

Riluzole Ameliorates Harmaline-induced Tremor in Rat

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ABSTRACT

Introduction: Excessive olivo-cerebellar burst-firing occurs during harmaline-induced tremor. We hypothesized that ant glutamatergic agents would suppress harmaline tremor. From this point of view, the aim of the present study was to investigate the effects of riluzole on harmaline-induced tremor in rat.

Methods: Four groups of Wistar rats weighing 80–100 g were injected with harmaline (30 mg/kg i.p.) for inducing experimental tremors. The rats in group 1 served as control, whereas the animals in groups 2, 3 and 4 were also given riluzole intraperitoneally at doses of 2, 4 and 8 mg/kg 30 min before and 90 min after harmaline administration. The onset latency, intensity and duration of tremor were recorded.

Results: The results of this study demonstrated that riluzole could significantly increase latency period, and reduce duration and intensity of tremor.

Discussion: It is concluded that pretreatment of riluzole can ameliorate harmaline-induced tremor in rats.

1. Introduction

Essential tremor (ET) is regarded as one of the most common neurological disorders of adults, with a prevalence similar to or greater than that of stroke, Alzheimer disease, migraine and lumbosacral pain syndromes and is as much as 20 times more prevalent than Parkinson's disease. The incidence of ET increases with advancing age, but it is fairly common in all age groups and almost equal in men and women. Although ET is often viewed as a benign problem, almost all the patients with ET are disabled to some extent (e.g., difficulty with or inability to perform daily activities such as writing, feeding, or dressing) and around 15% are sufficiently motorically impaired due to continuous high amplitude shaking that they are unable to continue to work (Tariq et al., 2002; Bain et al., 1994). Essential tremors result

from both physiologic and pathologic processes in the nervous system, and always involve the interaction of central and peripheral nervous systems. Due to lack of understanding of the basic mechanism and origin of tremors, it is difficult to develop pharmacological agents with selective and specific antitremor activity.

The cerebellum is generally accepted to be involved in the control and integration of motor processes as well as of cognitive functions. Several studies suggested an important role of this structure in pathological processes underlying different forms of tremor, schizophrenia, attention deficit, and Parkinson's disease (PD). Abnormal activation of climbing glutamatergic fibers, arising from the inferior olive, which induces synchronous firing of Purkinje cells of the cerebellar cortex, assumed to be a "pacemaker" responsible for development of essential tremor (DeLong, 1978).

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Harmaline, a derivative of β -carboline is a well-known tremorigenic compound suggested as a model for essential tremor in animals (Miwa, 2007). Harmaline induces the action and postural tremor in several animal species which is manifested by the tremor of fore and hind limbs, head tremor or generalized tremor of the whole body (Miwa, 2007; Milner et al., 1995; Wang, & Fowler, 2001; Kolasiewicz et al., 2009). Oscillation frequency in this tremor decreases with increasing the weight of an animal and is equal to 11 – 14 Hz in mice, 10 – 12 Hz in rats and 8 – 10 Hz in monkeys (Miwa, 2007). However, synchronous activation of the olivo-cerebellar pathway and release of glutamate in the cerebellum which acts at NMDA and AMPA receptors is suggested to be a primary cause of the harmaline-induced tremor (Miwa, 2007; Beitz & Saxon, 2004; Paterson, et al., 2009).

Much attention focused on neuroprotection as a strategy in therapies for neurodegenerative diseases as means of preserving the synaptic and/or intrinsic properties of neurons. Riluzole (2-amino-6-trifluoromethoxybenzothiazole), a Ca^{2+} -dependent K^+ channel opener with anti-glutamatergic activity can slow the progression of disease in patients with amyotrophic lateral sclerosis and is approved for treatment of this disorder in several countries (Bensimon et al., 1994; Lacomblez et al., 1996). Riluzole is neuroprotective in animal models of acute and chronic neurodegenerative disease (Stutzmann & Doble, 1994; Mary et al., 1995), including rat and primate models of PD (Benazzouz et al., 1995) and 3-acethyl pyridine model of ataxia in rats (Janahmadi et al., 2009).

Riluzole inhibits the presynaptic release of excitatory amino acids both in vitro (Martin et al., 1993) and in vivo (Cheramy et al., 1992). This mechanism proposed as the one responsible for the drug's neuroprotective effects. However, riluzole is shown to have modulatory effects on the membrane ion channels.

Thus, the beneficial neuroprotective action of riluzole on the motor diseases and its anti-glutamatergic effect, prompted us to examine the role of riluzole on harmaline-induced tremor in rats.

2. Methods

2.1. Drugs and Chemicals

Harmaline HCl and Riluzole were purchased from Sigma-Aldrich, Germany. All compounds were prepared freshly on the day of the experiment. Harmaline HCl and riluzole dissolved in normal saline and solution of 0.1N HCl, respectively.

2.2. Animals

Male Wistar rats (weighing 80-100 g) were purchased from Pastur Institute of Iran. The animals were kept under standard laboratory conditions with a 12-h light/dark cycle and given ad libitum access to food and water throughout the experiments. The experimental procedures approved by the Animal Ethics Committee of Damghan University.

2.3. Tremor Induction

On the day of experimentation, the animals were transferred to the testing room and left 1 h for acclimatization. They were randomly assigned to experimental groups and experimental tremors produced in four groups (ten rats each) of animals by a single injection of harmaline (30 mg/kg) intraperitoneally as previously described (Krahl et al., 2004). The rats in group 1 served as control (harmaline only), whereas, animals in group 2, 3, and 4 were given riluzole (i.p) at doses of 2, 4 and 8 mg/kg 30 min before and 90 min after harmaline administration. Riluzole dosage was selected on the basis of earlier reports which demonstrated its neuroprotective effects in ataxic rat (Janahmadi et al., 2009). The occurrence of tremors was rated by an observer blinded to treatment protocol. The period between the injection of harmaline and the appearance of the first symptoms of tremors was recorded as the time of onset of tremors. The duration of tremors was recorded as the time between onset and complete disappearance of tremors. The intensity of tremors at tremor onset and then every 30 min after harmaline administration over a 240-min period (until the tremors completely subsided and the animals became normal) were recorded. The clinical grading of tremor intensity was done according to Arshaduddin et al. method (2004) as follows: 0: no tremor, 1: mild tremor, 2: moderate intermittent tremor, 3: moderate persistent tremor and 4: pronounced severe tremor (Arshaduddin et al., 2004).

2.4. Statistical Analysis

The results expressed as the means \pm S.E.M. The analysis of intensity, duration, and latency period were undertaken using one-way ANOVA followed by Dunnett's multiple comparison tests. Differences with p-value <0.05 were considered significant.

3. Results

Harmaline administration to rats induced the characteristic pattern of tremors starting within 2.67 ± 0.23 min following administration and lasted for more than 4 h.

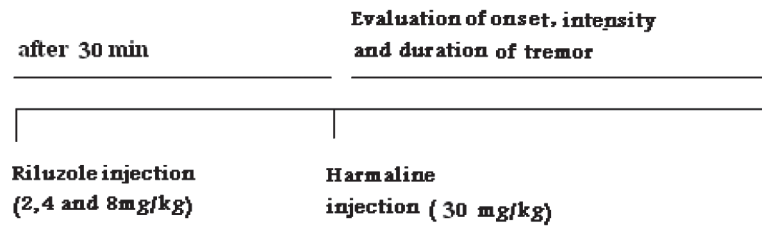


Figure 1. Timeline of the behavioral procedures.

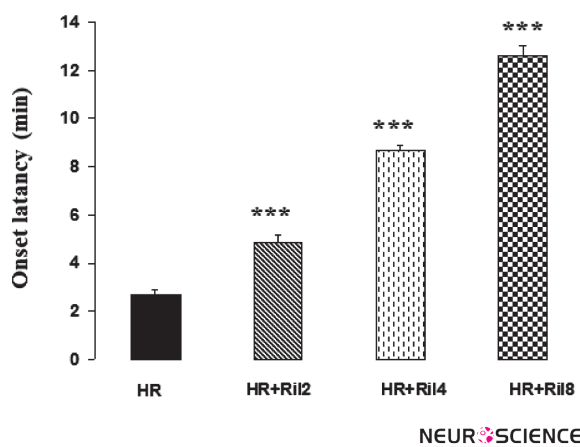


Figure 2. Effect of riluzole on onset latency of harmaline induced tremor.

Riluzole significantly reduced onset latency of tremor than harmaline treated group. The values are mean ± SEM. HR, Harmaline; Ril2, Riluzole 2mg/kg; Ril4, Riluzole 4mg/kg; Ril8, Riluzole 8 mg/kg.

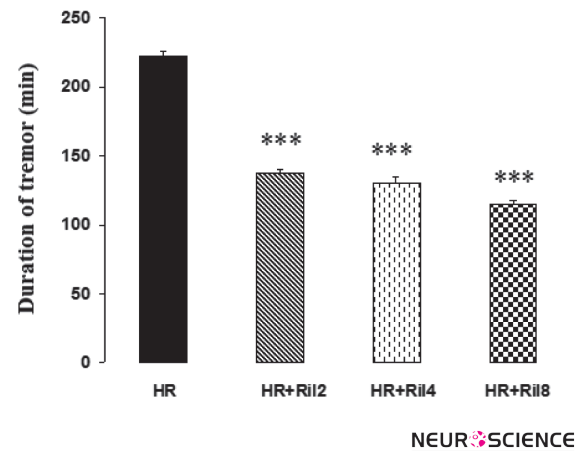


Figure 3. Effect of riluzole on duration of harmaline induced tremor.

Riluzole significantly reduced duration of tremor in comparison to harmaline group. The values are mean ± SEM. HR, Harmaline; Ril2, Riluzole 2mg/kg; Ril4, Riluzole 4mg/kg; Ril8, Riluzole 8 mg/kg.

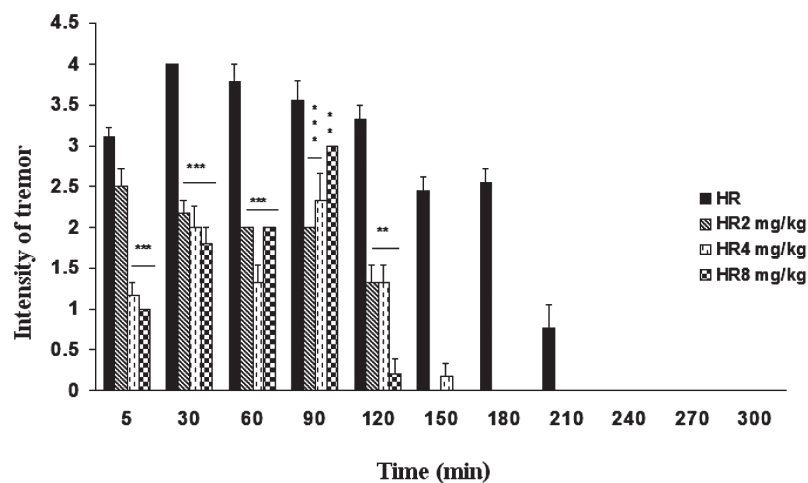
The tremor intensity at 5 min following harmaline administration was 3.1 ± 0.11 . The data showed that treatment of rats with riluzole (2, 4 and 8 mg/kg) produced significant decrease (137.5 ± 3.35 , 130.5 ± 3 , 115 ± 2.7 min; $p < 0.01$, dose 2, 4 and 8 mg/kg, respectively) in the duration of harmaline-induced tremor (222 ± 3.4 min). Riluzole also increased duration of onset of tremor ($p < 0.01$) in harmaline treated rats (Fig. 1, 2 and 3).

The intensity of the tremor at 30, 60 and 120 min following harmaline remained unchanged throughout this period. Treatment with riluzole in the doses of 2, 4 and 8 mg/kg resulted in a significant reduction in the intensity of the tremor at 30 min ($p < 0.001$), 60 min ($p < 0.001$), 90 min ($p < 0.001$, dose 2 and 4 mg/kg; $p < 0.01$, dose 8 mg/kg, respectively), 120 min ($p < 0.001$) and 150 min ($p < 0.001$, dose 4 mg/kg) (Fig. 4).

4. Discussion

The results of this study showed significant reduction of harmaline-induced tremors by riluzole. Reduction of harmaline-induced tremors was evident from delay in onset, decreased duration and severity of tremors in the rats treated with riluzole (Figs. 2, 3 and 4).

Administration of harmaline to rats induces severe tremor as previously reported beginning within a few minutes, and lasted for at least 3.5 h (Arshaduddin et al., 2004). The tremor was more pronounced upon movement and when the animals were not leaning against the wall of the cage. The motor activity was reduced and urination and defecation were increased. Harmaline produces tremors in the 8–12 Hz frequency range that is believed to be due to an enhancement of neuronal synchrony and rhythmicity in the inferior olive (Wilms et al.,



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Figure 4. Effect of riluzole on tremor intensity (total score over the 210 min period in 30 min bins) after harmaline administration (30 mg/kg, i.p.). The values are mean \pm SEM, ** $P < 0.01$ and *** $P < 0.001$ as compared with harmaline treated animals. HR, Harmaline; Ril2, Riluzole 2mg/kg; Ril4, Riluzole 4mg/kg; Ril8, Riluzole 8 mg/kg.

1999). Among other experimental models of essential tremor, the harmaline model is one of particular interest since it presents behavioral, metabolic imaging and pharmacological similarities to essential tremor. Furthermore, β -carbolines related to harmaline, are detected in patients suffering from essential tremor and induce severe tremors in humans (Pennes & Hoch, 1957).

Riluzole in the doses of 2, 4 and 8 mg/kg produced a highly significant reduction in the duration and intensity of harmaline induced tremor (Fig. 3 and 4). Our results are in agreement with earlier investigator who observed beneficial effects of memantine (NMDA receptor antagonist) on the harmaline model of transient action tremor (Iseri et al., 2011).

It is thought that genetic factors, age, ethnicity, and several toxic agents (such as β -carboline alkaloids and lead) are the risk factors for the occurrence of ET (Louis, 2005); however, the underlying mechanisms are not clearly identified. A widely accepted hypothesis for its etiology is the functional disturbance of olivo-cerebellar pathways (Wills et al., 1995; Deuschl and Elble, 2000). This hypothesis was derived from clinical and animal studies (treated by β -carbolines) showing over activity of cerebellar cortex and deep nuclei (Wills et al., 1995; Wilms et al., 1999). On the other hand, the results of recent studies (e.g., swelling of purkinje cell axons and purkinje cell loss) supported the idea that ET may have neurodegenerative features (Louis, 2010).

Experimental and clinical evidences implicated the role of glutamatergic system in ET (Eblen et al., 1996; Málly et al., 1996). Harmaline preferentially enhances synaptic activity of climbing fibers which originate in the inferior olive (Ryder et al., 2006). The activation of the climbing fiber system increases the level of excitatory amino acids, nitric oxide, and cGMP in the cerebellum that is believed to be responsible for molecular mechanism underlying harmaline-induced tremor, increased cerebellar blood flow, and neurotoxicity (O'Hearn and Molliver, 1997; Yang and Iadecola, 1998; Zhang et al., 2003; Beitz and Saxon, 2004).

As excessive synchrony among olivo-cerebellar ensembles could underlie ET, we hypothesized that glutamate release enhancement resulting of olivo-cerebellar synchrony following of harmaline administration can probably underlie tremor. Therefore, we hypothesized that glutamate content reduction by inhibitor of glutamatergic neurotransmission may suppress tremor.

Riluzole (2-amino-6-trifluoromethoxy benzothiazole) has neuroprotective, anticonvulsant, anxiolytic and anesthetic qualities. These effects are mediated by blockade of glutamate transmission, stabilizing of sodium channels and blockade of γ -aminobutyric acid (GABA) reuptake. Riluzole-induced neuroprotection was observed in various animal models of injury (Malgouris et al., 1989; Stutzmann et al., 1997) and in neurodegenerative pathologies, such as ALS and Parkinson's disease (Estevez &

Stutzmann, 1995; Jacquin & Gruol, 1999; Noh et al., 2000). Janahmadi et al. (2009), reported that combined riluzole (4 mg/kg) and 3-AP treatment can relatively improve 3-AP induced ataxia in rat (Janahmadi et al., 2009).

Also, there are several reports that riluzole can inhibit voltage gated Na⁺ channels (Herbert et al., 1994; Wang et al., 2008). Na⁺ channel blockage is proposed to be responsible for prevention of epilepsy and cellular death induced by this neuroprotective agent (Herbert et al., 1994).

Our previous study demonstrated that riluzole had therapeutic effects on harmaline-induced tremor and ataxia in rats. Riluzole could significantly reduce cerebellar glutamate content and Purkinje cell loss in harmaline-treated rat (Rahimi Shoormasti et al., 2012). In the present study, antiglutamatergic action of riluzole may presumably counteract the neurotoxic glutamatergic effect of harmaline and following tremor.

In conclusion, we suggest that riluzole might be a potentially useful choice in the treatment of essential tremor. The protective effect of riluzole probably related to its inhibitory effect on glutamatergic neurotransmission or its modulatory effect on ion channels. Therefore, the mechanism of action of riluzole in harmaline-induced tremor should be further investigated in more details.

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