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# Diversity of Potassium Channels in Human Umbilical Artery Smooth Muscle Cells: A Review of Their Roles in Human Umbilical Artery Contraction

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**Abstract**

Through their control of cell membrane potential, potassium ($K^+$) channels are among the best known regulators of vascular tone. This article discusses the expression and function of $K^+$ channels in human umbilical artery smooth muscle cells (HUASMCs). We review the bibliographic reports and also present single-channel data recorded in freshly isolated cells. Electrophysiological properties of big conductance, voltage- and $Ca^{2+}$-sensitive $K^+$ channel and voltage-dependent $K^+$ channels are clearly established in this vessel, where they are involved in contractile state regulation. Their role in the maintenance of membrane potential is an important control mechanism in the determination of the vessel diameter. Additionally, small conductance $Ca^{2+}$-sensitive $K^+$ channels, 2-pore domains $K^+$ channels and inward rectifier $K^+$ channels also appear to be present in HUASMCs, while intermediate conductance $Ca^{2+}$-sensitive $K^+$ channels and ATP-sensitive $K^+$ channels could not be identified. In both cases, additional investigation is necessary to reach conclusive evidence of their expression and/or functional role in HUASMCs. Finally, we discuss the role of $K^+$ channels in pregnancy-related pathologies like gestational diabetes and preeclampsia.

**Keywords**

human umbilical artery, $K^+$ channels, patch-clamp

## Introduction

The blood vessels of the umbilical cord are necessary for the communication between the growing fetus and the placenta, so a thorough knowledge of the mechanisms and structures responsible for their contractile state is fundamental for the study of the physiology and the pathophysiology of fetal–placental blood flow. Two umbilical arteries deliver blood with low $O_2$ pressure from the fetus to the placenta, while the umbilical vein returns nutrient-rich blood with a higher $O_2$ pressure to the fetal circulatory system. Umbilical vessels are unique in that they are not innervated by the autonomic nervous system,[1,2] so the contraction and relaxation of their vascular smooth muscle layer depends on blood-borne vasoactive substances or on locally produced factors, secreted either by the endothelial cells or by the cells in the Wharton jelly.

Among the most important regulators of vascular tone are potassium ($K^+$) channels, because through their role in cell membrane potential (Vm) regulation, they can determine the activity of voltage-operated calcium ($Ca^{2+}$) channels and hence the degree of vessel contraction.[3,4] In brief, closure of $K^+$ channels induces membrane depolarization, activation of voltage-operated $Ca^{2+}$ channels, and extracellular $Ca^{2+}$ influx, which can be accompanied by $Ca^{2+}$ release from intracellular $Ca^{2+}$ stores (sarcoplasmic reticulum and mitochondria). This increases cytosolic free $Ca^{2+}$ concentration producing smooth muscle contraction, which leads to vasoconstriction. On the other hand, opening of $K^+$ channels causes hyperpolarization and hence, closure of $Ca^{2+}$ channels. The $Ca^{2+}$ removing mechanisms then reduce the cytosolic free $Ca^{2+}$ concentration: plasma membrane $Ca^{2+}$-ATPase and the forward mode of $Na^+/Ca^{2+}$ exchanger transport $Ca^{2+}$ out of the cell, while sarcoplasmic reticulum and mitochondrial $Ca^{2+}$-ATPases pump $Ca^{2+}$ back into those intracellular $Ca^{2+}$ reservoirs. This reduction in cytosolic $Ca^{2+}$ causes smooth muscle cell relaxation, producing vasodilation. Figure 1 summarizes the link between $K^+$ channel activity, membrane potential, and smooth muscle

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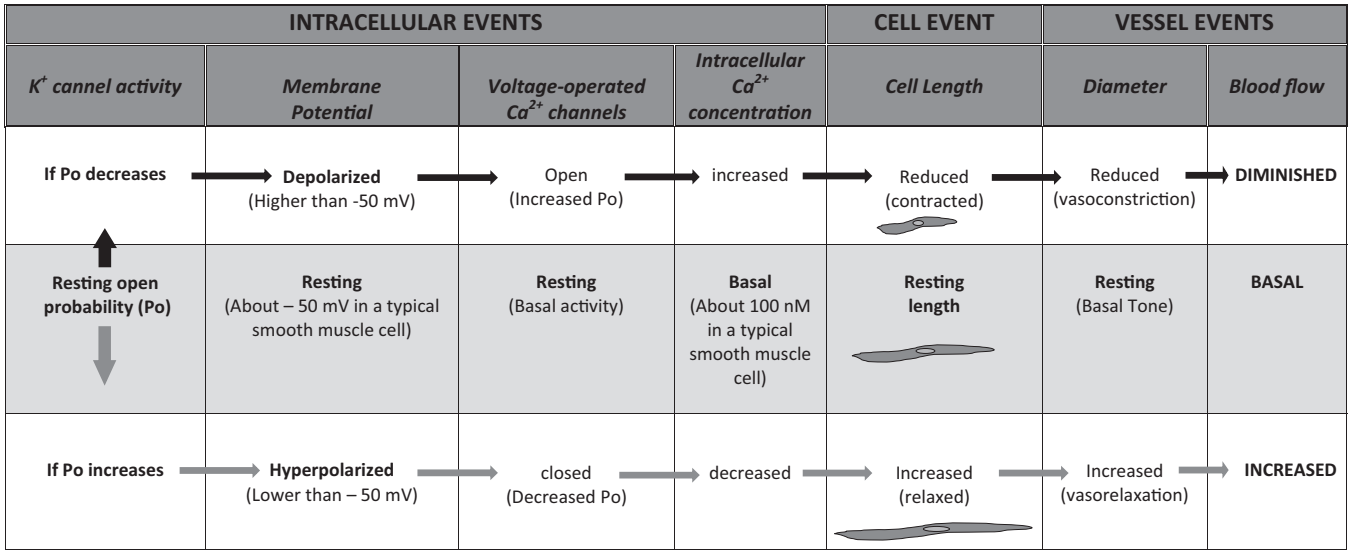



**Figure 1.** Summary of the link between $K^+$ channel activity, membrane potential, and smooth muscle contractile state which, in turn, determines a blood vessel diameter.

contractile state which, in turn, determines the blood vessel diameter.

We studied the electrophysiological properties of different types of ion channels in human umbilical artery (HUA) smooth muscle cells (HUASMCs) and found that they present several different types of $K^+$ channels, which are clearly observed in patch-clamp single-channel recordings.[5,6] However, in the literature, in contrast to human placental vessels,[7-9] there are no review articles addressing the different types of $K^+$ channels functionally expressed in HUA. The present review summarizes our current knowledge on $K^+$ channels in these vessels.

We first present a brief summary of the structure, classification, and role of $K^+$ channels in vascular smooth muscle cells. Afterward, we show electrophysiological evidence of the variety of $K^+$ channels found in freshly dispersed HUASMCs recorded in the single-channel configuration of the patch-clamp technique. Finally, based on this information, we discuss the evidence found in the literature pointing to the presence and the characteristics of the different types of $K^+$ channels in these cells, comparing it with our single-channel data and bibliographic reports from other fetoplancental vessels. We shall evaluate direct or indirect evidence obtained either in isolated cells or in intact tissue through the use of different experimental techniques, such as electrophysiology, isometric force measurement, and specific messenger RNA (mRNA) or protein detection.

## Potassium Channels in Vascular Smooth Muscle

Potassium channels are widely expressed cell membrane structures that allow the selective flux of $K^+$ ions between the intracellular and extracellular compartments. They are mainly involved in cell membrane potential regulation and electrical cell excitability and also in other important cellular functions like proliferation and differentiation.[3,4,10] A typical $K^+$ channel is formed by a homo- or heterotetrameric protein of $\alpha$ subunits, which includes a conserved region constituting a permeation pathway for $K^+$ fluxes, namely, the channel pore. There are many different $\alpha$ subunits, and they present variable numbers of transmembrane domains (TMDs). One of the existing classifications of $K^+$ channels is based on the number of TMDs of their $\alpha$ subunits:

(1) The 6 TMDs (S1-S6) with 1-pore domain formed by the S4-S5 protein region. This is the most numerous group and can be divided into 3 different families. The first family is the classic voltage-dependent $K^+$ channels ($K_V$), comprising several subfamilies. The second family includes $Ca^{2+}$-sensitive but voltage-insensitive channels of small ($SK_{Ca}$) and intermediate ($IK_{Ca}$) conductance values. The third family corresponds to the Slo $K^+$ channels (Slo1 to 3), of which the most common and most studied member is the big conductance, voltage- and $Ca^{2+}$-sensitive $K^+$ channel ($BK_{Ca}$), also known as $K_{Ca}1.1$, Slo1, or maxi-$K^+$ channel. It must be noted that the Slo channels have an additional transmembrane segment ($S_0$), so they would really be 7 TMD channels.

(2) The 4 TMDs with 2-pore domains, which have been named $K_{2P}$ channels. This is a newly described family characterized by channels with 2 $\alpha$ subunits of 4 TMDs with 2 pore-forming loops in each of them formed by the S1-S2 (P1 domain) and S3-S4 (P2 domain) segments.

(3) The 2 TMDs with 1-pore domain, being the most frequently observed members of this family, the typical inward rectifier $K^+$ channels ($K_{IR}$) and the ATP-sensitive $K^+$ channels ($K_{ATP}$).

Table 1 summarizes this classification and provides additional electrophysiological data as well as the most common pharmacological tools used to characterize each type of $K^+$ channel discussed in this article.

Many of these $K^+$ channels have been described in vascular smooth muscle cells.[3,4] Of those, the presence and functions of $BK_{Ca}$ and $K_V$ channels[3,4] have been clearly demonstrated in many vascular beds. Physiological studies of vascular function have shown that these channels are mainly involved in vessel diameter regulation and consequently in the regulation of blood pressure. This is achieved by different mechanisms, like myogenic response, or vasodilatation induced by endothelial factors, as the most important. The presence and functions of members of other $K^+$ channel families were also demonstrated in smooth muscle cells of different blood vessels: the classic $K_{IR}$ and $K_{ATP}$, and several $K_{2P}$ channels (TALK2, TASK1 and 2, TRAAK, and TREK1 and 2).[3,4,11,12] On the other hand, the $SK_{Ca}$ and $IK_{Ca}$ channels are weakly expressed in vascular smooth muscle cells but are generally expressed in endothelial cells.[13]

## Diversity of $K^+$ Channels in Freshly Dissociated HUASMCs: The Electrophysiological Properties

With the patch-clamp technique,[14] it is possible to record the current flowing through a single or multiple ionic channels in biological membranes. In contrast to other techniques used to study ion channels such as Western blot, immunochemistry, and reverse transcriptase–polymerase chain reaction (RT-PCR), which only show the expression of the protein or its mRNA transcription, patch-clamping has the advantage of directly measuring protein activity (as the transmembrane ion current) in their native membrane lipid environment. Moreover, it allows a precise control of the conditions that could affect the channel activity, like membrane potential and the composition of the solution bathing both sides of the plasma membrane (depending on the patch-clamp configuration). In the whole-cell configuration, the measured current is the product of ions flowing through all the channels present in the cell membrane. These types of recordings are known as macroscopic or whole-cell currents. In the other configurations, cell-attached (CA), inside-out (IO), and outside-out (OO), only a few single channels are recorded, which is seen as discrete jumps in the current value when they open and as zero current when they are closed. In the CA configuration, the cell remains practically intact, so it is regarded as more physiological, while in the IO and OO configurations the membrane is isolated from the cell, but this allows a complete control of both intracellular and extracellular conditions.

Figure 2A shows a typical CA recording obtained in freshly isolated HUASMCs. By measuring channel activity at different membrane potentials, one can obtain 2 basic characteristics of the channel: the voltage dependence of the open probability and the unitary conductance value. The open probability is the fraction of time that the ionic channel stays in the open state (allowing ion flux) and represents a measure of channel activity. The unitary conductance, also named slope conductance, is an elementary empirical parameter of each ionic channel that reflects the ease with which the ion flows through the channel pore. This value can be obtained from the slope of the (generally) linear relationship between single-channel current amplitude and the value of transmembrane potential, which is known as a current–voltage (IV) curve. In Figure 2A, the single-channel openings present different current amplitude values, suggesting the presence of different ionic conductances. Figure 2B depicts the distribution histogram of the slope conductance values obtained from 73 HUASMCs. It can be seen that these cells express a wide range of conductance values, ranging from 10 to 293 pS. The histogram was well fitted by a 6-component Gaussian function, indicating the presence of at least 6 different subgroups of ion channels. The mean conductance values of the different channels in these subgroups and its dispersion, as well as their frequency of apparition, can be seen in the box chart of Figure 2C.

After applying to the extracellular side of the membrane patch 5 mmol/L 4-aminopyridine (4-AP), a blocker of $K_V$ channels, the recorded conductance values showed a similar distribution, where 6 groups of ion channels could be identified, but the frequency of apparition of several types of channels was significantly reduced (see Figure 3). The comparison of these 2 situations suggests that those channels that almost disappeared after 4-AP treatment are probably members of the $K_V$ family.

Based on these results, we can propose that HUASMCs present several functional $K^+$ channels that could be involved in the regulation of their contractile state as well as in other cellular functions. In particular, those channels that are open at a membrane potential value of about $-50$ mV[5] could be involved in resting Vm maintenance, while those that are voltage dependent and begin to activate at more depolarized potential values would be involved in a feedback mechanism opposing depolarization as has been clearly established in different vascular smooth muscle cells.[3] So, this technique contributes to the knowledge of ion channel expression showing functional evidence of such proteins and their putative participation in HUASMC physiology.

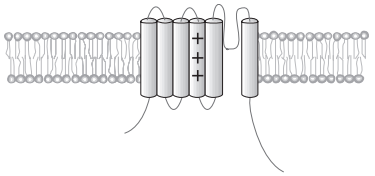
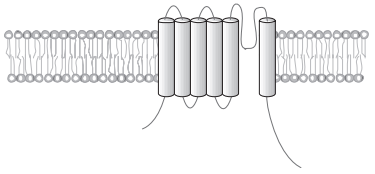
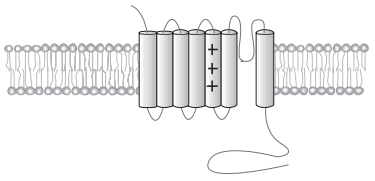
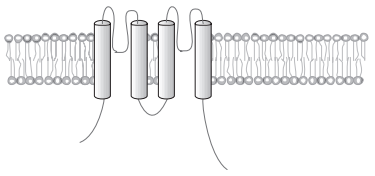
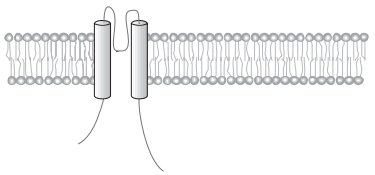
In the following sections, we review the different reports found in the literature where the function of $K^+$ channels was directly or indirectly demonstrated in human umbilical vessels, comparing this information to what is known about these channels in placental vessels.

## Published Reports About $K^+$ Channels in Human Umbilical Arteries

### *Big Conductance, Voltage- and $Ca^{2+}$-Sensitive $K^+$ Channels*

Several reports implicate these channels in the control of HUA contractile state. For instance, the $K^+$ channels blocker tetraethyl ammonium (TEA; 5 mmol/L) induced vascular contraction on

**Table 1.** Structure and Characteristics of the Different $K^+$ Channels Discussed in this Article.[a]

| $K^+$ Channel Families | α-Subunit Membrane Topology | Summary of Electrophysiological and Pharmacological General Characteristics of the $K^+$ Channels Discussed in This Review | | |
|---|---|---|---|---|
| $K_V$, voltage-dependent $K^+$ channels (KCNA1-10 KCNB1-2 KCNC1-4 KCND1-3 KCNF1 KCNG1-4 KCNQ1-5 KCNV1-2 KCNS1-3 KCNH1-8) | 6 TMDs: 42 isoforms; 12 subfamilies  | Macroscopic conductance: outward rectification.<br>The $K_V$1.4 (KCNA-4), $K_V$3.3-3.4(KCNC3-4), and the $K_V$4 (KCND) subfamily present fast inactivation (A-type $K^+$ current).<br>Single-channel conductance: 5-80 pS.<br>Classical inhibitor: 4-AP (except for $K_V$7.x (KCNQ) and $K_V$10.x-$K_V$12.x (KCNH) subfamilies | | |
| $SK_{Ca}$ & $IK_{Ca}$, small and intermediate conductance calcium-activated $K^+$ channels (KCNN1-4) | 6 TMDs: 4 isoforms; 2 subfamilies  | | $SK_{Ca}$ subfamily | $IK_{Ca}$ subfamily |
| | | Macroscopic conductance | voltage independent* | |
| | | Single-channel conductance | 10-14 pS | ≈ 45pS |
| | | Classical inhibitors | apamin | clotrimazole, charibdotoxin |
| | | Classical activators | ↑$[Ca^{2+}]_{intracellular}$ | |
| Slo $K^+$ channels, high conductance voltage-operated $K^+$ channels sensitive to $[ion]_{intracellular}$ ($Ca^{2+}$, $Cl^-$, $Na^+$ or $OH^-$; KCNMA1, KCNT1-2, KCNU1) | 7 TMDs: 4 isoforms; 3 subfamilies  | $BK_{Ca}$, Slo1, or MaxiK (big conductance, voltage- and $Ca^{2+}$-sensitive $K^+$ channel, KCNMA1).<br>Macroscopic conductance: outward rectification. Single-channel conductance ≈250 pS.<br>Classical inhibitors: iberiotoxin, paxilline, charibdotoxin, 0.1 mmol/L TEA.<br>Classical activators: phloretin, ↑$[Ca^{2+}]_{intracellular}$ | | |
| $K_{2P}$, 2-pore domain $K^+$ channels, also named background $K^+$ channel (KCNK1-7, 9,10,12,13,15-18) | 4 TMDs: 15 isoforms; 6 subfamilies  | Macroscopic conductance: voltage independent.<br>Single-channel conductance <40 pS, except TREK subfamily (≈100 pS).<br>Insensitive to classical $K^+$ channels Inhibitors: TEA, 4-AP.<br>Classical inhibitors: none | | |
| $K_{IR}$, Inward rectifier $K^+$ channels (KCNJ1-6,8-16) | 2 TMDs: 15 isoforms; 7 subfamilies  | | $K_{IR}$ subfamily | $K_{ATP}$ subfamily |
| | | Macroscopic conductance | strong inward rectification | intermediate inward rectification |
| | | Single-channel conductance | <30 pS and inward rectification | |
| | | Classical inhibitors | 500 μmol/L $Ba^2+$ | glibenclamide, ↑$[ATP]_{intracellular}$ |
| | | Classical activators | | Pinacidil, cromakalim |

Abbreviations: ATP, adenosine triphosphate; TEA, tetraethyl ammonium; TMD, transmembrane domain.

[a] The first column lists the different families of potassium ($K^+$) channels stating their most common denomination as well as their HUGO Gene Nomenclature Committee (HGNC) designations. The second column indicates the number of transmembrane domains (TMDs) constituting the α subunits of each type of channel, as well as how many isoforms of this subunits have been found up to date. The third column gives several electrophysiological and pharmacological characteristics for each type of $K^+$ channels, which will be used in the review of their role in human umbilical artery smooth muscle.

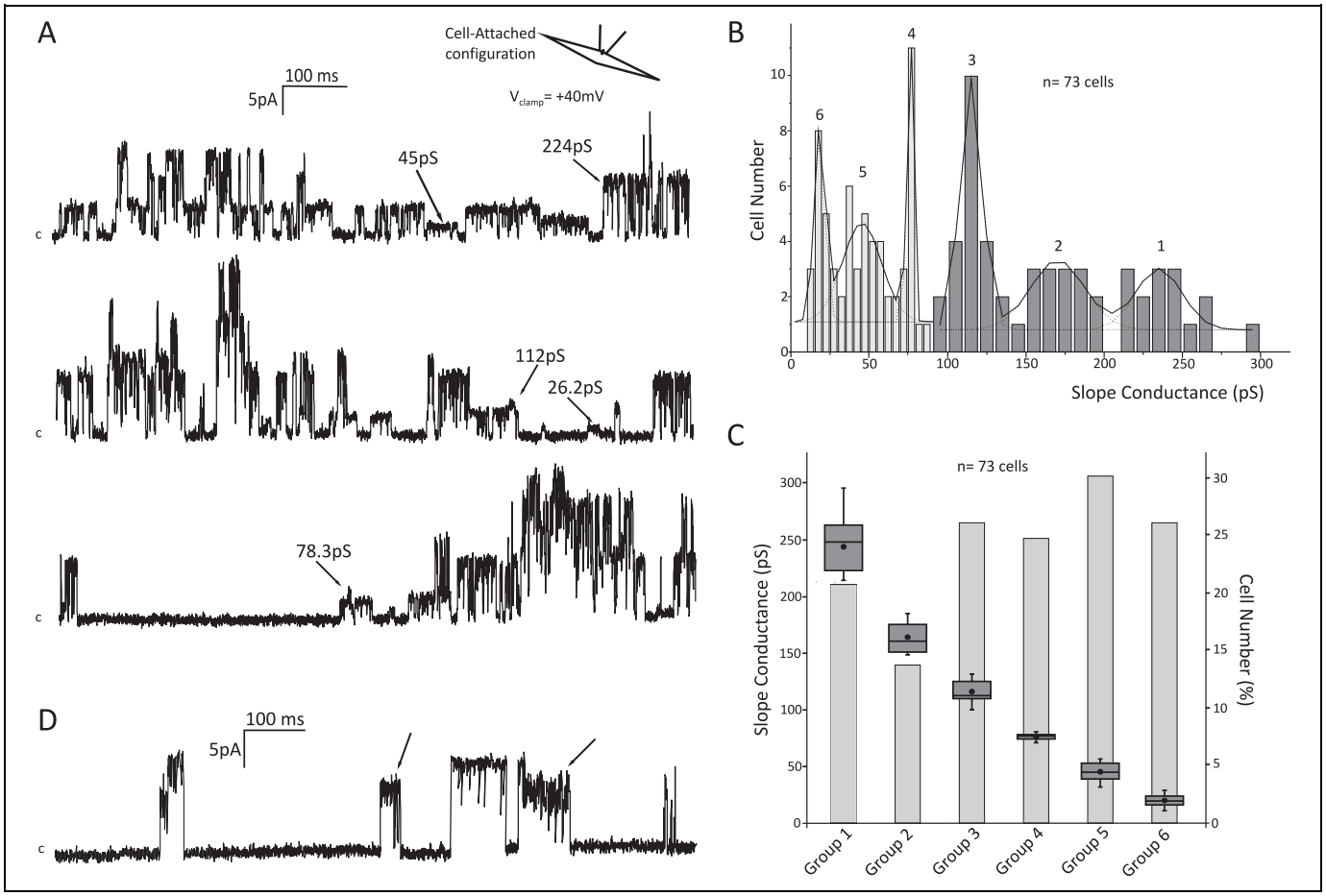



**Figure 2.** Diversity of $K^+$ channels in dispersed human umbilical artery (HUA) smooth muscle cells. A, Typical cell-attached single-channel recording in the stationary state (recordings of 30-60 seconds) using symmetrical $K^+$ concentrations (assuming an intracellular concentration $K^+$ of 140 mmol/L). The arrows indicate the ionic channel conductance values obtained from the slope of the respective current–voltage (IV) curves. B, Distribution of all single-channel conductances observed in HUA smooth muscle cells constructed from records similar to those shown in (A). The resulting histogram was well fitted by a 6-component Gaussian curve (indicated by the solid line; the dotted lines define each of these 6 groups). C, Box diagrams showing the properties of the 6 groups of conductances obtained in (B). For each of the groups are shown the mean conductance value (solid dot inside the box), the median (line inside the box), and the range of conductance values between the 25 and 75 percentiles (box) and between the 5 and 95 percentiles (capped lines). The bars show the relative frequency of appearance of the components of each group. D, Typical cell-attached single-channel recordings obtained as described in (A), showing $BK_{Ca}$ channel activity and probable subconductance levels (indicated by arrows) for this channel. Bath solution (in mmol/L): 140 potassium chloride (KCl); 0.5 magnesium chloride ($MgCl_2$); 10 HEPES; 6 glucose; 1 ethylene glycol tetraacetic acid, pH adjusted to 7.4 with potassium hydroxide (KOH). Pipette solution (in mmol/L): 140 KCl; 0.5 $MgCl_2$; 10 HEPES; 6 glucose; 1 calcium chloride; pH adjusted to 7.4 with KOH.

nonstimulated HUA rings, whereas the $BK_{Ca}$ channels activator phloretin (50 μmol/L) produced relaxation, suggesting the participation of these channels in the regulation of HUA basal tone.[5] Moreover, the relaxation of precontracted HUA rings induced by nitric oxide (NO),[15] testosterone,[16] and levosimendan[17] was inhibited by low TEA concentrations, so $BK_{Ca}$ channels may also be involved in the relaxation induced by endogenous and exogenous substances. Supporting the findings reported by Lovren et al,[15] we demonstrated that $BK_{Ca}$ channels are activated by NO at the single-channel level through a mechanism that most probably involves protein kinase G (PKG) activation.[18]

Some of the inferences about the functional role of $BK_{Ca}$ channels in HUA could be controversial, since they derive from results obtained with TEA, which is not a selective $BK_{Ca}$ channel blocker. However, electrophysiological data obtained from freshly dispersed HUASMCs support the above-mentioned suggestions inferred from intact tissue. The whole-cell currents evoked by depolarizing voltage steps showed no apparent inactivation during the 500 ms of the step and had a reversal potential close to −50 mV.[5] This current was reduced by 1 mmol/L TEA (~71% inhibition) and 2 $BK_{Ca}$-specific blockers: ~65% inhibition by 200 nmol/L iberiotoxin[5] and ~85% inhibition by 500 nmol/L paxilline[6] (both measured at +60 mV), showing that more than half of the total current is carried by $BK_{Ca}$ channels. Similar results were also observed in HUA-derived cultured smooth muscle cells,[19] where $BK_{Ca}$ channels together with $K_V$ channels are the main contributors to macroscopic $K^+$ current.

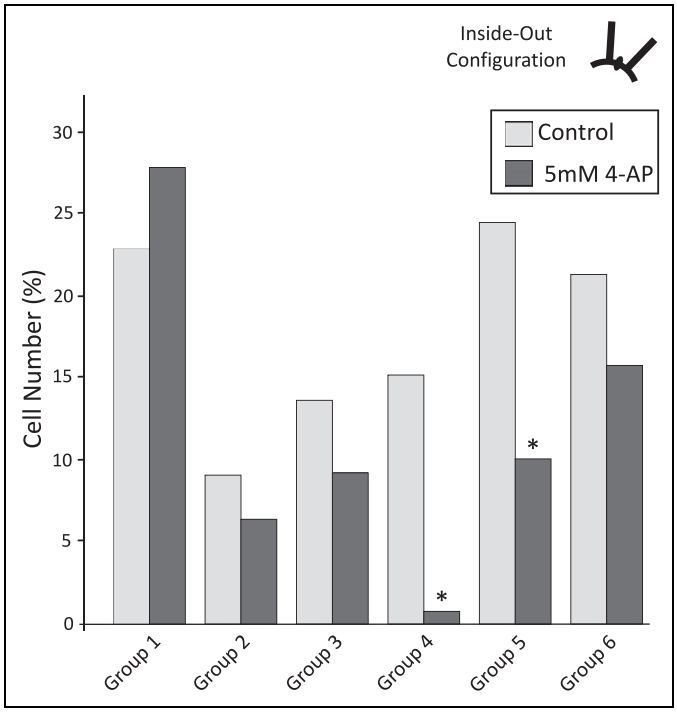



**Figure 3.** 4-AP sensitivity of ion channel conductances in human umbilical artery (HUA) smooth muscle cells. Frequency of appearance of the different single-channel conductances recorded in the inside-out configuration in control conditions (n = 65 cells) and with 5 mmol/L 4-AP (n = 100 cells) added to the pipette solution. The symbol * indicates statistically significant difference from control (chi-square test). Bath solution (in mmol/L): 140 potassium chloride (KCl); 0.5 magnesium chloride ($MgCl_2$); 10 HEPES; 6 glucose; 1 ethylene glycol tetraacetic acid, pH adjusted to 7.4 with potassium hydroxide (KOH). Pipette solution (in mmol/L): 140 KCl; 0.5 $MgCl_2$; 10 HEPES; 6 glucose; 1 calcium chloride; pH adjusted to 7.4 with KOH.

Our laboratory had extensively characterized $BK_{Ca}$ properties at the single-channel level,[5,6] and they are in accordance with those observed in the whole-cell configuration as well as in intact tissue.

Among the ionic conductances presented in the previous section, the one in group 1 displays the classical properties of the $BK_{Ca}$ channel: high conductance value (241.0 $\pm$ 6.8 pS) and insensitivity to 4-AP. The $BK_{Ca}$ identity of this channel was confirmed by the use of the selective blocker paxilline.[6] The $BK_{Ca}$ channel found in HUASMCs presents all the typical biophysical properties displayed in other tissues, such as a high selectivity to $K^+$ and an increase in its open probability (Po) due to membrane depolarization and intracellular $Ca^{2+}$ concentration increase.[5,6]

Additionally, there is a 4-AP-insensitive conductance present in group 2, which always appears immediately before or after a $BK_{Ca}$ channel opening. This suggests that it is actually a subconductance level of this channel (Figure 2D), as it has been reported before.[20]

The functions of $BK_{Ca}$ channels in this vessel may be altered in physiopathological situations. For instance, Radenkovic et al have reported that in HUA coming from neonates whose mothers presented pregnancy-induced hypertension, the $BK_{Ca}$ channels seem to have a different role in bradykinin-developed force than the HUA from normal pregnancies, because 0.5 mmol/L TEA increased bradykinin contractions in the former case, while it did not affect HUA from normotensive pregnancies.[21]

In placental vessels, $BK_{Ca}$ channel expression has also been demonstrated by electrophysiological techniques[22] and by RT-PCR and Western blotting.[8] Similar to what occurs in HUA, these channels seem to be involved in NO-induced vascular relaxation[23] and attenuate agonist-induced contractions in placental vessels.[8]

## *Voltage-Dependent $K^+$ Channels*

It seems that $K_V$ channels are not involved in determining basal contractile tone in HUA, since the $K_V$ blocker 4-AP (5 mmol/L) had no effect on in vitro resting tone of these arterial rings.[5] However, the relaxations of precontracted HUA rings induced by NO,[15] testosterone,[16] or levosimendan[17] are partially inhibited by 4-AP. These results are indicative that $K_V$ channels, together with $BK_{Ca}$ channels, as already discussed, are involved in the vasorelaxation mechanism of these substances.

Electrophysiological results obtained in enzymatically dispersed single HUASMCs show that 5 mmol/L 4-AP blocked part of a noninactivating whole-cell $K^+$ current elicited by a depolarizing voltage step protocol.[5] These results suggest the presence of members of the $K_V$ family sensitive to this blocker and that do not show inactivation. Similar results were reported in primary cultured smooth muscle cells obtained from HUA.[19]

Our single-channel data give more information about the properties of 4-AP-sensitive ionic conductances. In the 2 groups of conductances whose frequency of apparition in the stationary state is reduced by 4-AP, there are 2 channels that are activated by membrane depolarization, presenting conductance values of 76.3 $\pm$ 1.2 pS (included in group 4) and 45.7 $\pm$ 2.6 pS (included in group 5), respectively. The behavior of these 2 noninactivating, 4-AP-sensitive conductances suggests that they may be members of one of the several $K_V$ subfamilies: $K_V1$ (except $K_V1.4$ that presents inactivation), $K_V2$, or $K_V3$ subfamilies of voltage-dependent channels. The use of more specific blockers is needed for the final identification of the different types of $K_V$ channels, although this would be further complicated in native cells by the existence of heteromultimers formed by combination of different kinds of subunits.[10]

Not all $K_V$ channels are blocked by 4-AP (see Table 1), so the voltage-dependent, 4-AP-insensitive $K^+$ channels in groups 3 and 6 may also be members of the $K_V$ family, but, up to now, there is no enough information to link them to specific $K^+$ channels.

In the placental vasculature, several subtypes of $K_V$ channels are expressed, namely, different members of the $K_V1$, $K_V2$, $K_V7$, and $K_V9$ families. The $K_V3$ may also be present, but evidence is still incomplete (see Wareing et al[9] for a complete review). Furthermore, the presence of functional 4-AP-sensitive $K_V$ ion channels was demonstrated in isolated smooth cells of peripheral

fetoplacental arteries by the patch-clamp technique.[7] In this vascular bed, in particular, 4-AP-sensitive $K_V$ channels contribute to basal vessel tone,[8] modulate agonist-induced responses,[8] and partly mediate tissue response to hypoxia.[7]

## Small Conductance $Ca^{2+}$-Sensitive $K^+$ Channels and Intermediate Conductance $Ca^{2+}$-Sensitive $K^+$ Channels

Small conductance $Ca^{2+}$-sensitive $K^+$ channels seem to be present and functional in HUA, although up to now the evidence is not conclusive as to their vessel layer localization (smooth muscle cell or endothelium). Radenkovic et al showed that apamin (2 μmol/L), a selective blocker of $SK_{Ca}$ channels, augments the maximal contractile responses to bradykinin in HUA rings, so $SK_{Ca}$ channels seem to be present and functional in this vessel.[24] Lovren and Triggle have shown that $SK_{Ca}$ are not activated during NO-induced relaxation of HUASMCs.[15] On the other hand, $SK_{Ca}$ channels do not contribute to whole-cell $K^+$ currents in freshly dispersed HUASMCs, since 100 nmol/L apamin had no effect.[5] A similar result was obtained in cultured cells derived from HUASMCs, because, in this case, a higher apamin concentration (10 μM) also had no effect on $K^+$ currents recorded at +60 mV.[19] However, since $SK_{Ca}$ are not voltage-sensitive channels, they can only contribute to voltage-evocated $K^+$ currents if they are basally active. If not, as it seems to be in our conditions, they could still play a role in modifying bradykinin-induced HUA contractions. Alternatively, the $SK_{Ca}$ channels reported to be involved in modulating bradykinin responses may be situated not in the smooth muscle but in the endothelial layer as it is observed in other vessels.[13]

Regarding the role of $IK_{Ca}$ channels in HUA, we could not find any report in the literature. In our experience, clotrimazole (400 nmol/L-1 μmol/L), an inhibitor of $IK_{Ca}$ channels, does not affect $K^+$ whole-cell currents in HUASMCs (V. Milesi, unpublished results). In the single-channel recordings mentioned earlier, we did not find any small or intermediate conductances activated by an increase in intracellular $Ca^{2+}$ concentration. This suggests that $SK_{Ca}$ and $IK_{Ca}$ are not present in HUASMCs. So, the results related to $SK_{Ca}$ from arterial rings discussed earlier may in fact reflect the role of these channels in the endothelial layer. Analysis involving the search for mRNA or protein expression in both types of cells is needed for a definitive answer. To our knowledge, neither $SK_{Ca}$ nor $IK_{Ca}$ channels were investigated in placental vessels.

## Inward Rectifier $K^+$ Channels and ATP-Sensitive $K^+$ Channels

A blocker of $K_{IR}$ channels (500 μmol/L $Ba^{2+}$) induced the contraction of nonstimulated HUA rings, suggesting that these channels participate in the maintenance of basal contractile state in this artery (V. Milesi, unpublished results). On the other hand, $Ba^{2+}$ (3 μmol/L) had no effect on top of bradykinin contractions of HUA rings coming from normal pregnancies, indicating that these channels are not involved in this mechanism.[24] However, this agent increased bradykinin-induced force in those rings coming from gestational diabetes mellitus pregnancies, showing that the relative importance of the different $K^+$ channels may change in pathological states.[24]

Channels present in groups 5 and 6 of the ionic conductance discussed earlier, generally displaying activity at negative membrane potentials (eg, −40 mV), could represent inward rectifier $K^+$ channels. However, their characteristics are also compatible with $K_{2P}$ channels, as mentioned in the following section, so a definite characterization using specific blockers is required to confirm their identity.

No evidence of the participation of $K_{ATP}$ channels in contraction/relaxation of intact HUA rings could be found in the literature. The $K_{ATP}$ blocker glibenclamide (1 μmol/L) had no effect in HUA rings when applied on top of bradykinin-[24] or serotonin-induced contractions.[21] Moreover, glibenclamide (10 μmol/L) did not modify NO- or levosimendan-induced relaxations, ruling out the participation of $K_{ATP}$ in such contractile and relaxing mechanisms.[15,17]

The $K_{ATP}$ channels have also been looked for in primary cultured HUASMCs. It has been reported that $K_{ATP}$ channels do not contribute to total $K^+$ currents (measured at +60 mV) because glibenclamide had no effect on them.[19] In contrast, a recent report also about cultured HUASMCs by Bai et al suggests the presence of $K_{ATP}$ protein (by Western blot).[25] The authors then ascribe to $K_{ATP}$ channels a whole-cell current measured under ionic and voltage conditions (symmetrical $K^+$ concentrations and 0 mV) where there actually is no driving force to carry any $K^+$ current,[25] so this last results must be approached carefully.

None of the single-channel conductances discussed earlier could be definitely ascribed to a $K_{ATP}$ channel. Those presenting low conductance values (around 20 pS), which would be compatible with $K_{ATP}$, also showed strong inward rectifications, which is a typical property of a $K_{IR}$ channel, but not of $K_{ATP}$ channels, because these last ones present only intermediate inward rectification (see Table 1).

Unlike what we reviewed for HUA, $K_{ATP}$ channels seem to be present and functional in placental vessels. In this vascular bed, the presence of $K_{IR}6.1$, the pore-forming subunit of vascular $K_{ATP}$ channels, has been demonstrated by molecular biological techniques (RT-PCR and Western blot).[8,26] Moreover, functional experiments showed that pinacidil and cromakalim, the 2 drugs that increase $K_{ATP}$ activity, relaxed precontracted chorionic plate arteries and veins. Furthermore, pinacidil also decreased their basal tone, suggesting functional roles for $K_{ATP}$ channels in these vessels.[8,27] There is no evidence in the literature about the presence of classical $K_{IR}$ channels in placental vessels.

## Two-Pore Domain $K^+$ Channels

Although $K_{2P}$ channels are beginning to be described in different vessels, there is still no definitive evidence of their presence in HUA, mainly because there are no specific blockers for these channels, which makes their study difficult. However, we have

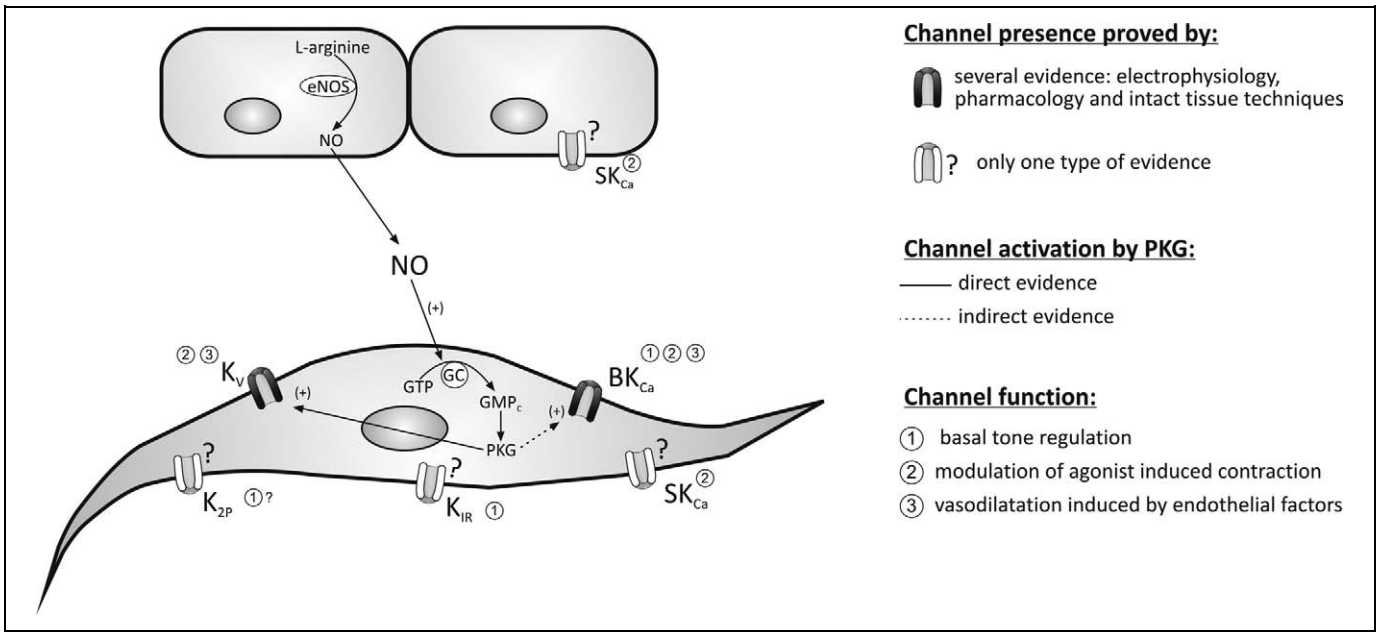



**Figure 4.** Summary of current knowledge regarding $K^+$ channel expression and function in human umbilical artery smooth muscle cells. Channels are depicted in different colors depending on the weight of the evidence pointing to their presence and function; black means there is fairly conclusive evidence obtained from different types of experimental techniques, while white (with a question mark) denotes that there is suggestive evidence present (only one type of experimental technique used) but definitive confirmation is needed. In the case of $SK_{Ca}$, a possible location in the endothelial cells is also suggested. The numbers 1 through 3 denote possible functions for these channels in these cells. NO,nitric oxide; $K_V$, voltage-dependent $K^+$ channels (different subfamilies); $BK_{Ca}$, big conductance, voltage- and $Ca^{2+}$-sensitive $K^+$ channel; $SK_{Ca}$, small conductance $Ca^{2+}$-sensitive $K^+$ channels; $K_{2P}$, 2-pore domains $K^+$ channels; $K_{IR}$, inward rectifier $K^+$ channels. Intermediate conductance $Ca^{2+}$-sensitive $K^+$ channels ($IK_{Ca}$), and ATP-sensitive $K^+$ channels ($K_{ATP}$) are not include because the evidence about their presence in HUA is either weak ($K_{ATP}$) or altogether not present in the literature ($IK_{Ca}$).

preliminary data showing that mRNA for TRAAK and mRNA for TREK1 are detectable in HUASMCs. One of the channels belonging to group 2 of single-channel conductance in HUASMCs has some characteristics compatible with a TREK member of the $K_{2P}$ channels (voltage independence, high conductance value, and 4-AP insensitivity), although no final identification can be made at present. There are also several voltage-independent channels in groups 5 and 6 showing heterogeneous characteristics regarding open probability and conductance values, which may represent channels of the $K_{2P}$ family, but further and more specific characterization is needed in order to ascertain the presence and the functional role of these channels in HUA intact tissue.

Similar to what we found in HUA, the presence of $K_{2P}$ channels in placental vessels is suspected but proofs are preliminary. Wareing et al reported detection of TASK1 mRNA in chorionic plate arteries and presented results involving possible block of these channels with anandamide, but they also remarked that more investigations are required due to the nonspecificity of this compound.[8]

### *Implication of $K^+$ Channels in Pregnancy-Related Pathologies*

It is well known that alterations in expression and/or function and modulation of $BK_{Ca}$ and $K_V$ channels are involved in several pathologies that present vessel dysfunction. Among the prevalent and most investigated ones are hypertension, diabetes, and atherosclerosis.[28,29] Focusing on pregnancy-associated pathologies, $K^+$ channels have been implicated in preeclampsia, gestational diabetes, and fetal growth restriction. The majority of these reports are focused on placenta,[30] fetoplacental vessels,[26] and maternal uterine vessels,[31] the knowledge of umbilical cord vessels being more scarce, regardless of the fact that their morphometric characteristics as well as blood flow parameters have been demonstrated to be altered in these pathologies.[32,33] In particular, it has been reported that the role of $K_{IR}$ and $BK_{Ca}$ channels may be altered in HUA coming from gestational diabetes mellitus and pregnancy-induced hypertension, respectively.[21] More investigation on human umbilical cord vessels is needed to complete the knowledge in this important field of human health, taking into account that in the past years there are many reports that indicate that the fetal environment could determine the presentation of adult cardiovascular diseases.[34]

## Conclusions

To the best of our knowledge, this is the first report compiling the existing information about $K^+$ channels expressed in HUASMCs. Figure 4 presents a graphical summary showing those types of $K^+$ channels for which there is any type of evidence about their expression and function in this tissue. In brief,

the functional presence of $BK_{Ca}$ and $K_V$ channel subtypes has been clearly demonstrated in this vessel. They are involved in the regulation of its contractile state, since the role of $K^+$ channels in determining membrane potential is an important cell control point to finally establish vessel diameter. Moreover, our single-channel data give additional supporting evidence for this information. Regarding the expression and function of other $K^+$ channels, $SK_{Ca}$, $K_{2P}$, and $K_{IR}$ channels appear to be present in HUASMCs, while $IK_{Ca}$ and $K_{ATP}$ channels could not be identified in this vessel, neither at the single-channel level nor by using pharmacological tools in intact tissue. Furthermore, there are no reports in the literature about the presence of 4-AP-insensitive $K_V$ channels in HUA, but our preliminary single-channel data suggest their existence. Hence, more investigation is necessary to reach conclusive evidence of the expression and/or functional role of several types of $K^+$ channels in HUA, making this an interesting open research field in human vascular physiology.

Finally, the expression and functions of these channels could be altered in pregnancy-related pathologies, so they could be relevant for the understanding of the etiology of these diseases as well as to design new pharmacological treatments aimed at modifying the activity of $K^+$ channels.

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## References

1. Spivack M. The anatomic peculiarities of the human umbilical cord and their clinical significance. *Am J Obstet Gynecol*. 1946; 52:387-401.
2. Reilly RD, Russell PT. Neurohistochemical evidence supporting an absence of adrenergic and cholinergic innervation in the human placenta and umbilical cord. *Anat Rec*. 1977;188(3):277-286.
3. Nelson MT, Quayle JM. Physiological roles and properties of potassium channels in arterial smooth muscle. *Am J Physiol*. 1995;268(4 pt 1):C799-C822.
4. Ko EA, Han J, Jung ID, Park WS. Physiological roles of K+ channels in vascular smooth muscle cells. *J Smooth Muscle Res*. 2008; 44(2):65-81.
5. Milesi V, Raingo J, Rebolledo A, Grassi de Gende AO. Potassium channels in human umbilical artery cells. *J Soc Gynecol Investig*. 2003;10(6):339-346.
6. Martin P, Enrique N, Palomo AR, Rebolledo A, Milesi V. Bupivacaine inhibits large conductance, voltage- and Ca2+- activated K+ channels in human umbilical artery smooth muscle cells. *Channels (Austin)*. 2012;6(3):174-180.
7. Hampl V, Bibova J, Stranak Z, et al. Hypoxic fetoplacental vasoconstriction in humans is mediated by potassium channel inhibition. *Am J Physiol Heart Circ Physiol*. 2002;283(6): H2440-H2449.
8. Wareing M, Bai X, Seghier F, et al. Expression and function of potassium channels in the human placental vasculature. *Am J Physiol Regul Integr Comp Physiol*. 2006;291(2):R437-R446.
9. Wareing M, Greenwood SL. Review: potassium channels in the human fetoplacental vasculature. *Placenta*. 2011;32(suppl 2): S203-S206.
10. González C, Baez-Nieto D, Valencia I, et al. K+ channels: function-structural overview. *Compr Physiol*. 2012;2(3):2087-2149.
11. Gardener MJ, Johnson IT, Burnham MP, Edwards G, Heagerty AM, Weston AH. Functional evidence of a role for two-pore domain potassium channels in rat mesenteric and pulmonary arteries. *Br J Pharmacol*. 2004;142(1):192-202.
12. Bryan RM Jr,You J, Phillips SC, et al. Evidence for two-pore domain potassium channels in rat cerebral arteries. *Am J Physiol Heart Circ Physiol*. 2006;291(2):H770-H780.
13. Marchenko SM, Sage SO. Calcium-activated potassium channels in the endothelium of intact rat aorta. *J Physiol*. 1996;492(pt 1): 53-60.
14. Hamill OP, Marty A, Neher E, Sakmann B, Sigworth FJ. Improved patch-clamp techniques for high-resolution current recording from cells and cell-free membrane patches. *Pflugers Arch*. 1981;391(2):85-100.
15. Lovren F, Triggle C. Nitric oxide and sodium nitroprusside-induced relaxation of the human umbilical artery. *Br J Pharmacol*. 2000;131(3):521-529.
16. Cairrao E, Alvarez E, Santos-Silva AJ, Verde I. Potassium channels are involved in testosterone-induced vasorelaxation of human umbilical artery. *Naunyn Schmiedebergs Arch Pharmacol*. 2008;376(5):375-383.
17. Yildiz O, Nacitarhan C, Seyrek M. Potassium channels in the vasodilating action of levosimendan on the human umbilical artery. *J Soc Gynecol Investig*. 2006;13(4):312-315.
18. Rebolledo A, Raingo J, Rinaldi G, Grassi de Gende AO, Milesi V. Cyclic GMP activates the big Ca-sensitive K channel in smooth muscle cells of human umbilical artery. *J Mol Cell Cardiol*. 2002;34(9): A3.
19. Cairrao E, Santos-Silva AJ, Verde I. PKG is involved in testosterone-induced vasorelaxation of human umbilical artery. *Eur J Pharmacol*. 2010;640(1-3):94-101.
20. McManus OB, Magleby KL. Kinetic states and modes of single large-conductance calcium-activated potassium channels in cultured rat skeletal muscle. *J Physiol*. 1988;402:79-120.
21. Radenkovic M, Radunovic N, Momcilov P, Grbovic L. Altered response of human umbilical artery to 5-HT in gestational diabetic pregnancy. *Pharmacol Rep*. 2009;61(3):520-528.
22. Guiet-Bara A, Ibrahim B, Leveteau J, Bara M. Calcium channels, potassium channels and membrane potential of smooth muscle cells of human allantochorial placental vessels. *Bioelectrochem Bioenerg*. 1999;48(2):407-413.
23. Sand A, Andersson E, Fried G. Nitric oxide donors mediate vasodilation in human placental arteries partly through a direct effect on potassium channels. *Placenta*. 2006;27(2-3):181-190.
24. Radenkovic M, Grbovic L, Radunovic N, Momcilov P. Pharmacological evaluation of bradykinin effect on human umbilical

artery in normal, hypertensive and diabetic pregnancy. *Pharmacol Rep*. 2007;59(1):64-73.
25. Bai XJ, Tian HY, Wang TZ, et al. Oleic acid inhibits the KATP channel subunit Kir6.1 and the KATP current in human umbilical artery smooth muscle cells[Published online October 30, 2012]. *Am J Med Sci*. 2012.
26. Corcoran J, Lacey H, Baker PN, Wareing M. Altered potassium channel expression in the human placental vasculature of pregnancies complicated by fetal growth restriction. *Hypertens Pregnancy*. 2008;27(1):75-86.
27. Jewsbury S, Baker PN, Wareing M. Relaxation of human placental arteries and veins by ATP-sensitive potassium channel openers. *Eur J Clin Invest*. 2007;37(1):65-72.
28. Sobey CG. Potassium channel function in vascular disease. *Arterioscler Thromb Vasc Biol*. 2001;21(1):28-38.
29. Hu XQ, Zhang L. Function and regulation of large conductance Ca(2+)-activated K+ channel in vascular smooth muscle cells. *Drug Discov Today*. 2012;17(17-18):974-987.
30. Riquelme G, de Gregorio N, Vallejos C, Berrios M, Morales B. Differential expression of potassium channels in placentas from normal and pathological pregnancies: targeting of the K(ir) 2.1 channel to lipid rafts. *J Membr Biol*. 2012;245(3): 141-150.
31. Wu YY, Singer CA, Buxton IL. Variants of stretch-activated two-pore potassium channel TREK-1 associated with preterm labor in humans. *Biol Reprod*. 2012;87(4):96.
32. Maulik D, Lysikiewicz A, Sicuranza G. Umbilical arterial Doppler sonography for fetal surveillance in pregnancies complicated by pregestational diabetes mellitus. *J Matern Neonatal Med*. 2002;12(6):417-422.
33. Di Naro E, Ghezzi F, Raio L, Franchi M, D'Addario V. Umbilical cord morphology and pregnancy outcome. *Eur J Obstet Gynecol Reprod Biol*. 2001;96(2):150-157.
34. Thompson JA, Regnault TR. In utero origins of adult insulin resistance and vascular dysfunction. *Semin Reprod Med*. 2011; 29(3):211-224.