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Novel treatment strategies for smooth muscle disorders: Targeting Kv7 potassium channels

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ABSTRACT

Smooth muscle cells provide crucial contractile functions in visceral, vascular, and lung tissues. The contractile state of smooth muscle is largely determined by their electrical excitability, which is in turn influenced by the activity of potassium channels. The activity of potassium channels sustains smooth muscle cell membrane hyperpolarization, reducing cellular excitability and thereby promoting smooth muscle relaxation. Research over the past decade has indicated an important role for Kv7 (KCNQ) voltage-gated potassium channels in the regulation of the excitability of smooth muscle cells. Expression of multiple Kv7 channel subtypes has been demonstrated in smooth muscle cells from viscera (gastrointestinal, bladder, myometrial), from the systemic and pulmonary vasculature, and from the airways of the lung, from multiple species, including humans. A number of clinically used drugs, some of which were developed to target Kv7 channels in other tissues, have been found to exert robust effects on smooth muscle Kv7 channels. Functional studies have indicated that Kv7 channel activators and inhibitors have the ability to relax and contact smooth muscle preparations, respectively, suggesting a wide range of novel applications for the pharmacological tool set. This review summarizes recent findings regarding the physiological functions of Kv7 channels in smooth muscle, and highlights potential therapeutic applications based on pharmacological targeting of smooth muscle Kv7 channels throughout the body.

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1. Introduction

Smooth muscle is distinguished from skeletal and cardiac muscle by its lack of striations when viewed under a microscope, as well as its anatomical locations, such as within the walls of the digestive and urinary tracts, the vasculature, male and female reproductive tracts, and the airways of the lungs. Contraction of smooth muscle serves two main functions: to produce visceral motility and propel contents forward, e.g. in the gastrointestinal tract, or to create a resistance to flow, as is the case in the vasculature and airways.

Smooth muscle contractility is modulated physiologically by locally released or circulating substances, by mechanical forces such as stretch, and by the autonomic nervous system. These modulators generally induce an increase or a decrease in cytosolic calcium (Ca^{2+}) concentration to stimulate smooth muscle contraction or relaxation, respectively (Webb, 2003). The contractile mechanism (actin–myosin filament cross-bridge cycling) of smooth muscle is activated by an increase in

Abbreviations: COPD, chronic obstructive pulmonary disease; CPA, chorionic plate artery; FDA, Food and Drug Administration; hERG, human Ether-à-go-go related gene; IBS, irritable bowel syndrome; PAH, pulmonary arterial hypertension; RT-PCR, quantitative reverse transcriptase polymerase chain reaction; VDCCs, voltage-dependent calcium channels.

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cytosolic Ca^{2+} concentration. Ca^{2+} is delivered to the cytosol from two sources: it is released from intracellular Ca^{2+} stores and it comes from the extracellular space, traversing the plasma membrane primarily through L-type voltage-dependent Ca^{2+} channels (VDCCs). The latter process requires opening (activation) of L-type VDCCs, which occurs when there is a positive change in membrane voltage (membrane depolarization). Membrane voltage is largely determined by potassium (K^+) channel activity, and hence, K^+ channels are often central mediators in the physiological modulation of smooth muscle contractility. Multiple types of K^+ channels are expressed in smooth muscle cells; several excellent review articles have been written describing their contribution to the regulation of smooth muscle cell contractility (Kotlikoff, 1993; Nelson and Quayle, 1995; Vogalis, 2000; Khan et al., 2001; Brainard et al., 2007; Greenwood & Ohya, 2009; Petkov, 2011; Jepps et al., 2013; Greenwood and Tribe, 2014; Stott et al., 2014). This review will focus on Kv7 (KCNQ) K^+ channels, a specific subtype of K^+ channels recently recognized for its important contributions to the regulation of smooth muscle cell contractility in viscera, vasculature, and airways. Additional consideration will be given to the potential for therapeutic targeting of smooth muscle Kv7 channels to treat a variety of medical conditions involving impaired or altered smooth muscle function.

2. Kv7 potassium channels: a historical perspective

In the late 1960s and early 1970s, a number of investigators observed that muscarinic acetylcholine receptor agonists decreased K^+ conductance in neurons (Kobayashi and Libet, 1968; Weight and Votava, 1970; Krnjevic et al., 1971). However it was not until 1980 that specific muscarinic agonist-sensitive currents were isolated and characterized (Brown and Adams, 1980). Named for their suppression in response to activation of muscarinic receptors, "M-currents" are outwardly rectifying K^+ currents which characteristically activate very slowly, in a voltage-sensitive manner, and exhibit little if any inactivation during sustained membrane depolarizations. They have a relatively negative threshold of activation at about −60 mV, which is near the resting membrane potential of neurons, enabling the channels to function as stabilizers of negative membrane voltage and suppressors of cell excitability (Brown and Adams, 1980).

The gene products responsible for producing the M-current were finally identified in the late 1990s. Messenger RNAs encoding human KCNQ2 (Kv7.2) and rat KCNQ3 (Kv7.3) were co-injected into *Xenopus* oocytes, resulting in a current that resembled neuronal M-currents. M-currents in sympathetic neurons were also sensitive to the Kv7 channel blocker linopirdine and its more potent analog XE991, providing additional evidence that Kv7 channels are responsible for mediating the M-current (Wang et al., 1998).

Five mammalian Kv7 channel α-subunits have now been identified (Kv7.1–Kv7.5, encoded by 5 genes, KCNQ1–KCNQ5 respectively). The channels are formed as homo- or heterotetramers of the individual gene products (Jentsch, 2000). Kv7.2/Kv7.3 heteromeric channels, which conduct M-currents, have been extensively studied in neurons and in expression systems (Jentsch, 2000; Delmas and Brown, 2005; Brown and Passmore, 2009). In cardiomyocytes, Kv7.1 homotetramers (with auxiliary subunits—see below) mediate I_{KS}, a slow delayed rectifier current, which contributes to repolarization of the cardiac action potential (Barhanin et al., 1996; Sanguinetti et al., 1996). In the sensory outer hair cells of the cochlea, Kv7.4 channels mediate I_{KN}, a Kv current with a very negative threshold for activation which serves to oppose the excitability of these specialized sensory neurons (Kubisch et al., 1999; Kharkovets et al., 2000). In addition, multiple Kv7 channel subtypes have been implicated in the regulation of proliferation and differentiation of skeletal muscle cells (Roura-Ferrer et al., 2008; Iannotti et al., 2010).

Auxiliary β-subunits are often important determinants of the expression and function of Kv channels (Brueggemann et al., 2013a). With regard to Kv7 channels, there is considerable evidence that KCNE1 (also called minK) and KCNE2–5 (or minK-related peptides), can associate with and regulate channel expression and function. KCNE1 is the best studied due to its partnership with Kv7.1 to mediate I_{KS} in cardiac myocytes. Two KCNE1 subunits are thought to associate with a homotetramer of Kv7.1 subunits to form functional channels which give rise to the slowly activating I_{Ks} current (Morin and Kobertz, 2008). The presence of KCNE1 within the channel oligomer is responsible for the slow channel activation (Sanguinetti et al., 1996) and it also increases channel conductance (Pusch, 1998). There is a paucity of information on the roles of natively expressed KCNE subunits in modulating Kv7 channel expression and function in tissues other than the heart, but KCNE subtypes have been found to modulate the function of Kv7 channel subtypes in expression systems. For example, KCNE2 decreases Kv7.1 current density (Tinel et al., 2000a) and KCNE3 greatly enhances Kv7.1 current density (Schroeder et al., 2000; Bendahhou et al., 2005), while KCNE4 and 5 both suppress Kv7.1 currents (Angelo et al., 2002; Grunnet et al., 2002; Grunnet et al., 2005). KCNE1 enhances Kv7.5 currents while slowing their activation (Roura-Ferrer et al., 2009), KCNE2 accelerates the activation and deactivation kinetics of Kv7.2/3 currents (Tinel et al., 2000b), KCNE3 suppresses Kv7.4 (Schroeder et al., 2000) and Kv7.5 (Roura-Ferrer et al., 2009) currents, whereas KCNE4 increases Kv7.4 current amplitude (Strutz-Seebohm et al., 2006; Jepps et al., 2015).

The function of Kv7 channels natively expressed in various tissues has been elucidated primarily through the development of pharmacological agents that selectively block or activate these channels. Linopirdine (1,3-Dihydro-1-phenyl-3,3-*bis*(4-pyridinylmethyl)-2*H*-indol-2-one) and XE991 (10,10-bis(4-pyridinylmethyl)-9(10H)-anthracenone) are the most widely used Kv7 channel blockers, effectively blocking all five Kv7 subtypes, with minimal effects on other ion channels at concentrations up to 10 μM (Aiken et al., 1995; Schnee and Brown, 1998; Wang et al., 2000; Wladyka and Kunze, 2006). Flupirtine (ethyl-*N*-{2-amino-6-(4-fluorophenylmethylamino) pyridine-3 yl cabamate) and retigabine (ethyl-*N*-{(2-amino-4-[(4-fluorophenyl)methylamino]phenyl} carbamate) are prototypical activators of the neuronal Kv7 channels; these drugs activate all Kv7 subtypes except for Kv7.1, which lacks a critical tryptophan residue required for activation by these compounds (Tatulian et al., 2001; Schenzer et al., 2005). L-364,373 (also known as R-L3; (3R)-5-(2-fluorophenyl)-3-(1H-indol-3-ylmethyl)-1-methyl-3H-1,4-benzodiazepin-2-one) reportedly activates only Kv7.1 channels (Salata et al., 1998; Seebohm et al., 2003) while chromanol 293B (*trans*-*N*-[6-Cyano-3,4-dihydro-3-hydroxy-2,2-dimethyl-2*H*-1-benzopyran-4-yl]-*N*-methyl ethanesulfonamide) and HMR-1556 (N-[(3R,4S)-3-hydroxy-2,2-dimethyl-6-(4,4,4-trifluorobutoxy)-3,4-dihydrochromen-4-yl]-N-methylmethanesulfonamide) are Kv7.1 channel inhibitors (Busch et al., 1996; Bleich et al., 1997; Thomas et al., 2003).

Smooth muscle has recently emerged as an important player in the Kv7 channel story. Sims, Singer and Walsh were the first to identify a K^+ current in isolated toad gastric smooth muscle cells that resembled the M-current previously described in neurons (Sims et al., 1985). Their ground-breaking studies can be more fully appreciated as the molecular components behind the M-current and its regulation in smooth muscle are now becoming more fully elucidated. In the past 10–15 years, the roles of Kv7 channels in the regulation of smooth muscle contractility have become an exciting area of discovery. Here we will summarize what is currently known about the Kv7 family K^+ channels in smooth muscle and discuss their potential as novel therapeutic targets for diseases associated with smooth muscle dysfunction. Table 1 lists the Kv7 channels and auxiliary KCNE subunits that have been detected in smooth muscle from various tissues.

3. Kv7 channels in visceral smooth muscle

3.1. Gastrointestinal tract

The expression of Kv7 channels was initially observed in rat gastric antrum smooth muscle cells over a decade ago (Ohya et al., 2002a).