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Antidepressant-like effect of harmane and other β-carbolines in the mouse forced swim test

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Abstract The purpose of the present study was to determine the effects of harmane, norharmane and harmine on the immobility time in the mouse forced swim test (FST) – an animal model of depression. After 30 min of the β-carbolines injections, mice were placed individually in a vertical glass cylinder (height, 25 cm; diameter, 12 cm) containing water about 15 cm deep at 22 ± 1 °C and forced to swim.

Treatment of animals with harmane (5–15 mg/kg, i.p.), norharmane (2.5–10 mg/kg, i.p.) and harmine (5–15 mg/kg, i.p.) reduced dose-dependently the time of immobility. Their antidepressant-like effects were not affected by pretreatment with reserpine at the dose of 5 mg/kg, i.p., 18 h before the test, which did not modify the immobility time. Conversely, when flumazenil (5 mg/kg, i.p.) was administered 30 min before the test, it was able to antagonize completely the antidepressant-like effects of harmane, norharmane and harmine. It was concluded that harmane, norharmane and harmine reduce the immobility time in this test, suggesting an antidepressant-like effect, via an inverse-agonistic mechanism located in the benzodiazepine receptors.

1. Introduction

β-Carboline alkaloids, also known as harmala's alkaloids because they were first isolated from *Peganum harmala* (Zygophillaceae), are natural products that have been found in a number of medicinal plants, tobacco smoke, well-cooked foods (Poindexter and Carpenter, 1962; Nishigata et al., 1980) and endogenously in mammalian tissues (Airaksinen and Kari, 1981; Beck and Faull, 1986). These alkaloids have a wide spectrum of pharmacological actions, including convulsive or anticonvulsive actions (Loew et al., 1985), tremorogenesis (Lutes et al., 1988), antioxidative action (Tse et al., 1991) and immunomodulatory effects (Li, 1996; Wang et al., 1996). β-Carbolines bind with high affinity to benzodiazepine site of the γ-aminobutyric acid type A ($GABA_A$) receptors as inverse agonists; some possess anxiogenic character and others display anxiolytic properties, depending on the dose (Muller et al., 1981; Rommelspacher et al., 1981, 1985; Prado de Carvalho et al., 1983; Barbaccia et al., 1986; Hollinshead et al., 1990; Allen et al., 1992; Adell et al., 1996; Sällström-Baum et al., 1996). These alkaloids also bind to other neurotransmitter receptors in the brain, including 5-hydroxytryptamine (5-HT) and dopamine receptors (Muller et al., 1981; Pawlik and Rommelspacher, 1988) and imipramine/citalopram recognition site

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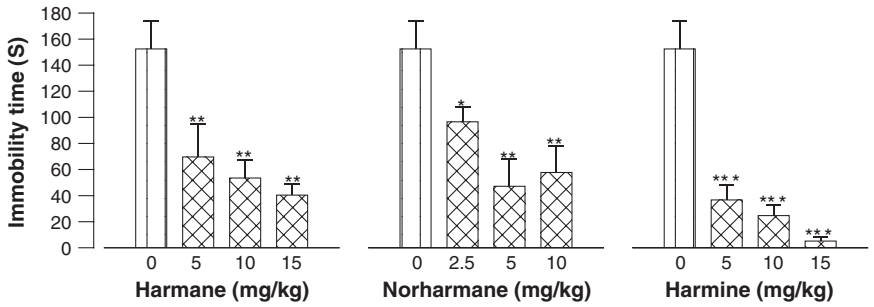

Figure 1 Antidepressant-like effects of harmane (5—15 mg/kg, i.p.), norharmane (2.5—10 mg/kg, i.p.) and harmine (5—15 mg/kg, i.p.) in the mouse forced swim test. Drugs and saline (10 ml/kg, i.p.) were injected 30 min before the test. Results are expressed as mean + S.E.M. ($n = 7$—8 mice/group). *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$, different from control groups.

(Langer et al., 1984; Pähkla et al., 1997). β-Carboline alkaloids increase the extracellular norepinephrine, dopamine and 5-HT levels in several brain regions via inhibition of monoamine reuptake systems (Komulainen et al., 1980; Kleven and Woolverton, 1993; Tella, 1995; Sällström-Baum et al., 1996). These compounds also increase the levels of monoamines after monoamine oxidase (MAO) A or B inhibition (Fuller et al., 1986; Fernandez de Arriba et al., 1994; Rommelspacher et al., 1994, 2002; Adell et al., 1996). The results of above studies suggest a possible importance of β-carbolines in control of depressive states. The present study was carried out to examine the antidepressant-like effects of β-carbolines harmane, norharmane and harmine in mice by an animal model of depression, the forced swim test.

2. Experimental procedures

2.1. Animals

All experiments were carried out on male Swiss—Webster mice (20—25 g). The animals were housed nine per plastic cage in an animal room maintained at 21 ± 2 °C on a 12-h light / dark cycle (lights on 0700—1900 h). Food and water were available at all times except during the experiments. Each animal was used once only. The experimental protocol was approved by the Research and Ethics Committee of Mazandaran University of Medical Sciences.

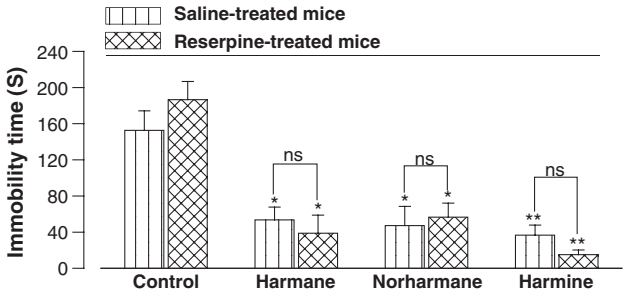

Figure 2 Antidepressant-like effects of harmane, norharmane and harmine in the reserpine-treated mice in the forced swim test. The ED_{50}s of harmane (11.5 mg/kg, i.p.), norharmane (8.5 mg/kg, i.p.) and harmine (8 mg/kg, i.p.) were injected in animals pretreated with saline (10 ml/kg, i.p.) or reserpine (5 mg/kg, i.p., 18 h before the test), 30 min before the test. Results are expressed as mean + S.E.M. ($n = 8$ mice/group). *$P < 0.01$, **$P < 0.001$, different from control groups.

2.2. Forced swim test procedure

The procedure was use described by Porsolt et al. (1977), with some modification. Mice were placed individually in a vertical glass cylinder (height, 25 cm; diameter, 12 cm) containing water about 15 cm deep at 22 ± 1 °C and forced to swim. The total duration of immobility was measured with a stopwatch during the 8 min. A decrease in the duration of immobility is indicative of an antidepressant-like effect.

2.3. Drugs

The following drugs were used:
Flumazenil (Sigma, USA), Harmane HCl (Sigma, USA), Harmine HCl (Sigma, USA), Norharmane HCl (Sigma, USA) and Reserpine (Sigma, USA). In all cases, the drug doses reported are for the base. The drugs were dissolved in saline, except for reserpine, which was dissolved in a drop of acetic acid and then diluted with saline. In this case, the vehicle control was acetic acid in saline. Reserpine (5 mg/kg, i.p.) was injected to animals around 18 h before test to deplete of monoamines from the nerve terminals (Zarrindast and Minaian, 1991). Drug concentrations were prepared so that the necessary dose could be injected in a volume of 10 ml/kg by i.p. route. In general, the doses of drugs and pretreatment time were usually those used previously and shown to be pharmacologically active (Pimpinella and Palmery, 1995; Sällström-Baum et al., 1996; Ruiz-Durántez et al., 2001; Cappendijk et al., 2001).

2.4. Statistical analysis

One-way analysis of variance (ANOVA), followed by Newman—Keuls test, was used for statistical analysis. Differences with $p < 0.05$ between experimental groups at each point were considered statistically significant. All data were