Mode of action of tremorgens

S.C. FINCH[1], W.L. IMLACH[2], J. DUNLOP[2], A.L. MEREDITH[3], R.W. ALDRICH[3] and J.E. DALZIEL[2]
[1]*AgResearch Ltd, Ruakura Research Centre, PB 3123, Hamilton, New Zealand*
[2]*AgResearch Ltd, Grasslands Research Centre, PB 11008, Palmerston North, New Zealand*
[3]*Department of Molecular and Cellular Physiology and the Howard Hughes Medical Institute, Stanford University, California 94305, USA.*
sarah.finch@agresearch.co.nz

Ryegrass staggers is a neurotoxic disorder of livestock grazing perennial ryegrass (*Lolium perenne* L.) infected with the endophytic fungus *Neotyphodium lolii*. Ryegrass staggers was first reported in 1880 (Anonymous 1880) although at this stage the cause of the observed tremorgenic disorder was unknown. The presence of an endophyte within perennial ryegrass was first recognised in 1935 (Neill 1941) but it was not until 1981 that the correlation between endophyte infection and the incidence of ryegrass staggers was established (Fletcher & Harvey 1981; Mortimer *et al*. 1982). The tremorgenic compound, lolitrem B, produced by the endophyte was then isolated and implicated in the disease (Gallagher *et al*. 1981, 1982, 1984). The future then seemed clear: eradicate the endophyte and solve the ryegrass staggers problem. Endophyte-free plots, however, showed little growth and suffered severe damage from the larvae of Argentine Stem Weevil (Mortimer *et al*. 1982). The correlation between endophyte levels, weevil numbers and tiller damage was then made (Prestidge *et al*. 1982) and a few years later the beneficial effects of endophyte were shown to be due to peramine, an anti-feedant produced by the fungus (Gaynor & Rowan 1986; Rowan *et al*. 1986).

Determining the mechanism of action of ryegrass staggers proved even more elusive. Initial research focussed on amino acid neurotransmitters (Norris *et al*. 1980; Selala *et al*. 1989) but this proved inconclusive. Furthermore, classical techniques to investigate receptor sites such as using brain slices or synaptosomes were unsuccessful due to the high lipophilicity of the lolitrems (Munday-Finch & Garthwaite 1999). Recent work has been more successful. The structurally related indole-diterpenoid, paxilline, which is also tremorgenic, was shown to inhibit large conductance calcium-activated potassium (BK) channels (Knaus *et al*. 1994). More recently, lolitrem B has similarly been shown to potently inhibit these channels (Dalziel *et al*. 2005). BK channels are widely distributed throughout vertebrates and are important in the regulation of cellular excitability, having roles in the regulation of blood pressure (Brenner *et al*. 2000), urinary bladder function (Meredith *et al*. 2004) and motor coordination (Sausbier *et al*. 2004). Both paxilline and lolitrem B inhibit the pore-forming subunit of the BK channel protein (known as the α subunit or *Slo*) but each subunit can co-assemble with one of four differentially expressed accessory ß subunits that contribute to the functional diversity of these channels in different tissue types (Wallner *et al*. 1999). Of particular interest is the ß4 subunit which is highly expressed in the brain.

The purpose of this study was to investigate whether the ataxia and tremor induced by lolitrem B and paxilline is due to the inhibition of BK channels. Using BK channel knockout mice we have shown that this is indeed the case. Therefore, 127 years after the first report of ryegrass staggers, the biological site of action of lolitrem B has been identified.

Keywords: BK channel, endophyte, lolitrem, paxilline, mechanism

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