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REVIEW

New tricks for old dogs: KCNQ expression and role in smooth muscle

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Ion channels encoded by the KCNQ gene family (K_v7.1–7.5) are major determinants of neuronal membrane potential and the cardiac action potential. This key physiological role is highlighted by the existence of a number of hereditary disorders caused by mutations to KCNQ genes. Recently, KCNQ gene expression has been identified in vascular and non-vascular smooth muscles. In addition, experiments with an array of pharmacological modulators of KCNQ channels have supported a crucial role for these channels in regulating smooth muscle contractility. This article will provide an overview of present understanding in this nascent area of KCNQ research and will offer guidance as to future directions.
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Abbreviations: $[Ca^{2+}]_i$, intracellular calcium concentration; EAG, *ether-a-go-go*; K_v, voltage-gated K^+ channels; K_v7, proteins encoded by KCNQ genes; RT-PCR, reverse transcription polymerase chain reaction; VDCCs, voltage-dependent Ca^{2+} channels

Introduction

Smooth muscle contraction drives most involuntary activity with smooth muscle cells being found in blood vessels, gastrointestinal tract, airways, reproductive organs and urinary bladder. As a consequence, deregulation of smooth muscle contraction is manifest in a number of clinical disorders including hypertension, asthma, urinary incontinence, constipation and impotence. Smooth muscle cells contract following a rise in intracellular calcium concentration ($[Ca^{2+}]_i$) that interacts with calmodulin to activate myosin light chain kinase. The subsequent phosphorylation of myosin light chain at ser19 results in cross-bridge formation and force development (Kamm and Stull, 2001; Somlyo and Somlyo, 2003). There is an array of intracellular signals that impact upon the state of contraction (Somlyo and Somlyo, 2003), but ultimately smooth muscle contraction is instigated by a rise in $[Ca^{2+}]_i$. Smooth muscle cells are rather heterogeneous with fundamental signalling pathways differing between visceral and vascular myocytes, arterial and venous cell types or even within the same organ (e.g. circular and longitudinal gastrointestinal muscles). However, in general there are three direct mechanisms that result in an increase in $[Ca^{2+}]_i$ (Figure 1) namely promotion of Ca^{2+} release from inositol trisphosphate-dependent or independent internal stores, opening of various non-selective cation channels (see Albert and Large, 2006 for overview) and opening of voltage-dependent Ca^{2+} channels (VDCCs) of which there are two major types, Ca_V1.2 (dihydropyridine-sensitive L-type) and Ca_V3.1–3.3 (T-type, See Cribbs, 2006). In most smooth muscles contractions are generated largely by the influx of Ca^{2+} through VDCCs. As the open probability of these channels is steeply dependent upon the membrane potential (see Nelson *et al.*, 1990 for discussion), then factors that regulate the resting membrane potential are key determinants of smooth muscle contraction (see Figure 1). Ultimately this is dictated by the dominant K^+ conductance, and research over the years has focussed on which K^+ channels contribute to setting the resting membrane potential or are recruited to blunt different spasmogenic events. The K^+ channel gene superfamily is extremely large (Gutman *et al.*, 2005). A combination of single-cell electrophysiology, reverse transcription polymerase chain reaction (RT-PCR) and Western blot analysis has shown that smooth muscle cells express an array of K^+ channels with the dominantly expressed K^+ channel being dependent on the tissues, species, pathophysiology and age.

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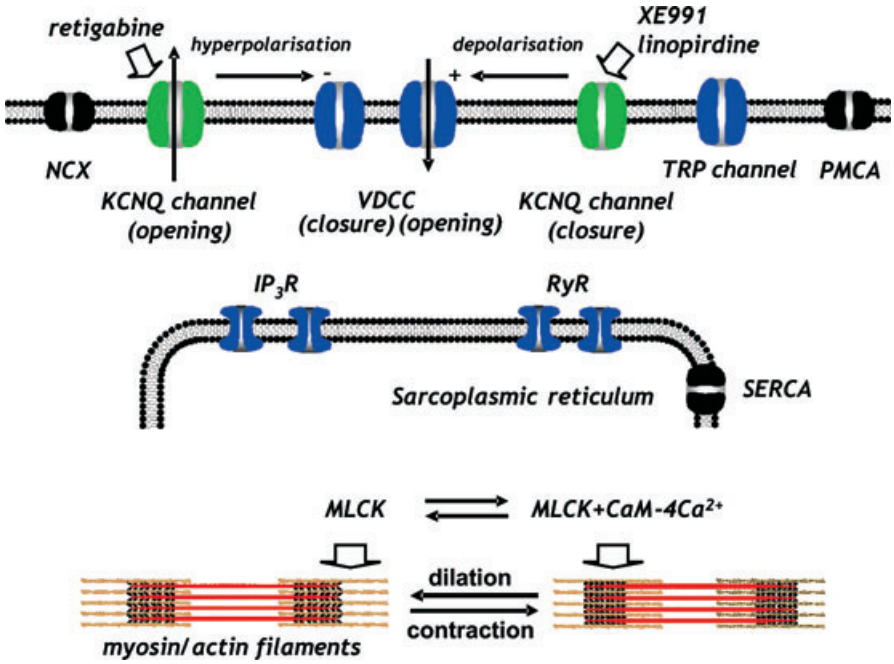

Figure 1 Schematic diagram of the physiological roles of K_v7 channel in smooth muscle cells. The major mechanism underlying a rise in $[Ca^{2+}]_i$ in smooth muscle are highlighted in blue. The K_v7 is highlighted in green. $[Ca^{2+}]_i$, intracellular calcium concentration; CaM, calmodulin; IP_3R, IP_3 receptor Ca^{2+}-releasing channel; K_v7, proteins encoded by KCNQ genes; MLCK, myosin light chain kinase; NCX, Na^+-Ca^{2+} exchanger; PMCA, plasma membrane Ca^{2+}-ATPase; RyR, ryanodine receptor Ca^{2+}-releasing channel; SERCA, sarco/endoplasmic reticulum Ca^{2+}-ATPase; SR, sarcoplasmic reticulum; TRP, transient receptor potentials; VDCC, voltage-dependent Ca^{2+} channel.

Smooth muscle cells present an array of contractile and electrical phenotypes (e.g. electrically silent vs. exhibiting spiking behaviour; low basal tone vs. myogenic). Voltage-gated K^+ (K_v) channel activity is associated with the regulation of the resting membrane potential and the control of action potential shape and frequency in smooth muscles. Much of this behaviour is determined by the properties of the K_v channels active in the myocytes in question. Consequently, K_v1 (KCNA) and K_v2 (KCNB) channels have been implicated in suppressing myogenic tension development in cerebral and other resistance arteries (Cox *et al.*, 2001; Thorneloe *et al.*, 2001; Albarwani *et al.*, 2003; Fountain *et al.*, 2004; Plane *et al.*, 2005; Amberg and Santana, 2006). K_v4 (KCND) channels underlie the transient 'A-type' currents found predominantly in spiking visceral muscles (Ohya *et al.*, 1997; Song *et al.*, 2001; Amberg *et al.*, 2003) as well as the portal vein (Yeung *et al.*, 2006). In addition, accessory β and γ subunits of K_v1 and 2 channels ($K_v\beta$ and K_v5–9) generate the diversity of K_v current kinetics in various types of smooth muscles (Patel *et al.*, 1997; Thorneloe and Nelson, 2003; Cox, 2005), and K^+ channel-interacting proteins regulate membrane trafficking and kinetics of K_v4 channels (Amberg *et al.*, 2003). Ca^{2+}-activated K^+ channels also have negative feedback effects on contractility in smooth muscles (Nelson and Quayle, 1995; Bolton and Imaizumi, 1996; Jaggar *et al.*, 2000). However, there is still considerable doubt about the molecular identity of the K^+ channels that set the resting membrane potential in smooth muscle. These channels need to be active at low membrane potentials and exhibit little inactivation to present a sustained conductance at negative potentials. A number of possible candidates have been identified, but it is not known whether the channels responsible for the leak conductance are a unified species or not.

KCNQ gene expression

Five KCNQ genes-encoding K_v7 proteins have been identified in mammalian cells (Jentsch, 2000), and the expression profile of these genes has been determined mainly in neurons and cardiac tissues. KCNQ1-encoded proteins ($K_v7.1$) contribute to the slow component of delayed rectifier K^+ currents, I_{Ks} in cardiac muscles (Sanguinetti *et al.*, 1996). $K_v7.2$ and $K_v7.3$ contribute to M-currents in neuronal cells (Brown and Adams, 1980; Wang *et al.*, 1998; Selyanko *et al.*, 1999), and the defects in their function caused by mutations are manifest as idiopathic epilepsies or peripheral nerve hyperexcitability (Cooper *et al.*, 2000; Maljevic *et al.*, 2008). KCNQ4 expression is considered to be restricted to the inner ear and auditory nerves (however, see following), and defects of KCNQ4 underlie some progressive deafness (Kharkovets *et al.*, 2000). KCNQ5 is broadly expressed in nerves and can also contribute to M-channels (Schroeder *et al.*, 2000a). To date no hereditary disorder has been correlated with a mutation in this gene. KCNQ channel properties are influenced by a number of factors including heterotetramer formation and $PtdInd(4,5)P_2$ hydrolysis (Delmas and Brown, 2005; Haitin and Attali, 2008). In addition, auxiliary subunits encoded by five KCNE (KCNE1–5) genes modulate the expression and biophysical or pharmacological properties of KCNQ channels especially KCNQ1 (Abbott and Goldstein, 2001; Grunnet *et al.*, 2002; McCrossan and Abbott, 2004).

KCNQ gene expression in vascular smooth muscle

Evidence for KCNQ gene expression in smooth muscle cells is a relatively new area of research. Consequently, there are few cases where KCNQ expression has been demonstrated definitively. KCNQ expression in a vascular smooth muscle was first identified by Ohya *et al.* (2003) in the mouse portal vein. In this tissue KCNQ1 message was by far the most abundant, being comparable to KCNQ1 in the heart, with the translated protein located predominantly in the cell membrane. More recently, single-cell RT-PCR and immunocytochemistry have shown that message for KCNQ4 and KCNQ5 are also detectable in mouse portal vein and the expression products locate to the cell membrane (Yeung *et al.*, 2008a,b). Yeung *et al.* (2007) examined the KCNQ gene expression in several arterial smooth muscles: thoracic aorta, carotid artery and femoral artery of mice. In these vessels the most abundant transcripts in all vessels examined were KCNQ1 and KCNQ4 with message for KCNQ5 also readily detectable, and immunocytochemistry confirmed the presence of K_v7.1, 7.4 and 7.5 in the cell membrane of aortic myocytes. This expression profile was remarkably consistent in all arterial preparations studied. In contrast, the expression of KCNE genes appeared to be much more vessel-specific (Ohya *et al.*, 2002b; Yeung *et al.*, 2007). For instance KCNE4 message was considerably more abundant than any other KCNE in the murine aorta, whereas KCNE3 and KCNE5 dominated in the mouse portal vein and femoral artery respectively (Ohya *et al.*, 2002b; Yeung *et al.*, 2007).

Brueggemann *et al.* (2007) also showed that adult rat aorta expressed KCNQ1, KCNQ4 and KCNQ5 and that A7r5 cells, derived from embryonic rat aorta, only expressed KCNQ5. Moreover, RNA interference targeted to KCNQ5 significantly decreased K_v currents in A7r5 cells (Brueggemann *et al.*, 2007). They also suggested that blockade of KCNQ5 channels may underlie the physiological constrictor effects of vasopressin (Brueggemann *et al.*, 2007). More recently, this group observed that rat mesenteric artery smooth muscle cells express KCNQ1, KCNQ4 and KCNQ5 transcripts (Mackie *et al.*, 2008) corroborating the findings in murine mesenteric artery (Yeung *et al.*, 2007).

Recently, Yeung *et al.* (2008a) reported that the KCNQ5 expressed by vascular smooth muscle was a shorter spliced variant, missing exon 9, which led to a 9 amino acid truncation. This KCNQ5 splice variant was also present in a number of visceral tissues. Alternative splicing is a common mechanism for generating K^+ channel diversity. In KCNQ2, over 10 different splice variants have been reported (Nakamura *et al.*, 1998), and the longer variants contribute to neuronal M-currents, whereas shorter variants function as dominant negatives (Pan *et al.*, 2001; Smith *et al.*, 2001). In a Xenopus oocyte expression system, KCNQ5-Δexon9 showed identical electrophysiological and pharmacological properties to the full-length KCNQ5 normally expressed in neurons (Yeung *et al.*, 2008a). Blood vessels also express a truncated form of KCNQ1, identified initially in murine portal vein by Ohya *et al.* (2003). This spliced variant, termed KCNQ1b, has a considerably shorter C-terminal domain due to missing exons 12–15. KCNQ1b was significantly expressed in portal vein at same levels as full-length KCNQ1 but was absent in the heart (Ohya *et al.*, 2003). The C-terminal of K_v7.1, like all K_v7 channels, is crucial for channel assembly and membrane trafficking (Haitin and Attali, 2008; Wiener *et al.*, 2008). Consequently, the KCNQ1b may constitute a dominant negative variant. Although the function of these truncations still needs to be defined, the existence of splice variants of KCNQ1 and KCNQ5 in vascular smooth muscle suggests that KCNQ transcription is tailored to the demands of the smooth muscle cell. Overall, the KCNQ expression profile in vascular smooth cells is very consistent. Message for KCNQ1 and KCNQ4 appear the most abundant with KCNQ5 also being present. However, KCNE expression is highly tissue-specific. This raises the possibility that alterations in the expression of auxiliary subunits rather than the K_v7 proteins may underlie some aspects of smooth muscle diversity.

Functional role of KCNQ channels in smooth muscle

The working hypothesis for investigating the role of KCNQ-encoded K^+ channels (K_v7) in regulating smooth muscle was described in the *Introduction* (see Figure 1). The assumption is that, like neurons, K_v7 channels are open at rest and the K^+ efflux through these channels contributes to the formation of the resting membrane potential. Blockade of these channels will lead to reduced hyperpolarization and an increase in the open probability of VDCCs (Figure 1). If the influx of Ca^{2+} through VDCCs is sufficient to overpower the inherent homeostatic mechanisms (i.e. Ca-ATPases, Na/Ca exchangers), then a contraction or enhanced response to a low concentration of a vasoconstrictor will occur (see Figure 1). Pharmacological dissection of a functional role relies on good probes, ideally with high selectivity for a certain target *ie* ion channel. For K_v7 channels the most common research tools are XE991 (10,10-bis(4-pyridinylmethyl)-9(10H)-anthracenone), which inhibits K_v7.1–7.4 with IC_{50} values ~1–5 $\mu mol \cdot L^{-1}$ (see Yeung *et al.*, 2007) and K_v7.5 with an IC_{50} ~60 $\mu mol \cdot L^{-1}$ (Jensen *et al.*, 2005; Yeung *et al.*, (2008a) or the less potent linopirdine (3,3-bis(4-pyridinylmethyl)-1-phenylindolin-2-one, DUP996; Dupuis *et al.*, 2002). Any pharmacological experiment is undertaken with a consideration of the necessary caveats *ie* selectivity. To date XE991 has been shown to have no effect on K_v1.2, K_v4.3 or *ether-a-go-go* (EAG)-encoded channels at concentrations below 100 $\mu mol \cdot L^{-1}$ (Wang *et al.*, 2000), reduces K_v2.1 channels by only 20% at 100 $\mu mol \cdot L^{-1}$ (Wladyka and Kunze, 2006) and inhibits heterologously expressed ERG channels with an IC_{50} of 107 $\mu mol \cdot L^{-1}$ (Elmedyb *et al.*, 2007). Consequently, at concentrations that inhibit K_v7 channels XE991 appears to be selective. However, it is possible that XE991 may have an, as yet unreported, effect on other ion channels in the micromolar range where K_v7 block occurs. There are also other compounds, such as chromanol 293B, HMR 1556 or L-768-673, which preferentially block K_v7.1 containing channels (see Seebohm *et al.*, 2003; Bett *et al.*, 2006; Dong *et al.*, 2006; Lerche *et al.*, 2007). In addition to K_v7 channel blockers there are a few compounds, namely retigabine, flupirtine and the acrylamide S-1 that enhance K_v7 channel activity (see Miceli *et al.*, 2008 for overview). These agents provide an alternative mechanism to probe for a functional role for K_v7 channels. Moreover, because they only activate the so-called neuronal K_v7 channels (encoded by KCNQ2–5, Schenzer *et al.*, 2005;

Wuttke *et al.*, 2005) they provide a tool to discriminate $K_v7.1$-mediated function from that generated by $K_v7.2$–7.5 channels. In fact, the acrylamide (S)-1 appears to have greater efficacy activating $K_v7.4$ or 7.5 rather than $K_v7.2$ or 7.3 (Bentzen *et al.*, 2006).

Electrophysiology. The electrophysiological effects of XE991 and linopirdine have been assessed in studies on myocytes from mouse portal vein (Ohya *et al.*, 2003; Yeung and Greenwood, 2005), rat mesenteric artery (Mackie *et al.*, 2008) and A7r5-cultured aorta cells (Brueggemann *et al.*, 2007). In the mouse portal vein depolarizing voltage steps evoked a time-dependent current superimposed on a linear, time-independent current. Application of 5 mmol·L^{-1} 4-aminopyridine effectively blocked the time-dependent current but had minimal effect on the linear current. In comparison, XE991 (1–30 μmol·L^{-1}) was a more effective inhibitor of the linear current rather than the time-dependent component. The XE991-sensitive current was well sustained, showing no decrease in current amplitude during a 45 s depolarizing pulse (Yeung and Greenwood, 2005). XE991 (30 μmol·L^{-1}) also inhibited completely the augmented holding current at –60 mV produced by bathing the cells in an external solution containing 36 mmol·L^{-1} KCl (Yeung and Greenwood, 2005). Mackie *et al.* (2008) employed a different protocol where they held at –4 mV to allow inactivation of the majority of K_v channels and calculated current activation in the absence and presence of linopirdine. Using this protocol these authors calculated that the K_v7 channels in rat mesenteric arteries were 50% activated at –34 mV. These findings are consistent with a role for K_v7 channels in setting the resting membrane potential. This was confirmed by the observation that blockade of K_v7 channels by either XE991 or linopirdine produced a marked depolarization in murine portal vein (Yeung and Greenwood, 2005), rat mesenteric artery (Mackie *et al.*, 2008) and A7r5-cultured myocytes (Brueggemann *et al.*, 2007). The vascular effects of K_v7 channel enhancers have only been reported in murine portal vein myocytes, rat mesenteric arteries and A7r5 cells (Brueggemann *et al.*, 2007; Mackie *et al.*, 2008; Yeung *et al.*, 2008b). In mouse portal vein myocytes retigabine and flupirtine augmented currents at potentials between –60 and 0 mV, suppressed spontaneous membrane depolarizations and elicited a membrane hyperpolarization of ~12 mV (Yeung *et al.*, 2008b). However, at more depolarized potentials the K_v7 activators inhibited the voltage-dependent K^+ currents. The inhibitory and stimulatory effects of retigabine were abrogated by prior application with XE991 consistent with retigabine having a bi-modal effect on vascular K_v7 currents. In A7r5 cells flupirtine (10 μmol·L^{-1}) augmented K^+ currents markedly and reversed completely the membrane depolarization produced by 25 pmol·L^{-1} vasopressin (Brueggemann *et al.*, 2007). Taken together, it is clear that KCNQ currents are essential components of delayed rectifier K^+ currents in vascular smooth muscle cells. Further studies with gene-silencing technology might help to confirm major contributors to vessel KCNQ channels: KCNQ1a/b, KCNQ4 and/or KCNQ5. Dysfunction of KCNQ channels might be associated with development of vascular disorders such as hypertension as reported for other K_v channels.

K_v7 channels and contractility. Figure 1 describes the working model underlying the functional impact of K_v7 channels in smooth muscle cells. Consequently, blockade of these channels should generate contraction of quiescent vessels and increased contractile activity in spontaneously active tissues. Conversely, enhancement of K_v7 channel activity should lead to membrane hyperpolarization with a concomitant reduction of the contractile response. An effect of K_v7 channel blockers on smooth muscle contraction was observed initially in whole mouse portal veins (Yeung and Greenwood, 2005). This blood vessel generates spontaneous, phasic contractile activity manifest as bursts of contractions ~5 s apart (see Yeung and Greenwood, 2005). XE991 (10 μmol·L^{-1}) and linopirdine increased the frequency and amplitude of contractions considerably, an effect also seen in the presence of 4-AP at a concentration that would block most non-KCNQ Kv channels, as well as glibenclamide and paxilline to block ATP-sensitive and Ca-activated K^+ channels (Yeung and Greenwood, 2005). Interestingly, chromanol 293B at a concentration that would block $K_v7.1$ channels (see Bett *et al.*, 2006) had little effect on portal vein contractions. This initial work was followed by a study by Joshi *et al.* (2006) showing that XE991 and linopirdine were effective spasmogens of rat pulmonary artery segments but not mesenteric arteries. The contractions of pulmonary artery were completely reversed by reducing the entry of Ca^{2+} through voltage-dependent channels either directly, with a dihydropyridine, or indirectly by hyperpolarizing the membrane potential with the ATP-sensitive K^+ channel opener cromakalim. Subsequently, Yeung *et al.* (2007) performed an exhaustive analysis of the functional effect of K_v7 channel blockers in a variety of murine arterial preparations. They reported that XE991 and linopirdine contracted segments of murine aorta (thoracic and abdominal), carotid artery and femoral artery. Contraction of mesenteric artery was also observed especially if a small degree of pre-tone was supplied by a very low concentration of phenylepherine. Mackie *et al.* (2008) also saw a reduction of rat mesenteric artery diameter using perfusion myography. The variable effect of XE991 on the mesenteric artery seen by Joshi *et al.* (2006), Yeung *et al.* (2007) and Mackie *et al.* (2008) likely reflects the need for a depolarizing drive to generate contraction that is supplied either by a low concentration of vasoconstrictor or by perfusion pressure. Interestingly, Mackie *et al.* (2008) showed that linopirdine increased the mean arterial pressure and mesenteric vascular resistance *in vivo*. Mackie *et al.* (2008) also postulated that the contraction of mesenteric arteries evoked by a low concentration of vasopressin was mediated by an inhibition of K_v7 channels, reminiscent of the effect of muscarinic agonists on neuronal activity (e.g. Brown and Adams, 1980). However, Brueggemann *et al.* (2007) showed that K_v7 activator flupirtine was able to reverse the membrane depolarization produced by vasopressin, which would not be expected if the vasoconstrictor had blocked the K_v7 channels (see Yeung *et al.*, 2008b). Moreover, it is worth considering that in many vessels the constrictor effect of XE991 is augmented by a low concentration of vasoconstrictor such as phenylepherine, 5-HT or angiotensin II (Yeung *et al.*, 2007). Future experiments will explore this interesting mechanism further.

While the pan-K_v7 channel blockers, XE991 and linopirdine contract blood vessels no $K_v7.1$-selective blocker (i.e. chromanol 293B, HMR 1556 or L-768-673) has been shown to be spasmogenic (see Yeung *et al.*, 2007). This suggests that while KCNQ1 is expressed in all blood vessels the expression product has little impact on the resting membrane potential in these cells. This proposal is supported by the ability of retigabine to relax precontracted murine aortae, carotid artery, femoral artery and mesenteric artery (Yeung *et al.*, 2007). This agent does not affect $K_v7.1$ channels but augments the activity of $K_v7.2$–7.5 by shifting the voltage-dependence of activation to more negative potentials through an interaction with a key tryptophan residue in the S5 transmembrane domain (Schenzer *et al.*, 2005; Wuttke *et al.*, 2005). Retigabine relaxed aortic segments precontracted by phenylepherine or 4-aminopyridine to the same extent as nicardipine but had no effect on contractions evoked by blockade of K_v7 channels by XE991. Moreover, the vasorelaxant effects of retigabine were reversed by application of XE991 but not 4-AP. These data are consistent with retigabine working solely on K_v7 channels. Similar effects were observed with flupirtine as well as meclofenamic acid, a cyclooxygenase inhibitor that also activates K_v7 channels (Peretz *et al.*, 2005). Flupirtine also lowers mean arterial pressure in rats *in vivo* (Mackie *et al.*, 2008). These findings consolidate the view that K_v7 channels other than $K_v7.1$ are major players in defining vascular reactivity. It also lends credence to the proposal that calling these KCNQ genes 'neuronal' may need to be refined.

KCNQ expression and function in non-vascular smooth muscle. Early electrophysiological studies showed that acetylcholine increased the excitability of toad and guinea-pig stomach partially through suppression of a resting K^+ conductance (e.g. Sims *et al.*, 1985; Lammel *et al.*, 1991), similar to the situation in central neurons (Brown and Adams, 1980). Logically if the M-channels in neurons are encoded by KCNQ genes then similar resting K^+ conductances in gastric smooth muscle would have a similar molecular identity. However, in contrast to the research on KCNQ genes in vascular smooth muscle there is a dearth of data in non-vascular cell types. A number of groups have shown KCNQ1/KCNE3 expression in the epithelial cells of the gastrointestinal tract (Schroeder *et al.*, 2000b; Dedek and Waldegger, 2001), but none investigated expression beyond these cells in the smooth muscle layers. However, Ohya *et al.* (2002a) showed that KCNQ1, KCNQ3, KCNE1 and KCNE2 transcripts were expressed in rat gastric antral smooth muscle layers. Due to the limitation of rat KCNQ and KCNE homologues in the DNA database at the time, this study only examined KCNQ1–3 and KCNE1–2 expression, however recently message for KCNQ4, KCNQ5 and KCNE4 were detected in murine proximal and distal colon (Jepps *et al.*, 2007). Message for the truncated KCNQ5 was also detected in murine oesophagus, colon and ileum (Yeung *et al.*, 2008a). In contrast to Ohya *et al.* (2002a) where no functional experiments were performed, Jepps *et al.* (2007) showed that XE991 increased the spontaneous contractions of the murine colon. More recently, KCNQ and KCNE expression was detected in murine myometrial smooth muscles throughout the oestrus cycles (McCallum *et al.*, 2008). Real-time PCR and Western blot analyses showed the most readily detected message throughout the oestrus cycle was for KCNQ1, KCNQ5 and KCNE4. However, there was no indication about whether the KCNQ5 expressed was the shorter variant described in vascular smooth muscle (Yeung *et al.*, 2008a). The level of expression for all genes was remarkably consistent over the oestrus cycle except for KCNQ1, KCNQ5 and KCNE1, which were up-regulated in metestrous. XE991 augmented and retigabine decreased the spontaneous contractility at all parts of the oestrus cycle. Again, chromanol 293B had no effect on contractility, even at metestrous when KCNQ1 and KCNE1, whose expression products co-associate to produce a channel with a higher sensitivity to chromanol, were raised. These data provided new insight into the control of uterine activity and presented a possible new therapeutic target for disorders due to uterine dysfunction (e.g. preterm labour).

Several patents granted to American Home Products, Wyeth and ICAgen describe KCNQ activators for the use of disorders in urinary bladder such as overactive bladder, bladder incontinence, bladder spasms and bladder outflow obstruction. KCNQ channels expression has been detected in the bladder, with KCNQ4, KCNQ5 and KCNE4 predominant in murine urinary bladder (S. Ohya, unpubl. obs.). Moreover, retigabine completely inhibited acetic acid-induced micturition, decreased baseline and maximal bladder pressures and increased voided and infused volumes (Streng *et al.*, 2004; Wickenden *et al.*, 2004; Argentirei and Butera, 2006). In guinea-pig bladder detrusor strips XE991 and linopirdine increased and flupirtine decreased spontaneous contractility (Carson and McCloskey, 2007). Interestingly, the same group observed XE991-sensitive currents in the interstitial cells that coexist with the smooth muscle cells and influence their activity (Anderson and McCloskey, 2007). This raises the intriguing possibility that KCNQ-encoded channels located in these pacemaker cells as well as the smooth muscle cells may regulate smooth muscle function.

Cell-cycle regulation and post-translational modification of KCNQ channels

A KCNQ/KCNE complex has recently been shown to be involved in cell proliferation and differentiation in skeletal muscle and germ cells (Tsevi *et al.*, 2005; Roura-Ferrer *et al.*, 2008). As K_v channel dysfunction underlies not only vasoconstriction but also cell proliferation (Burg *et al.*, 2008), then aberrant KCNQ/KCNE activity may also underlie vascular smooth muscle cell phenotypic modulation in atherosclerosis and re-stenosis.

Recently, it has been reported that the ubiquitin ligase Nedd4-2 is involved in the mechanisms underlying the activity and cell surface expression of K_v7 channels, and the Nedd4-2 activity is regulated by serum- and glucocorticoid-regulated kinase-1 (SGK-1) (Ekberg *et al.*, 2007; Jespersen *et al.*, 2007; Schuetz *et al.*, 2008). BelAiba *et al.* (2006) have suggested that the SGK-1-signalling pathway might play a critical role in vascular remodelling in pulmonary artery. The roles of Nedd4-2 in smooth muscles remain to be determined;

however, a signalling pathway involving proteaseome-ubiquitin cascades may be involved in KCNQ channel regulation under physiological and pathophysiological conditions in these cells.

Conclusion

K_v7 channels are the new players in regulating smooth muscle contractility. The reports so far have shown that blood vessels express predominantly KCNQ1, KCNQ4 and KCNQ5 with the latter existing as a truncated variant. An array of pharmacological approaches has revealed an important role for K_v7 channels in the vasculature. The situation in visceral smooth muscle is less well defined, but there are a few studies showing a similar functional impact. However, little is known beyond these findings. For instance, K_v7 channels in neurons are regulated by local changes in PIP_2 concentration (Delmas and Brown, 2005; Brown and Passmore, 2009) and by calmodulin, but nothing is known about the influence of these regulators on smooth muscle K_v7 channel activity. Finally, $K_v7.1$ seems to have a rather redundant role in smooth muscles. So why is KCNQ1 expression in smooth muscles as high as in cardiac myocytes (Ohya *et al.*, 2003; Yeung *et al.*, 2007) and the protein present in the cell membrane? $K_v7.1$ does not form heteromultimers with any other K_v7 protein so the function of $K_v7.1$ remains a mystery. There are a number of hereditary disorders due to mutations in KCNQ genes, so are any smooth muscle dysfunctions associated with these diseases? In general there appears to be no report of smooth muscle disturbance in these cases, but this does not mean the functional role of KCNQ expression products in smooth muscle should be dismissed. First, of all the common hereditary mutations are to KCNQ1, which appears to have little functional role in smooth muscles, or KCNQ2/3 that are not present in myocytes. Second, most clinicians focus on the disease in hand and not other manifestations so that smooth muscle dysfunctions may be missed. For instance, a KCNQ4 knockout mouse has been created (Kharkovets *et al.*, 2006) that is deaf but there is no comment about the blood pressure in these animals. Finally, mutations in KCNQ genes whose expression products suppress vascular reactivity may not lead to hypertension per se but may provide a predisposition for blood vessels to contract exaggeratedly in response to normal physiological spasmogens. The constantly disproportionate response to vasoconstrictors may then lead to idiopathic hypertension.

The identification of KCNQ expression in smooth muscle cells and the establishment of a role for K_v7 channels in smooth muscle activity is a new development in KCNQ research. It will be interesting to see whether these channels hold any other surprises.

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Conflict of interest

Neither author has any conflict of interests.

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