MINIREVIEW

Cardiovascular KCNQ (Kv7) Potassium Channels: Physiological Regulators and New Targets for Therapeutic Intervention

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ABSTRACT

Potassium channels play an important role in electrical signaling of excitable cells such as neurons, cardiac myocytes, and vascular smooth muscle cells (VSMCs). In particular, the KCNQ (Kv7) family of voltage-activated K^+ channels functions to stabilize negative resting membrane potentials and thereby opposes electrical excitability. Of the five known members of the mammalian Kv7 family, Kv7.1 was originally recognized for its role in cardiac myocytes, where it contributes to repolarization of the cardiac action potential. Kv7.2 to Kv7.5 were first discovered in neurons, in which they play a well characterized role in neurotransmitter-stimulated action potential firing. Over the past 5 years, important new roles for Kv7 channels have been identified. Kv7 channels have been found to be expressed in VSMCs from several vascular beds where they contribute to the regulation of vascular tone. There is evidence that Kv7.5 channels in VSMCs are targeted by the hormone vasopressin to mediate its physiological vasoconstrictor actions and evidence that neuronal Kv7 channels in the baroreceptors of the aortic arch adjust the sensitivity of the mechanosensitive neurons to changes in arterial blood pressure. These newly identified physiological roles for Kv7 channels in the cardiovascular system warrant increased attention because pharmacological modulators of this family of channels are being used clinically to treat a variety of neurological disorders. This raises questions about the cardiovascular side effects associated with existing therapies, but there is also obvious potential to capitalize on the established and evolving pharmacology of these channels to develop new therapies for cardiovascular diseases.

Mammalian cells are surrounded by a lipid membrane that functions as a barrier to the diffusion of many substances, including ions. However, proteinaceous channels integrated into these membranes allow ions to cross the membrane when the channels are open; a subset of these channels is selective for potassium ions (K^+). K^+ is usually approximately 25-fold more concentrated inside the cell compared with outside as a result of the activity of Na^+/K^+-ATPases. When potassium channels open (activate), K^+ tends to leak out of the cell through these channels, producing a measurable electrical current that establishes a charge difference across the membrane (membrane potential). This polarization of the membrane is the basis of cellular electrical signaling.

KCNQ voltage-activated K^+ channels (also known as the Kv7 family) play an important role in regulating the membrane potential of many excitable tissues (Robbins, 2001; Delmas and Brown, 2005). The Kv7 family is composed of five subtypes (Kv7.1–7.5) encoded by five genes (KCNQ1–5). Channels formed as tetrameric assemblies of Kv7 protein subunits (homotetramers or heterotetramers) have distinct tissue localizations and functions. The importance of these channel proteins in regulating membrane potential was established by the discovery of several mutations in KCNQ genes that can be linked to human diseases arising from altered cellular excitability (Brown, 2008).

Although Kv7 channels are most commonly recognized for their importance in the nervous system, where they repre-

ABBREVIATIONS: ACh, acetylcholine; AD, Alzheimer's disease; AVP, arginine vasopressin; IPA, intrapulmonary artery; MASMC, mesenteric artery smooth muscle cell; PKC, protein kinase C; PVM, portal vein myocyte; PVR, peripheral vascular resistance; SAH, subarachnoid hemorrhage; V_M, membrane potential; VSMC, vascular smooth muscle cells; BMS204352, (5-chloro-2-methoxyphenyl)-1,3-dihydro-3-fluoro-6-(trifluoromethyl)-2H-indol-2-one; 4-AP, 4-aminopyridine; XE991, 10,10-bis(4-pyridinylmethyl)-9(10H)-anthracenone; (S)-1, (S)-N-[1-(3-morpholin-4-yl-phenyl)-ethyl]-3-phenyl-acrylamide.

sent the structural correlate of the well described "M-current," recent evidence suggests that they also play a pivotal role in the regulation of cardiovascular function. The purpose of this review is to highlight what is known regarding Kv7 channels and their role in cardiovascular physiology. We will also discuss the pharmacology of both neuronal and vascular Kv7 channels from the point of view that therapies targeting the former may have cardiovascular side effects, whereas the latter may be important new targets for the treatment of cardiovascular disorders such as hypertension or stroke.

Historical Perspective

During the late 1960s and throughout the 1970s, several groups established that in sympathetic, cortical, and hippocampal neurons, muscarinic acetylcholine (ACh) receptor agonists induced slow membrane depolarization and a decrease in potassium conductance (Kobayashi and Libet, 1968; Weight and Votava, 1970; Krnjević et al., 1971; Kuba and Koketsu, 1976a,b; Dodd et al., 1981). It was not until 1980 that Brown and Adams identified a voltage-sensitive K^+ current that could account for the decreased potassium conductance and named it the "M-current" (also known as I_M) for its propensity to be inhibited by muscarinic receptor activation (Brown and Adams, 1980). Brown and Adams determined that the current they isolated was activated in a time- and voltage-dependent manner and that it was intimately involved in maintaining the electrical stability of the cell. The M-current is activated at relatively negative potentials (~-60 mV, close to the resting potential for many excitable cells), providing a resting outward current that renders cells more resistant to the impact of depolarizing stimuli. Conversely, when M-currents are suppressed, cells are more depolarized at rest, and excitatory inputs are capable of producing greater depolarization, which can lead to enhanced membrane excitability.

In the 15 years after Brown and Adams' findings, research from several laboratories established that the M-current is not exclusively regulated by muscarinic receptor activation but is also sensitive to activation of a number of other G-protein-coupled receptors. G-protein-coupled receptor agonists capable of modulating the M-current include muscarinic (M_1/M_3) (Brown and Adams, 1980; Constanti and Brown, 1981; Halliwell and Adams, 1982), substance P (Jones, 1985), luteinizing hormone releasing hormone (Adams and Brown, 1980), purinergic (P2Y) (Adams et al., 1982), β-adrenergic (Akasu, 1988), angiotensin II (AT_1) (Shapiro et al., 1994; Zaika et al., 2006), serotonin (5-hydroxytryptamine-2) (Colino and Halliwell, 1987), opiates (κ and δ) (Moore et al., 1994), and glutamate (metabotropic glutamate receptors 1/5) (Charpak et al., 1990). It is interesting that agonists such as angiotensin II and serotonin are also hormones that play a significant role in cardiovascular homeostasis through direct actions on vascular smooth muscle cells.

Identification of a gene product that could account for the long-established M-current came in 1996. Wang and colleagues showed that injection of KCNQ2 and KCNQ3 potassium channel subunit cDNA into *Xenopus laevis* oocytes was sufficient to produce a current with striking electrophysiological similarity to the aforementioned M-current. In addition, they observed pharmacological similarities between native neuronal M-currents and oocyte-expressed KCNQ2/

KCNQ3 currents—both currents were sensitive to the Kv7 channel-selective blockers, linopirdine and XE991 (Wang et al., 1998). Around the same time, a number of genetic studies individually identified mutations in KCNQ genes that lead to pathological conditions such as benign familial neonatal convulsions (KCNQ2–3), idiopathic deafness (KCNQ4), and long QT syndrome (KCNQ1). All of these conditions are related to a loss of control of membrane excitability (Brown, 2008).

Functional Kv7 channels form as homomeric or heteromeric tetramers of the individual gene products. Kv7.1 (KvLQT1) is distinct from the other four members in that it is not believed to be expressed in neurons (Jespersen et al., 2005) and it cannot form heteromeric channels with any of the other Kv7 channel subtypes (Schwake et al., 2003). Kv7.1 is also insensitive to drugs (e.g., flupirtine, retigabine, and *N*-ethyl maleimide) that activate the other Kv7 channel subtypes (Gamper et al., 2005; Munro and Dalby-Brown, 2007). In many neurons, heteromeric complexes of Kv7.2 and Kv7.3 subunits underlie ACh-regulated M-currents. Kv7.4 and Kv7.5 channels are expressed in some neurons and form functional ACh-regulated channels in expression systems (Schroeder et al., 2000; Selyanko et al., 2000). Kv7.5 channels are also expressed in other excitable tissues, including visceral smooth muscle (Jensen et al., 2005) and skeletal muscle (Lerche et al., 2000; Schroeder et al., 2000).

The importance of Kv7.1 (KvLQT1) in cardiac electrophysiology is now well established. In cardiac myocytes, Kv7.1 tetramers combine with two KCNE1 (MinK) β-subunits to form functional channels. These channels underlie I_{Ks}, a slow delayed-rectifier K^+ current that is involved in repolarization of the cardiac action potential and an important determinant of the QT interval of the electrocardiogram. Mutations that alter the function of either Kv7.1 or MinK subunits result in long QT syndrome, which can lead to arrhythmias, ventricular fibrillation, and cardiac arrest. For further reading, several excellent reviews on this subject area are available (Chiang and Roden, 2000; Herbert et al., 2002; Jespersen et al., 2005). Here, we focus on Kv7 channel expression and function in vascular smooth muscle, along with a brief description of their involvement in neurons that regulate cardiovascular function, including the baroreceptor reflex.

Kv7 Channel Pharmacology

Relatively selective blockers and activators of Kv7 channels have been developed. These drugs have helped to define the importance of Kv7 channels in many tissue types, including vascular smooth muscle. All of the studies described in the subsequent section have used one or more of these compounds, and they therefore warrant a brief introduction. Two potent Kv7 channel blockers have been developed: linopirdine [DuP 996, 1,3-dihydro-1-phenyl-3,3-bis(4-pyridinylmethyl)-2*H*-indol-2-one] and its more potent analog XE991. At concentrations of 10 μM or less, linopirdine and XE991 are effective and relatively selective blockers of all five subtypes of Kv7 channels, although effects of these drugs on other types of K^+ channels have been reported, particularly at higher concentrations (Schnee and Brown, 1998; Wladyka and Kunze, 2006). Flupirtine [ethyl-*N*-[2-amino-6-(4-fluorophenylmethylamino)pyridin-3-yl] carbamic acid] and retigabine [D-23129, *N*-(2-amino-4-(4-fluorobenzylamino)-phenyl) carbamic acid ethyl ester] are commonly used activators of the Kv7 channels. They activate

all of the subtypes except for Kv7.1, which lacks a transmembrane tryptophan residue that is believed to be essential for the actions of these drugs (Schenzer et al., 2005; Wuttke et al., 2005; Bentzen et al., 2006). The clinical usefulness of Kv7 channel modulators is discussed in a following section.

Kv7 Channels in Vascular Smooth Muscle Cells

The first report of KCNQ expression in vascular smooth muscle cells (VSMCs) came from Ohya et al. (2003), who discovered the expression of KCNQ1 in murine portal vein myocytes (PVMs) (Ohya et al., 2003). A KCNQ1 truncation splice variant was also detected within the same tissue, although its functional significance has yet to be determined. Ohya et al. (2003) recorded native PVM delayed-rectifier potassium currents that were inhibited by the selective Kv7 channel blocker linopirdine, providing the first functional evidence for Kv7 channels in vascular myocytes (Ohya et al., 2003). Both the duration and amplitude of evoked depolarizations in PVM were increased in the presence of linopirdine, suggesting a role for Kv7 channels in VSMC repolarization (Ohya et al., 2003). A follow-up study (Yeung and Greenwood, 2005) further defined the electrophysiological role for Kv7 channels in murine PVMs. Although Kv7 currents were not electrophysiologically isolated from other whole-cell currents, the authors were able to pharmacologically distinguish Kv7 currents from 4-aminopyridine (4-AP)-sensitive voltage-activated K^+ currents using an analytical current subtraction method. The Kv7 channel blockers XE991 and linopirdine induced membrane depolarization, providing evidence that Kv7 currents contribute to the resting conductance of PVM. In addition, XE991 and linopirdine increased the excitability of isolated portal veins (Yeung and Greenwood, 2005).

Further evidence of a functional role of vascular Kv7 channels as regulators of vasoconstriction came from a study by Joshi et al. (2006), who found that intrapulmonary arteries (IPA) from rat and mouse could be constricted by linopirdine and XE991 in an endothelium- and nerve terminal-independent manner. Joshi et al. also established that IPA constriction in response to Kv7 channel inhibition was dependent on voltage-sensitive L-type calcium channels: linopirdine- or XE991-induced IPA constriction was abolished in the presence of nifedipine, an L-type calcium channel blocker. This finding provided an important link between membrane depolarization (by blocking Kv7 channels) and activation of voltage-sensitive Ca^{2+} channels, which results in the elevation of cytosolic $[Ca^{2+}]$ needed for VSMC contraction and vasoconstriction.

Another significant advance came in early 2007 with the discovery that Kv7.5 channels can serve as signal transduction intermediates in the actions of physiological concentrations of the vasoconstrictor hormone Arg^8-vasopressin (AVP). By optimizing electrophysiological recording conditions in A7r5 cells (a rat aortic smooth muscle cell line), Brueggemann et al. (2007) were successful in isolating Kv7 channel-mediated currents from the many other ionic conductances present in VSMCs. This enabled the first detailed electrophysiological and pharmacological characterization of VSMC Kv7 currents and an investigation of the signaling mechanisms involved in their regulation. AVP binds to G_q-coupled

V_{1a} vasopressin receptors on VSMCs to induce vasoconstriction. Kv7 currents in A7r5 cells were suppressed by a physiological vasoconstrictor concentration (100 pM) of AVP, an effect that was associated with membrane depolarization, action potential firing, and generation of repetitive Ca^{2+} transients in A7r5 cells. The effects of AVP were apparently protein kinase C (PKC)-dependent because they were abolished by the PKC inhibitor calphostin C and mimicked by the PKC activator 4β-phorbol 12-myristate 13-acetate (Fan and Byron, 2000; Brueggemann et al., 2007).

The isolated Kv7 currents in A7r5 cells were inhibited by linopirdine and XE991 and enhanced by flupirtine as expected for Kv7.2–7.5 (KCNQ2, -3, -4, and -5) subtypes. Of these, only the expression of KCNQ5 was detected in A7r5 cells by reverse-transcriptase polymerase chain reaction, whereas adult rat aorta expressed both KCNQ1 and KCNQ5. Knocking down KCNQ5 expression by RNA interference resulted in a decrease in the AVP-sensitive Kv7 current, suggesting that Kv7.5 was an essential channel subunit in A7r5 cells (Brueggemann et al., 2007). This was the first report of KCNQ5 expression in VSMCs and the first evidence that vascular Kv7 family channels may serve as targets of vasoconstrictor hormones. In a recent report, Iain Greenwood's laboratory identified a splice variant of KCNQ5 in murine thoracic aorta, carotid and mesenteric arteries, and portal vein (Yeung et al., 2008). Yeung et al. (2008) found that all KCNQ5 transcripts in murine VSMCs encode protein subunits with a 9-amino acid deletion located approximately 55 amino acids from the C-terminal end of the sixth transmembrane domain (Schroeder et al., 2000). It is not yet apparent whether this deletion has implications for channel regulation. The pharmacological and electrophysiological characteristics of the truncated Kv7.5 channels seem to be indistinguishable from those of full-length Kv7.5 channels (Yeung et al., 2008).

Another study by Yeung et al. shed further light on the expression patterns of KCNQ genes by quantitatively evaluating mRNA levels in VSM tissues from several vascular beds of the mouse. KCNQ4 and KCNQ5 were the most abundantly expressed subtypes as detected by quantitative reverse-transcriptase polymerase chain reaction in murine carotid, femoral, and mesenteric arteries and thoracic aorta (Yeung et al., 2007). KCNQ1 expression was also detected in each of the artery types, but KCNQ2 and KCNQ3 were not appreciably expressed.

Expression of the KCNE K^+ channel accessory subunit family was also evaluated in the same murine arteries (Yeung et al., 2007). KCNE expression patterns varied widely among the different arteries, but all five KCNE subtypes were detected, with abundant expression of KCNE4 or KCNE5 in each of the arteries examined (Yeung et al., 2007). The KCNE family of K^+ channel accessory subunits is encoded by five mammalian genes (KCNE1–KCNE5). The subunits have in common a structure that includes a single membrane-spanning domain and an ability to interact with a variety of K^+ channel types, including Kv7.1, human *ether-a-go-go*-related gene, and several members of the Kv1–Kv4 families, to modulate their intracellular targeting and functional characteristics (McCrossan and Abbott, 2004; Cai et al., 2006). All five KCNE family members can interact with Kv7.1, with varying effects on channel function. It is noteworthy that KCNE4 (MiRP3) and KCNE5 (MiRP4) essen-

tially abrogate Kv7.1 activation at physiological potentials (McCrossan and Abbott, 2004; Jespersen et al., 2005).

Following up on the A7r5 cell results that implicated Kv7.5 channels as intermediates in vasopressin signal transduction, the study by Mackie et al. (2008) examined the contributions of Kv7 channels to AVP-induced rat mesenteric artery vasoconstriction (Mackie et al., 2008). Mesenteric artery smooth muscle cells (MASMCs) were found to express KCNQ1, KCNQ4, and KCNQ5, in agreement with the study of murine mesenteric artery KCNQ expression (Yeung et al., 2007). As in A7r5 cells, a physiological concentration of AVP (100 pM) was sufficient to suppress the MASMC Kv7 currents, and this effect was PKC-dependent.

Both AVP and linopirdine caused membrane depolarization in MASMCs and constricted pressurized rat mesenteric artery segments, but these effects of AVP and linopirdine were not additive, suggesting a common mechanism (Mackie et al., 2008). In agreement with previous findings (Joshi et al., 2006; Yeung et al., 2007), the vasoconstrictor effects of linopirdine were prevented by pretreatment with an L-type Ca^{2+}-channel blocker. Considering an earlier study that concluded that the vasoconstrictor actions of a physiological concentration of AVP (30 pM) on rat mesenteric arteries were dependent on PKC and L-type Ca^{2+} channels (Henderson and Byron, 2007), it is likely that Kv7 channels serve as an intermediate in this signaling pathway.

Kv7 channel activators would be expected to relax vascular smooth muscle cells by opposing membrane depolarization, reducing their excitability and preventing Ca^{2+} responses associated with activation of voltage-gated Ca^{2+} channels, as shown in A7r5 cell cultures (Brueggemann et al., 2007). Functional studies of murine arteries using a wire myograph demonstrated that the Kv7 channel activators retigabine, flupirtine and meclofenamic acid relaxed precontracted aortic segments (Yeung et al., 2007). Retigabine also produced dilation in murine femoral, carotid and mesenteric arteries using the same technique. The Kv7 channel activator flupirtine was also found to dose-dependently dilate preconstricted pressurized mesenteric arteries (Mackie et al., 2008). The possibility that this class of drugs may be useful as antihypertensive or antivasospastic agents is discussed below.

The consequences of vascular Kv7 channel modulation for the regulation of systemic blood pressure and/or local blood flow have been addressed to some extent in the study by Mackie et al. (2008), who found that intravenous administration of linopirdine produced dose-dependent increases in systemic blood pressure and mesenteric vascular resistance in anesthetized rats; intravenous flupirtine had the opposite effects. The study did not rule out the possibility that the observed changes in mesenteric vascular resistance could be the result of systemically administered linopirdine or flupirtine acting to modulate local sympathetic nerve function. Sympathetic nerves are known to express functional Kv7 channels, and Kv7 channel modulators are known to affect neurotransmitter release by influencing membrane potential (Hernandez et al., 2008). It also remains to be determined to what extent changes in systemic blood pressure, which will also reflect the activity of baroreceptor reflexes, are influenced by the effects of systemically administered Kv7 channel modulators acting on neuronal Kv7 channels. This possibility is considered in more detail below.

Function of Kv7 Channels in the Context of Multiple Kv Channel Subtypes Expressed in Vascular Myocytes

It is well documented that the electrical resistance of VSMCs is extremely high, such that very small changes in ionic current can result in relatively large fluctuations in membrane potential (Nelson and Quayle, 1995). Considering the activity of Kv7 channels at very negative membrane potentials, the resulting outward K^+ currents are probably playing a much larger role in regulating resting membrane potential (V_M) and vascular tone than has been recognized previously.

VSMCs express many types of Kv channels (composed of Kv1–Kv11 families) in varying proportions and combinations that differ among vascular beds and the size of the vessel examined (Nelson and Quayle, 1995; Cox, 2005; Ko et al., 2008). Kv1–Kv4 family K^+ channels have been well characterized and are considered the primary mediators of outwardly rectifying Kv currents in VSMCs (Cox, 2005). Unlike the Kv7 family channels, the Kv1 to Kv4 families are blocked by millimolar concentrations of 4-AP, and they activate at much more positive voltages than do Kv7 channels (Nelson and Quayle, 1995; Cox, 2005). This was demonstrated directly in mesenteric arterial myocytes, in which Kv7 currents were insensitive to 2 mM 4-AP and were found to a have a $V_{0.5}$ value (voltage that produces 50% activation) of -34 mV, whereas 4-AP-sensitive Kv currents activated with a $V_{0.5}$ value of $+5$ mV (Mackie et al., 2008).

The 40-mV difference in voltage dependence of activation has important functional implications considering that the resting V_M value of vascular myocytes is typically between -40 and -60 mV (Nelson and Quayle, 1995). Kv7 channels are expected to be active at resting V_M, and therefore any stimulus that leads to the suppression of Kv7 currents should increase membrane resistance and enhance cellular excitability, as demonstrated with AVP in A7r5 cells (Brueggemann et al., 2007). The 4-AP-sensitive channels have a very low probability of opening at such negative resting potentials and cannot therefore be suppressed to increase excitability in resting myocytes. However, 4-AP-sensitive Kv currents measured in rat mesenteric artery myocytes are much larger than Kv7 currents (Mackie et al., 2008), suggesting that when these channels do open (e.g., after membrane depolarization to potentials positive to -20 mV), they will rapidly overwhelm the small Kv7 currents and provide a powerful hyperpolarizing effect.

Thus, the different Kv channels may play essentially opposite roles in regulating myocyte excitability: Kv7 channels can be closed to increase myocyte excitability, activate L-type Ca^{2+} channels, and constrict arteries. In contrast, 4-AP-sensitive Kv channels can open in response to this increased excitability. This provides a negative feedback to limit the extent of membrane depolarization and Ca^{2+} influx and thereby opposes vasoconstriction (Fig. 1). This model is supported by the finding that 4-AP did not constrict pressurized rat mesenteric arteries but enhanced the vasoconstrictor responses of the same arteries to the Kv7 channel-blocker linopirdine (Mackie et al., 2008).

It should be noted that the results of Mackie et al. from large conduit arteries may not reflect the contributions of Kv channels in small arteries or arterioles that have significant

resting tone. In the latter cases, the resting V_M value of the arterial myocytes is likely to be more depolarized such that 4-AP-sensitive channels are more active. The membrane resistance would be considerably reduced, and the V_M value of such arterial myocytes would reflect a balance between strongly depolarizing influences that are being countered by the hyperpolarizing effects of the 4-AP-sensitive Kv currents. In this scenario, suppression of the 4-AP-sensitive currents (e.g., with 4-AP itself) would be expected to constrict the arteries, as has been observed in many studies (Nelson and Quayle, 1995; Cox, 2005).

Indirect Effects of Kv7 Channel Modulation on the Cardiovascular System

The cardiovascular system is intensively regulated by the nervous system. It is therefore a distinct possibility that Kv7 channels in the nervous system play an important role in modulating the magnitude or sensitivity of cardiovascular responses to neuronal regulation.

Arterial baroreceptors of the aortic arch and the carotid sinuses detect the arterial pressure and convey signals to the central nervous system that contribute to increases or decreases in heart rate and peripheral vascular resistance (PVR). A study by Wladyka and Kunze (2006) demonstrated that M-currents contribute to the maintenance of the resting membrane potential in baroreceptor neurons. Nodose neurons projecting to the aortic arch express KCNQ2, KCNQ3, and KCNQ5 at baroreceptor terminals where pressure sig-

nals are detected. Using an isolated aortic arch-baroreceptor nerve preparation, Wladyka et al. (2008) found that the Kv7 channel activator retigabine increased the pressure threshold for firing of nodose neurons by nearly 20 mm Hg. Presumably, retigabine activated Kv7 channels, stabilizing the resting potential and rendering the neurons less likely to fire action potentials.

Based on these studies, the effects of Kv7 channel modulators at the arterial baroreceptors will be opposite to the direct effects of these drugs acting on the peripheral vasculature (Fig. 2). Kv7 channel activators such as retigabine and flupirtine would reduce baroreceptor sensitivity, such that at higher blood pressures within the aortic arch, release of norepinephrine from sympathetic nerve terminals in the artery walls would be sustained, with a consequent α-adrenergic receptor-mediated increase in PVR. However, results from our laboratory and others suggest that retigabine or flupirtine acting directly on the peripheral vasculature will tend to hyperpolarize the vascular myocytes to oppose vasoconstrictor actions and decrease PVR. Considering that PVR is a primary determinant of systemic blood pressure, what net change in systemic blood pressure would result from the opposing effects of Kv7 channel modulators? The Kv7 channel activator flupirtine administered on a short-term basis to rats (Mackie et al., 2008) or on a long-term basis to patients (Herrmann et al., 1987) decreased blood pressure, suggesting that the vascular effects of the Kv7 channel activators override their baroreceptor effects. Systemic administration of
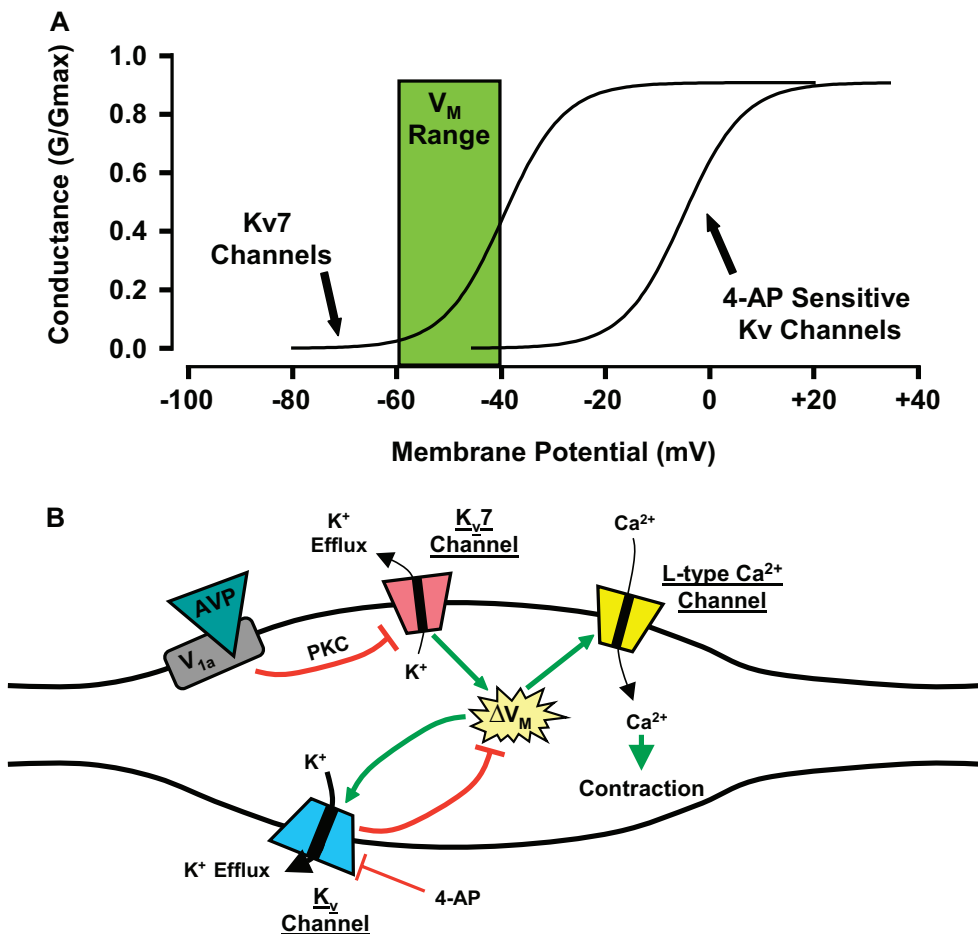
Fig. 1. Distinction between Kv7 and 4-AP-sensitive Kv channels. A, in MASMCs, the voltage-dependent activation of 4-AP-sensitive Kv channels occurs at significantly more depolarized potentials than Kv7 channels. The activation of Kv7 channels occurs very close to the resting V_M range of vascular smooth muscle cells (indicated by the green box), making them important regulators of membrane potential. B, PKC-dependent inhibition of Kv7 currents by AVP causes membrane depolarization of MASMCs and the activation of L-type Ca^{2+} channels, resulting in Ca^{2+} entry and cellular contraction. Opposing this membrane excitability are 4-AP-sensitive Kv channels, which become activated at membrane potentials positive to -40 mV and produce a large outward current that limits further depolarization, thereby reducing Ca^{2+} entry and contraction. Red lines indicate an inhibitory action, whereas green lines indicate a stimulatory action. ΔV_M refers to a change in the membrane potential (depolarization of the cell).

the Kv7 channel-blocker linopirdine in rats did not affect heart rate but produced a sustained increase in systemic blood pressure and mesenteric vascular resistance (Mackie et al., 2008), again suggesting that the vascular effects predominate.

M-currents have a well characterized role in mediating the effects of ACh to promote postsynaptic neuronal excitation. There is also evidence that suppression of Kv7 currents can enhance neurotransmitter release presynaptically (Hernandez et al., 2008). On the other hand, Kv7 channel openers effectively inhibit neurotransmitter release, which may account for a resulting decrease in dopaminergic and serotonergic activity in the central nervous system (Hansen et al., 2008). Kv7.4 channels in particular have been implicated in the control of somatodendritic neuronal excitability in monoaminergic neurons (Hansen et al., 2008). This finding has suggested a possible use of Kv7.4 channel openers in the treatment of disease states characterized by dopaminergic or serotonergic overactivity, including schizophrenia, drug abuse, and anxiety (Hansen et al., 2008). It is not clear to what extent modulation of these neurotransmitter systems might also influence the cardiovascular system. Serotonin and dopamine have complex effects on the cardiovascular system, including modulatory actions at the level of the baroreceptor reflex (van den Buuse, 1998; Raul, 2003; Villalón and Centurion, 2007), and both are released locally in the peripheral vasculature (Murphy, 2000; Villalón and Centurion, 2007). It remains to be determined whether systemically administered Kv7 channel modulators alter plasma levels or local release of monoamine neurotransmitters to affect peripheral vascular tone.

## Cardiovascular Kv7 Channels as Therapeutic Targets

As soon as Kv7 channels were recognized to limit the excitability of neurons, they became the targets of drug development for the treatment of diseases that involve increased or decreased neuronal activity. One such condition is epilepsy, which involves excessive or abnormal synchronous neuronal activity in the brain. Retigabine, a neuronal Kv7 channel activator (Wickenden et al., 2000; Tatulian et al., 2001), is currently in phase III clinical trials, with promising results as an antiepileptic agent (Porter et al., 2007). Kv7

Fig. 2. Kv7 channel activators decrease PVR by a direct action on vascular smooth muscle Kv7 channels but oppose the indirect baroreceptor-mediated decrease in PVR by activating nodose neuron Kv7 channels. The solid line denotes the effect of Kv7 channel activators to oppose membrane depolarization of nodose neurons. HR, heart rate.

channel activators were also found to be effective as analgesics. In fact, flupirtine has been approved as a treatment for pain in Europe since 1981 and has been approved recently for clinical trials in the United States as a treatment for fibromyalgia. Although flupirtine was originally classified as an N-methyl-D-aspartate receptor antagonist (Schwarz et al., 1994; Osborne et al., 1998), ligand binding studies did not support that classification (Osborne et al., 1998). Flupirtine is now believed to exert its beneficial analgesic effects by activating neuronal Kv7 channels. Diclofenac, a U.S. Food and Drug Administration-approved nonsteroidal anti-inflammatory drug, is also a Kv7.2/7.3 channel activator (Peretz et al., 2005). Diclofenac is widely used to treat inflammation and pain associated with arthritis and acute injury. BMS204352 (MaxiPost), which has a dual role as a Kv7 channel activator (Schröder et al., 2001) and an opener of large-conductance Ca^{2+}-activated K^+ channels (Gribkoff et al., 2001), was evaluated in clinical trials as a neuroprotective agent in ischemic stroke (Bozik et al., 2001). Kv7 channel blockers were also evaluated for clinical utility related to their ability to increase neuronal excitability. Linopirdine and XE991 generated interest for their potential to increase brain activity under conditions of dementia. Linopirdine was tested in clinical trials with the hope that it would reverse age-associated memory impairment or the cognitive deficit in patients with Alzheimer's disease (AD). Unfortunately, very little clinical benefit of linopirdine has been observed so far (Rockwood et al., 1997; Börjesson et al., 1999).

The discovery that Kv7 channels are expressed and functional in arterial smooth muscle cells and that they serve to regulate arterial diameter raises several important questions about the therapeutic use of Kv7 channel activators or blockers. Which Kv7 channel subtypes in the human vasculature may be affected by these drugs? What are the effects of clinically used Kv7 channel modulators on blood pressure and blood flow in human subjects? Are the uses of Kv7 channel modulators previously approved, which target the central nervous system or peripheral nervous system, associated with an increased risk of cardiovascular complications? Can therapeutic regimens be improved based on the knowledge that drugs affecting the neuronal Kv7 channels may also have vascular effects? Are there any drugs that act selectively on either neuronal or vascular Kv7 channels? Finally, and perhaps most importantly, would Kv7 channel modulators be useful for the treatment of cardiovascular diseases?

The studies described above from the laboratories of Greenwood, Gurney, and Byron have revealed that several clinically used Kv7 channel modulators are also potent modulators of vascular tone. Considering the consistent findings that these drugs are vasoactive substances and that they can alter vascular resistance and blood pressure in live animals, it seems somewhat surprising that their cardiovascular side effects are not well documented. Unfortunately, there is little published information about the cardiovascular effects of Kv7 channel modulators that have been used for the clinical applications described above.

It has been reported that linopirdine was well tolerated in patients and did not appreciably alter measured cardiovascular parameters in human volunteers (Pieniaszek et al., 1995). It was also relatively ineffective as a cognition enhancer in patients with AD (Rockwood et al., 1997; van Dyck
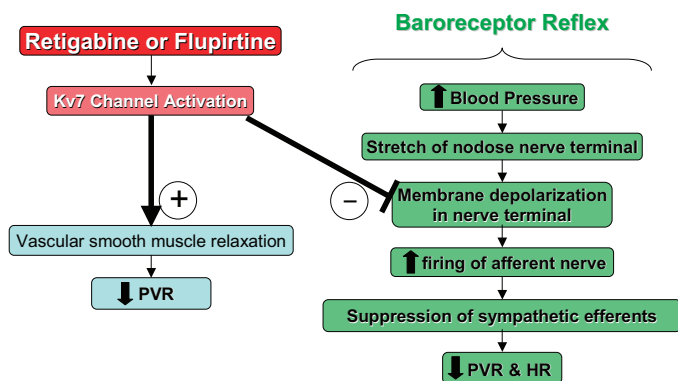
et al., 1997). The use of linopirdine in AD has largely been based on its ability to enhance cholinergic neurotransmission. Enhanced release of acetylcholine may account for an observed increase in regional cerebral blood flow in patients with AD who were treated with linopirdine (van Dyck et al., 1997). ACh acting on endothelial muscarinic receptors may be expected to induce vasodilation and increase blood flow. Although a vasodilatory effect of linopirdine seems contradictory to the vasoconstrictor actions observed with isolated arteries (Joshi et al., 2006; Yeung et al., 2007; Mackie et al., 2008), a partial reversal of AD-associated hypoperfusion was observed in some but not all brain regions examined (van Dyck et al., 1997). This suggests a localized vasodilatory effect that may correspond to sites in which ACh release is enhanced, perhaps overwhelming any direct constrictor actions of linopirdine at these sites.

Flupirtine has been in clinical use in Europe for more than 25 years without drawing much attention to its cardiovascular effects. A modest increase in systolic blood pressure 2 h after a single administration of flupirtine (200 mg p.o.) was observed in one study (Hummel et al., 1991), whereas 55 patients treated for 52 weeks with flupirtine (300 mg average daily dose) experienced on average a small sustained decrease in systolic blood pressure without any significant change in diastolic pressure or heart rate (Herrmann et al., 1987).

The paucity of information available regarding the effects of Kv7 channel activators on systemic blood pressure in patients or animal models precludes an informed prediction as to the utility of these agents as treatments for hypertension. A reduction in PVR is the basis for using many other classes of antihypertensive agents. Kv7 channel activators are expected to reduce PVR, and they have already proven to be well tolerated when used clinically for other indications. Systematic in vivo testing of Kv7 channel activators is required to determine whether their effects on PVR, on a background of elevated systemic blood pressure, are beneficial in terms of lowering blood pressure without inducing other adverse effects that would be contraindications for their clinical use.

The potential to use Kv7 channel activators as antivasospastic agents may be more immediately promising. For example, cerebral vasospasm is the most common cause of morbidity and mortality in patients admitted to the hospital after suffering aneurysmal subarachnoid hemorrhage (SAH) (Kassell et al., 1985). Vasospasm of the basilar arteries can lead to ischemic damage of the brain (i.e., stroke). SAH is associated with elevated AVP levels (Doczi et al., 1981), and studies in rat models of SAH-induced basilar artery vasospasm have revealed that elevated local AVP levels contribute to the vasospastic condition (Delgado et al., 1988; Trandafir et al., 2004). Vascular potassium channels have been suggested as promising therapeutic targets to alleviate cerebral vasospasm (Sobey, 2001), but as yet, no effective clinical therapy has emerged. If Kv7 channel openers can oppose the vasoconstrictor effects of AVP on basilar arteries, as demonstrated in vitro with rat mesenteric arteries (Mackie et al., 2008), they may be of therapeutic benefit in the prevention of vasospasm and stroke after SAH.

There is continued interest in the pharmacology of neuronal Kv7 channels, with hopes of developing better agents for the treatment of epilepsy, neuropathic pain, and other central or peripheral nervous system disorders. Several selective modulators of Kv7 channel subtypes have been investigated or are currently in development (Munro and Dalby-Brown, 2007; Gribkoff, 2008; Xiong et al., 2008). As drugs become available that selectively activate subtypes of Kv7 channels, it may become clear whether the vascular channels can also be selectively targeted for therapeutic benefit. Most of the pharmacological characterization of Kv7 channel modulators is derived from experimental results obtained using expression systems in which one or more exogenous Kv7 channel subtypes is overexpressed. Expression systems may have limited utility for predicting vascular effects of these drugs because it is likely that subunit stoichiometry, including KCNE family β subunits and other auxiliary components of the native vascular channels, will determine the effects of Kv7 channel modulators. Nonetheless, based on the KCNQ subtype expression pattern observed in rat and mouse arteries (Yeung et al., 2007; Mackie et al., 2008), it may be reasonable to predict that drugs which affect Kv7.4 and/or Kv7.5 channels in expression systems will be most likely to have vascular effects, whereas drugs that selectively modulate Kv7.2/7.3 channel activity may have relatively few direct vascular effects. Along these lines, it is of interest that a Bristol-Myers Squibb compound, (S)-N-[1-(3-morpholin-4-yl-phenyl)-ethyl]-3-phenyl-acrylamide [(S)-1], was found to be a much more effective activator of Kv7.4 and/or Kv7.5 currents compared with Kv7.2 or Kv7.2/7.3 currents in a *X. laevis* oocyte expression system (Bentzen et al., 2006). The ability to enhance the Kv7 currents is likely dependent on the presence of a conserved tryptophan residue in the fifth transmembrane domain of Kv7.2 to Kv7.5 that is missing in Kv7.1; mutating this residue abrogated the enhancement of Kv7.4 currents by (S)-1, as well as that by retigabine and BMS204352 (Bentzen et al., 2006). BMS204352 has also been found to exhibit some selectivity for Kv7.4 and Kv7.5 versus Kv7.2/7.3 channels (Korsgaard et al., 2005); this may relate to its ability to abolish inactivation of the Kv7.4 channels (Jensen et al., 2007).

Conclusions

Kv7 channels in vascular smooth muscle cells and in baroreceptor neurons have been demonstrated recently to serve as important regulators of cardiovascular function. Although the vascular Kv7 channel studies have so far been limited to animal models, if human arteries express a similar complement of functional Kv7 channels, the implications are broad. Cardiovascular side effects of currently used Kv7 channel modulators may not have been thoroughly evaluated, and the potential to use these drugs to treat cardiovascular diseases has yet to be explored. It is also a distinct possibility that U.S. Food and Drug Administration-approved drugs of other classes might have previously unrecognized effects on vascular Kv7 channels. Suppression or activation of vascular Kv7 channels could account for unexplained differences in efficacy and/or unwanted side effects of some widely used therapeutic agents. Elucidating Kv7 channel-dependent cardiovascular effects may lead to the development of improved therapeutic regimens for drugs already in clinical use and new applications for this class of drugs for treatment of cardiovascular diseases such as hypertension and stroke.

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