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Mibefradil inhibition of the Cole-Moore shift and K$^+$-conductance of the tumor-related Kv10.1 channel

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The KCNH1 gene codes for the K$^+$-selective, non-inactivating, voltage-dependent channel, referred to as Kv10.1 or Eag1.[1,2] Outside the central nervous system Eag1 expression is associated with a cancerous phenotype.[3,4] This was first demonstrated by the laboratory of Luis A Pardo and Walter Stühmer who showed that CHO cells upon stably expressing Eag1, but not other K$^+$ channels, acquire the hallmark characteristic of cancer cells; and that injection of Eag1 expressing cells to immunosuppressed mice leads to the development of massive tumors in these animals.[4]

Naturally, the question arises as to what particular structural characteristic(s), as compared with other K$^+$ channels, endow(s) Kv10.1 with its tumor promoting features, and which of them could be pharmacologically targeted in an effort to lessen the tumorous effect of Kv10.

The Eag family presents a unique structural characteristic, namely a cytoplasmic, Per-Arnt-Sim (PAS) domain, which interacts with the voltage-sensor (S1 to S4 segments) domain.[5,6] PAS domains are present in proteins engaged in pathways that regulate cellular responses to environmental stimuli,[7] as such these domains mediate protein-protein interactions and bind diverse chemical molecules.[7,8] These properties suggest that PAS domain could be an interesting target to test drugs aimed to lessen the tumor promoting properties of Kv10.1.

In addition, Eag1 channels present other unique structural, and functional, features, for example:

1) Eag1 channels present a long pore-loop that forms glycosylated helical structures which surround the pore opening. It has been proposed that this arrangement may hinder the binding of peptide toxins.[9] This means that in the quest to find pharmacological tools against Kv10.1 studies using non-peptide compounds, as mibefradil, are instrumental.

2) The pore lumen presents a rather uniform diameter along its full length. In other words Eag1 channels do not present the pore widening, or central cavity of KscA and Shaker-related channels.[9,10] The central cavity is the place where internally added TEA,[11] and numerous hydrophobic cations, as for example quinidine (e.g., reference 12 and references therein cited), blocks the open-pore of Shaker-related channels.[11]

3) Eag1 channels endure a voltage-dependent gating even in the absence of a covalent linkage between S4 and S5 segments.[13] This shows that communication between voltage-sensor and pore modules presents characteristics not shared with canonical Kv channels.[9,13,14]

The aforementioned properties indicate that care must be taken to translate drug-interaction models, initially developed on canonical Shaker-related K$^+$ channels, to Eag1 channels.

In addition to the above mentioned features, a functional hallmark of Eag channels is the presence of both, a noticeably lag or time-shift for current surge,[15] and the development of a markedly sigmoidal time course of current activation, as the holding potential (HP) becomes more negative;[16,17] the latter arises from a rate limiting transition that

accompanies the passage from deep closed states to sates near the open state (e.g; references 9,16,17 and references therein cited). This combined HP effect is customarily referred to as the Cole-Moore shift of Eag channels.

Recently, we studied the effect of the anti-hypertensive drug mibefradil on Kv10.1, and found that it exerts 2 major effects, depending on the initial conditions and on the time window of channel activity.[18] Upon prolonged depolarizations, externally added mibefradil induces an apparent open-state inactivation, that inhibits the K^+ conductance, and shifts the conductance (G) vs. voltage (V) curve to the left. On the other hand, by looking at the first tens of milliseconds of channel activity it was found that, strikingly, mibefradil inhibits the Cole-Moore shift of Kv10.1 (see Fig. 1), pointing out to the voltage-sensor domain as a site of action of mibefradil.[18]

The exhaustive Competition-plot of Cornish-Bowden,[19] performed between quinidine and mibefradil, along with other observations, excluded open-pore block as the mechanism underlying mibefradil-induced inactivation. Hence, we concluded that both the mibefradil inhibition of the Cole-Moore effect and its induced inactivation were the result of the drug binding within the voltage-sensor domain.
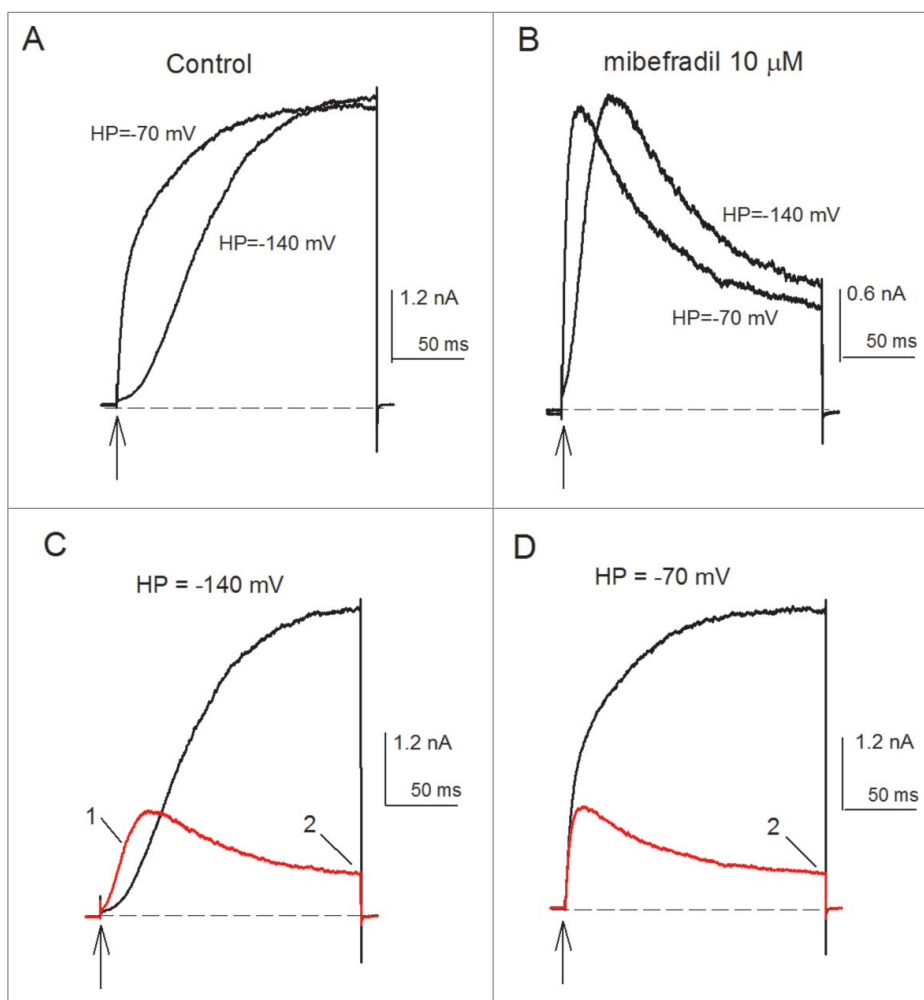

Figure 1. Mibefradil inhibition of the Cole-Moore shift and K^+-conductance of Kv10.1 channels. (A) Control I_K evoked by a 200-ms pulse to +30mV applied from the HP of either -70 or -140 mV, as indicated. The rate of I_K activation depends on the HP from which channels are activated. I_K activated from -140 mV is markedly slower than I_K evoked from the HP of -70mV. (B) As in A, but in the presence of 10 μM mibefradil. (C) Superposed I_K in control (black trace) vs. 10 μM mibefradil conditions (red trace). The currents were activated as in A from the HP of -140 mV. Notice the clear mibefradil inhibition of the Cole-Moore shift. As a consequence of this, the initial I_K of mibefradil-modified channels surpasses control I_K (labeled 1). Also note the marked mibefradil inhibition of G_K at pulse end (labeled 2). (D) As in C but currents were activated from the relatively depolarized HP of -70 mV. Note that, as expected, in this case mibefradil only inhibits G_K at pulse end. The vertical arrows indicate the time of delivery of the constant +30 mV activation pulse. The dashed line is the zero current level.

Regarding the above, and supporting our proposal of the voltage-sensor domain as the site of action of mibferdil, it has been reported that (a) amino-acid deletions of the PAS domain inhibit the Cole-Moore effect, shift the G vs. V curve to the left, and induce an open-state inactivation,[6] similar to the effects of mibefradil on Kv10.1; on the other hand, regarding the prototypical Shaker B channel it was reported that, (b) progressive elimination of functional S4 segments, performed by neutralizations of arginine residues, shifts the G vs. V curve to the left, and inhibits the Cole-Moore shift. The authors hypothesized that the introduced mutations left S4 segments in a pre-activated like state.[20] Similar to our hypothesis regarding the gating modification of Kv10.1, namely that mibefradil binds to the voltage-sensor domain inducing an apparent inactivation and accelerating the rate limiting transition that accompanies the passage from deep closed states to states near the open state.[17,18] It remains to be determined where on the voltage-sensor domain mibefradil binds, thus exerting the aforementioned effects, and how this relates to the particular way of communication between voltage-sensor and pore domains of Kv10.1.

Disclosure of potential conflicts of interest

No potential conflicts of interest were disclosed.

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