Fungal toxins as pharmacological tools to investigate BK ion channel function

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Large conductance calcium-activated potassium (BK) channels are widely distributed throughout vertebrates, having roles including regulation of blood pressure and in urinary bladder function, where they limit cellular excitability. In the brain they are important for motor coordination, contributing to action potential repolarization and regulation of neuronal firing patterns. How pharmacological modulation of BK channels affects motor function is not well understood. The fungal indole diterpene, lolitrem B, isolated from endophyte-infected ryegrass, potently inhibits BK channels. It is also known to impair motor function as it is the main causative agent in ryegrass staggers, a neurotoxic disorder in grazing animals characterised by tremors and uncoordinated movement. We therefore used lolitrem B as a pharmacological tool to investigate the role of BK channels in motor coordination and explored its mechanism of BK channel inhibition. Using other structurally related lolitrem compounds we have identified key structural characteristics of lolitrems that are important for BK channel inhibition. In patch-clamp experiments, macroscopic currents were recorded using inside-out membrane patches from FLP-in 293 cells stably expressing *hSlo*. Channels were activated by depolarizing membrane voltages and intracellular calcium. The degree of inhibition by lolitrem B was not voltage-dependent, but was affected by the intracellular calcium concentration. Our results suggest that the inhibitory action of lolitrem B is state-dependent and is affected by the conformational state of the channel when different calcium binding sites are occupied. Experiments using BK channel knock-out mice demonstrated that BK channels are required for the neurotoxic effects of lolitrem B.