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Involvement of C-type inactivation gating in the actions of voltage-gated K^+ channel inhibitors

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ABSTRACT

Voltage-gated K^+ (Kv) channels serve multiple functions. Besides the most well known function of controlling membrane excitability, they may also play roles in cell death and differentiation. Pharmacological activators and inhibitors of Kv channels therefore offer potential therapeutic treatments for a variety of diseases. Inhibition of Kv channels by classical blockers such as tetraethylammonium and 4-aminopyridine, and toxin peptides such as scorpion toxins, are believed to result from a direct intervention or occlusion of the K^+ permeation pathway. During prolonged depolarization, most Kv channels undergo a process called slow or C-type inactivation, by which the selectivity filter destabilizes and thus limits K^+ flux. Increasing amount of evidence shows that there are certain compounds which inhibit Kv currents not by directly obstructing the K^+ conduction pathway, but by accelerating or intensifying selectivity filter destabilization once the channels open. This mode of block represents an alternative mechanism of Kv channel inhibition. Indeed, some of the classical Kv channel blockers are to some extent, or in certain circumstances, involved in hastening slow inactivation. This review begins with a brief description of structure–functions of Kv channels, and then discusses the multiple mechanisms of Kv channel inhibition by classical blockers and how certain compounds inhibit Kv channels by accelerating C-type inactivation. The pharmacological and therapeutic potentials of these C-type inactivation-dependent Kv channel inhibitors are discussed.

Contents

1. Introduction

K^+ channels are integral membrane proteins which provide selective conduction pathways for K^+ flux along the electrochemical gradient. Their general role is to stabilize the cell membrane electrically. Voltage-gated K^+ (Kv) channels open upon depolarization and the K^+ efflux accounts for the repolarization phase of an action potential or acts by reducing the neuronal spiking frequency (Hille, 2001). Blocking Kv channels pharmacologically prolongs the duration of an action potential. Lack of expression, mutation or reduced activities (loss-of-functions) of certain Kv channel members may lead to neuronal hyperexcitability in rodents or humans, manifested as neurological disorders such as ataxia and epilepsy (Browne et al., 1994; Bernard et al., 2004; Maljevic et al., 2008; Miceli et al., 2008).

Besides their most well known function in dampening cellular excitability, convincing evidence supports the involvement of Kv

Abbreviations: 4-AP, 4-aminopyridine; AHR, 6β-acetoxy-7α-hydroxyroyleanone; AVE0118, (2′-{[2-(4-methoxy-phenyl)-acetylamino]-methyl}-biphenyl-2-carboxylic acid (2-pyridin-3-yl-ethyl)-amide); KN-93, 2-[N-(2-hydroxyethyl)]-N-(4methoxybenzenesulfonyl)amino-N-(4-chlorocinnamyl)-N-methylbenzylamine; Kv channels, voltage-gated K^+ channels; QA, quaternary ammoniums; TEA, tetraethylammonium.

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channels in cell death and differentiation of a wide variety of cells. For instance, there is an overexpression of Kv channels during apoptosis of neurons, immune cells and muscle cells (Yu et al., 1997; Yu, 2003). The massive K^+ efflux through over-expressed Kv channels leads to loss of intracellular K^+ and the reduced $[K^+]_i$ results in relief of inhibition (thus activation) of pro-apoptotic caspases and nucleases (Hughes et al., 1997; Hughes & Cidlowski, 1999). Kv channels have also been implicated in the axon development in *Xenopus* retinal cells, as pharmacological inhibition of Kv channels suppresses axon extension and produces a prominent effect on guidance cues (McFarlane & Pollock, 2000; Pollock et al., 2005). More recently, it was found that K^+ efflux through Kv1.1, Kv1.4 and Kv2.1 was responsible for cAMP-stimulated neuritogenesis in mouse neuroblastoma N2A cells (Leung et al., 2011). All these evidence suggests that Kv channels are versatile molecules which have a variety of functions in addition to provision of K^+ efflux for repolarization.

Specific Kv channel blockers are useful tools for probing the functional roles of Kv channels. On the other hand, Kv channel blockers may offer therapeutic opportunities by their ability to enhance cellular excitability. Augmenting conductivity of demyelinated axons via inhibition of Kv channels by 4-aminopyridine (4-AP) is a potential therapeutic treatment of multiple sclerosis (Judge & Bever, 2006). Kv channel blockers such as 4-AP and tetraethylammonium (TEA) could be employed to prevent neuronal apoptosis by suppressing excessive K^+ efflux (Yu et al., 1997; Yu, 2003; Hu et al., 2006). The potential of using Kv channel blockers for neuroprotection is discussed in a recent review (Leung, 2010).

The block of Kv channels by classical blockers (TEA and 4-AP) is fast, and it is believed that these molecules directly interfere with the K^+ conduction pathway or even plug the channel pore. A process by which Kv channels limit K^+ efflux during continuous depolarization is destabilization or distortion of the selectivity filter (Hoshi et al., 1991; see Kurata & Fedida, 2006 for an excellent review). This has been coined slow inactivation or C-type inactivation (see next section). Increasing amount of evidence has shown that there are some compounds which do not appear to act by directly obstructing the pore; instead, they inhibit the channel by hastening or intensifying the destabilization of the selectivity filter. This review begins by describing the fundamental structure-function aspects of Kv channel gatings and then recapitulates some basic understanding of the actions of classical Kv channel blockers (especially TEA). The observations that some classical blockers, to certain extent or under special circumstances, are involved in promoting Kv channel slow inactivation are explained. The mode of actions of C-type inactivation-dependent blockers, as exemplified by several recently reported drugs, will be elaborated. Finally, the pharmacological and potential therapeutic values of C-type inactivation-dependent blockers are discussed.

2. Voltage-gated K^+ channel gating and C-type inactivation

2.1. Voltage-gated K^+ channel gating

Based on sequence homology, Kv channels are classified into Kv1 to Kv12 subfamilies; each Kv subfamily contains multiple subtype members. The α-subunit of Kv channel is the conducting subunit, which is a tetramer of four polypeptides clustering around a central pore (Choe et al., 1999). There are six transmembrane helices (S1–S6) in each polypeptide. The S5–S6 linker is a pore-forming P-loop, and the P-loops from the four polypeptides orientate to constitute the K^+ selectivity filter (Choe et al., 1999; Yellen, 2002). The selectivity filter possesses the T(V/I)GYG signature motif, and the carbonyl oxygen atoms lining it act as hydration shell surrogates for the dehydrated K^+ ions to pass through (Doyle et al., 1998). S4 is the major sensor of voltage (depolarization); it moves outward when the plasma membrane is depolarized and S5–S6 helices then undergo conformational changes, thus opening the cytoplasmic activation gate to allow K^+ efflux (Choe et al., 1999; Yellen, 2002).

On the basis of gating behaviors, Kv channels could be broadly classified into two major types, namely, A-type K^+ channels and delayed rectifiers. A-type K^+ channels, exemplified by Kv1.4, Kv3.3 and Kv4.2, are fast-inactivating Kv channels with low activation thresholds (around −60 to −40 mV) (Hollerer-Beitz et al., 1999; Hille, 2001; Sacco et al., 2006). These channels serve to control the spiking or firing frequency. The fast inactivation, usually with time constants around tens of milliseconds, results from the rapid occlusion by the cytoplasmic N-terminus at the internal vestibule of the opened channel — "ball-and-chain mechanism" (Hoshi et al., 1990; Zagotta et al., 1990; Hoshi et al., 1991; Kurata & Fedida, 2006). Therefore fast inactivation is also called N-type inactivation. Delayed rectifiers (e.g. Kv1.2, Kv2.1, Kv3.1) have higher activation thresholds (around −30 to −20 mV) and inactivate very slowly, usually with time constants in the range of seconds. Such slow inactivation (also termed C-type inactivation) is generally thought to involve a localized conformational change at the channel outer pore mouth, such as a distortion or destabilization of the selectivity filter during persistent depolarization (Hoshi et al., 1991; Kurata & Fedida, 2006; Cordero-Morales et al., 2007) (see Fig. 1). However, slow inactivation may also involve more global conformational change of the channel (see below).

2.2. C-type inactivation gating

In Shaker channels, the role of T449, a residue at the external pore mouth, in C-type inactivation has been studied in details. Mutation of this amino acid to arginine, lysine, alanine or glutamate accelerates C-type inactivation, while mutation to valine or tyrosine retards C-type inactivation (Lopez-Barneo et al., 1993). However, how pivotal this residue determines C-type inactivation rate is still questionable, as this site does not appear to produce similar effects in mammalian Kv1 C-type inactivation (Rasmusson et al., 1995; Fedida et al., 1999).

A mechanistic model was proposed in which the interaction strength (via hydrogen bonds) between residues in the selectivity filter and the adjacent pore helix is crucial for C-type inactivation (Cordero-Morales et al., 2007). These authors adopted the prokaryotic proton-activated KcsA channel as a model. Upon extracellular acidification, KcsA channels open and immediately undergo C-type inactivation at a decent rate. Interaction via hydrogen bonds between E71 and D80 (equivalent to Shaker 438 and 447) is important in controlling C-type inactivation rate. Mutating glutamate at 71 to several amino acids including glycine, alanine and valine strongly retards inactivation. Kv1.2 WT channels inactivate very slowly. Introducing equivalent mutations in Kv1.2 yields expected results. Thus, in Kv1.2, mutating Val370 into Glu370 greatly speeds up inactivation as this manipulation renders possible a stronger interaction with Asp379; such interaction acts like a spring to pull and collapse the selectivity filter (Cordero-Morales et al., 2007). Recent evidence reveals that hydrogen bonding between D80 and another amino acid, W67, is also crucial in determining the rate and extent of C-type inactivation in the KcsA channel (Cordero-Morales et al., 2011). Similarly, it was shown in the same work that W434–D447 interaction and W366–D379 interaction are crucial in controlling C-type inactivation in Shaker and Kv1.2, respectively.

It is interesting to note that the V370–D379 interaction (Fig. 2) in the Kv1.2 channel or the E71–D80 interaction in the KcsA channel does not only control C-type inactivation (pore stability), but also affects K^+ selectivity (Chao et al., 2010; Cheng et al., 2011). In these reports, it was shown that weakening such interactions (thus reduced C-type inactivation) results in increased Na^+ permeability. Therefore, mechanisms governing C-type inactivation appear to be intimately coupled to the control of cationic selectivity.