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Involvement of dopamine D_1/D_2 receptors on harmane-induced amnesia in the step-down passive avoidance test

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ABSTRACT

Ingestion of harmane and other alkaloids derived from plant *Peganum harmala* has been shown to elicit profound behavioural and toxic effects in humans, including hallucinations, excitation, feelings of elation, and euphoria. These alkaloids in the high doses can cause a toxic syndrome characterized by tremors and convulsions. Harmane has also been shown to act on a variety of receptor systems in the mammalian brain, including those for serotonin, dopamine and benzodiazepines. In animals, it has been reported to affect short and long term memory. In the present study, effects of dopamine D_1 and D_2 receptor antagonists on the harmane (HA)-induced amnesia and exploratory behaviors were examined in mice. One-trial step-down and hole-board paradigms were used for the assessment of memory retention and exploratory behaviors in adult male NMRI mice respectively. Intraperitoneal (i.p.) administration of HA (5 and 10 mg/kg) immediately after training decreased memory consolidation, while had no effect on anxiety-like behavior. Memory retrieval was not altered by 15- or 30 min pre-testing administration of the D_1 (SCH23390, 0.025, 0.05 and 0.1 mg/kg) or D_2 (sulpiride 12.5, 25 and 50 mg/kg) receptor antagonists, respectively. In contrast, SCH23390 (0.05 and 0.1 mg/kg) or sulpiride (25 and 50 mg/kg) pre-test administration fully reversed HA-induced impairment of memory consolidation. Finally, neither D_1 nor D_2 receptor blockade affected exploratory behaviors in the hole-board paradigm. Altogether, these findings strongly suggest an involvement of D_1 and D_2 receptors modulation in the HA-induced impairment of memory consolidation.

1. Introduction

A number of tremorogenic β-carboline alkaloids such as harmane (HA; 1-methyl-β-carboline), 1-methyl-7-methoxy-3, 4-dihydro-β-carboline (harmaline) and 9H-pyrido [3, 4-β] indole (norharmane) are naturally present in the human food chain. They have been found in common plant-derived foodstuffs (wheat, rice, corn, barley, soybeans, rye, grapes, mushrooms, and vinegar), plant-derived beverages (wine, beer, whisky, brandy, and sake), and plant-derived inhaled substances (tobacco)(Adachi et al., 1991). The β-carbolines HA, norharmane and harmine exist in the blood plasma, heart, kidney, liver and also in brain tissue (Hudson et al., 1999; May et al., 1994; Rommelspacher et al., 1980). Since, high plasma levels of these compounds have been found in heavy smokers (Spijkerman et al., 2002), alcoholics (Rommelspacher et al., 1991b), heroin-dependent humans (Stohler et al., 1996), patients with essential tremor (Louis et al., 2002) or Parkinson's disease (Kuhn et al., 1996), they are assumed to have a crucial role in the pathophysiology of various disorders of the CNS. Condensation reaction between an indoleamine and acetaldehyde forms the molecule in peripheral and brain tissue (Susilo et al., 1987). On the other hand, there is evidence showing that formation of β-carbonile can be achieved by enzymatic processes, because it only occurs in the presence of mammalian tissue. In particular, experiments performed in vitro under pseudo-physiological conditions with [3H]tryptamine and pyruvic acid failed to result in β-carboline formation, indicating that an enzymatic process was involved (Rommelspacher et al., 1991a). The β-carbolines have a mixed pharmacology and individual compounds have been shown to bind to a variety of different targets including monoamine oxidase A

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and B (MAO_A and MAO_B), benzodiazepine, imidazoline, dopamine and 5-hydroxytryptamine (5-HT) receptors (Glennon et al., 2000; Pahkla et al., 1997; Rommelspacher et al., 1980; Taylor et al., 1984). B-carboline alkaloids increase the extracellular norepinephrine, dopamine and 5-HT levels in several brain regions via inhibition of monoamine reuptake systems (Baum et al., 1996; Kleven and Woolverton, 1993; Komulainen et al., 1980; Tella, 1995). These compounds also increase the levels of monoamines after monoamine oxidase (MAO) $_A$ or $_B$ inhibition (Adell et al., 1996; Fuller et al., 1986; Rommelspacher et al., 1994).

Moreover, dopamine exerts its action by binding to specific membrane receptors (Gingrich and Caron, 1993). The dopamine receptors were classified as D_1-like and D_2-like based on sequence homology and pharmacology (Civelli et al., 1993; Missale et al., 1998; Seeman and Van Tol, 1993). A large number of different paradigms have been used to demonstrate that dopamine receptors play a critical role in the modulation of neuronal activities that are related to different forms of learning and memory (see (Jay, 2003) for a review). Adriani reported that dopamine receptors are also able to alter the capability to learn and store information (Adriani et al., 1998). A method based on the measurement of step-down latency in passive avoidance has been developed for the study of learning and memory in mice (Kameyama et al., 1986).

On the other hands, the research in anxiety is focused on serotonergic, GABAergic and adrenergic neurotransmitter systems but also dopamine has been discussed to be involved in anxiety. In conclusion, because of β-carboline presence in the food chain and that these alkaloids have a wide spectrum of pharmacological actions and immunomodulatory effects, in the present study, the effects of HA on memory consolidation/exploratory behaviors and involvement of D_1/D_2 receptors on these behaviors in the step-down passive avoidance and hole-board test in mice have been investigated.

2. Materials and methods

2.1. Animals

Male albino NMRI mice weighing 25–30 g were used. Animals were kept in an animal house with a 12/12-h light–dark cycle and controlled temperature (22 ± 2 °C). Animals were housed in groups of 10 in Plexiglas cages and they had free access to food and tap water except during the limited periods of experiments. Ten animals were used in each group; each animal was used once only. Behavioural experiments were done during the light phase of the light/dark cycle. All procedures were carried out in accordance with institutional guidelines for animal care and use.

2.2. Memory testing and apparatus

An inhibitory avoidance apparatus consisted of a wooden box ($30 \times 30 \times 40$ cm^3) with a floor that consisted of parallel stainless steel rods (0.3 cm in diameter, spaced 1 cm apart). A wooden platform ($4 \times 4 \times 4$ cm^3) was set in the center of the grid floor. Electric shocks (1 Hz, 0.5 s and 50 V DC) were delivered to the grid floor by an isolated stimulator (Grass S44, Quincy, MA, USA).

For testing, each mouse was gently placed on the wooden platform. When the mouse stepped down from the platform and placed all its paws on the grid floor, intermittent electric shocks were delivered continuously for 15 s (Zarrindast et al., 2009; Zarrindast et al., 2008). This training procedure was carried out between 9:00 a.m. and 2:00 p.m. Twenty-four hours after training, each mouse was placed on the platform again, and the step-down latency was measured with a stop-watch as passive avoidance behavior. An upper cut-off time of 300 s was set. The retrieval test was also carried out between 9:00 a.m. and 2:00 p.m.

2.3. Exploratory behavior testing and apparatus

The hole-board test as a simple method for examining the response of an animal to an unfamiliar environment was first introduced by Boissier and Simon (1962). This test has been used to evaluate emotionality, anxiety and/or responses to stress in animals (Rodriguez Echandia et al., 1987). Different behaviors which can be observed and measured in this test, makes possible a comprehensive description of the animal's behavior. The hole-board apparatus (Borj Sanat Co, Tehran, Iran) consisted of gray Perspex panels (40 cm × 40 cm, 2.2 cm thick) with 16 equidistant holes 3 cm in diameter in the floor made on the basis of method used previously (Vinade et al., 2003). The board was positioned 15 cm above a table.

For anxiety testing, 5 min after memory testing, animals were placed singly in the center of the board facing away from the observer and head-dip numbers were recorded by photocells arranged below the holes over 5 min. Increase or decrease in head-dips indicated anxiolytic-like or anxiogenic-like behavior respectively. Furthermore, locomotor activity was measured by an observer unaware of the treatments measured during the testing phase. For this purpose the ground area of the hole-board were divided into four equal sized squares. Locomotion was measured as the number of locomotor activity crossings from one square to another. Other behavioural performance such as latency to the first head-dipping, rearing, grooming and defecation was recorded by the experimenter during the test, manually.

2.4. Drugs

The drugs used in the present study were HA (1-methyl-9H-pyridol [3,4-b]indole, C12H10N2) from Sigma (St. Louis, MO), D_1 receptor antagonist, SCH23390 (R(+)-7-chloro-8-hydroxy-3-methyl-1-phenyl-2,3,4,5-tetrahydro-1H-3-benzazepine hydrochloride) and D_2 receptor antagonist, sulpiride from Sigma (St Louis, CA, USA). The time of injection and doses of compounds used in the experiments were chosen according to published work in scientific literature (Rezayof et al., 2006; Zarrindast and Rezayof, 2004). All the compounds were tested at three doses: harmane (HA) 2.5, 5 and 10 mg/kg; SCH23390, 0.025, 0.05 and 0.1 mg/kg; sulpiride, 12.5, 25 and 50 mg/kg. HA was dissolved in sterile 0.9% NaCl solution and the compound was stirred for 1 h before obtaining the final solution; SCH23390 was dissolved in 0.9% physiological saline, just before the experiments. Sulpiride was dissolved in a minimal volume of diluted acetic acid (1 drop; 5 µl; pH: 6.3 by Hamilton micro-syringe 10 µl) and made up to a volume of 5 ml with 0.9% physiological saline and was then diluted to the required volume with saline (0.9%w/v NaCl solution).

2.5. Drug treatment

Ten animals were used in each experimental group. In experiments where animals received two injections, control groups received either two saline (10 ml/kg) or vehicle (10 ml/kg) injections. Moreover, control groups for HA and SCH23390 treatment in Experiments 1 and 2 received saline 0.9% physiological saline while control groups for sulpiride in the Experiment 3 received 0.9% physiological saline or vehicle (PH = 7.2; solution used for sulpiride). All drugs were injected intraperitoneally (i.p.) in a volume of 10 ml/kg. Furthermore, the timing of the pre-test drug administration was selected based on pilot and previous studies (Rezayof et al., 2006; Zarrindast and Rezayof, 2004). The protocol has been summarized in Table 1.

2.5.1. Experiment 1: effects of post-training HA administration on memory consolidation and exploratory behaviors

In this experiment, four groups of animals received saline (10 ml/kg) or different doses of HA (2.5, 5 and 10 mg/kg, i.p.) immediately after training, and in the test's day all groups received saline (10 ml/