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REVIEW

One man's side effect is another man's therapeutic opportunity: targeting Kv7 channels in smooth muscle disorders

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Retigabine is a first in class anticonvulsant that has recently undergone clinical trials to test its efficacy in epileptic patients. Retigabine's novel mechanism of action – activating Kv7 channels – suppresses neuronal activity to prevent seizure generation by hyperpolarizing the membrane potential and suppressing depolarizing surges. However, Kv7 channels are not expressed exclusively in neurones and data generated over the last decade have shown that Kv7 channels play a key role in various smooth muscle systems of the body. This review discusses the potential of targeting Kv7 channels in the smooth muscle to treat diseases such as hypertension, bladder instability, constipation and preterm labour.

Abbreviations
ADRF, adipocyte-derived releasing factor; AVP, arginine vasopressin; Kv, voltage-gated potassium channel

KCNQ channels – the traditional view

Potassium (K^+) channels regulate the resting membrane potential in various cell types throughout the body. The voltage-gated potassium channels (Kv) constitute the largest and most diverse family among human K^+ channels. Kv channels are formed by homomeric or heteromeric assembly of four α-subunits that encircle a central pore. Each α-subunit consists of six transmembrane domains (S1–S6) with a single pore loop found between S5 and S6, and a voltage sensor in the S4 helix. The Kv superfamily consists of 12 subfamilies (Kv1–Kv12; see Gutman *et al.*, 2005 and Alexander *et al.*, 2011 for a general overview of the current nomenclature) within which five members of the Kv7 family have been identified (Kv7.1–Kv7.5), encoded by the genes *KCNQ*1 to *KCNQ*5. Expression of the different *KCNQ* gene transcripts varies between mammalian cell types although the majority of research into these channels has focused on their expression in the heart and brain.

KCNQ-encoded channels can associate with small, single transmembrane auxiliary proteins encoded by the *KCNE* genes. In the heart, *KCNQ*1 expression predominates and, co-assembled with KCNE1, it is responsible for the delayed-rectifier current that contributes to the late repolarization of the cardiac action potential (Barhanin *et al.*, 1996; Sanguinetti *et al.*, 1996). In epithelia, Kv7.1 associates with the KCNE3 expression product to generate a constitutively active, voltage-independent channel crucial for fluid secretion/accumulation (Schroeder *et al.*, 2000a).

In neuronal tissue, *KCNQ*2, *KCNQ*3 and *KCNQ*5 predominate and heteromultimeric combinations of Kv7.2/7.3 and Kv7.3/7.5 have been revealed as the molecular correlates of the 'M-channel' (Brown and Adams, 1980; Wang *et al.*, 1998, Selyanko *et al.*, 1999). The M-current is the slowly activating

and non-inactivating potassium current, identified initially in bullfrog sympathetic neurones, which maintains a sufficiently negative resting membrane potential to prevent continuous excitations of the neurones (Brown and Adams, 1980). The name 'M-channel' was derived from its first observation in 1980 when a muscarinic acetylcholine receptor agonist (muscarine) inhibited a voltage-sensitive K^+ current. The muscarinic acetylcholine receptors are coupled to Gq proteins, and it is now known that activation of other Gq protein-coupled receptors will also inhibit the 'M-channel' by depleting the levels of phosphatidylinositol-4,5-bisphosphate (PIP_2) in the membrane. PIP_2 is required for the Kv7 channels to enter the open state, therefore depletion of PIP_2 by phospholipase C hydrolysis results in channel closure (Suh *et al.*, 2006). Heteromeric Kv7.2/7.3 channels are viewed as constituting the major M-current but Kv7.5, the last of the Kv subfamily to be identified (Lerche *et al.*, 2000; Schroeder *et al.*, 2000b), has also been implicated as a molecular correlate of the M-current by forming heteromultimers with Kv7.3 (Schroeder *et al.*, 2000b; Shah *et al.*, 2002). Kv7.4 channels are expressed on the basal membrane of the outer hair cells of the inner ear and have an intrinsic role in the control of their electrical properties (Kharkovets *et al.*, 2000; Søgaard *et al.*, 2001).

Several inheritable disorders are due to loss-of-function mutations within the *KCNQ* gene family highlighting the physiological importance of the expression products in the regulation of membrane excitability. In the heart, approximately 20% of inheritable long QT arrhythmias are caused by mutations to Kv7.1 (*KCNQ*1) (Wang *et al.*, 1996). Defects in Kv7.2 and Kv7.3 are responsible for benign familial neonatal convulsions, which are an autosomal dominant epilepsy of the newborn characterized by the occurrence of focal, multifocal or generalized tonic-clonic convulsions starting soon after birth and spontaneously disappearing after a few weeks or months (Biervert *et al.*, 1998). Mutations in *KCNQ*4 gene expression have been implicated in the pathophysiology of autosomal dominant deafness (DFNA2) (Kubisch *et al.*, 1999; Beisel *et al.*, 2000).

Retigabine: a first-generation novel anticonvulsant

Epilepsy is a serious, life-threatening disease and one of the most common chronic neurological disorders. The mechanism of epilepsy and seizure generation differs between individuals, and despite the fact that there are already many approved drugs to treat epilepsy, up to a third of epileptic patients cannot fully control their seizures with the currently available medications. Consequently, treatments that target novel antiepileptic molecular mechanisms are needed to help control seizure generation in these patients.

Retigabine was developed in the 1980s as an analogue of flupirtine, a non-opioid analgesic that had demonstrated some anticonvulsant activity in the Antiepileptic Drug Development program supported by the U.S. National Institute of Health (Kupferberg, 1989). Initial studies revealed retigabine to be a potent anticonvulsant in a broad range of epilepsy and seizure models (Dailey *et al.*, 1995; Rostock *et al.*, 1996; Tober *et al.*, 1996); however, the mechanism of action was unknown. Several studies tried to pinpoint retigabine's mechanism of action by studying its effects on established antiepileptic targets such as GABA receptors, sodium channels and calcium channels. Apart from some enhancement of $GABA_A$ receptor activity at high concentrations, retigabine did not conform to a known anticonvulsant mechanism of action. However, in 1997 retigabine was shown to augment a potassium current in NG108-15 neuronal cells, primary cultures of mouse cortical neurones and in differentiated hNT cells, a cell line derived from human neuronal cells (Rundfeldt, 1997). Subsequently, retigabine was found to specifically open *KCNQ*-encoded Kv, and more specifically the channels underling the 'M-current' (Wang *et al.*, 1998) – KCNQ2 and KCNQ3 (Main *et al.*, 2000; Rundfeldt and Netzer, 2000; Wickenden *et al.*, 2000; Tatulian *et al.*, 2001). Electrophysiological experiments performed on *Xenopus* oocytes (Main *et al.*, 2000) and CHO cells (Rundfeldt and Netzer, 2000; Wickenden *et al.*, 2000) overexpressing the KCNQ2/3 heteromultimer showed that retigabine shifted the voltage dependence of channel activation to a more hyperpolarized membrane potential, increased the rate of channel activation, and slowed channel deactivation. These results identified the mechanism by which retigabine reduced neuronal excitability in the animal seizure model studies (Dailey *et al.*, 1995; Rostock *et al.*, 1996; Tober *et al.*, 1996). Retigabine's binding site has since been identified as a hydrophobic pocket containing a tryptophan residue (Trp-236 in Kv7.2; Trp-265 in Kv7.3) in the cytoplasmic part of S5 and S6 to stabilize the channel in the open state (Schenzer *et al.*, 2005; Wuttke *et al.*, 2005). Additionally, Leu-272 in S5, Leu-314 within the inner pore loop, and Leu-338 in S6 of the neighbouring subunit are of importance for the binding of retigabine (Lange *et al.*, 2009). These four amino acids are not found in Kv7.1, thus explaining why this subtype is insensitive to retigabine-induced enhancement (Wuttke *et al.*, 2005; Lange *et al.*, 2009). Retigabine is now widely used to study Kv7 channel activity and as such we have a better understanding of neuronal KCNQ activity and the pathway by which retigabine exerts its novel anticonvulsant effects.

Retigabine's mechanism of action is different from all other currently approved antiepileptic treatments, making it a first in class anticonvulsant. In the last 3 years, two large-scale phase III clinical trials with retigabine have been completed in patients with partial epilepsy [RESTORE 1 (Study 301; French *et al.*, 2011; retigabine titrated over 6 weeks to a dosage of 1200 mg day^{-1}) and RESTORE 2 (Study 302; Brodie *et al.*, 2010; retigabine titrated over 2–4 weeks to a dosage of 600 or 900 mg day^{-1})], in which the drug had a dose-dependent anticonvulsant efficacy. Both trials revealed retigabine produced significant reductions in seizure frequency versus the placebo control patients. Much excitement surrounds retigabine as a novel antiepileptic drug, highlighted by several reviews published in the last year alone (Fattore and Perucca, 2011; Gunthorpe *et al.*, 2012; Harden, 2012; Large *et al.*, 2012; Rejdak *et al.*, 2012); however, in this article we will discuss KCNQ channels as a therapeutic target of treatment of non-neuronal, smooth muscle diseases.

Kv7 activators: improving subtype specificity

Following the success of retigabine as an anticonvulsant there has been widespread interest in the development of drugs tailored to specifically target Kv7 channels. Retigabine (N-(2-amino-4-[fluorobenzylamino]-phenyl) carbamic acid) and flupirtine have been the predominant enhancers used to study Kv7 channels, however, several other Kv7-specific activators are now available including S-1 ((S)-N-[1-(3-morpholin-4-yl-phenyl)-ethyl]-3-phenyl-acrylamide), BMS-204352 ((3S)-(+)-(5-chloro-2-methoxyphenyl)-1, 3-dihydro-3-fluoro-6-(trifluoromethyl)-2H-indol-2-one) and ICA-27243 (*N*-(6-chloropyridin-3-yl)-3,4-difluorobenzamide). The same tryptophan residue essential for retigabine binding has been shown to be critical for S-1 and BMS-204352, although these drugs have a greater efficacy activating Kv7.4 and Kv7.5 rather than Kv7.2 or Kv7.3 (Bentzen *et al.*, 2006). ICA-27243 is more potent at activating heteromeric Kv7.2/3 channels (IC_{50} of 0.4 μM) than homomeric Kv7.4 channels (IC_{50} of 9.7 μM) and heteromeric Kv7.3/5 channels (IC_{50} of >10 μM; Wickenden *et al.*, 2008; Blom *et al.*, 2010). The activity of ICA-27243 does not map to the S5–S6 domain, rather the selective activity of ICA-27243 is determined by a novel site within the S1–S4 voltage-sensor domain of the Kv7 channels (Padilla *et al.*, 2009). The subtype-selective activity of ICA-27243 has been proposed to arise from high degree of sequence diversity within the Kv7 channel family at the ICA-27243 binding site. In the treatment of epilepsy, Kv7 activators that are more selective for Kv7.2/7.3 channels could offer a significant advantage over the modulators that enhance Kv7.2–7.5. ICA-27243 has demonstrated a promising antiepileptic activity in a broad range of rodent seizure models (Roeloffs *et al.*, 2008), and future work should continue to develop these more subtype specific Kv7 activators in order to determine their efficacy in humans.

Most of the attention in developing Kv7 activators has focused on the therapeutic effects in neurological disorders. However, the data that have emerged over the last 10 years suggest that targeting these channels may have therapeutic benefits in human diseases of the cardiovascular, urogenital, digestive and respiratory systems.

Kv7 channels in smooth muscle

The Kv7 potassium channels were first identified in the heart and then in the brain, which has given rise to the terms 'cardiac Kv7 channel' (Kv7.1) and 'neuronal Kv7 channels' (Kv7.2–7.5). Although the importance of these channels in both the heart and the brain cannot be overstated, in the last decade Kv7 channels have also been established as important regulators of contractility in vascular and non-vascular smooth muscle (see Greenwood and Ohya, 2009). *KCNQ* transcript and protein expression have been identified in an array of vascular and non-vascular smooth muscle where Kv7.1, 7.4 and 7.5 appear to predominate and Kv7.2 appears non-existent (see Soldovieri *et al.*, 2011).

Kv7 channels regulate vascular tone

In the vasculature, pharmacological modulation of these channels provokes profound changes in smooth muscle membrane potential and consequently vascular tone. Several studies have shown that the non-selective Kv7.1–Kv7.5 channel blockers, linopirdine and XE991, produce membrane depolarization, and concomitant vasoconstriction by enhancing calcium influx through voltage-dependent calcium channels (Ohya *et al.*, 2003; Yeung and Greenwood, 2005; Joshi *et al.*, 2006; 2009; Yeung *et al.*, 2007; 2008; Mackie *et al.*, 2008; Zhong *et al.*, 2010; Jepps *et al.*, 2011; Ng *et al.*, 2011; Chadha *et al.*, 2012a). Conversely, Kv7 activators such as retigabine and S-1 hyperpolarize the membrane potential and by limiting voltage-dependent Ca^{2+} influx relax precontracted rodent or human arteries (Yeung *et al.*, 2007; 2008; Mackie *et al.*, 2008; Joshi *et al.*, 2009; Zhong *et al.*, 2010; Jepps *et al.*, 2011; Ng *et al.*, 2011; Chadha *et al.*, 2012a). Retigabine has displayed vasorelaxant effects in a variety of blood vessels including the mouse thoracic aorta, carotid, femoral and mesenteric arteries (Yeung *et al.*, 2007), and portal vein (Yeung *et al.*, 2008), as well as the rat pulmonary artery (Joshi *et al.*, 2009), thoracic aorta, mesenteric artery and coronary artery (Jepps *et al.*, 2011; see Table 1 and Figure 1). In the rat mesenteric artery, retigabine was found to have an IC_{50} of 14 μM after precontraction with the α_1-adrenoceptor agonist methoxamine. However, the effects of retigabine in the vasculature are less potent than those of S-1, which has an IC_{50} of 2.5 μM in the rat mesenteric artery (Jepps *et al.*, 2011). Kv7.1, 7.4 and 7.5 channels are also expressed in cerebral arteries where both S-1 and retigabine produce vasodilatation across a range of perfusion pressures and Kv7 blockers produce intense vasospasm (Zhong *et al.*, 2010; Mani *et al.*, 2011). Importantly, the work on rodent blood vessels has been translated into humans with *KCNQ* transcripts being detected in human mesenteric and visceral adipose arteries, and Kv7 modulators have marked effects on artery tone (Ng *et al.*, 2011) supporting a role for these channels in the maintenance of vascular tone.

Interestingly, celecoxib, a COX-2-specific inhibitor, has been shown to enhance Kv7.2–Kv7.5 currents overexpressed in HEK 293 cells with an EC_{50} of 2–5 μM (Du *et al.*, 2011). Previously, celecoxib had been shown to enhance Kv7.5 currents in A7r5 rat aortic smooth muscle cells and cause a vasodilatation of rat mesenteric arteries, whereas other COX-2-specific inhibitors, such as rofecoxib (Vioxx™; Merck & Co. Inc., Whitehouse Station, NJ, USA), had no effect on the currents (Brueggemann *et al.*, 2009). The vasodilator effect of celecoxib may explain why this drug has fewer deleterious cardiovascular effects than other COX-2-specific inhibitors, and emphasizes the importance of these channels in regulating the resting vascular tone. The general COX inhibitor diclofenac (100 μM) has also been shown to enhance currents generated by overexpression of KCNQ4 but inhibits currents generated by KCNQ5 expression or KCNQ4/5 co-expression (Brueggemann *et al.*, 2011). The effects of diclofenac on currents from the rat mesenteric artery myocytes closely resembled those of diclofenac on the KCNQ4/5 overexpressed currents (Brueggemann *et al.*, 2011).

Although Kv7.1 has been readily shown to be expressed in rodent and human vascular smooth muscle, the functional impact of this channel has remained enigmatic. In several

Table 1

List of Kv7 activators shown to have an effect on different smooth muscle systems in humans and rodents, and their effective concentrations in smooth muscle. See Figure 1 for an illustration of the different smooth muscle systems in which these Kv7 channel activators have been shown to have an effect. For a comprehensive pharmacological profile of activators, see Xiong *et al.* (2008)

Compound name	Channels enhanced	Effective concentration range tested in smooth muscle (μM)	References
Retigabine	Kv7.2–7.5	2–30	Yeung *et al.*, 2007; Yeung *et al.*, 2008; Jepps *et al.*, 2009; Joshi *et al.*, 2009; Ng *et al.*, 2011; Ipavec *et al.*, 2011; McCallum *et al.*, 2011; Jepps *et al.*, 2011; Brueggemann *et al.*, 2012
S-1	Kv7.2–7.5	1–30	Ng *et al.*, 2011; Jepps *et al.*, 2011; Chadha *et al.*, 2012a
BMS-204352	Kv7.2–7.5	1–10	Jepps *et al.*, 2011
Flupirtine		10–20	Brueggemann *et al.*, 2007; Mackie *et al.*, 2008; Joshi *et al.*, 2009; Morecroft *et al.*, 2009; Anderson *et al.*, 2009; McCallum *et al.*, 2011; Ipavec *et al.*, 2011; Brueggemann *et al.*, 2012
Celecoxib	Kv7.2–7.5	10–20	Brueggemann *et al.*, 2009; Mani *et al.*, 2011; Brueggemann *et al.*, 2012
Diclofenac	Kv7.2–7.4	100	Brueggemann *et al.*, 2011
R-L3	Kv7.1	0.3–3	Chadha *et al.*, 2012b
Mefenamic acid	Kv7.1	10–30	Chadha *et al.*, 2012b

smooth muscle studies, Kv7.1-specific blockers such as chromanol 293B, HMR1556, L-768 673 and JNJ39490282 have had no contractile effect (Chadha *et al.*, 2012b). However, Chadha *et al.* (2012b) recently showed that Kv7.1 activators, such as R-L3 (L-364373) and mefenamic acid, relax precontracted rat blood vessels, which is abolished by application of Kv7.1-specific blockers (Chadha *et al.*, 2012b). These findings suggest that Kv7.1 channels are functional in vascular smooth muscle but, because the Kv7.1-specific blockers have no effect, do not appear to contribute to resting vascular tone (Chadha *et al.*, 2012b).

It has also been shown that Kv7 channels are regulated by different physiological vasoconstrictors or vasodilators to determine vascular smooth muscle tone. In A7r5 cells (a rat aortic smooth muscle cell line), Brueggemann *et al.* (2007) found that the vasoconstrictor hormone arginine vasopressin (AVP) suppressed native Kv currents via PKC activation, an effect prevented by prior application of Kv7 blockers but not by the Kv1.5 blocker correolide. Additionally, AVP-induced constriction of rat mesenteric arteries was shown to be propagated through Kv7 channel suppression via PKC activation (Mackie *et al.*, 2008). More recently, Kv7 channels, and Kv7.4 in particular, have been shown to underlie β-adrenoceptor-mediated relaxation of rat renal arteries (Chadha *et al.*, 2012a). Thus, isoprenaline evoked a linopirdine-sensitive current and isoprenaline-induced relaxations of the rat renal artery were attenuated by linopirdine or KCNQ4-targeted siRNA. Other papers have proposed that Kv7 channels are involved in adipocyte-derived releasing factor (ADRF)-induced vascular relaxation (Schleifenbaum *et al.*, 2010). It has been proposed that periadventitial adipose tissue regulates vascular tone by releasing ADRF that activates Kv channels in vascular smooth muscle cells (Gollasch and Dubrovska, 2004). In the mesenteric arteries and aortas of rats and mice, XE991 inhibited the effects of ADRF indicating that Kv7 channels may play a role in ADRF-induced vasorelaxation (Schleifenbaum *et al.*, 2010; Gollasch, 2012). Overall, these data reveal that Kv7 channels are vital determinants of resting vascular tone that are regulated by physiological processes to control vasoconstrictor or vasodilator responses.

Kv7 channels in non-vascular smooth muscle

Outside the vasculature, Kv7 channel functionality has been shown in various other smooth muscle systems (Figure 1): the digestive system [stomach (Ohya *et al.*, 2002; Ipavec *et al.*, 2011) and colon (Jepps *et al.*, 2009)], the bladder (Streng *et al.*, 2004; Rode *et al.*, 2010; Svalø *et al.*, 2011), the uterus (McCallum *et al.*, 2009; 2011) and the airways (Brueggemann *et al.*, 2012).

In the murine gastrointestinal tract, the most abundant *KCNQ* transcripts in the smooth muscle are *KCNQ*4 and *KCNQ*5. In the mouse colon, Kv7 channel blockers significantly increased smooth muscle contractility and retigabine reduced spontaneous activity (Jepps *et al.*, 2009), suggesting these channels are important in limiting contractile activity in the gastrointestinal tract, and particularly in the colon. In addition, *KCNQ* expression has been shown in the smooth muscle of the rat stomach and Kv7 modulators have a pronounced effect on muscle tone (Ohya *et al.*, 2002; Ipavec *et al.*, 2011).

In the rat bladder, retigabine given intravenously, intracerebroventricularly and intravesically increased micturition volume and voiding intervals (Streng *et al.*, 2004). Moreover, Rode *et al.* (2010) used retigabine to isometrically relax rat bladders *ex vivo* suggesting Kv7 channels regulate bladder smooth muscle excitability, directly. Also, bladder interstitial

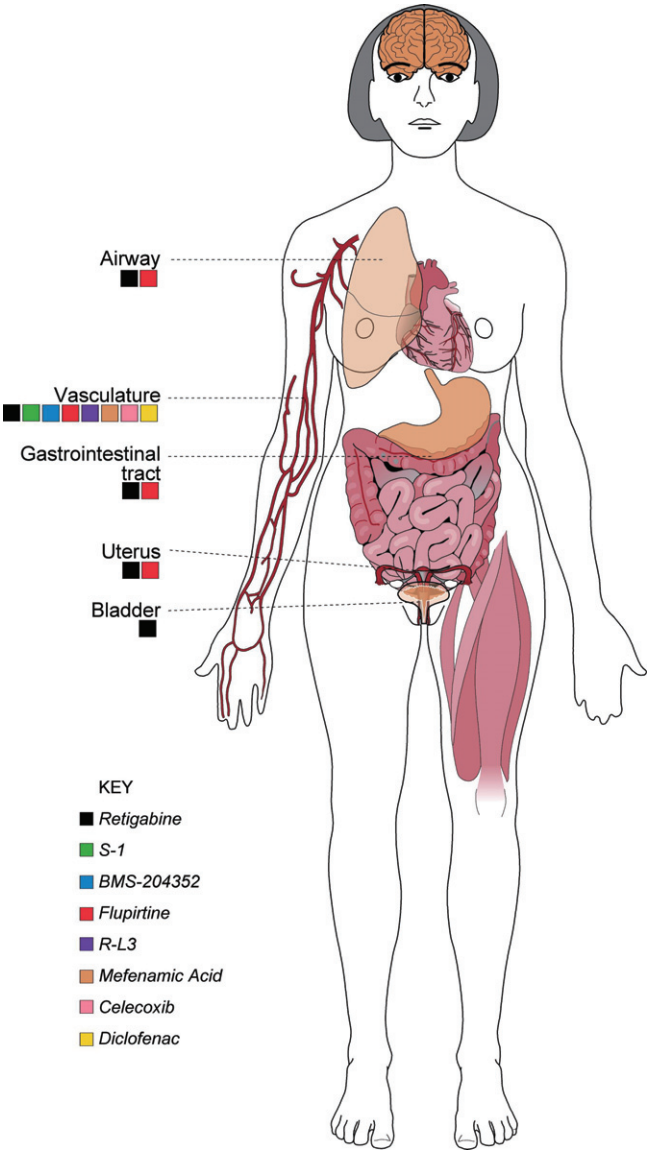

Figure 1

Summary of where Kv7 activators have been shown to have effects in different smooth muscle systems of the body. This figure highlights different smooth muscle systems of the body as potential therapeutic targets for Kv7 activators or blockers. See Table 1 for a description and references for each activator. The illustration summarizes data from human, rat and mouse studies.

cells of Cajal display KCNQ currents that have been shown to regulate the resting membrane potential of these cells (Anderson *et al.*, 2009). This study found that flupirtine enhances outward currents from isolated interstitial cells of Cajal and KCNQ channel blockers decreased the current (Anderson *et al.*, 2009). This suggests the effect of KCNQ channel drugs may be amplified in the bladder by the interstitial cells of Cajal.

KCNQ expression has also been shown in the mouse and human uterus where *KCNQ*1 expression predominates throughout the oestrous cycle (McCallum *et al.*, 2009). *KCNQ* expression has also been found to change during pregnancy with the majority of *KCNQ* isoform expression decreases initially in early pregnancy before returning to robust levels at late pregnancy (McCallum *et al.*, 2011). Kv7 activators (flupirtine and retigabine) relaxed the human and mouse uterus *ex vivo*, but were more effective on the uteri of late pregnant mice and humans. These data present the Kv7 channels as a novel target in the treatment of pregnancy disorders such as preterm labour.

In airway smooth muscle, expression of *KCNQ*1, *KCNQ*4 and *KCNQ*5 appears to predominate in humans; however, *KCNQ*2 predominates in guinea pig with *KCNQ*1 being undetectable (Brueggemann *et al.*, 2012). This study found that both human and guinea pig airways were modulated by application of Kv7 activators and blockers, suggesting that Kv7 channels can regulate airway diameter and are likely to be responsible for maintaining the resting tone in the airways (Brueggemann *et al.*, 2012). Moreover, this study suggests that Kv7 enhancers may be useful bronchodilators in the treatment of airway diseases such as asthma.

Smooth muscle: an alternate target of Kv7 activators

It is now appreciated that Kv7 channels play a major functional role throughout the smooth muscle systems of the body, thus it is logical to suggest that these channels may provide novel therapeutic targets for a range of smooth muscle-associated diseases. Some of the observed side effects of retigabine confirm the potential of these channels as novel targets for smooth muscle diseases (Figure 1).

In 2008, over 4.3 million (~42%) deaths in the European Union were due to cardiovascular disease, highlighting there is an unmet need for new cardiovascular therapies. Kv7.2–7.5 channel activators improve coronary flow in rat hearts considerably without any effect on the cardiomyocytes (Jepps *et al.*, 2011), suggesting that targeting Kv7.4 or 7.5 could be a new therapeutic rationale for the treatment of ischaemic heart disease. Alternatively, Kv7 activators could be developed in the treatment of stroke and specific Kv7 blockers could theoretically serve as anti-migraine medications. However, there is a caveat to these therapeutic interventions in that it has recently been shown that the functional effect of Kv7 activators S-1, retigabine and BMS-204352 was impaired in the mesenteric arteries from spontaneously hypertensive rats and angiotensin II-infused hypertensive mice (Jepps *et al.*, 2011). Moreover, the effectiveness of S-1 was completely lost in the renal artery of spontaneously hypertensive rats, which was associated with an abrogation of the β-adrenoceptor–mediated responses (Chadha *et al.*, 2012a). In all arteries, the loss of Kv7 function was concomitant with a reduction in Kv7.4 protein. These findings corroborate those of Morecroft *et al.* (2009) which showed that the effect of flupirtine to relax precontracted mouse pulmonary arteries was attenuated in a mouse genetic model of pulmonary artery hypertension. The mechanisms that produce the impairment of Kv7 function are unknown, but these findings suggest that in addition to augmenting Kv7 channel activity, correcting the Kv7.4 deficit could be a practical and effective therapy in the treatment of hypertension.

In the two phase III double-blind placebo-controlled clinical trials of retigabine [RESTORE 1 (Study 301; French *et al.*, 2011) and RESTORE 2 (Study 302; Brodie *et al.*, 2010)], the most common adverse side effect was dizziness. In RESTORE 1 (French *et al.*, 2011), 40.5% of patients receiving retigabine (1200 mg day^{-1}) reported dizziness compared with 13.8% in the placebo group; and in RESTORE 2 (Brodie *et al.*, 2010), 17.1 and 26.4% of patients receiving 600 and 900 mg day^{-1} of retigabine, respectively, reported dizziness compared with 6.7% in the placebo group. In 1987, a study to investigate the efficacy and safety of long-term treatment with flupirtine, a structural analogue of retigabine, in patients with chronic pain found a slight trend for lowering systolic blood pressure but no changes in diastolic blood pressure (Herrmann *et al.*, 1987). This study also found the most frequently observed side effect to be dizziness (11%). Retigabine has been shown to effect myogenic control of cerebral blood vessels in rats (Zhong *et al.*, 2010; Mani *et al.*, 2011) and cause relaxation of human blood vessels *ex vivo* (Ng *et al.*, 2011). Although no clinically significant changes in blood pressure were found in the clinical trials, it could be suggested that decreased blood supply to the brain caused by increased orthostatic hypotension is a contributing factor to the dizziness observed with these drugs.

The findings in animal model studies suggest that Kv7 channel activators might be a novel treatment for urinary disorders such as incontinence. In the clinical trials, patients receiving retigabine displayed an increased risk of urinary hesitation, urinary retention, residual urine volume and decreased urine flow compared with the placebo controls (see Brickel *et al.*, 2012). These findings confirm that Kv7 channels in the smooth muscle of the urinary bladder could provide a novel target to treat diseases such as incontinence. Also, consistent with the effects of retigabine on the colon in animal models, constipation was identified as another side effect in the clinical trials. In RESTORE 1, 5% of patients receiving 1200 mg day^{-1} retigabine were affected by constipation compared with 1.4% receiving placebo (French *et al.*, 2011). These findings suggest that Kv7 channels found in the digestive system, or at least the colon, may provide another therapeutic target for Kv7 channel activators and blockers, which could be administered as a suppository to treat diarrhoea or constipation respectively.

In the uterus, animal and human studies have found KCNQ channels to play a functional role in uterine wall contractions, especially during late pregnancy. Retigabine has yet to be fully tested in pregnant woman, although it should be noted that five pregnant women had taken retigabine, of which four delivered healthy babies. The remaining patient received eight doses of retigabine before finding out she was pregnant and withdrew from the trial, but had several birth complications including a premature birth (28 weeks). Before adequate, well-controlled trials are conducted to assess the effect of retigabine in pregnancy, it has been advised that retigabine should only be used during pregnancy if the potential benefit justifies the potential risk to the fetus. Based on the work from our laboratory (McCallum *et al.*, 2009; 2011), retigabine has the potential to relax uterine smooth muscle which may produce complications during pregnancy and labour but importantly could provide a novel therapy to treat preterm labour.

Consistent with the *in vitro* work in animal models, retigabine has demonstrated relaxant effects on smooth muscle in the clinical trials, including the urogenital and digestive systems and possibly also in the vasculature. Retigabine shows greater efficacy for Kv7.2 and Kv7.3, which predominate in the brain, whereas Kv7.1, Kv7.4 and Kv7.5 predominate in smooth muscle. Therefore, to reduce the number of smooth muscle-associated adverse effects of retigabine, Kv7 activators more selective for channels formed by Kv7.2/3 subunit (possibly similar to ICA-27243) need to be developed. Conversely, generation of drugs with increased specificity for Kv7.4–7.5, such as S-1 and BMS-204352, may result in therapeutics of smooth muscle disorders such as preterm labour, constipation, bladder instability and angina pectoris. Alternatively, hydrophilic analogues of existing Kv7.2–5 activators being less blood-brain-barrier permeable may be used to target smooth muscle Kv7 channels primarily.

Conclusions

The novel mechanism of action of retigabine offers remarkable new hope to epileptic patients who cannot control their seizures with current medication. Retigabine hyperpolarizes the resting membrane potential of neurones by activating Kv7 channels, which prevents the bursts of action potentials developing that are associated with seizure generation. The recent clinical trials have translated the findings in animal seizure models and found that retigabine dose dependently reduces seizure generation in patients with partial epilepsy [RESTORE 1 (Study 301; French *et al.*, 2011) and RESTORE 2 (Study 302; Brodie *et al.*, 2010)].

However, in this review we have considered Kv7 channels in smooth muscle as novel therapeutic targets for other diseases as highlighted by some of retigabine's 'side effects' in these systems. Over the last decade, Kv7 channels have been strongly implicated in the control of smooth muscle contractility, emphasizing that these channels are not exclusively expressed in neurones and the heart. As such, what was perceived as side effects from retigabine may actually lead to novel therapeutics for a range of disorders.

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Conflict of interest

None.

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