The Role of γ-aminobutyric acid (GABA) and Glutamate in Harmaline-Induced Tremors in Mice

S.A. Malekiani, N.E. Paterson, M. Foreman* and T. Hanania
PsychoGenics Inc., Tarrytown, NY,*Formerly of JAZZ Pharmaceuticals, Palo Alto, CA

Introduction

> Essential Tremor (ET) is one of the most common movement disorders which is thought to be distinct from Parkinson’s disease. In general, ET is prevalent in the elderly and does not lead to serious complications. However, it has been shown that ET can be debilitating in a small percentage of patients.

> Though the causes of ET are unknown, genetics are thought to play a role in about 50% of ET cases. Three gene loci are known (ETM1 on 3q13, ETM2 on 6p23, and a locus on 1p21p2) have been identified in a familial form of ET. In addition, post mortem studies of ET patients revealed higher levels of glutamate and decrease levels of γ-aminobutyric acid (GABA) and serine in brains from ET patients (Kralic et al., 1990).

> Animal models for ET are scarce. Two rodent models that have been used to study ET are the hamster animal model and the GABA receptor agonist 3-amino-butyric acid (GABA). The hamster model is associated with increased risk to develop ET (Jankovic et al., 1990, but see Blair et al., 2000).

> Harmaline is a beta-carboline derivative that causes generalized tremor in mice and has a frequency at 11 – 14 Hz (Miller et al., 1995). Harmaline acts on the neurons of the inferior olivary nucleus (ION) to modulate their rhythmic-generating ionic currents and thereby resulting in generalized tremor. Harmaline-induced tremors in mice can be alleviated by beta2-adrenergic receptor agonist such as doxepin (Rappaport et al., 1984, Martin et al., 2003) as well as propranolol (Martin et al., 2003) suggesting an adrenergic component.

Hypothesis

GABAergic, glutamatergic and dopaminergic systems underlie some of the mechanisms of harmaline-induced tremors in mice.

Methods

Make ICR mice from Taconic Laboratories (Germantown, NY) were used in this study. Upon receipt, mice were assigned unique identification numbers (tail marked) and group housed in IVC ventilated cages. All animals remained housed in groups of four for the remainder of the study. Mice were given access to food and water ad libitum. The light on at 0500 am EST. The room temperature was maintained between 20 and 23°C, with a 12-hr light/dark cycle with the light on at 6:00 a.m. EST. Mice were maintained on an ad libitum diet.

Group housed mice were brought to the experimental room for at least one hour to acclimate prior to testing. Following a 20 min pretreatment with vehicle or test compounds, mice were injected with harmaline (30 mg/kg) and placed into the Tremor Monitor (Tremor Technology, S1D) chamber for 10 minute acclimation period following. Habituation, tremor activity of the mice was measured for approximately 5 min. The recorded frequencies (164 herz) of activity and the number of tremor events were captured electronically.

Data are analyzed by the tremor monitor software (SD1 in a two part process. Using a Fast Fourier Transform (FFT), an output is provided showing the percentage of activity recorded at each frequency. A center frequency of activity between 15 - 15 Hz is chosen, along with a bandwidth of 15 Hz. Using these parameters, tremor events are tabulated as total tremor events.

Statistical Analysis

Data were analyzed using analysis of variance (ANOVA) followed by Fisher’s LSD test when appropriate. An effect was considered significant if p < 0.05.

Figure 1: Fast fourier transform output for the effects of propranolol on harmaline-induced tremors

Figure 2: Lithium and Carbamazepine dose-dependently decrease harmaline-induced tremors

Figure 3: GABA and GABA receptor agonists muscimol and baclofen decrease harmaline-induced tremors

Figure 4: N-methyl-aspartate receptor antagonists decrease harmaline-induced tremors

Figure 5: The dopamine agonist apomorphine decreases harmaline-induced tremors

Table 1: Effects of AMPA and mGlu5 receptor antagonists on harmaline-induced tremors

Mice were injected with vehicle, the mood stabilizer lithium, or the anticonvulsant carbamazepine 30 min prior to harmaline. Data represent mean ± SEM. *significantly different from vehicle (p<0.05)

Summary

> In ICR mice, injection of harmaline produces tremors that can be quantified based on the frequency range of 10 – 14 Hz.

> The most stabilizer lithium and the anticonvulsant carbamazepine showed a dose-dependent attenuation of harmaline-induced tremors.

> The GABAergic involvement in harmaline-induced tremors is supported by our findings showing attenuation of harmaline-induced tremors by GABA, GABAa, and GABAb receptor agonists.

> These data also support the possibility of glutamatergic mechanisms underlying harmaline-induced tremors. Both competitive and non-competitive NMDA receptor antagonists decrease harmaline-induced tremors.

> SKF and MK-801 can increase dopaminergic tone and we have previously shown that the dopamine transporter inhibitor GBR12909 decreased harmaline-induced tremors, we investigated some of the dopaminergic systems involved. The data presented show that the dopamine agonist apomorphine attenuated harmaline-induced tremors. This effect could be primarily on activation of D2 receptors since quinpirole, a D2 receptor agonist, significantly decreased harmaline-induced tremor at all doses tested. Interestingly, D1 receptors do not appear to play a role in the decrease of harmaline tremor via D2 receptor agonist SKF25858 had no effect on harmaline-induced tremors.

> The harmaline-induced tremor model in mice continues to provide a valuable and functional model for screening novel compounds for treatment of ET.

References