The effect of memantine in harmaline-induced tremor and neurodegeneration

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A R T I C L E   I N F O

Article history:
Received 9 April 2011
Received in revised form 12 May 2011
Accepted 16 May 2011

Keywords:
Memantine
Harmaline-induced tremor
Neuroprotection
Tremor and locomotion analysis system

A B S T R A C T

Essential tremor (ET) is one of the most common and most disabling movement disorders among adults. The drug treatment of ET remains unsatisfactory. Additional therapies are required for patients with inadequate response or intolerable side effects. The current study aims to investigate the anti-tremogenic and neuroprotective effects of memantine (NMDA receptor antagonist) on the harmaline model of transient action tremor. The effects of memantine were further compared with ethanol. Three separate groups of male Wistar rats were injected either with saline, ethanol (1.5 gr/kg), or memantine (5 mg/kg) 15 min prior to a single intraperitoneal injection of harmaline (20 mg/kg). Tremor and locomotion were evaluated by a custom-built tremor and locomotion analysis system. After 24 h of harmaline injection, cellular viability, and apoptosis were assessed using crystal violet staining, and caspase-3 immunostaining, respectively. Harmaline caused neuronal cell loss and caspase-3 mediated apoptosis in cerebellar granular and purkinje cells as well as the inferior olivary neurons. Despite a reduction in tremor intensity and duration with ethanol, this compound resulted in cell loss in cerebellum and olivary nucleus. Memantine exhibited neuroprotective efficacy on cerebellar and inferior olivary neurons albeit weaker anti-tremor effect compared to ethanol. In conclusion, anti-tremogenic and neuroprotective effects do not necessarily overlap. Memantine is a potential treatment for ET particularly given its neuroprotective efficacy.

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1. Introduction

Essential tremor (ET) is one of the most common movement disorders with a pooled prevalence of (all ages) = 0.9%, and crude prevalence of (all ages) = 0.4% (Louis et al., 1998; Louis and Ferreira, 2010). It is characterized by a tremor of posture and action mostly involving the upper limbs. ET usually emerges in adulthood and progresses with age. Despite being classified as benign the tremor may be severe enough to disrupt occupational and social activities in some patients (Elble, 2001; Sullivan et al., 2004). Unfortunately, the pharmacological treatments of this disorder are still limited and ineffective. The oldest anti-tremogenic agent in the management of ET is ethanol. A recent study showed the beneficial effects of ethanol on ET via the NMDA receptors, which are notably expressed in the cerebellum (Manto and Laute, 2008). However, this therapy is impractical because of the risk of addiction and serious drawbacks with chronic ethanol use (Pahwa and Lyons, 2003). For instance, the long-term consumption of moderate doses of daily ethanol in humans has been shown to result in pathological changes in the cerebellum including purkinje cell degeneration and apoptosis (Karhunen et al., 1994). It has been postulated that higher levels of chronic ethanol intake increases the risk of developing ET (Louis et al., 2009).

It is thought that genetic factors, age, ethnicity, and several toxic agents (such as β-carbolines and lead) are the risk factors for the occurrence of ET (Louis, 2005), however the underlying mechanisms have not been 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; Deuschl, 2000). This hypothesis was derived from clinical and animal (treated by β-carboline) studies showing over activity of cerebellar cortex and deep nuclei (Batini et al., 1979; Wills et al., 1995; Yang and Iadecola, 1998; 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).
N-Methyl-D-aspartate (NMDA) receptors constitute a major class of heteromeric glutamate-gated ion channels that play an important role in neurodegeneration related to excitotoxicity (Waxman and Lynch, 2005). It has been established that the inferior olive and cerebellum contain a high density of NMDA receptors (Shaw et al., 1992; Liansola et al., 2005). Based on these results, it may be hypothesized that NMDA receptor functions abnormally in ET (Klebe et al., 2005) and ET can be treated by NMDA antagonists. Experimental evidence indicates that the NMDA receptor complex is involved in animal models of essential tremor (Ehlen et al., 1996; Du et al., 1997; Kralic et al., 2005).

Harmaline-induced tremor is one of the animal models of transient action tremor (Miwa, 2007; Kralic et al., 2005). In this model, the activation of the inferior olive results in enhanced rhythmic activity, which is then conveyed to the cerebellar purkinje cells via climbing fibers. Studies investigating the pharmacological profile (Paterson et al., 2009; Martin et al., 2005), affected brain areas (Miwa et al., 2000; Deuschi and Elble, 2000), and the phenomenological properties of harmaline-induced tremor (Tarig et al., 2001; Martin et al., 2005) revealed that this model might be useful in assessing the clinical efficacy of putative therapies in ET.

The aim of this study was to evaluate the effect of memantine, a non-competitive antagonist of NMDA receptors, on tremor activity. We also examined the degeneration of cerebellar and olivary neurons induced by harmaline and the effect of ethanol in this model. The tremor activity has been evaluated by the custom-built computer aided image and force analysis system. Neuronal survival was assessed after 24 h by counting the number of crystal violet stained alive neurons and caspase-3 immunostained apoptotic neurons.

2. Materials and methods

2.1. Animals and drugs

Subjects were adult male Wistar rats weighing 290–370 g. Animals were maintained under standard laboratory conditions on a 12/12-h light/dark cycle (lights on at 7:00 a.m.), and they had free access to food and water. All experimental procedures were approved by Kocaeli University Ethical Committee for Experimental Animals (AEX 1/7). Harmaline hydrochloride (Sigma-Aldrich, St Louis, MO, USA), memantine hydrochloride (Sigma-Aldrich, St Louis, MO, USA), and ethanol (Riedel de Haen) were dissolved in physiological saline and injected intraperitoneally at a dose volume of 1 ml/kg, except for ethanol which was administered at the volume of 10 ml/kg. Drug solutions were freshly prepared prior to use.

2.2. Experimental procedure

On the test day, animals were transferred to the testing room, and were left there for an hour for acclimatization. They were randomly assigned to four groups of eight rats. Following an 15 min pretreatment with saline (saline + harmaline group, n = 8), 1.5 g/kg dose of ethanol (ethanol + harmaline group, n = 8) or 5 mg/kg dose of memantine (memantine + harmaline group, n = 8), rats received a single intraperitoneal (ip) injection of harmaline at the dose of 20 mg/kg. Animals were placed in the recording cage, and were observed for tremor and locomotor activity. Three different 10 min-long recordings were taken every half an hour (0–10, 30–40, and 60–70th min after the harmaline injection). A separate group of rats received saline for histopathological examination (sham). 24 h after the harmaline treatment, rats were anesthetized with ketamine (100 mg/kg, i.p.) and xylazine (15 mg/kg, i.p.), and then were perfused intracardially with 0.05% heparin/0.9% NaCl followed by 10% formalin fixative, pH 7.4. Brains were removed and postfixed in the same fixative. The cerebellum of each rat was embedded in paraffin. For sectioning, we maintained a coronal orientation, and cut 5-μm thick sections which were mounted on glass slides, and stained with cresyl violet and caspase-3 immunostaining. Coronal sections of the cerebellum were selected at levels from bregma between approximate AP: –9.7 mm to 10 mm area (Paxinos and Watson, 1998). For each animal two tissue sections were prepared for the immunohistochemical examination or cresyl violet staining. Images were collected using a BX51 microscope and a Sony 3CCD video camera, and analyzed using Image Pro Plus.

2.3. Tremor assessment

The custom-built tremor and locomotion analysis system is composed of four force sensors, a color digital camera, and a computer. The system uses PCB/Piezotronics Inc. 206CD3 ICP® dynamic force sensors and National Instruments NI-9233 data acquisition module to acquire force data with a sampling rate of 2000 samples per second (2 KHz). The locomotion parameters are extracted from the images captured by a Basler A625fc digital color camera at a rate of 15 frames per second. The moving plate (300 mm × 300 mm) is of glass material, which was chosen because of its high flexural strength. The camera is mounted high enough to capture the moving plate entirely. Fig. 1 illustrates this tremor and locomotion analysis system.

The output of this system is analyzed in three parts: the force analysis, the image analysis, and their combination. In the force analysis, the total force data is obtained from four different channels to extract tremor information. Discrete Short-Time Fourier Transform (Discrete STFT) is a signal processing method used for analyzing non-stationary discrete time signals that gives frequency changes over time, and it is utilized for the time–frequency analysis of the force data. For each time interval, the distribution of frequency power is analyzed in the motion frequency range (Martin et al., 2005). In our work, 1 s time interval was chosen, and 0.5 s overlapping size was utilized for STFT calculation. The tremor ratio is computed by dividing the power over the tremor band [6.12 Hz] to the power over the full motion band [0.15 Hz] in the spectrum. In our system, we obtain the tremor data with high precision due to the dynamic force sensors and 2 Ks/sampling rate. Therefore, we can analyze the spectrum precisely than those studies that use systems with load cells. It has been observed that the tremor frequency generally occurs in the range of 8–12 Hz, but sometimes it appears in the 6 Hz and 7 Hz components. Hence, these frequency components were also included in the tremor band in our system, and the tremor ratio is calculated using a power ratio as given in Eq. (1).

Tremor ratio(n) = \frac{\sum_{j}^\infty X(n.k)^2}{\sum_{j}^\infty X(n.k)^2}

(1)

Here n shows STFT interval index, k shows frequency index, and X(n, k) shows spectrum coefficients. In our system, tremor is detected if this ratio exceeds 0.5. In some cases, the tremor ratio falls and then rises instantaneously. In these cases, the tremor detection is updated to exclude these abrupt changes. Fig. 2 shows the tremor ratio and the decision output for a time interval from a representative experiment. Furthermore, the dominant frequency component of the tremor is extracted in order to investigate the relation between tremor and frequency. Power amplitude of the dominant frequency component is used to determine the tremor stage. Five tremor degrees were defined for this purpose. Fig. 3 presents 1 min long raw data from an experiment (top panel), and the corresponding tremor detection and degree results. We also assessed the tremor duration, which was computed as the total tremor time during the three periods regardless of the stage.

In the image analysis, the instantaneous position and displacement of the animal are computed by analyzing the captured images (e.g., Fig. 4a). The color information is used to separate the animal from the red background. The Hue–Saturation–Intensity color transform is applied, and a thresholding operation is performed on the saturation image to distinguish the white pixels (animal region) from the red pixels (background). The animal region is extracted using morphological operations and connected component labeling. A sample image frame captured by the camera, and image processing stages are presented in Fig. 4c. Center of gravity of the extracted animal region gives the animal’s instantaneous position, and the amplitude of the position change is utilized to decide whether the animal is moving or not. The tremor and the locomotion information are combined to gather the tremor data in the presence of locomotor activity.

2.4. Caspase-3 immunohistochemistry

Apoptotic neurons were observed in the cerebellar vermis and the inferior olive by caspase-3 immunohistochemistry. Following deparaffinization and rehydration, sections were irradiated in 0.1 mol/L sodium citrate buffer (pH 6.0) in a microwave oven (medium low temperature) for 12 min. The sections were exposed to 3% H2O2 for 10 min to bleach endogenous peroxidases. The procedure has been followed by
rinsing them three times in phosphate-buffered saline (PBS) for 10 min. Sections were blocked in 5% goat serum in 0.3% Triton X-100 (PBS-T) for 1 h. Subsequently, the slides were incubated for 24 h at 4°C with rabbit polyclonal cleaved caspase-3 antibody (1:200, Cell Signaling) in blocking solution, washed, and incubated for 1 h at 25°C with a secondary goat anti-rabbit biotinylated IgG antibody (1:200, Vector Labs) in blocking solution. Sections were immunostained using the ABC method (Vectastain Elite Kit, Vector Labs, Burlingame, CA) and diaminobenzidine (DAB), counterstained with Methyl green, dehydrated in an ethanol gradient, immersed in xylene and coverslipped. As a negative control, the primary antibody was replaced with nonimmune serum, and no staining occurred.

2.5. Caspase-3 cell counting

Cell counts were carried out by an experienced observer who was blind to the source of groups, and were performed on digital images captured at 40× magnification. Cell counting was performed using a stereological method. The counting frame, 100 × 100 μm for the cerebellar vermis and the inferior olive, was randomly placed over the structure of interest. Measurements were performed at three locations for each slide (total of six measurements per animal).

2.6. Cresyl violet cell counting

Sections were stained with cresyl violet (CV) to visualize the purkinje cells, the granule cells, and the inferior nucleus neurons. For cerebellum, the lobule 4 was examined and a random field was chosen within layer 4. This field was identified by the size and the packing density of the constituent cells, and its characteristic large pyramidal neurons. Light microscope photographs of the inferior nucleus and the lobule 4 of cerebellum were taken from each rat at equivalent anatomical levels (as stated above). Measurements were performed at three locations for each slide. The purkinje cells, granule cells, and inferior nucleus neurons were counted by using the mechanical cell counting with UTHSCSA ImageTool v 3.00.

3. Statistical analysis

Data were statistically evaluated by analysis of variance (ANOVA), which was followed by the post-hoc Bonferroni tests. A two-way ANOVA was used to compare tremor duration, tremor...
ratio, and locomotion/tremor ratio among three different recording periods and experimental groups. A one-way ANOVA was used to compare the mean locomotor activity and peak frequency of tremor among the saline + harmaline, memantine + harmaline, and ethanol + harmaline groups. Paired t-test was used to analyze the differences between locomotor activity in the saline + harmaline group before and after harmaline treatments. A one-way ANOVA was used to compare the caspase-3 positive cells of cerebellar vermis and inferior olive, and the CV stained cells of cerebellar lobule and inferior olive among the control, saline + harmaline, memantine + harmaline, and ethanol + harmaline groups. The level of statistical significance was considered to be \( p < 0.05 \). Results expressed as mean ± S.E.M.

4. Results

4.1. Harmaline-induced tremor and neurodegenerative effects of harmaline

As previously reported, within 5 min following the harmaline administration, rats displayed the characteristic tremor patterns of trunk, head, and limbs. The tremor was expressed in the 8–12 Hz range, in some cases it contained 6 Hz and 7 Hz frequency components. The predominant peak of the spectrum was centered at 10 Hz (Fig. 5). Tremor duration did not differ among three recording periods (Fig. 6A). In slices collected 24 h after the harmaline administration, caspase-3 immunoactivity was observed in the Purkinje cells of the cerebellar vermis and paravermis, and the cells of the inferior olivary nucleus. The number of caspase-3 positive Purkinje cells in cerebellar vermis and neurons in inferior olive of saline + harmaline group were significantly higher than those in the sham group (\( p < 0.001 \)). Moreover, the number of CV stained granule cells and Purkinje cells in cerebellar lobule and CV stained neurons in olivary nucleus were significantly lower than those in the sham group (\( p < 0.001 \)).

4.2. Ethanol and memantine reduce tremor scores

The tremor frequency and peak location did not change with ethanol or memantine administration (Fig. 5). Fig. 6A shows the mean duration of tremor in all of the experimental groups during the first, second, and third 10 min recording periods. The tremor duration differed significantly among the three groups (\( F(2, 42) = 14.32, p < 0.001 \)). A significant difference was also found among the three groups regarding the time effect (\( F(2, 42) = 16.93, p < 0.001 \)). More importantly, the interaction effect (treatment × time point) was significant (\( F(4, 42) = 3.5, p < 0.05 \)), which suggests that the effects of memantine and ethanol on harmaline-induced tremor occurred at different time points. The post-hoc Bonferroni tests showed that this difference was due to the decrease of the tremor duration in the memantine group during the first 10 min recording period (\( p < 0.001 \)). On the other hand, it decreased during all three recording periods in the ethanol group (\( p < 0.001, p < 0.01 \)). Hence, in the second and third 10 min periods, there was no significant...
The mean tremor ratio of stage 1 are present between saline and ethanol treatments during all three recording periods, showing the harmaline-induced 8–12 Hz peak frequency.

difference between saline and memantine-treated groups. Fig. 6B shows the mean tremor ratio of stage 1–5 during three recording periods for all groups. None of the ethanol administered rats exhibited stage 5 tremor. Ethanol decreased the tremor intensity at stages 2, 3, and 4 (p < 0.001, p < 0.05), whereas memantine significantly reduced the tremor intensity in stages 4 and 5 compared to the saline pre-treated harmaline group (p < 0.001).

4.3. Ethanol and memantine have differential effects on locomotor activity

Harmaline treatment produced a significant effect on locomotor activity (p < 0.05); reducing the total distance relative to the baseline levels (Fig. 7A). There was a significant difference among saline + harmaline, memantine + harmaline, and ethanol + harmaline groups after harmaline administration (F(2, 69) = 3.5, p < 0.001). Memantine administration resulted in an increase in locomotor activity (p < 0.01), whereas ethanol decreased it significantly (p < 0.01) compared to the saline + harmaline group.

Given the well-established relation between harmaline-induced tremor and locomotion, the locomotion/tremor ratio was computed for all three recording periods by dividing the total distance values by the tremor duration. The locomotion/tremor ratio was higher in the memantine + harmaline and ethanol + harmaline groups compared to the saline + harmaline group only during the first recording period (p < 0.001, p < 0.05, respectively; Fig. 7B).

4.4. Neuroprotective effects of memantine is superior to ethanol

Memantine markedly prevented the harmaline-induced neurodegeneration in the cerebellum. The number of caspase-3 positive purkinje cells in cerebellar vermis and caspase-3 positive neurons in inferior olive in the memantine + harmaline group were significantly lower than the ethanol + harmaline and saline + harmaline groups (p < 0.001). Moreover, the number of caspase-3 positive cells in all investigated areas did not show significant differences between the sham and memantine + harmaline groups (Fig. 8). Similarly, there were no significant differences in the number of CV stained purkinje

Please cite this article in press as: Iseri, P.K., et al., The effect of memantine in harmaline-induced tremor and neurodegeneration, Neuropharmacology (2011), doi:10.1016/j.neuropharm.2011.05.015
and granular cells of the cerebellum and the inferior olivary neurons between the sham control and memantine + harmaline groups (Fig. 9). In contrast, ethanol significantly reduced the number of CV stained cells compared to the sham and memantine + harmaline groups \( (p < 0.001) \). Fig. 10 shows representative photos of the cerebellum and inferior olive of the three groups with caspase-3 staining.

5. Discussion

This study revealed new findings regarding the harmaline model of tremor, and the effects of ethanol and memantine on this pathology. Harmaline-induced apoptosis was shown by increased caspase-3 activity. Ethanol decreased the tremor intensity and reduced the locomotion more effectively than the memantine. The neuroprotective efficacy of memantine on cerebellar purkinje cells, granular cells, and inferior olivary neurons were observed. Finally, although ethanol decreased caspase-3 mediated apoptosis, it caused cell loss in cerebellum and olivary nucleus.

Fig. 8. (A) Caspase-3 positive purkinje cells in cerebellar vermis and (B) caspase-3 positive neurons in inferior olive in the control group and in saline, memantine, and ethanol-treated animals 15 min before the harmaline administration. The number of caspase-3 positive purkinje cells and caspase-3 positive neurons are significantly higher in saline + harmaline and ethanol + harmaline groups \( (**p < 0.001) \) compared to the control animals. Administration of memantine and ethanol prior to harmaline-induced tremor significantly reduced the number of caspase-3 positive purkinje cells and caspase-3 positive neurons in cerebellum \( (**p < 0.001, *p < 0.01) \). Significant differences are also present between memantine + harmaline and ethanol + harmaline groups \( (**p < 0.001) \).

Fig. 9. The number of (A) CV stained granule and (B) purkinje cells in cerebellar lobule and (C) CV stained neurons in inferior olive in four groups. Significant differences are present between control vs. saline + harmaline groups, and between control vs. ethanol + harmaline groups \( (**p < 0.001) \). Pound signs indicate significant differences compared to the saline + harmaline group \( (**p < 0.001) \). There were also significant differences between memantine + harmaline vs. ethanol + harmaline groups \( (**p < 0.001) \).
5.1. The characteristics of harmaline-induced tremor and apoptosis

The results of caspase-3 immunostained and CV stained slides indicated that harmaline causes cell loss in the cerebellar vermis (including purkinje and granule cells) and neurons in the inferior olivary of the rat, and that caspase-3 mediated apoptotic mechanisms play a crucial role in harmaline-induced cell death. The harmaline-induced neurodegeneration in purkinje cells and inferior olivary neurons has already been reported in previous studies (O’Hearn and Molliver, 1993, 1997; Miwa, 2007). The role of caspase-3 mediated apoptosis in this process however has been shown for the first time in this study.

Harmaline is a β-carboline alkaloid that generates action tremor by enhancing the rhythmic bursting of the neurons in the inferior olivary nucleus and the purkinje cells of the cerebellum (Miwa, 2007). Systemic administration of harmaline and ibogaine (a substance that structurally and functionally resembles harmaline) lead to purkinje cell degeneration with microglial activation in the cerebellar vermis and paravermal regions of the rat brain (O’Hearn and Molliver, 1993, 1997; Miwa et al., 2006). Purkinje cell degeneration can be prevented by chemical ablation of olivocerebellar fibers. It has been hypothesized that the release of an excitatory amino acid from the climbing fiber synaptic terminals may lead to the excitotoxic degeneration of the purkinje cells (O’Hearn and Molliver, 1997). However, the excitotoxic neuronal death in the brain is not a uniform event. It is rather a spectrum of necrotic, apoptotic, and autophagic morphologies (Wang and Qin, 2010). It is related to several factors including the type of the neuron and the intrinsic vulnerability of the circuits. The identification of the cellular and molecular mechanisms leading to neuronal cell death under different conditions will provide an insight regarding the potential approaches to the therapeutic intervention targeting excitotoxic signaling pathways in neurological disorders.

A number of methods have been used to measure the tremor intensity, frequency, and peak location in the harmaline-induced tremor model (Fowler et al., 2001; Martin et al., 2005). The instrumental and analytical approaches that allow successful measurement and evaluation of different characteristics of tremor in experimental models are indeed required to advance the understanding and the management of motor disorders (Fowler et al., 2001; Martin et al., 2005). Because of the well-known relation between harmaline-induced tremor and locomotion, in this study we used a digital system composed of force sensors and a digital camera in order to capture and identify the locomotion in addition to the tremor. Tremor is expressed in the range of 8–12 Hz and the predominant peak of the spectrum was centered at 10 Hz. These results are consistent with the previous data obtained from rats (Martin et al., 2005; Wang and Fowler, 2001; Fowler et al., 2001). An interesting finding is that although the ethanol and memantine decreased the tremor intensity, neither the frequency interval nor the peak location was affected with drug treatment.

5.2. The effect of ethanol on the harmaline-induced tremor and neurodegeneration

Our results revealed that ethanol effectively decreased the tremor duration and intensity without changing the tremor frequency and peak location. Ethanol also decreased the locomotor activity, which might contribute to its anti-tremogenic efficacy. Sedative effect of alcohol can also play a partial role in its effects on locomotor activity. Besides its beneficial effects on the tremor,
ethanol did not prevent the loss of cerebellar and olivary neurons. Since the CV stained cell count in the ethanol + harmaline group was less than the saline + harmaline group, we concluded that neuronal cell loss was more abundant in the former group (Fig. 10). These data indicated that anti-tremogenic effects may not always contribute to cellular neuroprotective mechanisms. Similarly, it has been shown that although ethanol inhibits harmaline-induced tremor, it does not reverse harmaline-induced changes such as the increased cerebellar cyclic GMP (Rappaport et al., 1984) and elevated level of extracellular glycerol concentrations (a marker of membrane turn-over and damage) after intranuclear administration of harmaline (Manto and Laute, 2008).

Ethanol markedly suppresses the tremor in ET patients (Koller and Biary, 1984) and in harmaline treated animals (Martin et al., 2005). The effects of low blood levels of ethanol on ET may be even stronger than the effects observed with propranolol or primidone, two of the first-line agents for treating the ET (Koller and Biary, 1984; Louis, 2005). Consistently, it has been speculated that patients self-medicate with ethanol. Ethanol alters the neural activity of the cerebellar neurons by a number of mechanisms, such as inhibiting the NMDA or enhancing the GABA receptor response (Simson et al., 1991; Kelm et al., 2011). These systems have been hypothesized to function abnormally in the ET (Klebe, 2005). Although acute ethanol is symptomatically beneficial for the patients, the chronic ethanol use can be a continuous source of the underlying cerebellar neurotoxicity, and therefore it can be a contributor to the progression of the disease (Louis et al., 2009). Ethanol leads to a decrease in the number of both cerebellar granule cells and purkinje neurons (Oberdoerfer and Rabin, 1999).

In the developing brain, ethanol leads to cerebellar atrophy, typically most pronounced in the vermis, which is one of the most widely recognized structural changes caused by the long-term heavy ethanol consumption in the human nervous system (Fadda and Rossetti, 1998; Dikranian et al., 2005; Luo, 2010). These results are consistent with our findings, but not identical. In this study, we examined the acute effects of ethanol whereas previous studies reported its chronic effects. Experimental studies have shown that the mature cerebellum is less vulnerable to the alcohol-induced neuronal death in acute doses than the developing cerebellum (Karaçay et al., 2008). Even though, it has been emphasized that acute ethanol did not have neurotoxic effects in the adult rat brain, it might behave differently under abnormal conditions and exhibit an additive detrimental effect in presence of harmaline. It should be noted that, ethanol and harmaline both cause neuronal cell death in similar locations (e.g., cerebellar cortical and inferior olivary neurons) (Oberdoerfer and Rabin, 1999; Saito et al., 1999; Dikranian et al., 2005).

5.3. The effect of memantine on the harmaline-induced tremor and neurodegeneration

Our results indicate that memantine reduced the tremogenic and the neurotoxic effect of harmaline. This corroborates previous studies that investigated the effects of NMDA antagonists on harmaline-induced tremor. Experimental and clinical evidence implicated the role of glutamatergic system in ET (Ehlen et al., 1996; Mally et al., 1996; Du et al., 1997). Harmaline preferentially enhances synaptic activity of climbing fibers which originate in the inferior olive (Lamarre and Mercier, 1971; Batini et al., 1979; Ryder et al., 2006). The activation of the climbing fiber system increases levels of excitatory amino acids, nitric oxide, and cGMP in the cerebellum that is believed to be responsible molecular mechanism underlying harmaline-induced tremor, increased cerebellar blood flow, and neurotoxicity (Chan-Palay and Palay, 1979; O’Hearn and Moliver, 1997; Yang and Iadecola, 1998; Zhang et al., 2003; Beitz and Saxon, 2004). Harmaline also competitively inhibits MK-801 binding to the NMDA receptor in the rabbit brain (Du et al., 1997). It has been reported that competitive and non-competitive NMDA receptor antagonists decrease harmaline-induced NO and cGMP production as well as the tremor in different experimental setups (Ehlen et al., 1996; Paterson et al., 2009). There are however limited data from human studies. A randomized placebo-controlled trial with amantadine, a non-competitive antagonist of NMDA receptors, did not demonstrate efficacy in reducing tremor (Gironell et al., 2006). Adversely an increase in postural tremor was observed in one-third of the patients. The specificity of amantadine as a pure NMDA antagonist has been a matter of debate because of its effects on cholinergic, and adrenergic receptors (Moryl et al., 1993; Matsubayashi et al., 1997). A new clinical trial with a small sample size indicated that the average effect of memantine on tremor is mild or not significant (Handforth et al., 2010). However, in a small subset of patients, memantine may confer meaningful tremor benefit. NMDA receptors are highly expressed in cerebellum (mainly inhibitory interneurons in the cerebellar cortex, cerebellar nuclei, and inferior olive), but the most affected cells in both ET and harmaline-induced neurodegeneration, namely the purkinje cells, do not exhibit functional NMDA receptors (Renard et al., 1995; Perkel et al., 1993). Glutamate receptors that are postsynaptic to climbing fiber terminals include non-NMDA receptors (AMPA or kainate) (Konnerth et al., 1990; Perkel et al., 1990; Lambolle et al., 1992; Renard et al., 1995), or metabotropic receptor subtypes (Batchelor et al., 1997). Electrophysiological studies of the olivocerebellar projection revealed that the excitation of purkinje cells by climbing fibers is mediated primarily through non-NMDA receptors (Perkel et al., 1990; Farrant and Cull-Candy, 1991) and metabotropic receptors (Batchelor et al., 1997). Unexpectedly, it has been shown that AMPA/KA receptor antagonist (1-4-aminophenyl-methyl-7,8-methylenedioxy-5H-2,3-benzodiazepine [GYKI 52465]) significantly increase purkinje cell degeneration when co-administrated with ibogaine (O’Hearn and Moliver, 2004). Another study showed that a selective metabotropic receptor subtype 1 antagonists (JN 16259685) strongly enhanced tremor of forelimbs, head and trunk, hypolocomotion, and ataxia induced by harmaline (Kolasiewicz et al., 2009).

In conclusion, it is likely that anti-tremogenic effect may not always accompany a neuroprotective effect. Memantine might be a potentially useful choice in the treatment of essential tremor. Moreover, our data suggest that the role of the glutamatergic system in harmaline-induced tremor should be further investigated in more detail.

Acknowledgments

This work was supported by Lundbeck Inc. The authors wish to thank Lundbeck Company for their support. Authors would also like to thank Dr. Fuat Balcı for his suggestions and comments on the earlier drafts of this paper.

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