VSMCs at 1 and 10 μmol/L (n=4 in each group). The traces on the left show the effect of 10 μmol/L correolide in 1 cell at 3 different voltages and the subtracted correolide-sensitive portion of the current (labeled as dif.) B, Dose dependence of TEA block. Representative traces from 1 cell and the mean (±SEM) current density obtained in 6 to 10 cells for each condition are shown. C, Lack of effect of heteropodatoxin-2 (HTPx) (100 nmol/L) on the outward K^+ currents in a cultured VSMC. The data are representative of 4 experiments. D, Average I/V curves from n=4 cultured VSMCs in control (○) and in the presence of 2.5 μmol/L BDS-1 (●). The inset shows the actual traces in both conditions from 1 of the cells. E, Proportional contribution of Kv channel subfamilies to the total Kv current was obtained from the average inhibitory effect at +40 mV. Kv1 currents correspond to the fraction blocked by 10 μmol/L correolide, and Kv4 currents are the phrixotoxin- or HpTx-sensitive current. Kv3 component was the fraction of the current blocked by submillimolar TEA, and Kv2 current was the difference between this fraction and the fraction blocked by 20 mmol/L TEA.

determined protein expression by immunoblot and cellular location with immunocytochemistry. Representative Western blots both in freshly isolated tissues and in protein extracts from cultured cells are shown in Figure 5. Kv1α subunit proteins were detected in fresh tissues, being absent or expressed at much lower levels in cultured VSMCs, with the only exception of Kv1.3, which appears to be more abundant in protein extracts from cultured VSMCs, contrary to what we will predict according to mRNA levels. A similar inconsistency was found in the case of Kv3.4 protein, which seems to be more abundant in fresh VSMCs than in cultured cells. Finally, with regard to Kv4α subunit proteins, bands for Kv4.2 and Kv4.3 were observed in both tissue and cell cultures, with substantially higher levels of expression in protein extracts from tissues, revealing the presence in both preparations of Kv4α subunit proteins, whose functional contribution has been found to be very small or almost absent.

Next, we explored the subcellular location of some Kvα subunit proteins, which had levels of expression (as determined by Western blot) that did not match their estimated levels of activity (as determined by functional studies), namely Kv1.3, Kv3.4, and Kv4 subfamily members. Kv1.3 was expressed in patches of membrane in acutely dissociated VSMCs (Figure 6A), whereas Kv4.3 proteins were found to colocalize with 1,1′-dioctadecyl-3,3,3′,3′-tetramethylindocarbocyanine (DiI) in the membranes of intracellular compartments both in cultured (Figure 6E) and freshly dissociated (data not shown) VSMCs. Finally, Kv3.4 immunoreactivity was detected in both preparations but in different cellular locations. Whereas, in freshly dissociated VSMCs, Kv3.4 colocalized with calreticulin and was absent from the plasma membrane (Figure 6B), in cultured VSMCs, Kv3.4 was almost exclusively found in a plasma membrane location (Figure 6C and D). Interestingly, whereas Kv3.4 mRNA increased in proliferating cells, total Kv3.4α subunit protein decreased, although it seemed to be more efficiently located in the plasma membrane.

Role of Kv3.4 in VSMCs Proliferation

The upregulation of Kv3.4α subunit mRNA during VSMC dedifferentiation and its transposition from a cytoplasmic to a membrane location suggest that the activity of Kv3.4 channels may be related to proliferation. To explore this hypothesis, we studied the proliferation rate of cultured VSMCs

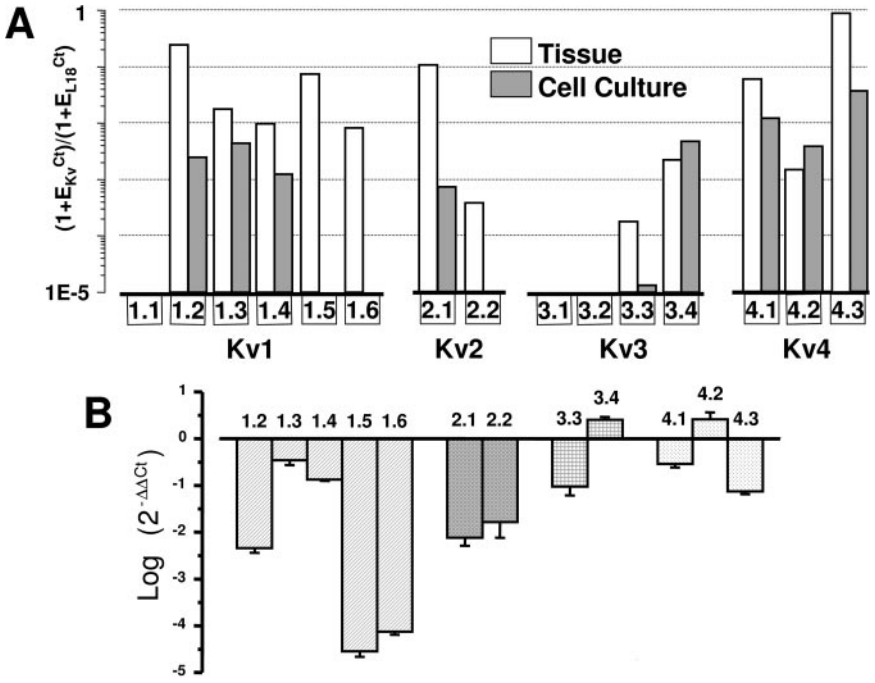

Figure 4. A, Relative abundance of Kv mRNA in contractile (Tissue, open bars) and synthetic (Cell Culture, gray bars) phenotypes. The relative abundance of mRNA was calculated from $(1+E_{Kv}^{CT})/(1+E_{L18}^{CT})$, where E represents efficiency for each PCR. Each determination was performed in 8 to 10 paired samples (the fresh tissue and its corresponding primary culture). B, Fold changes ($2^{-\Delta\Delta Ct}$) in the expression levels of each Kv mRNA in cultured VSMCs in relation to its tissue level. Note that the scale is logarithmic; therefore, −1 indicates a 10-fold decrease in mRNA levels and +1 indicates a 10-fold increase.

when the activity of the channels was blocked by low TEA concentrations (100 μmol/L) or BDS-I toxin (Figure 7A). TEA application led to a decreased proliferation rate that was already significant after 24 hours of treatment. The difference increased with time and reached a plateau around 96 hours after TEA treatment. The same results were obtained when Kv3.4 channels were blocked with 2.5 μmol/L BDS-I, whereas blockade of Kv4 channels with 70 nmol/L phrixotoxin did not affect proliferation rate (inset in Figure 7A). These experiments demonstrate a specific link between Kv3.4 (but not Kv4) channels and VSMC proliferation, although the contribution of other Kv channels to this process (ie, some Kv1 family members, as previously found in other preparations[22,23]) cannot be excluded.

The decreased number of cells in the presence of Kv3.4 channel blockers could reflect a decreased proliferation rate or an increase in the number of cells undergoing apoptosis. Because among the hallmarks of late-stage apoptosis is the fragmentation of nuclear chromatin, we performed a terminal deoxynucleotidyltransferase-mediated dUTP nick-end labeling assay to detect apoptosis-induced DNA fragmentation both in control cells and in 100 μmol/L TEA–treated cells (Figure 7B). There was no significant cell death in either of the 2 samples (2.1±0.9% in control versus 2.0±0.7% in TEA treated, n=10 fields from 2 independent experiments), indicating that TEA block of Kv3.4 channels does not induce apoptosis.

Discussion

In this work, we have characterized the Kvα subunit profile underlying Kv currents of VSMCs from human uterine

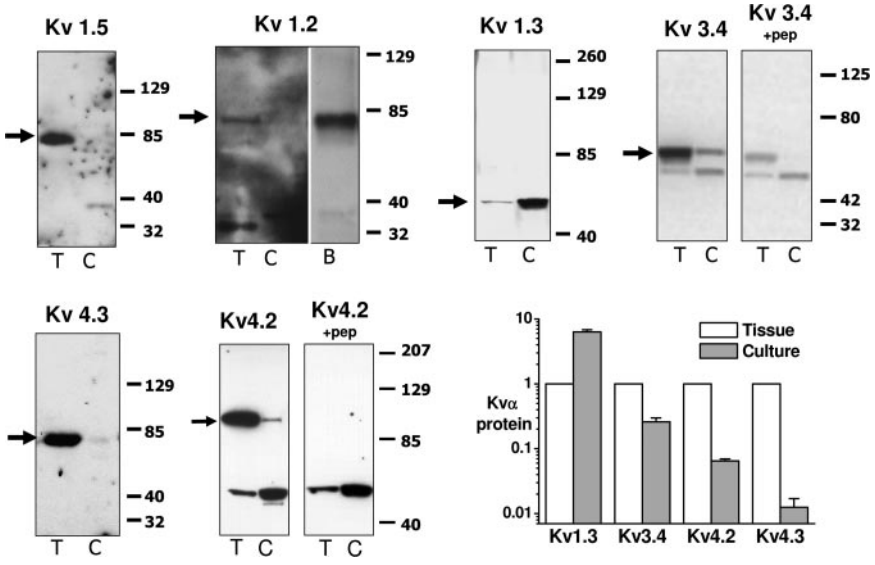

Figure 5. The presence of several Kvα subunits in contractile (T, left lanes) and proliferating phenotype (C, right lanes) was analyzed by Western blot. Control bands corresponding to mouse or human brain (B) were detected in all cases (only Kv1.2 is shown). Arrows indicate the bands corresponding to Kvα subunits. When available, blocking with a control peptide (pep) was performed, as shown for Kv4.2 and Kv3.4. Densitometry of the bands provides the relative amount of each Kvα protein, normalized to the amount in the tissue. Each experiment was performed 3 to 4 times with 3 different samples each time.

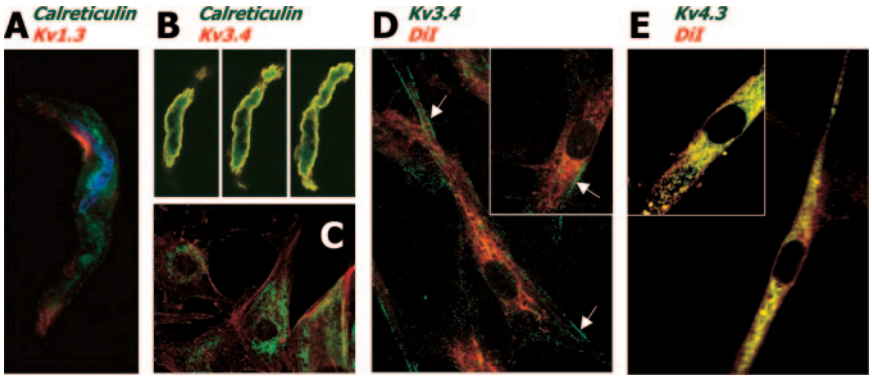

Figure 6. Intracellular distribution of Kv proteins studied with double immunocytochemical labeling and confocal microscopy. A, One acutely dissociated VSMC labeled with antibodies against Kv1.3 (red) and calreticulin (green). The image was obtained with a Zeiss ApoTome microscope. Nucleus was labeled with 4′,6-diamidino-2-phenylindole (blue). B, Kv3.4 (red) colocalizes with calreticulin (green) and is absent from the plasma membrane in contractile VSMC but not in cultured VSMC (C), where it appears restricted to the plasma membrane. D, Cultured VSMCs incubated with DiI (red) and Kv3.4 antibody (green). E. Kv4.3 (green) colocalize with DiI (red) staining in intracellular compartments. The same distribution pattern was found in acute dispersed cells (data not shown). B through D were obtained with a Bio-Rad confocal microscope. Each figure is representative of at least 3 experiments.

arteries and its changes when VSMCs switch from a contractile to a proliferating phenotype.

With the exception of pulmonary arteries, little is known regarding the expression profile of Kv channels in human resistance arteries.[24–27] However, it is well established that Kv channels play an important role in regulating contraction through their effects on E_m and by integrating a variety of vasoactive signals.[2] Moreover, abnormal expression profiles of Kv channels have been involved in the pathogenesis of arterial hypertension and vascular proliferation, leading to new opportunities for developing drugs for targeting disease-specific changes in ion channel expression.[28,29] Identification of the contributing Kv genes is crucial to pursuit this approach.

The large diversity of Kv channel genes, together with their possibilities of heteromultimerization, association with accessory subunits, and alternative splicing, leads to an enormous diversity in the molecular composition and properties of Kv channel complexes.[19] Often, the number of expressed Kv subunits appears to be much larger than the number of apparent Kv current components.[30] The oligomeric composition of Kv channels and the factors regulating their folding and trafficking to the cell membrane[31] could contribute to explain these discrepancies.

We have tried to correlate the electrophysiological and pharmacological properties of Kv currents with the expression of Kvα subunits, both at mRNA and protein levels. Although the role of BKCa currents in resistance arteries in vivo cannot be evaluated with this study, the apparent activation thresholds of BKCa and Kv currents (see Figure 1) suggests a small contribution of BKCa current to the resting E_m in relaxed cells, which is supported by the measured resting E_m values (Figure 1C) and its insensitivity to paxilline. Our observations echo the results obtained in other VSMC preparations, where Kv currents activated at potentials 20 to 30 mV more negative than BKCa currents.[15,32]

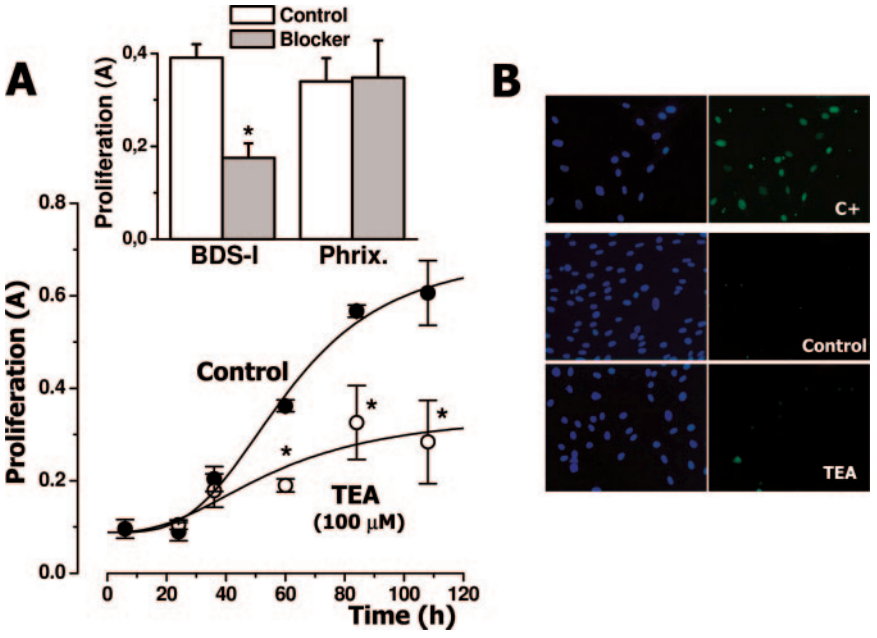

Figure 7. A, Absorbance at 490 nm (as an index of the number of viable cells) was taken at the indicated times in control VSMCs (●) and in cells incubated in the presence of 100 μmol/L TEA (○). TEA was added after 24 hours in culture. Each point is the mean±SEM of 4 determinations within the same experiment, and a total of 3 similar experiments where performed. Data were fitted to logistic functions. The inset shows the effect of 2.5 μmol/L BDS-1 and 70 nmol/L phrixotoxin (Phrix.) at 72 hours of culture (48 hours after drug application) obtained in a parallel experiment with n=4 data points in each condition. B, Terminal deoxynucleotidyltransferase-mediated dUTP nick-end labeling assay performed in control cells and in cells after 2 days in the presence of TEA. Top panels show images of an assay performed in DNase-treated cells (C+). Left images are 4′,6-diamidino-2-phenylindole–labeled nuclei. The data are representative of 3 experiments.

Our results showing that Kv1 members are the predominant components of the Kv currents are in good agreement with previous reports from other resistance vessels.[33–37] The pharmacological data from acute VSMCs also indicate the contribution of Kv2, Kv3, and Kv4 channels. Kv2.1 currents have been described in rat mesenteric artery VSMCs[38] and have been found to be the major contributor to the Kv current in VSMCs from conduit arteries such as rat aorta.[14,15,17] mRNA and even protein for Kv3.4 and Kv4 channels have been previously described in rat mesenteric SMCs, but their functional correlates were not found.[38]

Real-time PCR confirmed the presence of all the Kv studied but Kv1.1, Kv3.1 and Kv3.2. It also showed an inconsistency with the functional data, as the more represented Kvα subunit mRNAs (Kv4α subunits), represent only around 15% of the total Kv current. Western blot and immunocytochemistry data indicate that although Kv4 protein (mainly Kv4.3) is present in VSMCs, it is located mostly intracellularly and, therefore, cannot contribute to Kv currents.

On the other hand, the results from cultured VSMCs show remarkable differences, with relevant implications. First, our data demonstrate that VSMC proliferation leads to an important Kv channel remodeling; therefore, caution must be taken when interpreting data on ion channel expression or function obtained from cultured VSMCs, a common situation when studying human tissues. Also, the remodeling of Kv currents correlates well with the changes in mRNA, again with the notable exception of Kv4α subunits (see the online data supplement). Specific localization and trafficking mechanisms are relevant to Kv4 channel function in most cells where these channels have been studied. In addition, a surprisingly large number of ancillary subunits and scaffolding proteins that can interact with the primary subunits altering channel trafficking have been described (for a review, see Birnbaum et al[39]). The study of the expression pattern of these auxiliary subunits may contribute to understanding the lack of functional expression of Kv4 channels in fresh and cultured VSMCs. For the other Kvα subunits, we observed an overall reduction in the expression of Kv1 and Kv2 mRNA and an increase in the amount of Kv3.4 mRNA. These changes are congruous with the electrophysiological and pharmacological profile of cultured VSMCs (Figure 3), where the predominant Kv current is an inactivating current, highly sensitive to micromolar doses of TEA.

Since the first description by DeCoursey et al[40] of the possible role of K^+ channels in T-cell mitogenesis, many other K^+ channels have been implicated in normal and/or pathological cell proliferation in different preparations.[10] Proliferation associates with either changes in the expression of channels already present (ie, the switch between Kv1.3 and intermediate-conductance Ca^{2+}-activated K^+ [IKCa] channels in T cells,[40] between Kv1.5 and Kv1.3 in proliferating microglia,[22] or between BKCa and IKCa in rat aortic SMCs[41,42]) or with the concomitant appearance of channels absent in the native tissues, such as Kv10.1 channels in several tumor cell lines.[10,43] We have also explored the expression of Kv10.1 and IKCa1 in acute and cultured VSMCs, finding a significant upregulation of Kv10.1 mRNA in cultured VSMCs and no changes in IKCa mRNA (see the online data supplement).

Regulation during VSMC proliferation has been described for L- and T-type Cav channels[44–46] and for IKCa channels.[3,41,42] For Kv channels, a role in VSMC proliferation through their effects on E_m is well established in the pulmonary circulation, where the decreased activity of Kv1.5 (and also Kv2.1) in hypoxic pulmonary hypertension has been shown to contribute to vascular hypertrophy by decreasing apoptosis.[23,24]

Our results provide data in concordance with some of these previous reports and also some novel findings that increase the number of players contributing to VSMCs proliferation. (1) We demonstrate a marked decrease in the expression of all Kv1 channels. Downregulation of Kv1.5 is in agreement with the previous findings in pulmonary arteries, although its participation in the phenotypic change of human uterine artery SMCs was not further explored. (2) We found a decrease in Kv2 channel expression in proliferating VSMCs, an observation previously reported in immature rat aortic myocytes.[17] Interestingly, some of the functional changes observed in cultured VSMCs, such as the decreased in BKCa currents and the increase in the inactivating component of the Kv current, have also been reported in neonatal myocytes, suggesting a relationship between the proliferating phenotype and the immature VSMCs. (3) We show, for the first time, the presence and functional upregulation of Kv3.4 channels with the phenotypic change, most likely associated with their translocation from an intracellular location to the plasma membrane and their contribution to cell proliferation. This translocation of Kv3.4 protein could be associated with the stimulation of mitogen-activated protein kinase pathways or growth factors that are probably involved in VSMC proliferation and that have been shown to contribute to the membrane trafficking of BK channels in neurons.[47] Finally, the proliferation assays demonstrate that Kv3.4 channels are necessary for VSMCs to proliferate, as selective blockade of these channels decreases the number of viable cells without increasing apoptosis.

In summary, the present work provides a thorough functional and molecular characterization of the Kvα subunits expressed in VSMCs from human uterine artery and its modification by cell proliferation. We report Kv channel remodeling associated with the phenotypic change, and we found that Kv3.4 activity is related to cell proliferation; therefore, pharmacological modulation of this channel could become relevant for the regulation of VSMC proliferation associated with both physiological and pathological processes.

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